Eight New Anthocyanins, Ternatins C1−C5 and D3 and Preternatins A3 and C4 from Young *Clitoria ternatea* Flowers

Norihiko Terahara*

Department of Food Science and Technology, College of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan

Kenjiro Toki

Laboratory of Floriculture, College of Horticulture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan

Norio Saito

Chemical Laboratory, Meiji-gakuin University, Totsuka-ku, Yokohama 224, Japan

Toshio Honda

Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142, Japan

Toshiro Matsui and Yutaka Osajima

Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan

Received April 21, 1998

Eight new anthocyanins **1**−**8** (ternatins C1, C2, C3, C4, C5, and D3 and preternatins A3 and C4) were isolated from *Clitoria ternatea* flowers. By the application of chemical, UV−vis, and FABMS methods, the structures of **1**−**6** were postulated as delphinidin 3-malonylglucoside having 3′-GCGC-5′-G, 3′-GCGCG-5′-G, 3′-GC-5′-G, 3′-GCG-5′-G, 3′-G-5′-G, and 3′-GC-5′-GC, and compounds **7** and **8** as delphinidin 3-glucoside having 3′-GCG-5′-GCG and 3′-GCG-5′-G as side chains, respectively, in which Dp is delphinidin, G is D-glucose, and C is *p*-coumaric acid. The structures of the compounds **1**, **3**−**5**, and **7** were established completely by additional NMR methods.

Butterfly pea (*Clitoria ternatea* L.; Fabaceae) is a leguminous plant originally grown in Southeast Asia. Its flowers have a vivid blue color and a relatively large size, so it is used as an ornamental around the world. In Southeast Asia, the flower pigment is traditionally utilized as food colorant because of the high stability.[1−6]

Based on the flower developing period, *C. ternatea* flowers could be grouped into young and mature stage flowers. From mature flowers, nine anthocyanins (ternatins A1, A2, A3, B1, B2, B3, B4, D1, and D2) were isolated, and characterized as unique structural anthocyanins which were malonylated delphinidin 3,3′,5′-triglucosides having a series of 3′,5′-side chains with alternating D-glucose (G) and *p*-coumaric acid (C) units.[7−13] According to terminal patterns of the 3′,5′-side chains, ternatins were categorized into three groups, namely ternatin A series with -G and -G, ternatin B series with -G and −C, and ternatin D series with −C and −C. Their hydrophilicities depended on the glucose numbers of the 3′,5′-side chain's terminals such as an order of ternatin A series > B series > D series, which was parallel to the elution order in ODS−HPLC system.[14] These ternatins were very stable in aqueous solution, i.e., they retained the colors for about 20 days (half-life of T-B4) to about 90 days (half-life of T-D1) in neutral aqueous solution.[15] Compositions of anthocyanins in young and mature flower extracts were compared by HPLC analysis. Both extracts contained 20 or more common anthocyanins (Figure 1), but the contents of early eluted pigments in young flower extract were relatively higher than mature one. Since these anthocyanins had not been characterized yet, we tried to determine their structures through combination of chemical analyses, UV−vis, FABMS, and ^{1}H and ^{13}C NMR spectroscopies.

Results and Discussion

Young flowers of *C. ternatea* were extracted with 5% AcOH, and the extract was purified by column chromatographies such as XAD-2000, PVP, and preparative ODS−HPLC, successively. Finally, pigments **1**−**8** were isolated as reddish purple powders of trifluoroacetic acid (TFA) salts. On acid hydrolysis, **1**−**8** gave delphinidin (Dp) as the aglycon and D-glucose (G) as the sugar. When $AlCl_3$ was added to each 0.01% HCl−MeOH solutions of **1**−**8**, no bathochromic shift of the visible absorption maxima (λ_{VISmax}) around 530 nm was observed despite anthocyanins based on Dp with three vicinal OHs on B-ring. This suggested that 3′- and 5′-OH on the aglycon B-ring of **1**−**8** were both substituted. On alkaline hydrolysis, all pigments gave Dp 3,3′,5′-triglucoside (preternatin C5 (PT-C5)) as a completely deacylated ternatin, and **1**, **2**, **4**, **7**, and **8** gave 4-glucosyl-*p*-coumaric acid (CG) and **1**−**6** gave malonic acid (M) as the acyl components. Additionally, **1**, **3**, and **6** gave *p*-coumaric acid (C), indicating that C was linked through the ester bond to at least one terminal of 3′- and 5′-side chains of each ternatin.[8] In the UV region, **1**−**4** and **6**−**8** had large absorption due to acylation with *p*-coumaric acid(s) but **5** had no absorption. The number(s) of *p*-coumaryl residue(s) were estimated from E_{310}/E_{VISmax} values (the ratio of absorbance at 310 nm to absorbance at

* To whom correspondence should be addressed. Tel.: +81-983-22-6615. Fax: +81-983-22-0295. E-mail: terahara@nankyudai.ac.jp.

10.1021/np980160c CCC: $15.00
Published on Web 10/03/1998

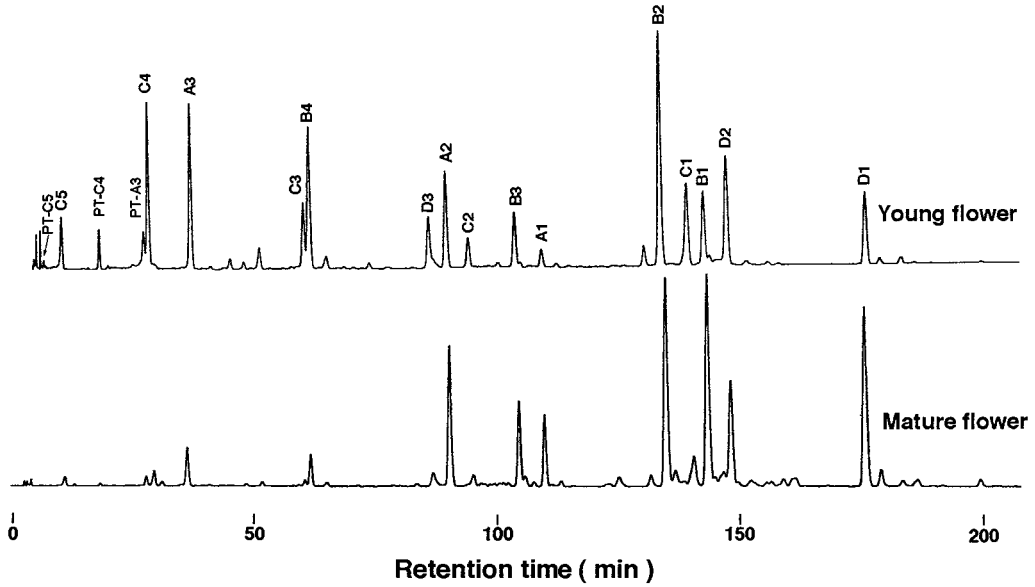

Figure 1. HPLC Chromatograms of the crude extract from *C. ternatea* young and mature flowers. (HPLC was run on an Inertsil ODS-2 column at 35 °C at 530 nm.)

λ_{VISmax}, and the ratios of nonacylated ternatin and *p*-coumaric acid itself were about 30% and 60%, respectively). The E_{310}/E_{VISmax} values indicated that **1**, **2**, **6**, and **7** had two molecules of C, **3**, **4**, and **8** had one molecule of C, and **5** had none of C, respectively.[16] On H_2O_2 oxidation, **1**−**6** gave 6-malonylglucose (MG), and **7** and **8** gave glucose (G) showing that **1**−**6**, **7**, and **8** had MG and G on Dp 3-position, respectively. Moreover, the fragment ions (M − G − malonate)$^+$ such as *m/z* 1081, 1233, 773, 935, 627, and 919 in respective FABMS spectra of **1**−**6** suggested the presence of MG residue in these ternatin molecules. However corresponding fragment ions were not observed in the spectra of **7** and **8**. The pigments **1**−**8** gave the clear molecular ion (M)$^+$ peaks *m/z* 1329, 1491, 1021, 1183, 875, 1167, 1405, and 1097 as the flavylium cations corresponding to $C_{60}H_{65}O_{34}^+$, $C_{66}H_{75}O_{39}^+$, $C_{45}H_{49}O_{27}^+$, $C_{51}H_{59}O_{32}^+$, $C_{35}H_{43}O_{25}^+$, $C_{54}H_{55}O_{29}^+$, $C_{63}H_{73}O_{36}^+$, and $C_{48}H_{57}O_{29}^+$, respectively. Moreover, the molecular ions decayed via three common routs to give fragments (M − G)$^+$, (M − G − malonate)$^+$, and (M − G − C)$^+$. These results showed that **5** was malonylated PT-C5, **6** had additional C and C on both side chains, and **1**−**4** had additional single side chain CGC, CGCG, C, and CG, respectively. Pigments **8** and **7** was composed of PT-C5 with one CG, and with two CGs, respectively. The amounts of pigments **2**, **6**, and **8** were too small to examine the next NMR experiment.

The precise 2D structures of **1**, **3**, **4**, **5**, and **7** were established through ^{1}H and ^{13}C NMR containing DQF−COSY, NOE difference spectroscopy (NOEDS), HOHAHA, HSQC, and HMBC techniques in DMSO-d_6: CF_3COOD = 9:1. One-dimensional ^{1}H NMR spectral data (Table 1) showed the separated proton signals of Dp and C in the low magnetic field region (δ_H >6 ppm) while overlapped signals of Gs and M were observed in the high magnetic field region (δ_H <6 ppm). Characteristic singlets of Dp ring protons appeared in the range of δ_H 6.60−8.92 ppm, in which singlet peaks of 2′ and 6′ protons on the Dp B ring of **5** and **7** were fused, but those of **1**, **3**, and **4** were split. This suggested that **5** and **7** have identical side chains on positions 3′ and 5′, whereas in the other compounds these are different, because the different 3′,5′-side chain structures brought a large extent nonequivalent environment around 2′,6′ protons.[12] One (**3**, **4**) and two (**1**, **7**) pair(s) of olefinic doublet signals with large coupling constants (J = 16 Hz) indicated for all Cs in **1**, **3**, **4**, and **7** to be trans (*E*) form. Assignment of aglycon and C protons was carried out by the aid of DQF−COSY, and sugar protons were assigned by DQF−COSY and HOHAHA techniques. In the high magnetic field region, four (**1**, **4**), three (**3** and **7**: G_a + two pairs of equivalent glucoses (G_b/G_c and G_d/G_e)), and two (**5**: G_a + one pair of equivalent glucoses (G_b/G_c)) downfield-shifted anomeric doublets and the ring proton signals with large coupling constants (J > 7 Hz) demonstrated all sugar components to be present as β-D-glucopyranosyl form in each pigment molecule. As one (**5**), two (**3**, **4**, **7**), and three (**1**) pairs of 6-methylene proton signals (6a and 6b) showed the downfield shifts (δ_H >4 ppm) arising from the proton deshielding, these sugars were proved to be acylated on the 6-CH_2OHs. In the highly overlapped region, the malonyl methylene protons of **1** and **3**−**5** but not **7** were observed as intense singlets at δ_H 3.39−3.42 ppm. Characteristic malonyl methylene carbons in **1** and **3**−**5** were also confirmed by ^{13}C NMR signals at δ_C 41.40, 41.30, 41.27, and 41.47 ppm, respectively.

Attachment positions of Gs and Cs in **1**, **3**−**5**, and **7** were ascertained by NOEDS. By irradiation of Dp-4 protons, NOEs were observed on the anomeric protons of glucose-a (G_a-1) in all pigments. Since for compounds **5**, **7** with identical 3′,5′-side chains, and **1**, **3**, and **4** with different ones, Dp-2′,6′ signals were fused and very close together in the spectra, thus their selective irradiation were impossible. So the irradiation at both Dp-2′ and -6′ signals resulted in enhancements at both G_b-1 and G_c-1 signals. This showed that G_a was linked through glycosyl bond to Dp 3-OH, and that G_b and G_c were linked to 3′-OH/ 5′-OH or 5′-OH/3′-OH, respectively. In **1**, **4**, and **7**, NOEs between C_I-3,5 and G_d-1 were observed but not in **3** and **5**. This showed that C_I-4-OH of **1** was glycosylated with G_d, i.e., a C_I-G_d unit was present in the Dp-3′-side chains, but **3** and **5** had no C_I-G_d unit. Irradiation on C_{II}-3,5 of **1** and C_I-3,5 of **3** did not furnish NOEs of anomeric protons in any glucoses, respectively. This explained that C_{II} of **1** and C_I of **3** attached at terminal position in each Dp-5′-side chain.

These findings were also verified by analogy between chemical shift values of *p*-coumaryl protons (olefinic-α,β, and ring-2,6 and -3,5 protons) in **1**, **3**, **4**, **7**, and the corresponding values of ternatin D1 (T-D1) bearing identical side chains 3′,5′-GCGC.[7,11,12] As depicted in Table 1,

Table 1. ^{1}H NMR Spectral Data of Ternatins and the Related Compounds (400−500 MHz, in DMSO-d_6−CF_3COOD = 9:1, δ ppm from TMS, and J Hz in Parentheses)[a]

H	T-C1 (1)	T−C3 (3)	T−C4 (4)	T−C5 (5)	PT-A3 (7)	T−D1[b]
Dp-4	8.75 (s)	8.70 (s)	8.71 (s)	8.92 (s)	8.54 (s)	8.59 (s)
Dp-2′	8.12 (s)	8.12 (s)	8.12 (s)	8.13 (s)	8.04 (s)	8.04 (s)
Dp-6′	8.09 (s)	8.03 (s)	8.08 (s)	8.13 (s)	8.04 (s)	8.04 (s)
Dp-6	7.00 (s)	6.97 (s)	7.00 (d, 1)	7.09 (brd, 2)	6.90 (s)	6.93 (s)
Dp-8	6.72 (d, 2)	6.72 (d,2)	6.69 (d, 2)	6.77 (brd, 2)	6.60 (s)	6.65 (s)
I-α	6.17 (d, 16)	6.09 (d, 16)	6.22 (d, 16)		6.18 (d, 16)	6.13 (d, 16)
II-α					6.18 (d, 16)	6.13 (d, 16)
III-α	6.31 (d,16)					6.31 (d,16)
IV-α						6.31 (d,16)
I-*β*	7.31 (d, 16)	7.22 (d, 16)	7.34 (d, 16)		7.30 (d, 16)	7.29 (d, 16)
II-*β*					7.30 (d, 16)	7.29 (d, 16)
III-*β*	7.45 (d, 16)					7.46 (d, 16)
IV-*β*						7.46 (d,16)
I-2&6	7.19 (d, 9)	7.05 (d, 9)	7.21 (d, 9)		7.20 (d, 8)	7.17 (d, 9)
II-2&6					7.20 (d, 8)	7.17 (d, 9)
III-2&6	7.42 (d, 9)					7.43 (d, 9)
IV-2&6						6.86 (d, 9)
I-3&5	6.88 (d, 9)	6.56 (d, 9)	6.85 (d, 9)		6.87 (d, 8)	6.86 (d, 9)
II-3&5					6.87 (d, 8)	6.86 (d, 9)
III-3&5	6.76 (d, 9)					6.75 (d, 9)
IV-3&5						6.86 (d, 9)
a-1	5.26 (d, 8)	5.22 (d, 8)	5.26 (d, 8)	5.41 (d, 8)	4.98 (d, 8)	5.08 (d, 8)
b-1	5.31 (d, 7)	5.31 (d, 7)	5.29 (d, 7)	5.07 (d, 7)	5.31 (d, 7)	5.27 (d, 7)
c-1	5.06 (d, 7)	5.07 (d, 7)	5.08 (d, 7)	5.07 (d, 7)	5.31 (d, 7)	5.27 (d, 7)
d-1	4.99 (d, 8)		4.93 (d, 8)		4.95 (d, 8)	4.96 (d, 8)
e-1					4.95 (d, 8)	4.96 (d, 8)
f-1						
a-2	3.58 (t, 7)	3.55 (t, 8)	3. 54 (t, 8)	3.49 (t, 8)	3.58 (t, 7)	
b-2	3.56 (t, 8)	3.48 (t, 8)	3.46 (m)	3.39 (m)	3.49 (t, 7)	
c-2	3.47 (m)	3.38 (t, 8)	3.39 (t, 7)	3.39 (m)	3.49 (t, 7)	
d-2	3.47 (m)		3.36 (t, 9)		3.33 (t, 8)	
e-2					3.33 (t, 8)	
f-2						
a-3	3.47 (m)	3.46 (t, 9)	3.46 (m)	3.43 (t, 8)	3.51 (t, 7)	
b-3	3.47 (m)	3.46 (t, 9)	3.46 (m)	3.43 (t, 8)	3.49 (t, 7)	
c-3	3.40 (m)	3.42 (m)	3.42 (m)	3.43 (t, 8)	3.49 (t, 7)	
d-3	3.40 (m)		3.42 (m)		3.38 (t, 9)	
e-3					3.38 (t, 9)	
f-3						
a-4	3.31 (m)	3.27 (t, 9)	3.29 (m)	3.23 (t, 9)	3.33 (t, 8)	
b-4	3.31 (m)	3.28 (t, 9)	3.29 (m)	3.28 (m)	3.32 (t, 8)	
c-4	3.31 (m)	3.26 (t, 9)	3. 26 (t, 9)	3.28 (m)	3.32 (t, 8)	
d-4	3.37 (t, 9)		3. 22 (t, 9)		3.24 (t, 9)	
e-4					3.24 (t, 9)	
f-4						
a-5	3.83 (m)	3.82 (m)	3.84 (t, 9)	3.84 (m)	3.40 (m)	
b-5	3.87 (m)	3.82 (m)	3.84 (t, 9)	3.57 (m)	3.87 (m)	
c-5	3.87 (m)	3.42 (m)	3.76 (m)	3.57 (m)	3.87 (m)	
d-5	3.74 (m)		3.76 (m)		3.43 (m)	
e-5					3.43 (m)	
f-5						
a-6a	4.18 (dd, 6, 12)	4.14 (dd, 8, 12)	4.14 (dd, 8, 12)	4.12 (dd, 8, 12)	3.32 (m)	
b-6a	4.22 (dd, 6, 12)	4.16 (dd, 8, 12)	4.16 (dd, 8, 13)	3.57 (m)	4.23 (m)	
c-6a	3.74 (m)	3.58 (dd, 8, 12)	3.46 (m)	3.57 (m)	4.23 (m)	
d-6a	4.08 (dd, 8, 12)		3.46 (m)		3.51 (m)	
e-6a					3.51 (m)	
f-6a						
a-6b	4.48 (brd, 11)	4.56 (brd, 11)	4.53 (brd, 11)	4.48 (brd, 10)	3.32 (m)	
b-6b	4.61 (brd, 11)	4.59 (brd, 10)	4.61 (brd, 11)	3.74 (brd, 10)	4.56 (brd, 11)	
c-6b	3.83 (m)	3.77 (brd, 10)	3.56 (m)	3.74 (brd, 10)	4.56 (brd,11)	
d-6b	4.46 (brd, 12)		3.56 (m)		3.79 (brd, 10)	
e-6b					3.79 (brd, 10)	
f-6b						
Ma-CH_{2-}	3.39 (s)	3.42 (s)	3.41 (s)	3.39 (s)		

[a] Abbreviations: T-D1, Dp, and Ma-CH_{2-}, ternatin D1, delphinidin, and malonyl methylene protons, respectively; s, d, t, m, dd, and brd, singlet, doublet, triplet, multiplet, double doublet, and broad doublet, respectively. [b] Only required values were cited from reference T-D1.[11]

chemical shifts of ring protons of C_I in **4**, C_I and C_{III} in **1**, and C_I and C_{II} in **7** agreed with one another within δ_H 0.09 ppm and also with those of T-D1, showing that the chemical environments around the side chains of **1**, **4**, and **7** were similar to those of T-D1. Therefore, C_I (and also C_{II} of **7**) was located on the inner site of the side chain(s) and C_{III} of **1** on the outer site, i.e., **1**, **4**, and **7** were confirmed to have the partial connectivities of 3′-GC_IGC_{III}, 3′-GC_IG, and 3′,5′-di-GC_IG, respectively. However C_I protons in **3** are considerably different from those of T-D1 with maximum

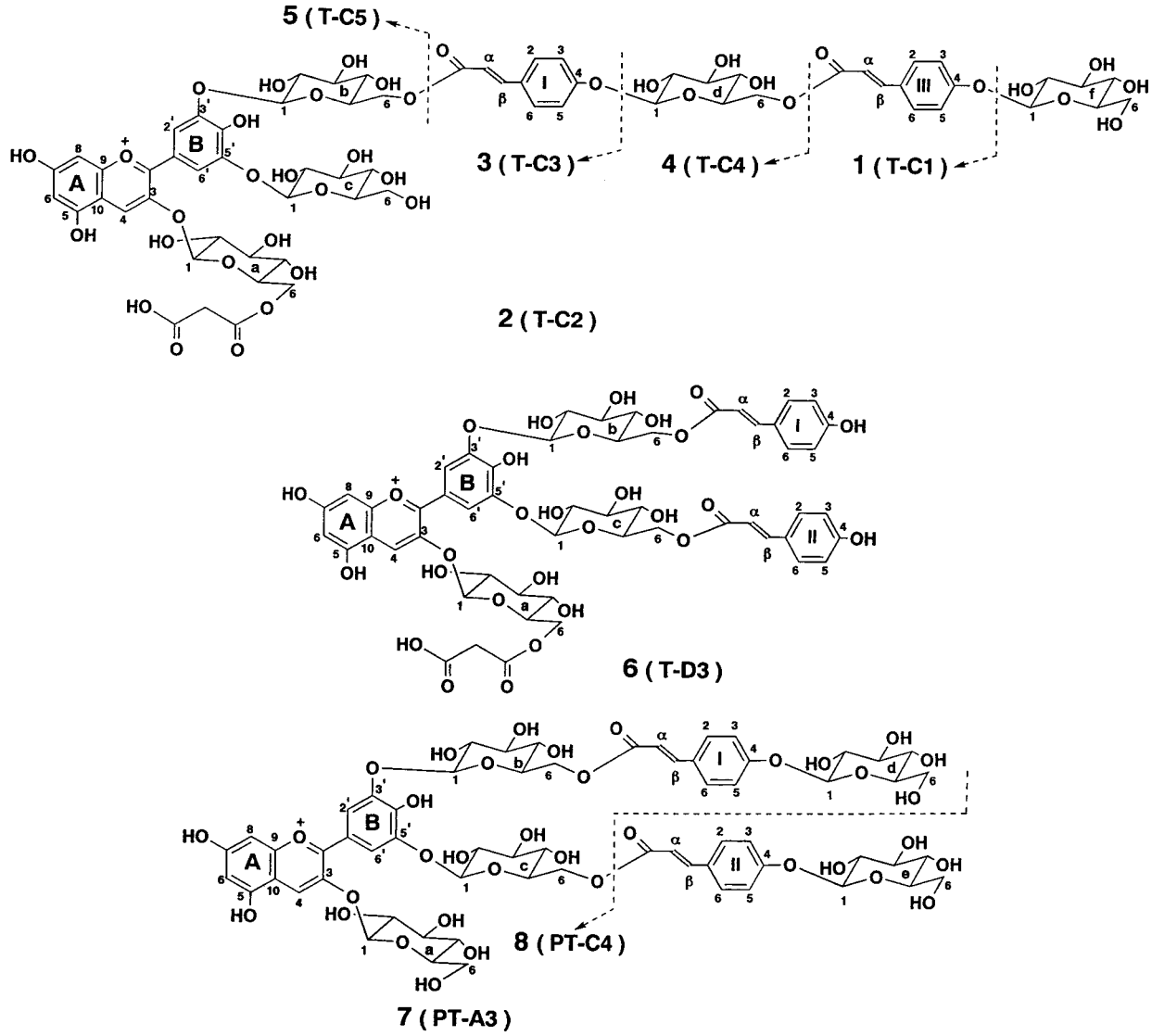

Figure 2. Structures of ternatins C1−C5, D3, and preternatins A3 and C4.

−0.30 ppm, indicating C_I of **3** to be located on a terminal position in the 3′-side chain with a different environment.

Consequently, structures of **1**−**6** have been estimated as delphinidin 3-malonylglucosides having 3′-GCGC-5′-G, 3′-GCGCG-5′-G, 3′-GC-5′-G, 3′-GCG-5′-G, 3′-G-5′-G, and 3′-GC-5′-GC, and **7** and **8** as delphinidin 3-glucosides having 3′-GCG-5′-GCG and 3′-GCG-5′-G side chains, respectively. Moreover **1**, **3**, **4**, **5**, and **7** were unambiguously determined as 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*-*β*-D-glucopyranosyl delphinidin, 3-*O*-(6-*O*-malonyl-*β*-D-glucopyranosyl)-3′-*O*-(6-*O*-((*E*)-4-*O*-*p*-coumaryl)-*β*-D-glucopyranosyl)-5′-*O*-*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*-*β*-D-glucopyranosyl delphinidin, 3-*O*-(6-*O*-malonyl-*β*-D-glucopyranosyl)-3′,5′-di-*O*-*β*-D-glucopyranosyl delphinidin, and 3-*O*-(6-*O*-*β*-D-glucopyranosyl)-3′,5′-di-*O*-(6-*O*-((*E*)-4-*O*-*β*-D-glucopyranosyl-*p*-coumaryl)-*β*-D-glucopyranosyl) delphinidin, respectively, the new ternatins (Figure 2). Pigment **1** and ternatin B4, and pigment **2** and ternatin A3, are isomers each other, respectively.

Anthocyanins **1**−**5** were named as ternatins C1−C5 (T-C1−C5), respectively, because they had no *p*-coumaryl residue(s) on one (5′-)side chain which were classified new ternatin C series. Pigments **7** and **8** were named as preternatins A3 and C4 (PT-A3 and -C4), since these were corresponding to demalonylated analogues of known T-A3 and T-C4, and they were also considered as the precursors for T-A3 and T-C4 biosynthesis. Pigment **6** was named as ternatin D3 (T-D3) belonging to T-D group with Cs both 3′,5′-side chain terminals. Ternatin C series and preternatins are a new type of pigments found for the first time in *C. ternatea* flowers.

Conformational structures of T-C1 (**1**), C3 (**3**), C4 (**4**), and PT-A3 (**7**) in solutions was also deduced from NOE and chemical shift data referring to T-D1. In the ^{1}H NMR spectra, weak NOEs were also observed between Dp-4 proton and C_I (and C_{II}) ring and olefinic protons which were present in the inner site of the 3′,5′-side chains. Moreover, the C_I (and C_{II}) ring protons shifted to upper field than those of outer C_{III} in T-C1 (Table 1). This tendency was clarified by a comparison of the chemical shift for *p*-coumaryl moieties of ternatins with 4-glucosyl-*p*-coumaric acid as the reference, which was a simple molecule[12] as shown in Table 2. Whereas chemical shift difference ($\Delta\delta_H$) values of sugar region were slightly plus (downfield shift) or 0 ppm (data not shown). Delphinidin ring protons of T-C1 (**1**), C3 (**3**), C4 (**4**), PT-A3 (**7**), and T-D1 but not T-C5 (**5**) were also observed with upper field shift from corresponding those of PT-C5. These phenomena could be attributable to diamagnetic anisotropy shielding effect of

Table 2. Proton Chemical Shift Differences between Ternatins and Related Compounds

	Δδ (proton chemical shift differences, ppm)						δ (ppm)	
H	T-C1 (**1**)	T-C3 (**3**)	T-C4 (**4**)	T-C5 (**5**)	PT-A3 (**7**)	T-D1[a]	PT-C5[a]	CG[a]
				Delphinidin moiety				
Dp-4	−0.29	−0.34	−0.33	−0.12	−0.50	−0.45	9.04	
Dp-2′	−0.05	−0.05	−0.05	−0.04	−0.13	−0.13	8.17	
Dp-6′	−0.08	−0.14	−0.09	−0.04	−0.13	−0.13	8.17	
Dp-6	−0.11	−0.14	−0.11	−0.02	−0.21	−0.18	7.11	
Dp-8	−0.05	−0.05	−0.08	0.00	−0.17	−0.12	6.77	
				p-Coumaryl moiety				
I-a	−0.21	−0.29	−0.16		−0.20	−0.25		6.38
II-a					−0.20	−0.25		
III-a	−0.07					−0.07		
IV-a						−0.07		
I-b	−0.2	−0.29	−0.17		−0.21	−0.22		7.51
II-b					−0.21	−0.22		
III-b	−0.06					−0.05		
IV-b						−0.05		
I-2&6	−0.41	−0.55	−0.39		−0.40	−0.43		7.6
II-2&6					−0.40	−0.43		
III-2&6	−0.18					−0.17		
IV-2&6						−0.17		
I-3&5	−0.16	−0.48	−0.19		−0.17	−0.18		7.04
II-3&5					−0.17	−0.18		
III-3&5	−0.28					−0.29		
IV-3&5						−0.29		

[a] Abbreviations: T-D1, PT-C5, CG, and Dp, ternatin D1, preternatin C5, 4-glucosyl-*p*-coumaric acid, and delphinidin, respectively. Chemical shift data were referred to T-D1,[11] PT-C5,[13] and CG.[8]

aromatic ring currents based on orientating the C_I (and also C_{II} in PT-A3) rings and the olefinic moieties above (and also below in PT-A3 and T-D1) the Dp ring as reported in the literature.[3] The fact proved the presence of an intramolecular (sandwich type in PT-A3 and T−D1) stacking between Dp ring and C_I (and also C_{II} in PT-A3 and T-D1) rings on the 3′,5′-side chains in T-C1, -C3, -C4, and PT-A3 solutions, and it blocks nucleophilic hydration of carbons on Dp-2 and -4 positions[17,18] leading to the colorless pseudobases.[19] Indeed, these ternatins showed higher stability in neutral aqueous solution than non-*p*-coumaryl analogues, T-C5 and PT-C5 (data not shown).

Possible ternatin biosynthesis was considered on the basis of the structures of isolated pigments and other known ternatins.[7−13] Now, fifteen ternatins are known in which T-C2 and -D3 have been partially determined described above. We could arrange them on the basis of the structural patterns of 3′,5′-side chains as illustrated in Figure 3. Ternatin C5 can be regarded as a starting material, T-A1 as a final compound and other ternatins as intermediates. Ternatin C5 is synthesized by acylation with malonic acid of PT-C5 served from flavonoid synthesis pathway and following glucosylation steps.[20,21] The ternatin pathway might begin acylation with *p*-coumaric acid of T-C5 and finally reach to T-A1 via 8 steps (four acylation with *p*-coumaric acid and four glucosylation steps). Especially the occurrence and amounts of low molecular weight ternatins may be more enhanced at young flower stage. This proposal is supported by the fact that the smaller molecular weight ternatins (T-C5, -C3, -C4, -A3, -B4, -B3, and -D3) are relatively superior in young flowers, while larger molecular weight ternatins (T-A2, -B1, -B2, -D1, -D2, -B3, -B3, and -A1) are dominant in mature flowers.

Simultaneously, preternatin transformations might occur through acylation with *p*-coumaric acid and glucosylation steps as the similar process of ternatins as shown in Figure 3. All fifteen preternatins may be contained in both young and mature flowers, since there are many small peaks regarding as preternatins in HPLC chromatograms. However they are hard to isolate and identify due to lower concentration, so structures of only PT-A3, -C5, and -D1 have been completely determined and PT-C2, -C4, and -D2 have been tentatively determined (PT-D1 and -D2: data not shown). Except acylation with *p*-coumaric acid and glucosylation, acylation with malonic acid of preternatins to corresponding ternatins occurs and is elucidated to perform on almost intermediate preternatins at appropriate timing. Production and transformation of low molecular weight preternatins may be also more active at young flower stage like ternatins. These pathways for ternatins and preternatins need to be confirmed by further biosynthetic investigations.

Experimental Section

General Experimental Procedures. TLC was carried out as noted in a previous publication[8] and open column chromatographies were applied on XAD-2000 (Amberlite) and PVP (poly(vinylpyrrolidone), Polyclar AT, GAF chemicals Co.). HPLC was performed on an L-6200 Intelligent pump system (Hitachi). Analytical HPLC was run on Inertsil ODS-2 column (4.6 i.d. × 50 mm + 4.6 i.d. × 250 mm, GL Sciences Inc.) 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 done on Inertsil ODS column (20 i.d. × 250 mm, GL Sciences Inc.) with 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 = 63:37−96:4 at 530 nm. 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. ^1H (400 or 500 MHz) and ^{13}C (100 or 125 MHz) NMR spectra were run on Alpha-400 (JEOL) or JMN GX-500 (JEOL) in DMSO-d_6: CF_3COOD = 9:1 with TMS as the internal standard.

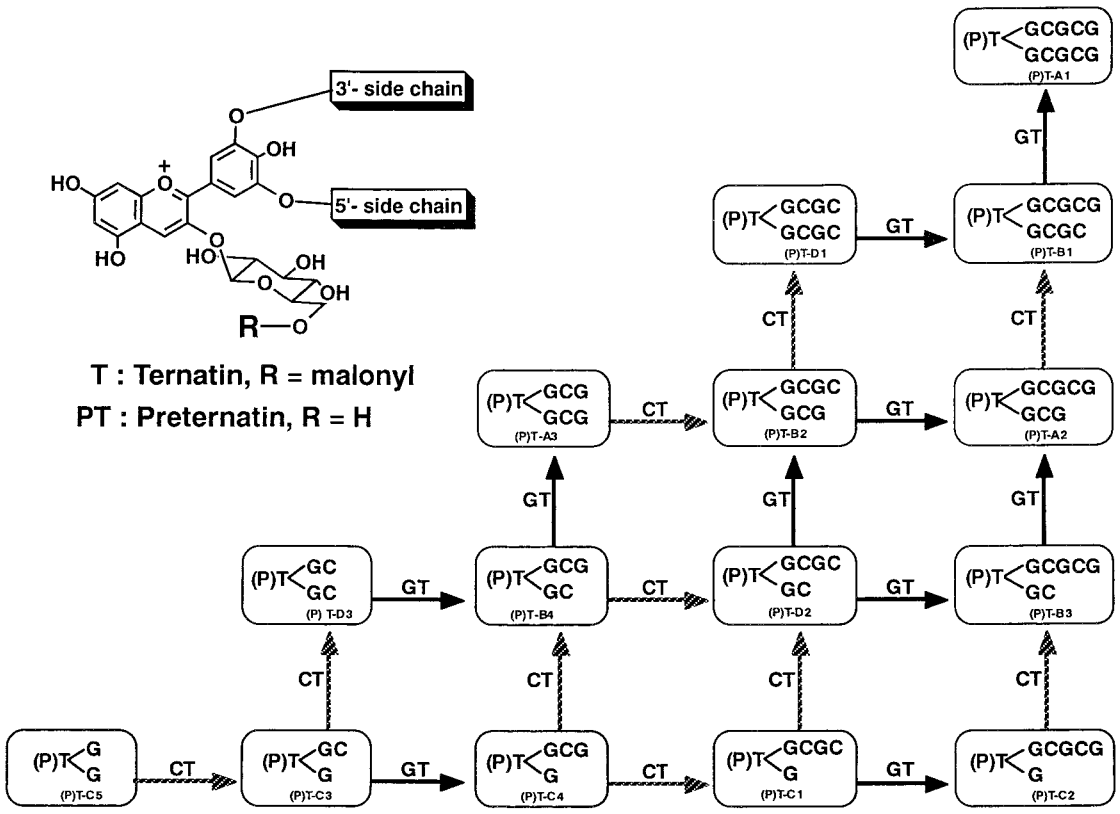

Figure 3. Possible ternatin and preternatin biosynthetic pathways. (Abbreviations: T, ternatin; PT, preternatin; G, D-glucose; C, *p*-coumaric acid; GT, glucosylation; CT, *p*-coumaric acid acylation. Symbolized structures in the scheme indicate 3′,5′-side chain constructions of corresponding ternatins or preternatins.)

Plant Materials. *C. ternatea* L. were grown in a farm of Minami-Kyushu University and the young and mature stage flower petals were collected during July and October 1996. They were dried at 45 °C overnight, and stored in a silica gel desiccator until use for extraction.

Isolation of Pigments. The dried petals of young flowers (25 g) were soaked in 1 L of 5% AcOH overnight and filtered. This operation was done three times (1 L × 3). The combined extract was adsorbed on XAD-2000 resin column (45 i.d. × 300 mm), washed with 2 L of 1% AcOH and eluted with 1 L of 1% AcOH in 70% EtOH. After evaporation, the residue was dissolved in 0.1% TFA−EtOH = 6:4 and chromatographed on a Polyclar AT column (45 i.d. × 230 mm) in the same solvent. Three fractions (fr) were obtained in elution order, in which preternatins A3 (**7**) and C4 (**8**), ternatins C2 (**2**), C4 (**4**), and A series (T-A1, -A2, and -A3) were contained in fr 1, and ternatins C4 (**4**) and C5 (**5**) and B series (T-B1, -B2, -B3, and -B4) in fr 2, and ternatins C1 (**1**), C3 (**3**) and D series (T-D1, -D2, and -D3 (**6**)) in fr 3, respectively. Each fraction was finally purified and isolated twice by preparative ODS−HPLC using an AcOH solvent system. Eight isolated anthocyanin fractions were evaporated to dryness in vacuo, dissolved in a small amount of TFA, and precipitated with excess Et_2O to give TFA salts of T-C1 (**1**, 25 mg), T-C2 (**2**, 3 mg), T-C3 (**3**, 11 mg), T-C4 (**4**, 31 mg), T-C5 (**5**, 17 mg), and T-D3 (**6**, 3 mg), and PT-A3 (**7**, 20 mg) and PT-C4 (**8**, 2 mg) as reddish purple powders.

Chemical Analyses. Acid and alkaline hydrolyses and H_2O_2 oxidation of isolated ternatins were performed according to the previous methods.[8]

Ternatin C1 (1). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 537 (not shifted bathochromically with $AlCl_3$), 288, $E_{440}/E_{VIS} = E_{440}/E_{537} = 31\%$, $E_{UV}/E_{VIS} = E_{288}/E_{537} = 187\%$, $E_{310}/E_{537} = 152\%$ (acyl = 2: *p*-coumaryl residue number(s) estimated from E_{310}/E_{VIS} values). FABMS: *m/z* 1329 ($M = C_{60}H_{65}O_{34}$)$^+$, 1243 (M − malonate)$^+$, 1167 (M − G)$^+$, 1081 (M − G − malonate)$^+$, 1021 (M − G − C)$^+$, 919 (M − 2G − malonate)$^+$, 713 (M − 2G − 2C)$^+$, 613. ^{13}C NMR: 168.23 (malonyl C═O), 166.73 (C_{II} C═O), 167.29 (Dp-7), 166.45 (C_I C═O), 160.16 (C_{II}-4, Dp-2), 160.56 (Dp-9), 158.98 (C_I-4), 155.66 (Dp-5), 146.36 (Dp-3′), 146.05 (Dp-5′), 145.21 (Dp-4′), 145.08 (C_{II}-β), 144.38 (Dp-3), 143.79 (C_I-β), 135.00 (Dp-4),130.44 (C_{II}-2,6), 129.78 (C_I-2,6), 127.70 (C_I-1), 125.25 (C_{II}-1), 118.54 (Dp-1′), 116.78 (C_I-α), 116.15 (C_I-3,5, C_{II}-3,5), 115.41 (C_{II}-α′), 114.38 (Dp-6′), 113.55 (Dp-2′), 112.58 (Dp-10), 103.10 (Dp-6), 102.37 (G_c-1), 102.13 (G_a-1), 100.66 (G_b-1), 99.93 (G_d-1), 94.69 (Dp-8), 77.72 (G_b-2), 76.59 (G_c-2), 73.65 (G_b-3), 73.44 (G_a-3), 73.22 (G_c, G_d-3), 70.48 (G_c-4), 70.25 (G_a-4), 69.96 (G_b-4), 69.84 (G_d-4), 67.79 (G_d-6), 64.13 (G_b-6), 63.58 (G_a-6), 60.95 (G_c-6), 41.40 (malonyl -CH_2-).

Ternatin C2 (2). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 538 (not shifted bathochromically with $AlCl_3$), 285, $E_{440}/E_{VIS} = E_{440}/E_{538} = 29\%$, $E_{UV}/E_{VIS} = E_{286}/E_{538} = 204\%$, $E_{310}/E_{538} = 156\%$ (acyl = 2). FABMS: *m/z* 1491 ($M = C_{66}H_{75}O_{39}$)$^+$, 1405 (M − malonate)$^+$, 1329 (M − G)$^+$, 1233 (M − G − malonate)$^+$, 1021 (M − 2G − C)$^+$, 763, 522.

Ternatin C3 (3). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 535 (not shifted bathochromically with $AlCl_3$), 282, $E_{440}/E_{VIS} = E_{440}/E_{535} = 35\%$, $E_{UV}/E_{VIS} = E_{282}/E_{535} = 113\%$, $E_{310}/E_{535} = 88\%$ (acyl = 1). FABMS: *m/z* 1021 ($M = C_{45}H_{49}O_{27}$)$^+$, 935 (M − malonate)$^+$, 859 (M − G)$^+$, 773 (M − G − malonate)$^+$, 713 (M − G − C)$^+$, 611 (M − 2G − C)$^+$, 459. ^{13}C NMR: 168.11 (malonyl C═O), 167.24 (Dp-7), 166.33 (C_I C═O), 160.08 (C_I-4), 159.73 (Dp-2), 154.78 (Dp-9), 155.93 (Dp-5), 146.33 (Dp-3′), 145.65 (Dp-5′), 144.53 (Dp-4′), 144.24 (Dp-3), 144.18 (C_I-β), 134.95 (Dp-4), 129.76 (C_I-2,6), 124.79 (C_I-1), 118.43 (Dp-1′), 115.69 (C_I-3,5), 114.31 (C_I-α), 113.72 (Dp-6′), 113.34 (Dp-2′), 112.48 (Dp-10), 102.21 (G_a-1, G_c-1), 102.06 (Dp-6), 100.08 (G_b-1), 94.75 (Dp-8),

73.48 (G_b-3), 73.41 (G_a-3), 73.23 (G_c-3), 70.61 (G_c-4), 69.78 (G_a-4, G_b-4), 63.56 (G_a-6, G_b-6), 60.86 (G_c-6), 41.30 (malonyl -*C*H_2-).

Ternatin C4 (4). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 534 (not shifted bathochromically with $AlCl_3$), 281, $E_{440}/E_{VIS} = E_{440}/E_{534} = 34\%$, $E_{UV}/E_{VIS} = E_{281}/E_{534} = 129\%$, $E_{310}/E_{534} = 91\%$ (acyl = 1). FABMS: *m*/*z* 1183 (M = $C_{51}H_{59}O_{32}$)$^+$, 1097 (M − malonate)$^+$, 1021 (M − G)$^+$, 935 (M − G − malonate)$^+$, 875 (M − G − C)$^+$, 1021 (M − 2G − C)$^+$, 613, 459. ^{13}C NMR (100 MHz): 168.04 (malonyl C═O), 167.16 (Dp-7), 166.22 (C_I C═O), 160.27 (Dp-2, Dp-9), 159.31 (C_I-4), 157.25 (Dp-5), 146.23 (Dp-3′), 145.75 (Dp-5′), 144.89 (Dp-4′), 144.19 (Dp-3), 143.68 (C_I-β), 134.97 (Dp-4), 129.47 (C_I-2,6), 127.46 (C_I-1), 118.43 (Dp-1′), 116.62 (C_I-3,5), 115.95 (C_I-α), 113.32 (Dp-6′), 112.37 (Dp-2′), 102.18 (G_c-1), 102.17 (Dp-6), 101.99 (G_a-1), 100.33 (G_b-1), 100.21 (Dp-10), 99.88 (G_d-1), 94.67 (Dp-8), 74.44 (G_b-3), 73.46 (G_a-3), 73.30 (G_c, G_d-3), 70.35 (G_c-4), 69.85 (G_a-4), 69.72 (G_b, G_d-4), 64.59 (G_a-6), 63.68 (G_b-6), 60.93 (G_c-6), 60.80 (G_b-6), 41.27 (malonyl -*C*H_2-).

Ternatin C5 (5). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 523 (not shifted bathochromically with $AlCl_3$), 277, $E_{440}/E_{VIS} = E_{440}/E_{523} = 32\%$, $E_{UV}/E_{VIS} = E_{277}/E_{523} = 77\%$, $E_{310}/E_{523} = 20\%$ (acyl = 0). FABMS: *m*/*z* 875 (M = $C_{36}H_{43}O_{25}$)$^+$, 789 (M − malonate)$^+$, 713 (M − G)$^+$, 627 (M − G − malonate)$^+$, 613, 459. ^{13}C NMR: 167.94 (malonyl C═O), 167.04 (malonyl C═O), 166.97 (Dp-7), 161.30 (Dp-2), 156.09 (Dp-9), 153.74 (Dp-5), 146.05 (Dp-3′,5′), 143.91 (Dp-4′,3), 135.68 (Dp-4), 130.64 (Dp-1′), 114.83 (Dp-2′,6′), 112.52 (Dp-10), 107.28 (Dp-6), 101.91 (G_b-5, G_c-5), 101.82 (G_a-1), 101.50 (G_b-1, G_c-1), 97.57 (Dp-8), 77.39 (G_a-5), 76.19 (G_a-2), 76.06 (G_b-2, G_c-2), 74.34 (G_a-3), 73.40 (G_b-3, G_c-3), 69.61 (G_a-4), 69.14 (G_b-4, G_c-4), 64.57 (G_a-6), 60.18 (G_b-6, G_c-6), 41.47 (malonyl -*C*H_2-).

Ternatin D3 (6). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 544 (not shifted bathochromically with $AlCl_3$), 299, $E_{440}/E_{VIS} = E_{440}/E_{544} = 34\%$, $E_{UV}/E_{VIS} = E_{299}/E_{544} = 159\%$, $E_{310}/E_{544} = 149\%$ (acyl = 2). FABMS: *m*/*z* 1167 (M = $C_{54}H_{55}O_{29}$)$^+$, 1081 (M − malonate)$^+$, 1021 (M − C)$^+$, 919 (M − G − malonate)$^+$, 859 (M − G − C)$^+$, 611 (M − 2G − C)$^+$.

Preternatin A3 (7). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 543 (not shifted bathochromically with $AlCl_3$), 286, $E_{440}/E_{VIS} = E_{440}/E_{543} = 33\%$, $E_{UV}/E_{VIS} = E_{286}/E_{543} = 186\%$, $E_{310}/E_{543} = 150\%$ (acyl = 2). FABMS: *m*/*z* 1405 (M = $C_{63}H_{73}O_{36}$)$^+$, 1243 (M − G)$^+$, 1097 (M − G − C)$^+$, 1081 (M − 2G)$^+$, 935 (M − 2G − C)$^+$, 773 (M − 3G − C)$^+$. ^{13}C NMR: 166.36 (C_I, C_{II} C═O), 159.54 (Dp-7), 159.09 (C_I, C_{II}-4, Dp-9), 157.40 (Dp-2), 155.54 (Dp-5), 145.98 (Dp-3′,5′), 144.29 (Dp-4′, Dp-3), 143.86 (C_I, C_{II}-β), 134.88 (Dp-4), 130.38 (Dp-1′), 129.74 (C_I, C_{II}-2,6), 119.55 (C_I, C_{II}-1), 116.44 (C_I, C_{II}-3,5), 115.92 (C_I, C_{II}-α), 112.48 (Dp-2′,6′), 112.01 (Dp-10), 108.17 (Dp-6), 102.30 (G_a-1), 100.41 (G_b, G_c-1), 99.99 (G_d-1, G_e-1), 94.50 (Dp-8, G_b-5, G_c-5), 77.87 (G_a-5), 77.21 (G_d-5, G_e-5), 76.69 (G_b-2, G_c-2), 76.47 (G_a-2), 75.96 (G_d-2, G_e-2), 74.57 (G_d-3, G_e-3), 73.44 (G_a-3, G_b-3, G_c-3), 70.46 (G_b-4, G_c-4), 69.92 (G_a-4, G_d-4, G_e-4), 63.77 (G_b-6, G_c-6), 61.20 (G_a-6), 60.98 (G_d-6, G_e-6).

Preternatin C4 (8). UV−vis λ_{max} (0.01% HCl−MeOH) nm: 532 (not shifted bathochromically with $AlCl_3$), 282, $E_{440}/E_{VIS} = E_{440}/E_{532} = 35\%$, $E_{UV}/E_{VIS} = E_{282}/E_{532} = 139\%$, $E_{310}/E_{532} = 95\%$ (acyl = 1). FABMS: *m*/*z* 1097 (M = $C_{48}H_{57}O_{29}$)$^+$, 935 (M − G)$^+$, 773 (M − 2G)$^+$, 627 (M − 2G − C)$^+$, 611 (M − 3G)$^+$, 459.

Acknowledgment. We are grateful to Mr M. Seki and Mr T. Arakawa of Minami-Kyushu University for their assistance of our experiment.

References and Notes

(1) Saito, N.; Abe, K.; Honda, T.; Timberlake, C. F.; Bridle, P. *Phytochemistry* **1985**, *24*, 1583−1586.
(2) Goto, T. *Fortschr. Chem. Org. Naturst.* **1987**, *52*, 114−158.
(3) Goto, T.; Kondo, T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 17−33.
(4) Lowry, J. B.; Chew, L. *Econ. Bot.* **1974**, *28*, 61−62.
(5) Tulyathan, V.; Duangmal, K.; Thunpittayakul, C. *Thai J. Agric. Sci.* **1993**, *26*, 171−183.
(6) Tulyathan, V.; Duangmal, K.; Thunpittayakul, C. *Thai J. Agric. Sci.* **1994**, *27*, 151−162.
(7) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Tetrahedron Lett.* **1990**, *31*, 2921−2924.
(8) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Phytochemistry* **1990**, *29*, 949−953.
(9) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Phytochemistry* **1990**, *29*, 3686−3687.
(10) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Heterocycles* **1990**, *31*, 1773−1776.
(11) Terahara, N.; Saito, N.; Honda, T.; Toki, K.; Osajima, Y. *Tetrahedron Lett.* **1989**, *30*, 5305−5308.
(12) Kondo, T.; Ueda, M.; Goto, T. *Tetrahedron* **1990**, *46*, 4749−4756.
(13) Terahara, N.; Oda, M.; Matsui, T.; Osajima, Y.; Saito, N.; Toki, K.; Honda, T. *J. Nat. Prod.* **1996**, *59*, 139−144.
(14) Amic, D.; Davidovic-Amic, D.; Trinajstic, N. *J. Chromatogr.* **1993**, *653*, 115−121.
(15) Terahara, T. *Bull. Fac. Hortic. Minami-Kyushu Univ.* **1993**, 1−132.
(16) Harborne, J. B. *Biochem. J.* **1958**, *70*, 22−28.
(17) Goto, T.; Kondo, T.; Tamura, H.; Imagawa, H.; Iino, A.; Takeda, K. *Tetrahedron Lett.* **1982**, *23*, 3695−3698.
(18) Brouillard, R. *Phytochemistry* **1983**, *22*, 1311−1323.
(19) Brouillard, R.; Dangles, O. In *The Flavonoids, Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 565−588.
(20) Heeler, W.; Forkmann, G. In *The Flavonoids, Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 499−535.
(21) Baker, D. C.; Dougall, D. K.; Glassgen, W. E.; Johnson, S. C.; Metzger, J. W.; Rose, A.; Seitz, H. U. *Plant Cell Tiss. Org. Cult.* **1994**, *39*, 79−91.

NP980160C