Structures of Campanin and Rubrocampanin, Two Novel Acylated Anthocyanins with *p*-Hydroxybenzoic Acid from the Flowers of Bellflower, *Campanula medium* L.

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The structures of campanin **1** and rubrocampanin **2**, major antocyanins from flowers of the purple and pink cultivars of *Campanula medium* L., have been identified as 3-*O*-[(6-*O*-α-L-rhamnosyl)-β-D-glucopyranosyl]-7-*O*-[6-*O*-(4-{6-*O*-[4-(6-*O*-*p*-hydroxybenzoyl-β-D- glucopyranosyloxy)benzoyl]-β-D-glucopyranosyloxy}benzoyl)-β-D-glucopyranosides] of delphinidin, and pelargonidin, respectively, by means of chromatography, FAB–MS, and NMR including negative NOE difference (DIFNOE) spectroscopy. The colour stabilities of both the *p*-hydroxybenzoylanthocyanins **1** and **2** were examined and found to be relatively unstable compared with that of platyconin, 3-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-7-*O*-{6-*O*-[(E)-4-*O*-{6-*O*-[(E)-4-*O*-(β-D-glucopyranosyl)-caffeoyl]-β-D-glucopyranosyl}caffeoyl]-β-D-glucopyranosyl}delphinidin, in neutral aqueous solution.

The flowers of the bellflower, *Campanula medium* L., vary in colour from white through pink to purple. In 1979 Asen *et al.*[1] reported a pigment from pink flowers, *Campanula* sp. cv. Rosea, and tentatively assigned this to be pelargonidin 3-bis-(*p*-hydroxybenzoyl)rutinoside 7-glucoside. Recently, a similar acylated antocyanin, violdelphin, was isolated from violet flowers of *Delphinium hybridum*[2] and was determined to be a delphinidin derivative containing two molecules of *p*-hydroxybenzoic acid.

As part of our study of flower-colour variation due to acylated antocyanins,[3–7] we now report the results of a further investigation of two acylated pigments isolated from the pink and purple flowers of *C. medium* L., and also the colour stabilities of both pigments and of platyconin, a related acylated anthocyanin. For the sake of convenience these two pigments were named campanin **1**, a delphinidin derivative from the purple flowers, and rubrocampanin **2**, a pelargonidin derivative from the pink flowers.

Results and Discussion

Major anthocyanins **1** and **2** were isolated from the purple and pink cultivar flowers of *C. medium* L. by preparative paper chromatography (PC) and a reversed-phase HPLC. The aromatic acylation in structures **1** and **2** was shown by their IR absorptions at 1706 and 1708 cm^{-1} (conjugated ester carbonyl bands), and by their UV–visible spectra, with intense absorptions in the UV region, respectively. The maximum absorptions at λ *ca.* 250 nm indicated that the acylating acids were not well known cinnamic acids (λ_{max} ~310 nm) but were similar to *p*-hydroxybenzoic acid (pHBA) as stated in the reports of Asen *et al.*[1,8] The acylating acid was clarified by alkaline hydrolysis of compounds **1** and **2** to afford pHBA as shown Table 2. On the basis of the values (**1** 1.36, **2** 1.61) of $\varepsilon_{acyl}/\varepsilon_{vismax}$, the number of pHBA moieties was eluciated to be ~2–4 in structures **1** and **2**.[1,9]

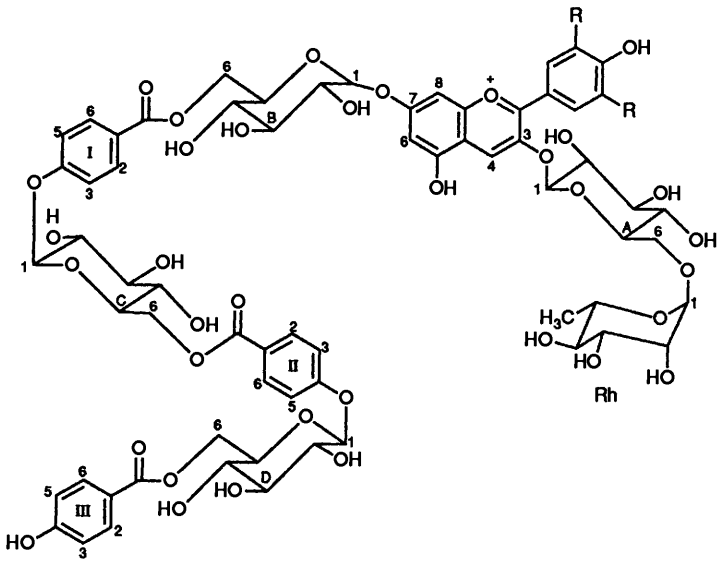
Table 1 Chromatographic data of campanin **1** and rubrocampanin **2**, their deacylanthocyanins, and aglycones

	TLC[a] (R_f × 100)				HPLC[a]
Sample	AHW	BAW	Forestal	Formic	t_R (min)
Campanin **1**	55	40			16.0
Deacylcampanin	53	17			4.6
Deacylplatyconin	56	19			4.7
Aglycone of **1**	5	47	24	15	13.2
Delphinidin	5	46	24	15	13.6
Rubrocampanin **2**	11	25			7.6
Deacyl-rubrocampanin	10	7			5.0
Aglycone of **2**	16	89	62	40	21.9
Pelargonidin	14	89	62	40	22.3
Cyanidin	8	58	43	28	17.9

Abbreviations: AHW (AcOH–HCl–water 15:3:82), BAW (Bu^nOH–AcOH–water 4:1:2), Forestal (HCl–AcOH–water 3:30:10), Formic (HCl–HCO_2H–water 2:5:3).

[a] TLC was carried out on microcrystalline cellulose plates (Avicel SF, Funakoshi) and HPLC was run on an Inertsil ODS-2 (4.6 i.d. × 250 mm) column at 35 °C with a flow rate of 1 cm^3 min^{-1} by linear gradient elution for 30 min from 40 to 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN in water) in solvent A (1.5% H_3PO_4 in water) (monitored at 520 nm). t_R Retention time.

Acid hydrolysis of compounds **1** and **2** gave delphinidin (Dp) and pelargonidin (Pg), respectively (Table 1) as aglycones, and also gave D-glucose (G), and L-rhamnose (Rh) as sugar components (Table 2), as determined by TLC and HPLC analyses. On the other hand, alkaline hydrolysis of compounds **1** and **2** gave the completely deacylated anthocyanins, which were named deacylcampanin and deacylrubrocampanin, respectively, in addition to pHBA and an unknown UV-absorbing



Campanula medium anthocyanins.

1, R = OH Campanin
2, R = H Rubrocampanin

Table 2 Chromatographic data of sugars (G and Rh), acyl groups (B and BG), and H_2O_2 oxidation product (Ru) of campanin **1** and rubrocampanin **2**

Sample	TLC[a] (R_f × 100)					Colour by		HPLC[a]
	BAW	APW	BBPW	BEW	PNW	UV	AHP	t_R (min)
G	35	23	26	35		nd	Br	
Rh	51	48	54	51		nd	YBr	
B	94			96	47	Va	nd	7.3
BG	71			76	35	Va	nd	3.6
Ru	31	18	20	31		nd	Br	
D-Glucose	35	24	27	36		nd	Br	
L-Rhamnose	51	49	54	52		nd	YBr	
Rutinose	31	19	20	32		nd	Br	
pHBA	93			97	49	Va	nd	7.4

Abbreviations: BAW (Bu^nOH–AcOH–water 4:1:2), APW (EtOAc–pyridine–water 15:7:5), BBPW (Bu^nOH–benzene–pyridine–water 5:1:3:2), BEW (Bu^nOH–EtOH–water 4:1:2.2), PNW (Pr^iOH–conc. NH_4OH–water 8:1:1); pHBA = *p*-hydroxybenzoic acid; Va = violet absorption, Br = brown, YBr = yellowish brown, nd = not detected.

[a] TLC was carried out on microcrystalline cellulose plates (Avicel SF, Funakoshi) and HPLC was run on an Inertsil ODS-2 (4.6 i.d. × 250 mm) column at 35 °C with a flow rate of 1 cm^3 min^{-1} by linear gradient elution for 30 min from 40 to 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN in water) in solvent A (1.5% H_3PO_4 in water) (monitored at 290 nm). t_R Retention time.

compound (BG) (Table 2). TLC and HPLC analysis of the deacylcampanin from campanin **1** was shown to be identical with deacylplatyconin, which had already been determined to be delphinidin 3-rutinoside 7-glucoside[3,10] (Table 1). By H_2O_2 oxidation,[11] campanin **1** and rubrocampanin **2** gave rutinose (6-*O*-α-L-rhamnosyl-D-glucose), showing that the rutinose attached at 3-OH of the Dp-and Pg-nucleus, respectively. On enzymic hydrolysis with β-glucosidase,[12] BG gave pHBA and glucose (1:1 molar ratio) as well as on partial acid hydrolysis. Thus BG was identified as 4-(β-D-glucopyranosyloxy)benzoic acid.

From the FAB–MS data, molecular (flavylium) ions of compounds **1** and **2** were determined as occurring at 1457 and 1425 mass units, corresponding to $C_{66}H_{73}O_{37}^+$ and $C_{66}H_{73}O_{35}^+$, respectively, containing one molecule of Dp and Pg, respectively, and also three molecules of D-glucose, one molecule of L-rhamnose, and three molecules of pHBA. Hence the structures **1** and **2** were estimated as 3-rutinoside 7-(acylated with two molecules of BG and one molecule of pHBA)-glucoside of Dp and Pg, respectively.

The detailed structure of compound **1** was elucidated by NMR measurements including DIFNOE spectra.[13] In the ^1H NMR spectra, four singlets, at δ 8.60 (4-H), 7.85 (2′- and 6′-H), 7.27 (6-H), and 6.90 (8-H), showed ring protons on Dp. Three pairs of $[AX]_2$ spin system (δ 7.76 and 6.83, 7.51 and 6.78, and 7.43 and 6.59) indicated the couplings between 2- and 6-H and 3- and 5-H on I (or II), II (or I) and III-pHBA moieties, respectively. In the sugar region, three doublets, at δ 5.29, 4.92 and 4.65, showed the anomeric protons of A and B, C (or D), and D (or C)-glucoses, respectively, whose coupling constants ($J_{1,2}$ 7.6, 7.6 and 7.3 Hz, respectively) showed that all glucose moieties were in the β-configuration. All methylene proton signals were shifted to lower magnetic field (lower than δ 4), showing the 6-CH_2OH groups of all glucoses to be substituted. Moreover the remaining characteristic doublet at δ 5.54 (*J* 6.43 Hz) showed the anomeric proton of L-rhamnose moiety in the 3-rutinoside. The presence of a rhamnosyl residue was confirmed by the doublet for the 5-methyl protons at δ 1.03 in the ^1H NMR spectra and δ_C 18.13 in the ^{13}C NMR spectrum (Fig. 1).

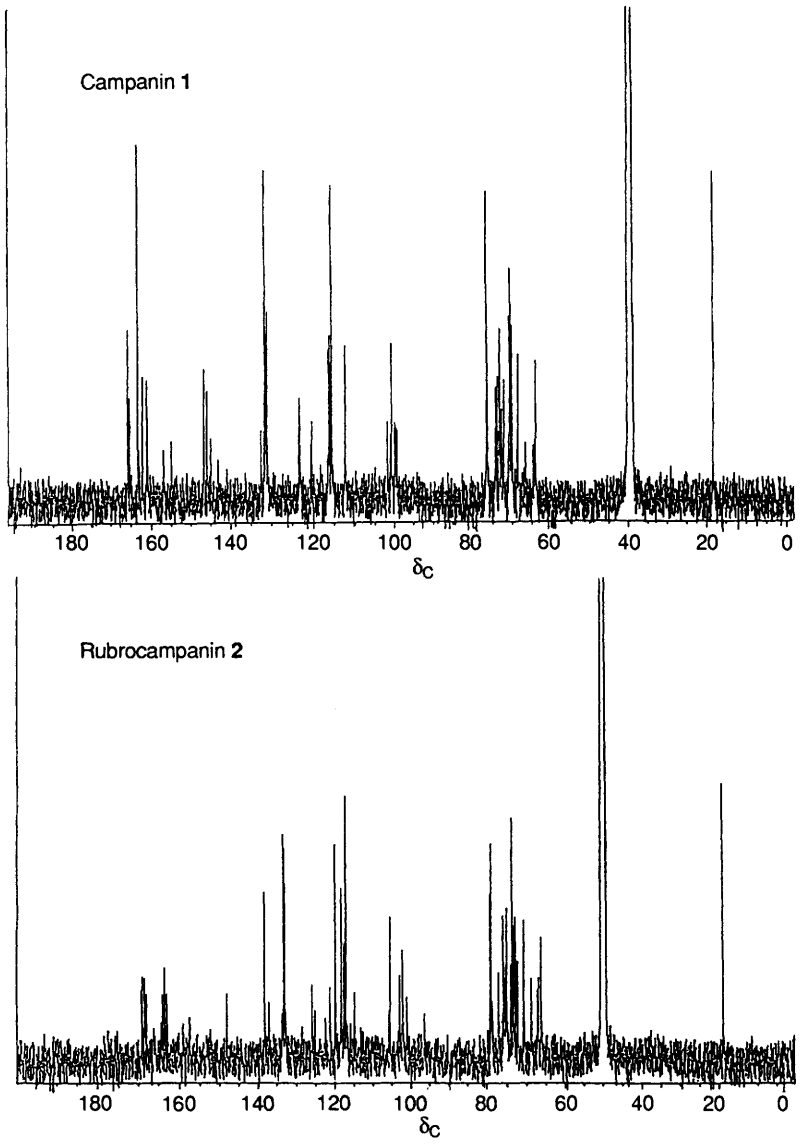


Fig. 1 ^{13}C NMR (100 MHz) spectra of *Campanula medium* anthocyanins in $(CD_3)_2SO$–CF_3CO_2D (9:1) (δ ppm from standard Me_4Si)

The linkage mode among the sugars and other residues in the campanin molecular were determined by DIFNOE measurement (Fig. 2). The negative NOE method[14] has played an important role in the determination of positions of attachment of the sugars on the aglycones in anthocyanins.[13,15] This well established analytical technique has already been applied to the structure determination of several anthocyanins with molecular weights of 600–2000 daltons. Observation of a negative NOE signal for 1-H of A-glucose by irradiation at 4-H of Dp showed that A-glucose was attached to the 3-OH of Dp through a glycosidic bond (Fig. 2b). Moreover, A-glucose had been confirmed to be substituted with rhamnose at 6-CH_2OH since rutinose was obtained by H_2O_2 oxidation of campanin **1** as described above. Similarly, B-glucose was indicated to glycosylate at 7-OH of Dp because of the presence of NOEs between 6-H (Dp) and 1-H (B) (Fig. 2c), or 8-H (Dp) and 1-H (B).

On irradiation at 2- and 6-H of I or II-pHBA, an NOE on 1-H of ring C or D was observed, besides those of 3- and 5-H of I or II. Similarly NOEs between 3- and 5-H of I (or II)-pHBA and 1-H of C or D was observed (Fig. 2d and 2e). These findings indicated that C- and D-glucoses were linked at 4-OH of I and II-pHBA through a glycosidic bond, respectively, and that rings I and II were located inside the C-7 side-chain of Dp, while the remaining pHBA moiety (III) was located at the terminal position, since irradiation of 3- and 5-H (III) gave no NOE other than that of its own 2- and 6-H, and hence the 4-OH of III was free (Fig. 2f). As irradiation of 2′- and 6′-H of Dp gave no NOE, 3′- and 5′-OH of Dp were also unsubstituted. Thus the structure of campanin was identified as 3-*O*-[(6-*O*-α-L-rhamnosyl)-β-D-glucopyranosyl]-7-*O*-{6-*O*-[4-(6-*O*-{4-[6-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyloxy]benzoyl}-β-D-glucopyranosyloxy)benzoyl]-β-D-glucopyranosyl}delphinidin.

The structure of rubrocampanin **2** was also determined by NMR spectroscopy. In the ^1H NMR spectra, five signals, at δ 8.76 (2′- and 6′-H), 8.68 (4-H), 7.75 (3′- and 5′-H), 7.45 (8-H) and 6.77 (6-H), showed ring protons on Pg. Three pairs of an $[AX]_2$ spin system (δ 7.39 and 6.66, 7.09 and 6.61, and 7.00 and 6.25) indicated couplings between 2- and 6-H and 3- and 5-H on I (or II), II (or I), and III-pHBA moieties, respectively. In the sugar region, four doublets, at δ 5.54, 5.42, 5.26 and 4.78, showed the anomeric protons of four (A-, B-, C- and D)-glucoses of compound **2**, whose coupling constants (all 8 Hz) showed that all glucose moieties were in the β-configuration. All methylene proton signals were shifted to lower magnetic field (lower than

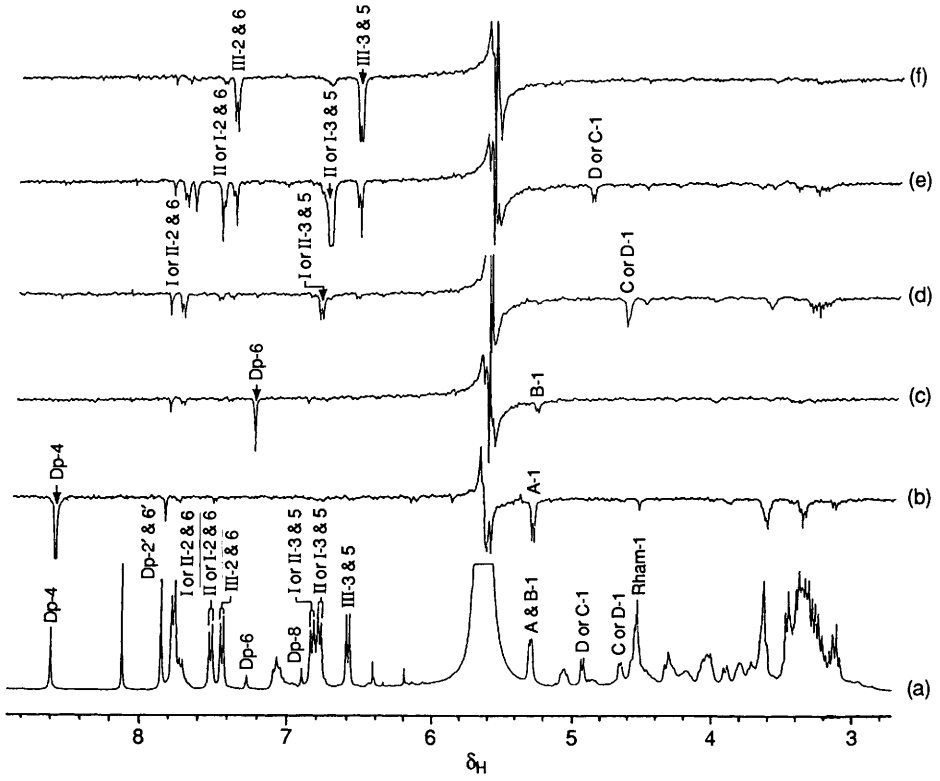


Fig. 2. ^{1}H NMR (400 MHz) DIFNOE spectra of campanin **1** in $(CD_3)_2SO$–CF_3CO_2D (9:1) at room temperature. (a) Normal spectrum; (b)–(f) DIFNOE spectra by irradiation at 4-H of Dp, 6-H of Dp, 3- and 5-H of I or II, 3- and 5-H of II or I, and 3- and 5-H of III, respectively (irradiation positions are indicated by arrows).

δ 4), showing the 6-OH groups of all glucoses to be substituted. Moreoever, the remaining characteristic signals, at δ 4.94 and 1.27 [δ 18.77 in ^{13}C NMR spectrum (Fig. 1)], showed the anomeric proton and 5-methyl protons, respectively, of the L-rhamnosyl moiety in the 3-rutinoside.

DIFNOE measurements showed A-glucose to glycosylate on the 3-OH group of Pg. Moreover, observation of NOEs between ring protons of I (or II)-pHBA and anomeric protons of glucose C (or D) indicated that C (or D)-glucose was linked at 4-OH of I (or II)-pHBA through a glycosidic bond, and that I and II were again located inside the C-7 side-chain of Pg. The remaining pHBA (III) was located at the terminal position since irradiation of 3- and 5-H (III) gave no NOE other than that of its own 2- and 6-H, and therefore the 4-OH group of III was free. NOE between 2′- and 6′-H of Pg and 3′- and 5′-H of Pg was also confirmed.

Thus the structure of rubrocampanin was estimated as 3-*O*-[6-*O*-(α-L-rhamnosyl)-β-D-glucosyl]-7-*O*-{6-*O*-[4-(6-*O*-{4-[6-*O*-(*p*-hydroxybenzoyl)-β-D-glucosyloxy)]benzoyl}-β-D-glucosyloxy)benzoyl]-β-D-glucosyl}pelargonidin.

These substitution patterns (3-rutinoside 7-acylated glucoside) are analogous to those of platyconin[3,6,10] and violdelphin,[2] 7-*O*-{4-[6-*O*-(4-hydroxybenzoyl)-β-D-glucopyranosyloxy]-benzoyl-β-D-glucopyranosyl}-3-*O*-(6-*O*-α-L-rhamnosyl-β-D-glucopyranosyl)delphinidin. In general, monomeric polyacylating anthocyanins are very stable in weakly acidic or neutral aqueous solutions by virtue of stacking interactions between their side-chain acyl groups and anthocyanidin nuclei.[4–7,16] Therefore, we examined the colour stability of two *Campanula medium* pigments in neutral aqueous solution (pH 7.0) in comparison with that of platyconin. As shown in Fig. 3 both *C. medium* anthocyanins were relatively less stable than platyconin which had a similar side-chain at the 7-position (but the absence of one acyl group and also substitution of a *p*-hydroxybenzoyl group for a caffeoyl group). Based on

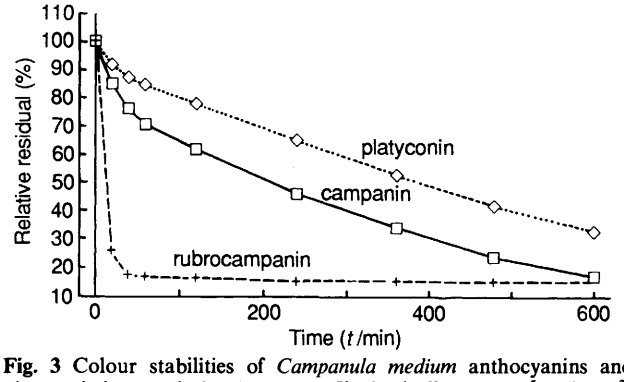


Fig. 3 Colour stabilities of *Campanula medium* anthocyanins and platyconin in aq. solution (pH 7.0 McIlvaine buffer 5×10^{-5} mol dm^{-3}) at *ca.* 30 °C for >600 min

these results we considered that the *p*-hydroxybenzoyl residues do not affect anthocyanin stability any more than do hydroxycinnamic groups such as caffeic acid because the benzoyl groups may be considered not to interact efficiently with the pyrylium nucleus, due to either the length and/or attachment position of the acyl groups.[17] Indeed, Asen *et al.* reported that larkspur flower anthocyanin acylated with *p*-hydroxybenzoic acid was unstable.[8] Moreover, in addition to the different types of acyl groups, the structures of the aglycones are also involved in the stability of these compounds since campanin (delphinidin base) was more stable than rubrocampanin (pelargonidin base) as demonstrated by Saito *et al.*[5]

Experimental

General Procedures.—TLC and PC were carried out on microcrystalline cellulose plates (Avicel SF, Funakoshi) and papers (Toyo No. 526), respectively, with solvent systems such

as AHW (AcON–conc. HCl–water 15:3:82), BAW (BuOH–AcOH–water 4:1:2), Forestal (conc. HCl–AcOH–water 3:30:10), Formic (conc. HCl–HCO_2H–water 2:5:3), APW (EtOAc–pyridine–water 15:7:5), BBPW (Bu^nOH–benzene–pyridine–water 5:1:3:2), BEW (Bu^nOH–EtOH–water 4:1:2.2), PNW (Pr^iOH–conc. NH_4OH–water 8:1:1). The sample spots on chromatograms were detected with a UV lamp for aromatic compounds, and aniline hydrogen phthalate (AHP) spray reagent for reducing sugars. HPLC was performed on an L-6200 Intelligent pump system (Hitachi). Analytical HPLC was run on Inertsil ODS-2 (4.6 i.d. × 250 mm, Gasukuro-Kogyo) or Asahipak ODP-50 (6 i.d. × 250 mm, Asahikasei-Kogyo) columns at 35 °C with a flow rate of 1 cm^3 min^{-1}, with monitoring at 290 nm for UV-absorbing compounds and 520 nm for anthocyanins. Solvent systems employed were as follows: a linear gradient elution for 30 min from 40 to 85% solvent B (1.5% H_3PO_4, 20% AcOH, 25% MeCN in water) in solvent A (1.5% H_3PO_4 in water). Preparative HPLC was performed on Inertsil ODS-2 (10.7 i.d. × 250 mm) or Asahipak ODP-50 (10 i.d. × 250 mm) columns, rate 2.5 cm^3 min^{-1} by isocratic elution using a mixture of solvent A (10% HCO_2H in water) and solvent B (10% HCO_2H, 40% MeCN in water). IR spectra were recorded on a 270–30 (Hitachi) spectrophotometer in KBr pellets, UV–visible spectra on an MPS-2000 (Shimadzu) spectrophotometer, FAB–MS spectra on JMX DX-300 and JMX SH-102 (JEOL) spectrometers, and ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra on a JNM GX-400 (JEOL) for solutions in $(CD_3)_2SO$–CF_3CO_2D (9:1) with Me_4Si as reference.

Plant Materials.—The seeds of purple cv. Caerulea and pink cv. Rosea of *Campanula medium* L. were purchased from SAKATA Seed Co. Ltd. (Japan) and cultivated in the flower garden of Minami-Kyushu University. The flowers were collected in May and were used for the investigation.

Isolation of Campanin **1** *and Rubrocampanin* **2**.—The two kinds of cultivar flowers (purple flowers: 2 kg; pink flowers: 1 kg) were immersed in 5% AcOH overnight and then filtered off. The crude pigment extracts were analysed by HPLC (ODP-50 column), which showed four antocyanin pigments at retention time (t_R) 5.2, 7.5, 14.3 and 15.2 min, which constituted 5, 15, 68 and 9%, respectively, of the extract from purple flowers and t_R 3.8, 5.8, 12.7 and 15.4 min, which constituted 14, 8, 76 and 3%, respectively, of the extract from pink flowers. Each pigment extract was applied to an HP-20 (Diaion) resin column (34 i.d. × 270 mm). The column was washed with 1% AcOH and then eluted with 1% AcOH in 70% MeOH. The pigment eluate was separated and purified by preparative HPLC with HCO_2H solvent system (A:B 9:1) and monitoring at 290 nm. Each major anthocyanin fraction was evaporated to dryness, dissolved in a small amount of trifluoroacetic acid (TFA), and precipitated with diethyl ether to give the TFA salt of campanin **1** (65 mg) or that of rubrocampanin **2** (36 mg) as a red powder.

Hydrolytic Analyses.—Acid and alkaline hydrolyses, and H_2O_2 oxidation, were performed by the usual methods.

Preparation of Deacylanthocyanins and BG.—An aliquot (10 mg) of a pigment (**1** or **2**) was dissolved in 2 mol dm^{-3} NaOH (5 cm^3) and kept for 60 min under N_2. The reaction mixture was acidified with TFA and evaporated to small volume. The residue was diluted with 1% TFA (3 cm^3), and the solution was applied to an HP-20 column (10 i.d. × 130 mm) and washed with 1% TFA. The washings were used for the preparation of BG. The HP-20 column was eluted with 1% TFA in 70% MeOH, and the eluate was evaporated. From the residue, BG and deacylanthocyanins were separated by HPLC on an ODP-50 with HCO_2H solvent system (A:B 9:1) with monitoring at 290 nm. The fractions of deacylcampanin or deacylrubrocampanin, and BG were taken and evaporated to dryness under reduced pressure.

Enzymic hydrolysis of BG.—BG (1 mg) was dissolved in 0.1% β-glucosidase (Sigma) in McIlvaine buffer solution (1 cm^3) (pH 4.5) and the solution was kept at 37 °C. At appropriate intervals, a small amount of the sample of reaction mixture was taken and analysed by TLC and HPLC at 270 nm for aromatic acids.

Campanin·TFA **1**.—M.p. >300 °C (blackened >200 °C); ν_{max}(KBr)/cm^{-1} 3428 (O–H), 2908 (C–H), 1706 (C=O), 1636 (aromatic C=C) and 1610 (aromatic C=C); λ_{max} (0.1% HCl–MeOH) (log ε) 550 (4.75, shifted with $AlCl_3$) and 248 nm (4.88), $\varepsilon_{248}/\varepsilon_{550} = \varepsilon_{acyl}/\varepsilon_{vismax}$ = 1.36, $\varepsilon_{440}/\varepsilon_{550}$ 0.11; FAB–MS (in water + glycerol as matrix), positive: *m/z* 1457 $[M]^+$, 1311 $[M - \text{rhamnose}]^+$, 1149 $[M - \text{rutinose}]^+$, 627 [Dp 3,7-glucoside = $C_{27}H_{31}O_{17}]^+$, 611 [Dp 3-rutinoside = $C_{27}H_{31}O_{16}]^+$; negative *m/z* 1547 $[M + \text{glycerol} - 2H]^-$, 1456 $[M - H]^-$; δ_H[400 MHz; $(CD_3)_2SO$–CF_3CO_2D (9:1); standard Me_4Si] 8.60 (1 H, s, 4-H or Dp), 7.85 (2 H, s, 2′-, 6′-H of Dp), 7.76 (2 H, d, *J* 8.61 Hz, 2-, 6-H of I or II), 7.51 (2 H, d, *J* 8.8 Hz, 2-, 6-H of II or I), 7.43 (1 H, d, *J* 8.6 Hz, 2-, 6-H of III), 7.27 (1 H, s, 6-H of Dp), 6.90 (1 H, s, 8-H of Dp), 6.83 (2 H, d, *J* 9.0 Hz, 3-, 5-H of I or II), 6.78 (2 H, d, *J* 8.8 Hz, 3-, 5-H of II or I), 6.59 (1 H, d, *J* 8.8 Hz, 3-, 5-H of III), 5.29 (2 H, d, *J* 7.6 Hz, 1-H or A and B), 4.92 (1 H, d, *J* 7.6 Hz, 1-H of C or D), 4.65 (1 H, d, *J* 7.3 Hz, 1-H of D or C), 4.54 (1 H, d, *J* 6.3 Hz, 1-H of rhamnose), 4.4–4.2 (3 H, m, 6-H of B, C, D), 4.17 (1 H, m, 6-H of A), 4.1–3.9 (4 H, m, 6-H of A, B, C, D), 3.89 (1 H, m, 2-H of rhamnose), 3.64 (1 H, m, 5-H of rhamnose), 3.5–3.05 (18 H, m, 2-, 3-, 4-, 5-H of A, B, C, D, and 3-, 4-H of rhamnose), and 1.03 (3 H, d, *J* 6.1 Hz, 5-Me of rhamnose).

Rubrocampanin·TFA **2**.—M.p. >300 °C (blackened >200 °C); ν_{max}(KBr)/cm^{-1} 3420 (O–H), 2924 (C–H), 1708 (C=O), and 1 610 (aromatic C=C); λ_{max}(0.1% HCl–MeOH) (log ε) 517 (4.26, not shifted with $AlCl_3$) and 251 nm (4.47), $\varepsilon_{251}/\varepsilon_{517} = \varepsilon_{acyl}/\varepsilon_{vismax}$ = 1.61, $\varepsilon_{440}/\varepsilon_{517}$ 0.33; FAB–MS (in 2% AcOH–MeOH + glycerol as matrix), positive *m/z* 1425 $[M]^+$, 1118 $[M - \text{rutinose} + H]^+$; δ_H[400 MHz; $(CD_3)_2SO$– CF_3CO_2D (9:1); standard Me_4Si] 8.78 (2 H, d, *J* 8.8 Hz, 2′-, 6′-H of Pg), 8.68 (1 H, s, 4-H of Pg), 7.75 (2 H, d, *J* 8.8 Hz, 2-, 6-H of I or II), 7.45 (1 H, s, 8-H of Pg), 7.39 (2 H, d, *J* 8.8 Hz, 2-, 6-H or II or I), 7.09 (2 H, d, *J* 8.8 Hz, 2-, 6-H or III), 7.00 (1 H, d, *J* 9.0 Hz, 3′-, 5′-H of Pg), 6.77 (1 H, s, 6-H of Pg), 6.66 (2 H, d, *J* 8.6 Hz, 3-, 5-H of I or II), 6.61 (2 H, d, *J* 8.8 Hz, 3-, 5-H of II or I), 6.25 (1 H, d, *J* 8.8 Hz, 3-, 5-H of III), 5.54 (1 H, d, *J* 7.6 Hz, 1-H of A), 5.42 (1 H, d, *J* 7.6 Hz, 1-H of B), 5.26 (1 H, d, *J* 7.6 Hz, 1-H of C or D), 4.94 (1 H, br d, *J* 10.0 Hz, 6-H), 4.91 (br s, 1-H of rhamnose), 4.78 (br d, *J* 8.8 Hz, 1-H of D or C), 4.54 (1 H, br d, *J* 9.5 Hz, 6-H), 4.38 (1 H, br t, *J* 11.7 Hz, 6-H), 4.20 (1 H, br t, *J* 11.2 Hz, 6-H), 4.17 (1 H, br t, *J* 10.2 Hz, 6-H), 4.1–3.3 (22 H, m, 2–5-H of A–D, rhamnose, and 2 × 6-H) and 1.27 (3 H, d, *J* 6.1 Hz, 5-Me of rhamnose).

Stability Tests of Compounds **1** *and* **2**.—Relative stabilities of compounds **1**, **2** and platyconin were compared. A test solution of each anthocyanin was prepared with a concn. 5 × 10^{-5} mol dm^{-3} in McIlvaine buffer (pH 7.0) and kept at ~30 °C. The absorptions at λ_{max} were measured at appropriate intervals for >10 h and the relative residual colour (initial absorbance as 100%) was plotted.

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