

## Characterization of C-Glycosylflavones and Anthocyanins in Several Species of Caryophyllaceae\*

By

Tsukasa IWASHINA\*\* and Shunji OOTANI\*\*\*

岩科 司\*\*・大谷俊二\*\*\*: ナデシコ科植物の数種に含まれる  
C-グリコシルフラボンとアントシアニンの定性

The chemotaxonomic survey in the family Caryophyllaceae has been confined chiefly to the occurrence of anthocyanins instead of betalains within the order Caryophyllales (or Centrospermae) (Mabry 1966). Recently, it was clarified that C-glycosylflavones are widespread rather than other class of flavonoids in anthocyanin-containing families, Caryophyllaceae and Molluginaceae (Richardson 1978), and many kinds of unique C-glycosylflavones and their O-glycoside, e. g., isovitexin 2"-O-arabinoside, 2"-O-rhamnoside, 7-O-galactoside-2"-O-rhamnoside, cerarvensin (6-C-xylosylapigenin), 6-C-galactosyl-8-C-arabinosylapigenin, 6-C-glucosyl-8-C-galactosylapigenin and so on, have been isolated from this family (Bouillant *et al.* 1979, Besson *et al.* 1979, Wagner *et al.* 1979, Dubois *et al.* 1982, 1984 etc). Since the Caryophyllaceae, however, is a large family consisting of about 80 genera with ca. 2000 species, information on flavonoids in the family has been comparatively scanty.

The present paper describes the flavonoid and anthocyanin profiles of the aerial parts, leaves, stems or flowers, of 12 caryophyllaceous species mostly native to Japan.

### Materials and Methods

#### *Plant materials*

The twelve plant species were collected as materials from various localities in 1983, which are arranged in Table 4.

#### *Isolation of flavonoids*

An appropriate amount of fresh leaves or flowers of each caryophyllaceous species was extracted with methanol, filtered and evaporated *in vacuo* to an aqueous concentrate,

\* Contribution from the Research Institute of Evolutionary Biology, No. 80.

\*\* Tsukuba Botanical Garden, National Science Museum, Ibaraki 305. 国立科学博物館 筑波実験植物園.

\*\*\* The Research Institute of Evolutionary Biology, Kami-Yōga 2-4-28, Setagaya-ku, Tokyo 158. (財)進化生物学研究所.

which was applied to two-dimensional paper chromatography (2D-PC) on Toyo No. 50 filter paper using two kinds of solvent systems, BAW (n-BuOH/AcOH/H<sub>2</sub>O=4:1:5, upper phase) on the one direction and 15% AcOH (AcOH/H<sub>2</sub>O=15:85) on the other. The individual crops separated were subjected to mass-paper chromatography (mass-PC) using the solvent systems described above or column chromatography, and were thoroughly purified in solution or obtained as crystals.

The anthocyanins were isolated with mass-PC using solvent systems, BuH (n-BuOH/conc. HCl/H<sub>2</sub>O=7:2:5, upper phase) and 1% HCl (conc. HCl/H<sub>2</sub>O=3:97) according to Takeda and Hayashi (1980).

#### *Identification of flavonoids*

This was made by the standard methods (Mabry *et al.* 1970, Markham 1982, Takeda and Hayashi 1980) such as complete or partial acid hydrolysis, direct comparisons with authentic specimens on UV spectral and chromatographic properties, or <sup>1</sup>H-NMR spectra.

#### *Identification of glycosidic sugars*

Glycosidic sugars which were liberated by acid hydrolysis of flavonoids were characterized by co-PC with the authentic samples according to Iwashina *et al.* (1984).

#### *UV spectral analysis*

UV spectra were measured on methanolic solutions throughout with a Hitachi EPS-3T type recording spectrophotometer, according to the methods of Mabry *et al.* (1970) and Hayashi *et al.* (1984).

#### *<sup>1</sup>H-NMR spectral analysis*

<sup>1</sup>H-NMR spectra were measured in DMSO-d<sub>6</sub> (dimethyl sulfoxide-d<sub>6</sub>) with JNM-GX400 FT NMR spectrometer using TMS (tetramethylsilane) as an internal standard.

## Results and Discussion

#### *Isolation of individual flavonoids*

*Flavonoid A and AG3*: Dried aerial parts (ca. 1 kg) of *Stellaria media* (Hakobe) were repeatedly extracted with methanol (6 l) overnight, filtered and concentrated *in vacuo* (ca. 500 ml). On standing for 3 days at room temperature, colorless inorganic impurities were filtered off and the mother liquor was repeatedly shaken with ethyl acetate (EtOAc), evaporated to dryness, and dissolved in a small volume of 5% acetic acid. This pigment fraction was applied to cellulose (Avicel SF, Funakoshi) column chromatography (4×25 cm) and eluted with 5% AcOH. Effluent fractions corresponding to flavonoid A, which were detected preliminarily by the method of 2D-PC, were combined, concentrated and subjected to the polyamide C-200 (Wako Pure Chemicals, Ltd.)

column chromatography (4×20 cm). After elution with methanol, the solution containing flavonoid A was evaporated and dissolved in a small volume of 70% methanol. This was applied to Sephadex LH-20 (Pharmacia) column chromatography (1.5×35 cm), eluted with 70% methanol and pure flavonoid solution was obtained. The eluent was evaporated *in vacuo* to dryness, dissolved in hot methanol and stood overnight in a refrigerator. The pale yellow minute needles (flavonoid A) were separated and air-dried. Yield, ca. 30 mg.

In parallel with column chromatographic separation described above, a mass-PC was also applied to a crude extract of *S. media* for the separation of flavonoid AG3. An aqueous concentrate which was washed with petroleum ether was treated with mass-PC using BAW, and then 15% AcOH, and the band corresponding to flavonoid AG3 (Rf 0.36 in BAW and 0.86 in 15% AcOH) was detected under UV light on the chromatograms and obtained as a pure pigment solution by elution with methanol.

*Flavonoid AG4*: Aerial parts (15.5 g) of *Pseudostellaria palibiniana* (Higene-wachigaisô) were extracted with methanol (900 ml). The extract was evaporated to a small volume, and applied to mass-PC using 15% AcOH. Thus, flavonoid AG4 (Rf 0.74) was obtained as a single band and eluted with methanol.

*Flavonoid AG1*: Fresh aerial parts (ca. 150 g) of *Myosoton aquaticum* (Ushi-hakobe) were extracted with methanol (ca. 2.5 l), filtered and concentrated *in vacuo* to a small volume of aqueous solution. After removal of greenish precipitate which was formed by the addition of methanol (200 ml), the solution was evaporated again to a small volume. Then, the crude flavonoid solution was subjected to a polyamide column chromatography (4×25 cm). Elution was carried out with methanol. The fractions still containing several kinds of flavonoids were combined and carefully applied to the mass-PC using BAW. Finally, flavonoid AG1 (Rf 0.66) was obtained as a single band, and eluted with methanol.

*Flavonoid B and AG2*: Fresh aerial parts (840 g) of *Cerastium glomeratum* (Oranda-miminagusa) were repeatedly extracted with methanol (6 l), filtered and concentrated *in vacuo* (ca. 1 l). After filtration to remove the precipitate of chlorophylls and other impurities, the filtrate was further evaporated to an aqueous solution (20 ml), which was shaken with petroleum ether and then with EtOAc to remove lipophilic substances. (By preliminary PC test, no flavonoid was present in these organic layers.) The mother liquor was evaporated to dryness, and the residue was taken in a small amount of water and subjected to polyamide C-200 column chromatography (4.5×30 cm) for the separation of flavonoids. The elution was effected by dist. H<sub>2</sub>O (300 ml), and then with a series of aqueous methanol of increasing concentration: namely, 10% MeOH (200 ml), 20% (200 ml), 40% (100 ml), 60% (200 ml), 80% (200 ml), and finally 100% methanol until flavonoids were completely liberated. Each fraction of effluent (ca. 15 ml, Nos. 1-105) was applied to 2D-PC (BAW and 15% AcOH).

By this means, the flavonoid AG2 was separated into frs. 10-40, and A and B into frs. 55-105. Frs. 10-40 containing AG2 were combined, concentrated to dryness and dissolved in hot 50% ethanol. The flavonoid AG2 was obtained as a yellow powder on standing at 5°C for two days. Frs. 55-105 having the flavonoid B together with a minor flavonoid A were combined and evaporated, and the residual yellow pigment was dissolved in hot methanol. After standing for a long time (ca. 2 months), the flavonoid B were separated as a yellow powder. Yield, ca. 20 mg.

*Flavonoid IV, IOR and Anthocyanin Cy1, Cy2*: Fresh petals (3.3 g) and leaves (ca. 20 g) of *Silene armeria* (Mushitori-nadeshiko) were extracted with methanol (100 ml and 500 ml, respectively). The petal extract was tested on mass-PC using BAW and 5% AcOH, and leaves with 15% AcOH and then BAW. The flavonoids IV and IOR from the former extract and only IOR from the latter were obtained.

Red-purple petals (4 g) were extracted with 1% methanolic HCl (100 ml). After concentration of crude extract *in vacuo*, the crop was applied to mass-PC using 1% HCl. Two kinds of anthocyanins (Cy1 and Cy2, Rf 0.05 and 0.14, respectively) were separated on the chromatograms. They were eluted with 5% methanolic AcOH and further purified by mass-PC using BuH.

#### Identification of C-glycosylflavones

*6, 8-Di-C-glucosylapigenin (vicenin-2, A)*: Flavonoid A which was isolated from *Stellaria media* as yellow minute needles, had  $\lambda_{\max}$  273, 333 nm in methanol showing the flavone nucleus but not flavonols. Bathochromic shift (65 nm) of long wavelength peak (Band I) with a remarkable increase in intensity relative to methanol spectrum

Table 1. Chromatographic properties of C-glycosylflavones isolated from Caryophyllaceae

Flavones	Rf-values				Colors
	BAW	15%AcOH	TBA	BEW	UV/NH <sub>3</sub>
6, 8-di-C-glucosylapigenin (vicenin-2, A)	0.38	0.60	0.36	0.45	dark yellow
6, 8-di-C-glucosylapigenin X''-O-glucoside (AG1)	0.66	0.81	0.65	0.57	dark yellow
6, 8-di-C-glucosylapigenin 4'-O-glucoside (AG2)	0.20	0.79	0.13	0.23	dark purple
6, 8-di-C-glucosylapigenin 4'-O-glycoside (AG3)	0.36	0.86	—	—	dark purple
6, 8-di-C-glucosylapigenin X''-O-glycoside (AG4)	0.53	0.74	0.48	0.31	dark yellow
6, 8-di-C-glycosylluteolin (B)	0.24	0.50	0.16	0.20	dark yellow
6-mono-C-glucosylapigenin (isovitexin, IV)	0.71	0.60	0.71	0.67	dark yellow
6-mono-C-glycosylluteolin (isoorientin, IOR)	0.53	0.28	—	—	dull yellow

BAW = n-BuOH/AcOH/H<sub>2</sub>O (4 : 1 : 5, upper phase), 15%AcOH = AcOH/H<sub>2</sub>O (15 : 85),  
TBA = t-BuOH/AcOH/H<sub>2</sub>O (3 : 1 : 1), BEW = n-BuOH/EtOH/H<sub>2</sub>O (4 : 1 : 2.2).

Table 2. UV spectral properties of C-glycosylflavones found in caryophyllaceous plants

Flavonoids in	$\lambda_{\max}$ (nm)				
	MeOH	+NaOMe	+AlCl <sub>3</sub>	+AlCl <sub>3</sub> /HCl	+NaOAc
A	273, 333	286, 329, 398 ↑	280, 306, 350, 384sh	281, 305, 345, 380sh	283, 382
AG1	277, 322	286, 333, 371 ↑	282, 307, 332, 386sh	283, 306, 333, 382sh	282, 316, 390
AG2	276, 324	283, 379 ↓	284, 304, 342, 385	284, 303, 338, 384	283, 380
AG3	277, 326	274, 374 ↓	284, 306, 338, 384sh	284, 304, 334, 380sh	284, 386
AG4	273, 333	284, 333, 398 ↑	281, 305, 349, 383sh	281, 305, 345, 382sh	283, 381
B	272, 348	284, 415 ↑	274, 410	280, 298sh, 359, 384sh	281, 329, 395
IV	272, 337	280, 328, 398 ↑	279, 304, 349, 380sh	280, 303, 345, 378sh	279, 366
IOR	272, 348	—	—	—	—

sh=shoulder, ↑ =remarkable increase in intensity relative to methanol spectrum,  
↓ =remarkable decrease in intensity.

indicated the presence of a free 4'-OH. And also, the presence of 5, 7-diOH and absence of 3, 3'-diOH were determined by UV spectral behaviors after addition of AlCl<sub>3</sub>, AlCl<sub>3</sub>/HCl or NaOAc (Mabry *et al.* 1970, Markham 1982, Hayashi *et al.* 1984). Under usual conditions for acid hydrolysis (Iwashina *et al.* 1984), the original compound remained unchanged in an aqueous mother liquor and no free sugar was liberated, showing that the pigment was C-glycosylflavonoid. However, a Wessely-Moser rearrangement did not occur by hot acid treatment, showing that flavonoid A was symmetrically substituted by di-C-glycosyl which have the same sugar at both C-6 and C-8 but not glycosylated either at C-6 or C-8 such as isovitexin (6-mono-C-glucosylapigenin), vitexin (8-mono-C-glucosylapigenin), isoorientin (6-mono-C-glucosylluteolin) and orientin (8-mono-C-glucosylluteolin) (Markham 1982). Thus, it was concluded that flavonoid A was 5, 7, 4'-trihydroxy-6, 8-di-C-glycosylflavone.

NMR data (400 MHz) also confirmed the above results: that is, two doublets corresponding to H-6 and H-8 protons do not occur in the range  $\delta$  6.0-6.5; in turn, two multiplet signals appear in range  $\delta$  4.6-4.9 (C-glucosyls, H-1's) and  $\delta$  3.5-3.9 (two glucosyls); and also two doublets  $\delta$  7.98 (2H,  $J=9.0$  Hz, H-2' and H-6') and  $\delta$  6.90 (2H,  $J=7.9$  Hz, H-3' and H-5'), and a singlet  $\delta$  6.85 (1H, H-3) occur in DMSO-d<sub>6</sub> (Fig. 1). These data were extremely similar to those of 6, 8-di-C-glycosylapigenin (Kawasaki *et al.* 1986).

Finally, flavonoid A was identified as 6, 8-di-C-glycosylapigenin (vicenin-2) by co-PC with the authentic specimen (Table 1, see Fig. 2).

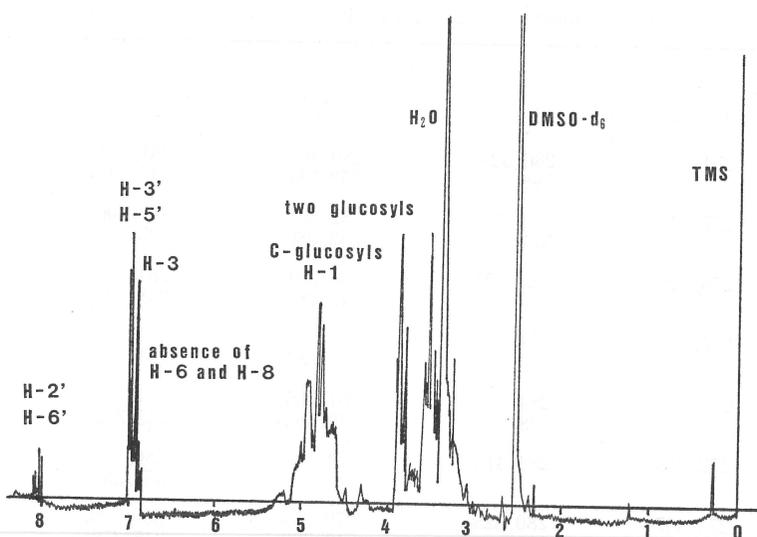


Fig. 1. NMR of TMS ether of 6,8-di-C-glucosylapigenin (vicenin-2, A) in DMSO- $d_6$ .

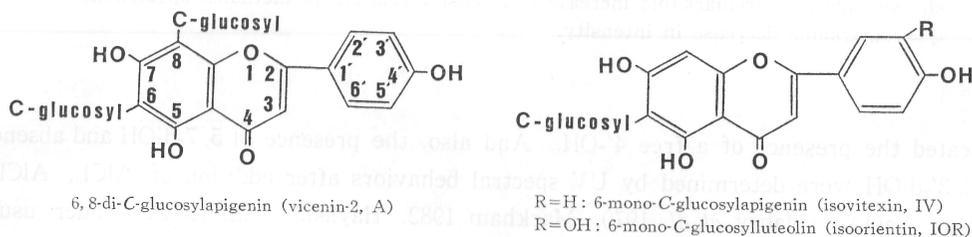


Fig. 2. The structures of three kinds of C-glycosylflavones.

*6,8-Di-C-glucosylapigenin X''-O-glucoside (AG1) and X''-O-glycoside (AG4)*: Flavonoid AG1 and AG4 represented the different R<sub>f</sub> values from each other in PC with BAW and 15% AcOH (Table 1), and UV spectral behaviors (Table 2) upon addition of NaOMe, AlCl<sub>3</sub>, AlCl<sub>3</sub>/HCl, or NaOAc indicated the presence of 5,7,4'-trihydroxyl groups (Mabry *et al.* 1970). Acid hydrolysis of flavonoid AG1 gave vicenin-2 (A), which also have 5,7,4'-trihydroxyl groups, and liberated glucose in mother liquor. Since both glycoside AG1 and its hydrolysate, vicenin-2, showed the presence of 5,7,4'-trihydroxyl groups, *O*-glucosyl must be attached to 6- or 8-C-glucosyl group. Accordingly, the flavonoid AG1 was regarded as 6,8-di-C-glucosylapigenin X''-O-glucoside.

On the other hand, flavonoid AG4 also gave, upon hot acid treatment, vicenin-2 which was characterized by co-PC with the authentic specimen. However, sugar which was attached to 6- or 8-C-glucosyl moiety failed to be determined on account of a scanty amount of the original glycoside.

*6,8-Di-C-glucosylapigenin 4'-O-glucoside (AG2) and 4'-O-glycoside (AG3)*: Flavonoid AG2 and AG3 having the distinctive R<sub>f</sub> values (Table 1) were also liberated

vicenin-2 upon acid hydrolysis in aqueous mother liquors. UV spectral measurements of their original components did exhibit a bathochromic shift of the Band I with decrease in intensity relative to methanolic spectrum after addition of NaOMe, showing the presence of substituted 4'-hydroxyl group, and also indicated the presence of free 5, 7-dihydroxyl groups after addition of  $\text{AlCl}_3$ ,  $\text{AlCl}_3/\text{HCl}$ , or NaOAc (Table 2, Mabry *et al.* 1970, Hayashi *et al.* 1984). Therefore, *O*-glycosyls of these flavonoids, which were identified as glucose by direct comparison with the authentic specimen at flavonoid AG2 and could not be characterized at flavonoid AG3, must be linked with 4'-hydroxyl group. The flavonoid AG2 and AG3, therefore, were proved as 6, 8-di-*C*-glucosylapigenin 4'-*O*-glucoside and 4'-*O*-glycoside, respectively.

*6, 8-Di-C-glycosylluteolin (B)*: The UV spectral properties of the flavonoid B upon addition of various reagents have shown the presence of 5, 7, 3', 4'-tetrahydroxyl groups (Table 2). On the other hand, this glycoside was neither hydrolyzed nor undergone a Wessely-Moser rearrangement upon hot acid treatment, which might be estimated by arrangement of the same *C*-glycosyl group between 6- and 8-positions (Markham 1982), as indicated by their  $R_f$  values in BAW and 15% AcOH (Table 1, Chopin and Bouillant 1975). Thus, the flavonoid B was presumed to be 6, 8-di-*C*-glycosylluteolin (probably, lucenin-2, 6, 8-di-*C*-glucosylluteolin).

*6-Mono-C-glycosylapigenin (isovitexin, IV)*: UV spectral properties of the minor component from *Silene armeria* have shown the presence of 5, 7, 4'-triOH groups (Table 2). The original glycoside was not degraded by acid treatment, but gave rise to an other spot on the chromatogram, owing to the Wessely-Moser rearrangement. Such data ascertained the structure of this compound as 5, 7, 4'-trihydroxy-6 or 8-mono-*C*-glycosylflavone. Finally, flavonoid IV was determined as 6-mono-*C*-glucosylapigenin (isovitexin, Fig. 2) by comparison of chromatographic behavior with an authentic sample (Table 1).

*6-mono-C-glycosylluteolin (isoorientin, IOR)*: It is apparent that a minor component of flavonoid IOR is 6- or 8-*C*-glycosylflavone having 5, 7, 3', 4'-tetraOH groups as indicated by spectral data ( $\lambda_{\text{max}}$  348 nm in methanol). The  $R_f$  values of co-PC using BAW and 15% AcOH were identical with those of the authentic 6-mono-*C*-glucosylluteolin (isoorientin, Table 1 and Fig. 2).

#### Identification of anthocyanins

*Cyanidin 3-O-rhamnosylglucoside (keracyanin, Cy1)*: The anthocyanins which have occurred in *Pseudostellaria palibiniana*, *Stellaria media*, *Myosoton aquaticum* and *Cerastium glomeratum*, and one of the two kinds of anthocyanins from *Silene armeria* had completely identical  $R_f$  values (BuH, 1% HCl, BAW and Forestal, Table 3) and absorption maxima, and liberated cyanidin, glucose and rhamnose by acid hydrolysis which were verified by co-PC with the authentic specimens. By partial hydrolysis, an anthocyanin

Table 3. Rf-values, absorption maxima and hydrolysates of anthocyanins occurring in the species of Caryophyllaceae

Anthocyanins	Rf-values				$\lambda_{\max}$ (nm)	hydrolysates	
	BuH	1% HCl	BAW	Forestal	0.01% MeOH-HCl	aglycones	sugars
Cy1	0.54	0.14	0.49	0.74	526*	cyanidin (glucose rhamnose)	glucose
Cy2	0.37	0.05	—	—	—		
Authentic specimens:							
cyanidin 3-O-rutinoside	0.54	0.14	0.49	0.74	523		
cyanidin 3-O-glucoside	0.37	0.06	—	—	525		
cyanidin 3, 5-O-diglucoside	0.22	0.20	—	—	525		

1% HCl = conc. HCl/H<sub>2</sub>O (3 : 97), BuH = n-BuOH/conc. HCl/H<sub>2</sub>O (7 : 2 : 5, upper phase), Forestal = AcOH/conc. HCl/H<sub>2</sub>O (30 : 3 : 10).

\* Bathochromic shift (6 nm) by the addition of AlCl<sub>3</sub>.

was produced from this component and identified as cyanidin 3-O-glucoside. Finally, anthocyanin Cy1 was determined as cyanidin 3-O-rhamnosylglucoside (keracyanin) by co-PC with the authentic sample (Table 3).

*Cyanidin 3-O-glucoside (chrysanthemine, Cy2)*: An anthocyanidin and a sugar component which were obtained by acid hydrolysis of anthocyanin Cy2 from *Silene armeria* were identified as cyanidin and glucose, respectively, by direct comparisons of chromatographic behaviors with the authentic specimens. Thus, the original anthocyanin was identified as cyanidin 3-O-glucoside (chrysanthemine) by co-PC with the authentic specimen (Table 3).

Until now, the majority of flavonoids which have been isolated from the species of Caryophyllaceae are C-glycosylflavones and their O-glycosides, such as vicenin-2, lucenin-2, isovitexin and its 7-O-galactoside, 2''-O-rhamnoside, cerarvensin and so on (Dubois *et al.* 1982, Besson *et al.* 1979, Wagner *et al.* 1979, Bouillant *et al.* 1979). Eight kinds of flavonoids which were identified or partially characterized in this experiment were also C-glycosylflavones and their O-glycosides, which were isolated for the first time from the following plants: *Pseudostellaria heterophylla*, *P. palibiniana*, *Arenaria serpyllifolia*, *Stellaria sessiliflora*, *S. alsine* var. *undulata*, *Myosoton aquaticum*, *Cerastium glomeratum*, *C. holosteoides* var. *hallaisanense*, *Sagina japonica*, *Silene armeria* and *Gypsophila elegans*. In *Stellaria media*, though isovitexin and vitexin has already been found (Richardson 1978), vicenin-2 (A) and its 4'-O-glycoside (A3) and 6, 8-di-C-glycosylluteolin (B) were detected in the present work (Table 4). The fact as such has indicated that the chemical geographic variations have occurred in this cosmopolitan weed. Although flavonol O-glycosides, e. g., isorhamnetin 3-O-rutinoside and 3-O-rutinosylglucoside, quercetin 3-O-rutinoside and kaempferol glycoside have also been reported in a few *Silene*, *Herniaria* and *Dianthus* species (Hegnauer 1963, Richardson 1978), it has proved that the

Table 4. Occurrence of flavonoids in the Caryophyllaceae species were used in this experiment

Species	Parts	Flavonoids	Localities
<i>Pseudostellaria heterophylla</i> (Miq.) Pax (ワダソウ)	aerial parts	A, AG4	Mt. Kushigata Yamanashi Pref.
<i>P. palibiniana</i> (Takeda) Ohwi (ヒゲネワチガイソウ)	{aerial parts stems	A, AG4 Cy1	Mt. Chichibu- Ontake, Saitama Pref.
<i>Arenaria serpyllifolia</i> L. (ノミノツヅリ)	aerial parts	A, AG1, B, IOR, IV	Ichinomiya Town, Yamanashi Pref.
<i>Stellaria sessiliflora</i> Yabe (ミヤマハコベ)	aerial parts and petals	A, AG1	Mt. Chichibu- Ontake, Saitama Pref.
<i>S. media</i> (L.) Villars (ハコベ)	{aerial parts stems	A, AG3, B Cy1	Setagaya-ku, Tokyo
<i>S. alsine</i> Grimm var. <i>undulata</i> (Thunb.) Ohwi (ノミノフスマ)	aerial parts	A, AG1, B, Cy1	Mt. Oono, Kanagawa Pref.
<i>Myosoton aquaticum</i> (L.) Moench (ウシハコベ)	{aerial parts stems	A, AG1 Cy1	Ichinomiya Town, Yamanashi Pref.
<i>Cerastium glomeratum</i> Thuill. (オランダミミナグサ)	{aerial parts stems	A, AG2, B Cy1	Setagaya-ku, Tokyo
<i>C. holosteoides</i> Fries var. <i>hallaisanense</i> (Nakai) Mizushima (ミミナグサ)	aerial parts	A	Mt. Kawanori, Tokyo
<i>Sagina japonica</i> (Sw.) Ohwi (ツメクサ)	aerial parts	A, AG1, B	Ichinomiya Town, Yamanashi Pref.
<i>Silene armeria</i> L. (ムシトリナデシコ)	{flowers leaves	IV, IOR, Cy1, Cy2 IOR	Setagaya-ku, Tokyo
<i>Gypsophila elegans</i> Bieb. (カスミソウ)	flowers	A	Setagaya-ku, Tokyo, (cultivated)

A=6, 8-di-C-glucosylapigenin (vicenin-2), AG1=6, 8-di-C-glucosylapigenin X<sup>n</sup>-O-glucoside, AG2=6, 8-di-C-glucosylapigenin 4'-O-glucoside, AG3=6, 8-di-C-glucosylapigenin 4'-O-glycoside, AG4=6, 8-di-C-glucosylapigenin X<sup>n</sup>-O-glycoside, B=6, 8-di-C-glycosylluteolin, IV=6-mono-C-gluco-sylapigenin (isovitexin), IOR=6-mono-C-glucosylluteolin (isoorientin), Cy1=cyanidin 3-O-rhamnosylglucoside (keracyanin), Cy2=cyanidin 3-O-glucoside (chrysanthemine).

presence of C-glycosylflavones is an ubiquitous feature in the Caryophyllaceae. Among their C-glycosylflavones, vicenin-2 and its O-glycosides have been found in most species (11 spp. except *Silene armeria*, Table 4), as described in this experiment.

The anthocyanins have been reported as major pigments in the flowers of *Dianthus* spp. (Terahara *et al.* 1986, Ootani and Miura 1961) and *Spergularia* (Beck *et al.* 1962). In the present survey, cyanidin 3-O-rhamnosylglucoside was newly found in the colored stems of *Pseudostellaria palibiniana*, *Stellaria media*, *Myosoton aquaticum*, *Cerastium glomeratum*, and keracyanin and cyanidin 3-O-glucoside from the flowers of *Silene armeria*, which was previously described as cyanidin glycoside (Yoshitama *et al.* 1980).

### Acknowledgements

The authors express their sincere gratitude to Dr. Kôzô Hayashi (the Research Institute of Evolutionary Biology) for his deep interest and continuous support throughout this experiment. Authors' thanks are also due to Dr. Fumiyuki Mitsumori (National Institute for Environmental Studies) for NMR measurements, and also to Mr. Masaru Kawasaki (Faculty of Pharmaceutical Science, Toyama Medical and Pharmaceutical University) for the supply of authentic sample of vicenin-2.

### Summary

The leaves, flowers and stems of 12 species from the family Caryophyllaceae mostly native to Japan were investigated for flavonoids involving anthocyanins. Eight kinds of C-glycosylflavones and their O-glycosides and two kinds of anthocyanins were determined by paper and column chromatography, and sometimes by crystallization. Final identification was made by chromatographic comparison with authentic specimens, UV spectral behaviors, <sup>1</sup>H-NMR data as usual. The following compounds were clarified: 6,8-di-C-glycosylapigenin (vicenin-2, A) and its X''-O-glucoside (AG1), 4'-O-glucoside (AG2), 4'-O-glycoside (AG3), X''-O-glycoside (AG4), 6,8-di-C-glycosylluteolin (B), 6-mono-C-glycosylapigenin (isovitexin, IV) and 6-mono-C-glycosylluteolin (isorientin, IOR) as C-glycosylflavones, and cyanidin 3-O-glucoside (chrysanthemine, Cy2) and 3-O-rhamnosylglucoside (keracyanin, Cy1) as anthocyanins.

Though flavonols have been found in a few caryophyllaceous plants, all the flavonoids which were characterized in this experiment were C-glycosylflavones and their O-glycosides. And, they were detected in *Pseudostellaria heterophylla*, *P. palibiniana*, *Arenaria serpyllifolia*, *Stellaria sessiliflora*, *S. alsine* var. *undulata*, *Myosoton aquaticum*, *Cerastium glomeratum*, *C. holosteoides* var. *hallaisanense*, *Silene armeria*, *Gypsophila elegans* and *Sagina japonica* excepting the cosmopolitan weed, *Stellaria media*. It has proved that the presence of C-glycosylflavones, especially vicenin-2, is a constant feature among the Caryophyllaceae.

### 摘 要

日本産を主とした12種のナデシコ科植物の地上部、花などに含まれるフラボン類とアントシアニン類を分離し、それらの同定を行なった。

濾紙およびカラムクロマト法、あるいは結晶化によって8種類のフラボン類と2種類のアントシアニンが得られた。それらの化合物はクロマトグラム上における基準標品との比較、UV吸収スペクトル、または<sup>1</sup>H-NMRスペクトルによって次のように定性することができた。すなわち、フラボンとしては6,8-di-C-glycosylapigenin (vicenin-2, A)とそのX''-O-glucoside (AG1)、4'-

*O*-glucoside (AG2), 4'-*O*-glycoside (AG3), X''-*O*-glycoside (AG4), 6, 8-di-*C*-glycosylluteolin (B), 6-mono-*C*-glucosylapigenin (isovitexin, IV) および 6-mono-*C*-glucosylluteolin (isoorientin, IOR), アントシアニンとしては cyanidin 3-*O*-rhamnosylglucoside (keracyanin, Cy1) および 3-*O*-glucoside (chrysanthemin, Cy2), である。

ナデシコ科植物に含まれるフラボノイドとしては、従来多くの *C*-グリコシルフラボンが知られており、今回定性することのできたフラボノイドも、上記のごとく、すべてアピゲニン型カルテオリン型の *C*-グリコシルフラボンであり、今までごく少数の種で知られていたフラボノール配糖体は検出できなかった。また、今回材料として用いた12種の植物のうち、ハコベ (*Stellaria media*) を除く11種の植物、すなわちワダソウ (*Pseudostellaria heterophylla*), ヒゲネワチガイソウ (*P. palibiniana*), ノミノツヅリ (*Arenaria serpyllifolia*), ミヤマハコベ (*Stellaria sessiliflora*), ノミノフスマ (*S. alsine* var. *undulata*), ウシハコベ (*Myosoton aquaticum*), オランダミミナグサ (*Cerastium glomeratum*), ミミナグサ (*C. holosteoides* var. *hallaisanense*), ムシトリナデシコ (*Silene armeria*), カスミソウ (*Gypsophila elegans*) およびツメクサ (*Sagina japonica*) では今回初めて *C*-グリコシルフラボンの存在が明らかになった。

従来、ハコベでは vitexin と isovitexin の存在が報告されていたが、今回の分析では、これらの成分は検出されず、代わりに vicenin-2 とその 4'-*O*-glucoside および 6, 8-di-*C*-glycosylluteolin が検出され、雑草として世界的に広く分布しているこの植物のフラボン成分に地理的変異の生じている可能性が示唆された。

## References

- Beck, E., H. Merxmüller und H. Wagner, 1962. Über die Art der Anthocyane bei Plumbaginaceen, Alsinoiden und Molluginaceen. *Planta* 58: 220-224.
- Besson, E., A. Besset, M.L. Bouillant, J. Chopin, J. van Brederode and G. van Nigtevecht, 1979. Genetically controlled 2''-*O*-glycosylation of isovitexin in the petals of *Melandrium album*. *Phytochemistry* 18: 657-658.
- Bouillant, M.L., F.F. de Arce, J. Favre-Bonvin, J. Chopin, A. Zoll et G. Mathieu, 1979. Nouvelles *C*-glycosylflavones extraites de *Spergularia rubra*. *Phytochemistry* 18: 1043-1047.
- Chopin, J. and M.L. Bouillant, 1975. *C*-Glycosylflavonoids. p. 680. In Harborne, J.B., T.J. Mabry and H. Mabry (ed.), *The flavonoids*. Chapman and Hall, London.
- Dubois, M., A. Zoll, M.L. Bouillant and J. Chopin, 1982. New *C*-glycosylflavones from *Cerastium arvense*. *Phytochemistry* 21: 1141-1143.
- , ———, K.R. Markham, M.L. Bouillant, G. Dellamonica and J. Chopin, 1984. 6-*C*- $\beta$ -D-Glucopyranosyl-8-*C*- $\beta$ -D-galactopyranosylapigenin from *Cerastium arvense*. *Phytochemistry* 23: 706-707.
- Hayashi, K., T. Iwashina, M. Kawasaki and S. Ootani, 1984. Flavonoids in the flowers of *Iris gracilipes*. *Sci. Rep. Res. Inst. Evolut. Biol.* 2: 75-83 (in Japanese).
- Hegnauer, R., 1964. *Chemotaxonomie der Pflanzen*. Vol. 3. p. 378-392. Birkhauser Verlag Basel und Stuttgart.
- Iwashina, T., S. Ootani and K. Hayashi, 1984. Neochilenin, a new glycoside of 3-*O*-methylquercetin, and other flavonols in the tepals of *Neochilenia*, *Neoporteria* and *Parodia* species (Cactaceae). *Bot. Mag. Tokyo* 97: 23-30.
- Kawasaki, M., T. Kanomata and K. Yoshitama, 1986. Flavonoids in the leaves of twenty-eight polygonaceous plants. *Bot. Mag. Tokyo* 99: 63-74.
- Mabry, T.J., 1966. The betacyanins and betaxanthins. p. 231-244. In Swain, T. (ed.), *Comparative phytochemistry*. Academic Press, London.
- , K.R. Markham and M.B. Thomas, 1970. The systematic identification of flavonoids. 1-175. Springer-Verlag, Berlin.

- Markham, K.R., 1982. Techniques of flavonoid identification. 1-113. Academic Press, London.
- Ootani, S. and T. Miura, 1961. Paper chromatographic survey of anthocyanins in carnation, *Dianthus caryophyllus*. Inst. Breed. Res. Tokyo Univ. Agr. 2: 22-27.
- Richardson, M., 1978. Flavonols and C-glycosylflavonoids of the Caryophyllales. Biochem. Syst. Ecol. 6: 283-286.
- Takeda, K. and K. Hayashi, 1980. Red flavonoids (Anthocyanins). p. 151-174. In Hayashi, K. (ed.), Plant pigments—An introduction to research and experiments—. Yokendo, Ltd., Tokyo (in Japanese).
- Terahara, N., M. Yamaguchi, K. Takeda, J.B. Harborne and R. Self, 1986. Anthocyanins acylated with malic acid in *Dianthus caryophyllus* and *D. deltoides*. Phytochemistry 25: 1715-1717.
- Wagner, H., G. Obermeier, O. Seligman und V.M. Chari, 1979. Neue Flavon-C-O-Glykoside aus *Melandrium album*. Phytochemistry 18: 907-910.
- Yoshitama, K., K. Ishii and H. Yasuda, 1980. A chromatographic survey of anthocyanins in the flora of Japan, I. J. Fac. Sci. Shinshu Univ. 15: 19-26.