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Flavonol Glycosides from *Clematis* Cultivars and Taxa, and Their Contribution to Yellow and White Flower Colors

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Abstract The flower pigments in two yellow *Clematis* cultivars, "Gekkyuden" and "Manshu-ki", and a yellow flower type of *C. patens* collected in Korea, were characterized. They were compared with those of three white *Clematis florida* varieties, var. *florida*, var. *florepleno* and var. *sieboidiana*. It was shown by UV-visible spectral survey of crude MeOH extract of their sepals that carotenoid pigment is apparently absent from yellow flowers. High performance liquid chromatographical and paper chromatographical survey of the flower pigments showed the presence of the flavonol glycosides. They were isolated and characterized by UV spectroscopy, acid hydrolysis, LC-MS, and direct HPLC and TLC comparisons with authentic samples. Quercetin 3-*O*-galactoside (**6**) and 3-*O*-glucoside (**7**) were isolated from two yellow cultivars and a yellow flower type of *C. patens* as major components together with minor quercetin 3-*O*-rutinoside (**8**). On the other hand, kaempferol 3-*O*-rutinoside (**9**) and 3-*O*-glucoside (**4**) were detected in the white *C. florida* varieties as major compounds. From the results described above, it was clear for the first time that yellow flower color of *Clematis* cultivars are due to much amount of quercetin glycosides. However, it was shown that kaempferol glycosides do not act as yellow pigments, even if they were abundantly accumulated. Apart from those results, the flavonol glycosides of *C. armandii* flowers were also isolated and identified as quercetin 3-*O*-rutinoside-7-*O*-glucoside (**1**), kaempferol 3,7-di-*O*-glucoside (**2**), kaempferol 3-*O*-galactoside (**3**), kaempferol 3-*O*-glucoside (**4**), kaempferol 3-*O*-glucuronide (**5**), quercetin 3-*O*-galactoside (**6**), quercetin 3-*O*-glucoside (**7**), Quercetin 3-*O*-rutinoside (**8**) and kaempferol 3-*O*-rutinoside (**9**).

Key words : *Clematis* cultivars, flavonol glycosides, quercetin glycosides, yellow flower color.

Introduction

The genus *Clematis* belongs to the family Ranunculaceae and consists of ca. 300 species (Matthews, 2002). *Clematis* is one of the world's important horticultural plants, valued as an ornamental garden subject. They have been extensively hybridized among five major *Clematis* species, i.e., *C. viticella* L., *C. florida* Thunb., *C. lanuginosa* Lindl., *C. patens* Morr. et Decne. and *C.* 'jackmanii' (Ino and Nakamura, 1986). A wide range of ornamental flower colors, white, red, purple and yellow, are available from many cultivars. Of their flower colors, it has been reported that red and purple are due to the anthocyanins, cyanidin and delphinidin glycosides, respectively (Hosoki *et al*., 2003). More recently, the anthocyanins and other flavonoids in the white, pink and purple flowers of *C. patens* were analyzed, and cyanidin and delphinidin glycosides and six flavonol glycosides, i.e., kaempferol 3-*O*-(caffeoylglucoside), kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-alloside, kaempferol glycoside and quercetin 3-*O*-glycoside, were found in pink flowers (Hashimoto *et al*., 2008). Two anthocyanidins and six

flavonol glycosides, i.e., kaempferol 3-*O*-gentiobioside, two kaempferol glycosides, kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside and kaempferol 3-*O*-alloside were detected in white flowers. Cyanidin and delphinidin glycosides were found in purple flowers without flavonol glycosides. However, the pigment components of the yellow flowers has not been known.

In this survey, the flavonol glycosides in two yellow cultivars, "Gekkyuden" and "Manshu-ki", and a yellow flower type of *C. patens* were characterized as yellow pigments, and compared with those of three white *C. florida* varieties, var. *florida*, var. *florepleno* G. Don and var. *sieboldiana* Morr.

Materials and Methods

Plant materials

Two yellow *Clematis* cultivars "Gekkyuden" (TBG 142597) and "Manshu-ki" (TBG 142589), a yellow flower type of *C. patens* Morr. et Decne. collected in Korea (TBG 134533), and three white *C. florida* Thunb. varietes, var. *florida* (TBG 141574), var. *florepleno* G. Don (TBG 142736) and var. *sieboldiana* Morr. (TBG 142734), and *C. armandii* Franch. (TBG 142744) were used as plant materials. They are cultivated at Tsukuba Botanical Garden, National Museum of Nature and Science, Japan.

Isolation of flavonoids

Fresh sepals of each cultivars and taxa were extracted with MeOH. After concentration, the extracts were applied to preparative paper chromatography using solvent systems: BAW (*n*-BuOH/HOAc/H_2O=4 : 1 : 5, upper phase), 15% HOAc and then BEW (*n*-BuOH/EtOH/H_2O= 4 : 1 : 2.2). Crude flavonoids were furthermore applied to preparative HPLC which was described below. The obtained flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

High performance liquid chromatography (HPLC)

Preparative HPLC was performed with Tosoh HPLC systems using Senshu Pak PEGASIL ODS column (I.D. 10×250 mm, Senshu Scientific Co. Ltd.), at a flow-rate of 1.5 ml min^{-1}; injection was 500 μl; detection was 350 nm and eluent was HCOOH/MeCN/H_2O (1 : 18–22 : 78–85). Qualitative and quantitative HPLC was performed with Shimadzu HPLC systems using PEGASIL ODS column (I.D. 6.0×150 mm, Senshu Scientific Co. Ltd.), at a flow-rate of 1 ml min^{-1}; injection was 10 μl; detection was 190–700 nm and eluents were MeCN/H_2O/H_3PO_4 (22 : 78 : 0.2) for glycosides and MeCN/H_2O/H_3PO_4 (35 : 65 : 0.2) for aglycones. Fresh sepals (0.2 g) were extracted with FMW (HCOOH/MeOH/H_2O=8 : 20 : 72) for quantitative HPLC.

Liquid chromatograph-mass spectra (LC-MS)

LC-MS were measured using a PEGASIL ODS column (I.D. 2.0×150 mm, Senshu Scientific Co. Ltd.) at a flow-rate of 0.2 ml min^{-1}; injection was 10 μl; eluent was HCOOH/MeCN/H_2O=0.2 : 18 : 82), ESI^+ 4.5 kV, ESI^- 3.5 kV, 250°C.

Identification of flavonoids

The isolated flavonoids were identified by UV spectral survey according to Mabry *et al*. (1970), acid hydrolysis and characterization of hydrolysates, LC-MS, ^1H and ^{13}C NMR and direct TLC and HPLC comparisons with authentic samples. TLC, HPLC, LC-MS and ^1H and ^{13}C NMR data of the isolated flavonoids are as follows.

Quercetin 3-*O*-rutinoside-7-*O*-glucoside (**1**). TLC: Rf 0.15 (BAW), 0.13 (BEW), 0.67 (15%HOAc); UV – dark purple, UV/NH_3 – light yellow. HPLC: *t*R (min) 3.55. UV: λmax (nm) MeOH 257, 266sh, 359; +NaOMe 270, 398 (inc.); +$AlCl_3$ 275, 438; +$AlCl_3$/HCl 270, 300sh, 367, 401; +NaOAc 270, 427; +NaOAc/H_3BO_3 267, 404. LC-MS: *m*/*z* 773 $[M+H]^+$ (quercetin+2 mol glucose and 1 mol rhamnose), 627 $[M-146+H]^+$ (quercetin+2 mol glucose), 611 $[M-162+H]^+$ (quercetin+each 1

mol glucose and rhamnose), 465 $[M-308+H]^+$ (quercetin+1 mol glucose) and 303 $[M-470+H]^+$ (quercetin). ^1H NMR (500 MHz, pyridine-d_5): δ 8.44 (^1H, *s*, H-2′), 8.06 (^1H, *d*, *J*=8.9 Hz, H-6′), 7.25 (^1H, *d*, *J*=8.2 Hz, H-5′), 6.92 (^1H, *s*, H-8), 6.78 (^1H, *s*, H-6), 6.00 (^1H, *d*, *J*=7.6 Hz, 3-glucosyl H-1), 5.79 (^1H, *d*, *J*=7.0 Hz, 7-glucosyl H-1), 5.32 (^1H, *s*, rhamnosyl H-1), 4.0–4.8 (*m*, sugar protons), 1.51 (3H, *d*, *J*=5.5 Hz, rhamnosyl OCH_3). ^{13}C NMR (125 MHz, pyridine-d_5): (quercetin) δ 158.5 (C-2), 135.2 (C-3), 178.8 (C-4), 162.1 (C-5), 100.4 (C-6), 164.0 (C-7), 95.2 (C-8), 156.9 (C-9), 105.2 (C-10), 122.6 (C-1′), 116.3 (C-2′), 146.8 (C-3′), 151.0 (C-4′), 117.9 (C-5′), 122.8 (C-6′); (3-glucose) δ 101.9 (C-1), 74.8 (C-2), 79.2 (C-3), 71.2 (C-4), 75.3 (C-5), 67.6 (C-6); (3-rhamnose) δ 101.7 (C-1), 72.1 (C-2), 72.3 (C-3), 73.2 (C-4), 69.6 (C-5), 18.5 (C-6); (7-glucose) δ 101.9 (C-1), 73.9 (C-2), 75.2 (C-3), 69.7 (C-4), 78.4 (C-5), 62.4 (C-6).

Kaempferol 3,7-di-*O*-glucoside (peonoside, **2**). TLC: Rf 0.34 (BAW), 0.24 (BEW), 0.65 (15%HOAc); UV – dark purple, UV/NH_3 – dark greenish yellow. HPLC: *t*R (min) 3.57. UV: λmax (nm) MeOH 267, 346; +NaOMe 275, 389 (inc.); +$AlCl_3$ 274, 300, 355, 395sh; +$AlCl_3$/HCl 274, 300, 351, 397sh; +NaOAc 267, 350; +NaOAc/H_3BO_3 267, 351. LC-MS: *m/z* 611 $[M+H]^+$ (kaempferol+2 mol glucose), 449 $[M-162+H]^+$ (kaempferol+1 mol glucose) and 287 $[M-324+H]^+$ (kaempferol).

Kaempferol 3-*O*-galactoside (trifolin, **3**). TLC: Rf 0.67 (BAW), 0.70 (BEW), 0.36 (15%HOAc); UV – dark purple, UV/NH_3 – dark greenish yellow. HPLC: *t*R (min) 7.24. UV: λmax (nm) MeOH 266, 350; +NaOMe 275, 318, 400 (inc.); +$AlCl_3$ 270, 304, 353, 395sh; +$AlCl_3$/HCl 271, 303, 355, 390sh; +NaOAc 270, 303, 358; +NaOAc/H_3BO_3 262, 298sh, 346. LC-MS: *m/z* 449 $[M+H]^+$ (kaempferol+1 mol galactose) and 287 $[M-162+H]^+$ (kaempferol).

Kaempferol 3-*O*-glucoside (astragalin, **4**). TLC: Rf 0.69 (BAW), 0.71 (BEW), 0.37 (15%HOAc); UV – dark purple, UV/NH_3 – dark greenish yellow. HPLC: *t*R (min) 7.95. UV: λmax (nm) MeOH 266, 349; +NaOMe 274, 325, 398 (inc.); +$AlCl_3$ 274, 305, 353, 390sh; +$AlCl_3$/HCl 275, 304, 351, 390sh; +NaOAc 271, 303, 359; +NaOAc/H_3BO_3 267, 295sh, 349. LC-MS: *m/z* 449 $[M+H]^+$ (kaempferol+1 mol glucose) and 287 $[M-162+H]^+$ (kaempferol).

Kaempferol 3-*O*-glucuronide (**5**). TLC: Rf 0.69 (BAW), 0.40 (BEW), 0.37 (15%HOAc); UV – dark purple, UV/NH_3 – dark greenish yellow. HPLC: *t*R (min) 7.94. UV: λmax (nm) MeOH 266, 349; +NaOMe 274, 325, 398 (inc.); +$AlCl_3$ 274, 305, 353, 390sh; +$AlCl_3$/HCl 275, 304, 351, 390sh; +NaOAc 271, 303, 359; +NaOAc/H_3BO_3 267, 295sh, 349. LC-MS: *m/z* 463 $[M+H]^+$ (kaempferol+1 mol glucuronic acid) and 287 $[M-176+H]^+$ (kaempferol).

Quercetin 3-*O*-galactoside (hyperin, **6**). TLC: Rf 0.54 (BAW), 0.57 (BEW), 0.27 (15%HOAc); UV – dark purple, UV/NH_3 – yellow. HPLC: *t*R (min) 5.97. UV: λmax (nm) MeOH 257, 265sh, 360; +NaOMe 273, 328, 413 (inc.); +$AlCl_3$ 274, 433; +$AlCl_3$/HCl 268, 301, 364, 398; +NaOAc 274, 410; +NaOAc/H_3BO_3 267, 393. LC-MS: *m/z* 465 $[M+H]^+$ (quercetin+1 mol galactose) and 303 $[M-162+H]^+$ (quercetin).

Quercetin 3-*O*-glucoside (isoquercitrin, **7**). TLC: Rf 0.54 (BAW), 0.57 (BEW), 0.27 (15%HOAc); UV – dark purple, UV/NH_3 – yellow. HPLC: *t*R (min) 6.13. UV: λmax (nm) MeOH 257, 267sh, 358; +NaOMe 273, 330, 411 (inc.); +$AlCl_3$ 275, 433; +$AlCl_3$/HCl 268, 301, 363, 396; +NaOAc 274, 414; +NaOAc/H_3BO_3 264, 385. LC-MS: *m/z* 465 $[M+H]^+$ (quercetin+1 mol glucose) and 303 $[M-162+H]^+$ (quercetin).

Quercetin 3-*O*-rutinoside (rutin, **8**). TLC: Rf 0.51 (BAW), 0.42 (BEW), 0.51 (15%HOAc); UV – dark purple, UV/NH_3 – yellow. HPLC: *t*R (min) 5.29. UV: λmax (nm) MeOH 257, 265sh, 357; +NaOMe 272, 330, 410 (inc.); +$AlCl_3$ 274, 431; +$AlCl_3$/HCl 268, 300, 363, 397; +NaOAc 272, 412; +NaOAc/H_3BO_3 267, 403. LC-MS: *m/z* 611 $[M+H]^+$ (quercetin+each 1 mol glucose and rhamnose), 465 $[M-146+H]^+$ (quercetin+1 mol glucose) and 303 $[M-308+H]^+$ (quercetin).

Kaempferol 3-*O*-rutinoside (nicotiflorin, **9**).

TLC: Rf 0.50 (BAW), 0.56 (BEW), 0.61 (15%HOAc); UV－dark purple, UV/NH_3－dark greenish yellow. HPLC: *t*R (min) 6.92. UV: λmax (nm) MeOH 266, 352; ＋NaOMe 275, 326, 400 (inc.); ＋$AlCl_3$ 274, 304, 354, 396; ＋$AlCl_3$/HCl 275, 303, 350, 392; ＋NaOAc 274, 306, 375; ＋NaOAc/H_3BO_3 267, 297sh, 351. LC-MS: *m*/*z* 595 $[M+H]^+$ (kaempferol＋each 1 mol glucose and rhamnose), 449 $[M-146+H]^+$ (kaempferol＋1 mol glucose) and 287 $[M-308+H]^+$ (kaempferol).

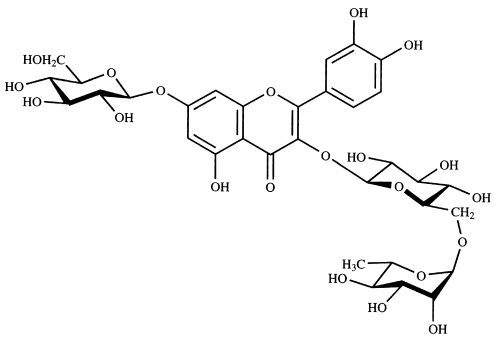

Fig. 1. Quercetin 3-*O*-rutinoside-7-*O*-glucoside (**1**).

Results and Discussion

Identification of flavonoids isolated from Clematis *flowers*

In this survey, nine flavonoids were isolated from the sepals of five *Clematis* taxa, *C. armandii*, *C. florida* var. *florida*, *C. florida* var. *florepleno*, *C. florida* var. *sieboldiana* and *C. patens*, and two *Clematis* cultivars, "Gekkyuden" and "Manshu-ki". Of their taxa and cultivars, all flavonoids were obtained from the extract of *C. armandii* flowers.

Flavonoid **1** was obtained as pale yellow powder. UV spectral properties of the compound were those of 5,3′,4′-trihydroxy-3,7-substututed flavone (Mabry *et al*., 1970). Quercetin, glucose and rhamnose were liberated by acid hydrolysis of the original glycoside. By LC-MS survey, molecular ion peak, *m*/*z* 773, showing the attachment of 2 mol glucose and 1 mol rhamnose to quercetin, was indicated together with fragment ion peaks, *m*/*z* 627 (quercetin＋2 mol glucose), 611 (quercetin＋each 1 mol glucose and rhamnose), 465 (quercetin＋1 mol glucose) and 303 (quercetin itself). Three intemediates were produced by partial acid hydrolysis (60°C, 120 min, in 1% HCl－MeOH : 18% aq.HCl＝1 : 1) and identified as quercetin 7-*O*-glucoside, quercetin 3-*O*-rutinoside and quercetin 3,7-di-*O*-glucoside by LC-MS and/or direct HPLC comparison with authentic sample. Finally, flavonoid **1** was identified as quercetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside-7-*O*-β-glucopyranoside, i.e., quercetin 3-*O*-rutinoside-7-*O*-glucoside by ^{1}H and ^{13}C NMR spectra (Fig. 1). The flavonoid has been found in the flowers of *Nicotiana tabacum* L. (Watanabe and Wender, 1965). Though flavonoid **1** has not been found in *Clematis* species until now, it was isolated from *C. armandii* in this survey.

Kaempferol and glucose were liberated by acid hydrolysis of flavonoid **2**. Since the presence of free 5- and 4′-hydroxyl groups was shown by UV spectral survey, glucose must be attached to 3- and 7-hydroxyl groups. Molecular ion peak, *m*/*z* 611 $[M+H]^+$ and two fragment ion peaks, *m*/*z* 449 $[M-162+H]^+$ and 287 $[M-324+H]^+$ appeared by LC-MS survey. Thus, flavonoid **2** was identified as kaempferol 3,7-di-*O*-glucoside (Fig. 2).

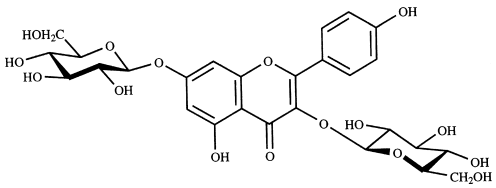

Fig. 2. Kaempferol 3,7-di-*O*-glucoside (peonoside, **2**).

It was shown by UV spectral survey that flavonoids **3** and **4** are 3-substituted 5,7,4′-trihydroxyflavones. Kaempferol was liberated by acid hydrolysis of both glycosides, and galactose and glucose as glycosidic sugars from flavonoids **3** and **4**, respectively. LC-MS survey of both compounds showed the molecular ion peak, *m*/*z* 449 $[M+H]^+$, showing the attachment of 1 mol hex-

ose to kaempferol. Finally, flavonoids **3** and **4** were identified as kaempferol 3-*O*-galactoside (Fig. 3) and kaempferol 3-*O*-glucoside (Fig. 4) by direct TLC and HPLC comparisons with authentic trifolin from the leaves of *Cornus canadensis* L. (Cornaceae) (Iwashina and Hatta, 1992) and astragalin from the fronds of *Cyrtomium falcatum* (L. f.) C. Presl. (Dryopteridaceae) (Iwashina *et al*., 2006). Of their glycosides, kaempferol 3-*O*-galactoside was found in *C. armandii*. On the other hand, kaempferol 3-*O*-glucoside was detected in all the *Clematis* taxa and cultivars used as plant materials in this survey. It has been isolated from the flowers of *C. patens* (Hashimoto *et al*., 2008).

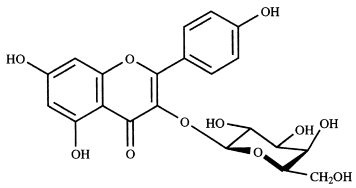

Fig. 3. Kaempferol 3-*O*-galactoside (trifolin, **3**).

UV spectral properties of flavonoid **5** was the same (3-substituted kaempferol) with those of flavonoids **3** and **4**. Acid hydrolysis of the original glycoside produced kaempferol and glucuronic acid as the hydrolysates. LC-MS survey of the compound was appeared the molecular ion peak, *m*/*z* 463 $[M+H]^+$, showing the attachment of 1 mol glucuronic acid to kaempferol. Flavonoid **5** was identified as kaempferol 3-*O*-glucuronide (Fig. 5) by direct TLC and HPLC comparison with authentic sample from the fronds of *Adiantum capillus-veneris* L. (Adiantaceae) (Iwashina *et al*., 1995). Kaempferol 3-*O*-glucuronide was found in *Clematis* species for the first time.

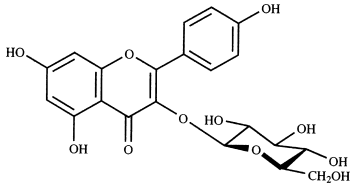

Fig. 4. Kaempferol 3-*O*-glucoside (astragalin, **4**).

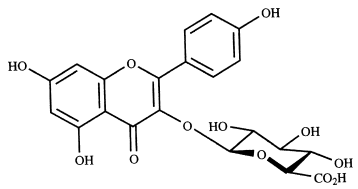

Fig. 5. Kaempferol 3-*O*-glucuronide (**5**).

Flavonoids **6** and **7** were isolated from all the *Clematis* taxa and cultivars used as plant materials in this experiment. Quercetin was liberated by acid hydrolysis of both glycosides and also galactose and glucose as glycosidic sugars from flavonoids **6** and **7**, respectively. Since UV spectral properties of both glycosides showed the presence of free 5-, 7-, 3′- and 4′-hydroxyl and a substituted 3-hydroxyl groups, galactose or glucose must be attached to 3-position of quercetin. Finally, flavonoids **6** and **7** were identified as quercetin 3-*O*-galactoside (Fig. 6) and quercetin

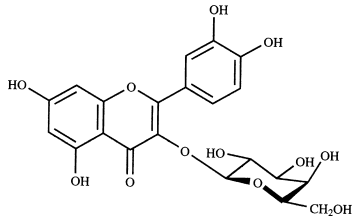

Fig. 6. Quercetin 3-*O*-galactoside (hyperin, **6**).

3-*O*-glucoside (Fig. 7) by direct TLC and HPLC comparisons with authentic hyperin from the flowers of *Notocactus ottonis* (Lem.) Berg. (Cactaceae) (Iwashina and Ootani, 1986) and isoquercitrin from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al*., 2008). Quercetin 3-*O*-galactoside was found in *Clematis* species for the first time, and quercetin 3-*O*-glucoside has been isolated from the leaves of *Clematis* stans Sieb. et Zucc. (Kizu *et al*., 1995) and of eight American *Clematis* taxa, i.e., *C. addisonii* Britton, *C. texensis* Buckley, *C. glaucophylla* Small, *C. versicolor* Small ex Rydb., *C. viorna* L., *C. reticulata* Walter, *C. pitcheri* Torr. et Gray var. pitcheri and *C. pitcheri* var. *dictyota* (Greene) Dennis (Dennis and Bierner, 1980).

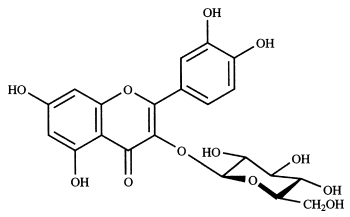

Fig. 7. Quercetin 3-*O*-glucoside (isoquercitrin, **7**).

Flavonoid **8** was also isolated from all the *Clematis* taxa and cultivars used as plant materials in this survey. Acid hydrolysis of this compound produced quercetin, glucose and rhamnose. Since the presence of free 5-, 7-, 3′- and 4′-hydroxyl groups was shown by UV spectral survey, it was indicated that both glucose and

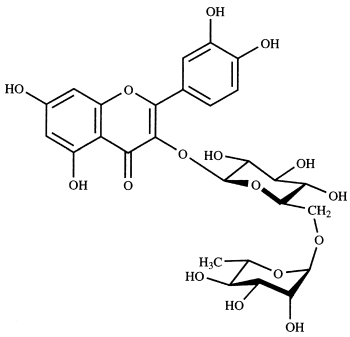

Fig. 8. Quercetin 3-*O*-rutinoside (rutin, **8**).

rhamnose are attached to 3-position of quercetin. Finally, flavonoid **8** was identified as quercetin 3-*O*-rutinoside (Fig. 8) by direct TLC and HPLC comparison with authentic rutin from the bracts of *Rheum nobile* Hook. f. et Thomson (Polygonaceae) (Iwashina *et al*., 2006). In *Clematis* species, this flavonoid has been reported from the leaves of *C. stans* (Kizu *et al*., 1995).

Flavonoid **9** was detected from three *C. florida* varieties, *C. armandii*, a yellow flower type of *C. patens* and a cultivar "Manshu-ki" but not from a cultivar "Gekkyuden". Kaempferol, glucose and rhamnose were liberated by acid hydrolysis. UV spectral properties of this glycoside indicated the presence of free 5-, 7- and 4′-hydroxyl and a substituted 3-hydroxyl groups, showing the attachment of glucose and rhamnose to 3-position of kaempferol. It was clear by appearance of a molecular ion peak, *m*/*z* 595 $[M+H]^+$ and a fragment ion peak, *m*/*z* 449 $[M–146+H]^+$ by LC-MS that each 1 mol glucose and rhamnose was attached to kaempferol. From the results described above, flavonoid **9** was characterized as kaempferol 3-*O*-rhamnosylglucoside. Finally, this glycoside was identified as kaempferol 3-*O*-rutinoside (Fig. 9) by direct TLC and HPLC comparison with authentic nicotiflorin from the flowers of *Glycine max* (L.) Merr. (Leguminosae) (Iwashina *et al*., 2007).

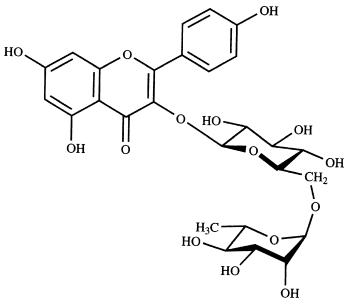

Fig. 9. Kaempferol 3-*O*-rutinoside (nicotiflorin, **9**).

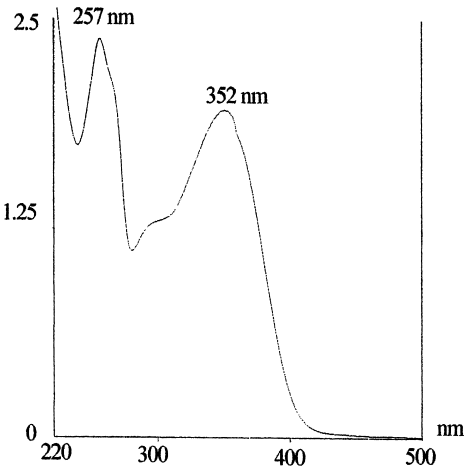

Fig. 10. UV-visible absorption spectra of crude MeOH extract from the yellow sepals of *Clematis* cultivar "Gekkyuden".

Quantitative comparison of flavonol glycosides between yellow and white Clematis *taxa and cultivars*

In UV-visible spectral survey of crude MeOH extract from the yellow sepals of *Clematis* culti-

Fig. 11. Yellow *Clematis* cultivar "Gekkyuden" (left) and white *C. florida* var. *florida* (right).

vars "Gekkyuden", the absorption maxima did not appear on the visible range (400–700 nm) (Fig. 10), showing the absence of visible pigments such as carotenoids. However, it showed absorption maxima at 352 nm. Quantitative HPLC analysis of two yellow *Clematis* cultivars "Gekkyuden" (Fig. 11) and "Manshu-ki", and a yellow flower type of *C. patens* collected in Korea showed much amount of quercetin 3-*O*-galactoside and 3-*O*-glucoside, together with minor kaempferol glycosides (Table 1). In contrast, those of three white *C. flarida* varieties, var. *florida* (Fig. 11), var. *florepleno* and var. *sieboldiana* indicated much amount of kaempferol glycosides together with minor quercetin glycosides. However, total flavonoid contents of white *Clematis* varieties have higher than those of yellow cultivars. From the results described above, it was shown for the first time in this survey that the yellow flower color of *Clematis* cultivars are due to much amount of quercetin glycosides, and kaempferol glycosides does not act as yellow pigments, even if they were abundantly accumulated.

It has been reporterd that 6- or 8-hydroxyflavonols such as patuletin and quercetagetin glycosides act as yellow pigments in *Centaurea* (Mishio *et al*., 2006a, 2006b), *Rudbeckia* and *Eriophyllum* species (Asteraceae) (Thompson *et al*., 1972; Harborne and Smith, 1978). The presence of common quercetin as yellow pigment has also been reported in yellow flowers of *Astrophytum* species (Cactaceae) (Iwashina *et al*., 1988). The red and purple flowers of *Clematis* species and cultivars are due to cyanidin and delphinidin glycosides and their isolation and identification are now in progress.

Table 1. Quantitative HPLC analysis of yellow and white *Clematis* taxa and cultivars.

Taxa and cultivars	Flower colors	Quercetin		Kaempferol		Total*
		8	**6** and **7**	**9**	**4**	
"Gekkyuden"	yellow	0.12	22.38	—	3.66	26.26 (1.00)
Yellow *C. patens*	yellow	0.23	19.22	0.08	1.10	20.63 (0.79)
"Manshu-ki"	yellow	0.16	2.28	0.02	3.10	5.55 (0.21)
C. florida var. *florida*	white	0.01	0.13	34.77	4.29	39.19 (1.49)
C. florida var. *florepleno*	white	0.01	0.12	32.15	4.95	37.50 (1.43)
C. florida var. *sieboldiana*	white	0.36	0.13	33.05	4.28	37.83 (1.44)

*mAU×10^6. ( )=Relative amount of total flavonoids as peak area of "Gekkyuden" is 1.00.

4=kaempferol 3-*O*-glucoside, **6**=quercetin 3-*O*-galactoside, **7**=quercetin 3-*O*-glucoside, **8**=quercetin 3-*O*-rutinoside and **9**=kaempferol 3-*O*-rutinoside.

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