

Flavonoids from the Leaves of *Chrysanthemum seticuspe* f. *seticuspe* in the Imperial Palace: —Chemotaxonomical Comparison with *Chrysanthemum seticuspe* f. *boreale*—

Ayumi Uehara^{1*}, Yuichi Kadota² and Tsukasa Iwashina²

¹Department of Chemistry, Hiyoshi Campus, Keio University,
4–1–1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223–8521, Japan

*E-mail: uehara@kahaku.go.jp

²Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan

Abstract. The leaves of *Chrysanthemum seticuspe* f. *seticuspe*, which is only growing in the Imperial Palace, Tokyo, was surveyed for flavonoid compounds. Four flavonoids were isolated by various chromatography and identified by HPLC, UV and LC-MS survey. Of their flavonoids, two flavone glycosides, acacetin 7-*O*-rutinoside (**1**) and apigenin 7-*O*-glucuronide (**4**) were identified, and other two flavone glycosides, acacetin 7-*O*-acetylramnosylglucosylglucoside (**2**) and acacetin 7-*O*-ramnosylglucosylglucoside (**3**), were partially characterized. Flavonoid comparison of the leaves of *C. seticuspe* f. *seticuspe* was compared with that of another forma, f. *boreale*, by HPLC. As the results, they were essentially the same with each other, and we presumed that their taxa are two forma of *C. seticuspe* or completely the same.

Key words: Acacetin 7-*O*-rutinoside, apigenin 7-*O*-glucuronide, Asteraceae, Chemotaxonomy, *Chrysanthemum seticuspe* f. *boreale*, *Chrysanthemum seticuspe* f. *seticuspe*.

Introduction

Chrysanthemum seticuspe (Maxim.) Hand.-Mazz. f. *seticuspe* (Asteraceae) put yellow-ligulated flowers (Fig. 1), which is only growing in the Imperial Palace, Tokyo. It was cultivated in Edo era and was published as a new taxa in 1872 (Sankei Shinbun Shakaibu, 2007). Though this taxa was considered to be extinct (Kitamura, 1940, 1967), it was rediscovered at the Imperial Palace in 1986 (Kitamura, 1987; Sankei Shinbun Shakaibu, 2007).

Kitamura (1987) describes that *C. seticuspe* f. *seticuspe* was derived from wild *C. seticuspe* f. *boreale* (Makino) H. Ohashi & Yonek. which is distributed in Japan, Korea and China. Though Ohashi and Yonekura (2004) supported his opinion, Lin *et al.* (2011) considered that *C. seticuspe* f. *seticuspe* and f. *boreale* are included in *C. lavandulifolium* (Fisch. ex Trautv.) Makino var.

lavandulifolium. However, the taxonomical characters of f. *seticuspe* have been hardly reported, because its distribution is very limited.

We have reported ten flavonoids from the leaves of *C. seticuspe* f. *boreale*, i.e. two flavonoid glycosides, apigenin 7-*O*-glucuronide and acacetin 7-*O*-rutinoside, and eight flavonoid aglycones luteolin, nepetin, 5,7,3',4'-tetrahydroxy-6,5'-dimethoxyflavone, apigenin, hispidulin, 5,7,4'-trihydroxy-6,3',5'-trimethoxyflavone, jaceosidin and eupatilin as external compounds (Uehara *et al.*, 2012). On the other hand, four flavonoids, acacetin, apigenin, luteolin and acacetin 7-*O*-rutinoside, were reported from the flowers of *C. boreale* (= *C. seticuspe* f. *boreale*) in Korea (Shin *et al.*, 1995). From the whole plants of *Dendranthema lavandulifolium* (= *C. lavandulifolium*) in China, luteolin apigenin, acacetin 7-*O*-rutinoside and acacetin 7-*O*-ramnosyl-(1→6)-[(2"-acetylglucosyl)-(1→2)-glucoside]



Fig. 1. *Chrysanthemum seticuspe* (Maxim.) Hand.-Mazz. f. *seticuspe*, at the Imperial Palace, Tokyo, Japan, 19 Nov. 2013, Photographed by A. Uehara.

have been reported (Shen *et al.*, 1997).

In this paper, we describe the flavonoids of the leaves of *C. seticuspe* f. *seticuspe*, and chemotaxonically compared with those of the leaves of *C. seticuspe* f. *boreale* and *C. lavandulifolium* var. *lavandulifolium*.

Materials and Methods

Plant materials

Chrysanthemum seticuspe (Maxim.) Hand.-Mazz. f. *seticuspe* was collected in the Imperial Palace in Tokyo. *Chrysanthemum seticuspe* f. *boreale* (Makino) H. Ohashi & Yonek. was transferred from the National Institute of Floricultural Science, Tsukuba, Japan, and cultivated in Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan. Voucher specimens were deposited in the Herbarium of the National Museum of Nature and Science, Japan (TNS).

Isolation of flavonoids

Fresh leaves (ca. 2.0 g) of *Chrysanthemum seticuspe* f. *seticuspe* were extracted with MeOH. The concentrated extracts were applied to preparative paper chromatography using solvent systems, BAW (*n*-BuOH/HOAc/H₂O = 4 : 1 : 5, upper phase)

and 15% HOAc. The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. The flavonoids were further purified by preparative HPLC.

High performance liquid chromatography (HPLC)

HPLC survey of the isolated flavonoids and their aglycones was performed with Shimadzu HPLC system using *L*-column 2 ODS column (I.D. 6.0 × 150 mm, Chemicals Evaluation and Research Institute, Tokyo) at a flow-rate of 1.0 mL min⁻¹, eluting with MeCN/H₂O/H₃PO₄ (25 : 75 : 0.2).

Liquid chromatograph–mass spectrometry (LC-MS)

The LC-MS survey of the isolated flavonoids was performed using *L*-column 2 ODS column (I.D. 2.1 × 100 mm, Chemicals Evaluation and Research Institute), at a flow-rate of 0.2 mL min⁻¹, eluting with MeCN/H₂O/HCOOH (20 : 78 : 2), ESI⁺ 4.5 kV, ESI⁻ 3.5 kV, 250°C.

Ultraviolet-visible (UV) spectra

UV spectra were recorded on a Shimadzu MPS-2000 multipurpose recording spectrophotometer (220–500 nm) according to Mabry *et al.* (1970).

Identification of flavonoids

The flavonoids were identified by UV spectroscopy, LC-MS and HPLC comparisons with authentic samples. UV, HPLC and LC-MS data of the isolated flavonoids were as follows.

Acacetin 7-*O*-rutinoside (**1**, Fig. 3–1). Color: UV (365 nm) and UV/NH₃ – dark purple. UV: λ max (nm) MeOH 268, 327; + NaOMe 289, 370 (dec.); + AlCl₃ 276, 300, 341, 380; + AlCl₃/HCl 277, 300, 338, 380; + NaOAc 269, 325; + NaOAc/H₃BO₃ 269, 328. HPLC: *t*R (min) 15.55. LC-MS: m/z 593 [M + H]⁺ (molecular ion peak, acacetin + each 1 mol of rhamnose and glucose), m/z 447 [M – rhamnosyl + H]⁺ (fragment ion peak, acacetin + 1 mol glucose) and m/z 285 [M – rhamnosylglucosyl + H]⁺ (fragment ion peak, acacetin).

Acacetin 7-*O*-acetylramnosylglucosylglucoside (**2**, Fig. 3–2). Color: UV (365 nm) and UV/NH₃ – dark purple. UV: λ max (nm) MeOH 269, 323; + NaOMe 286, 369 (dec.); + AlCl₃ 276, 300, 342, 380; + AlCl₃/HCl 276, 300, 339, 379; + NaOAc 270, 327; + NaOAc/H₃BO₃ 270, 339. HPLC: *t*R (min) 14.39. LC-MS: m/z 797 [M + H]⁺ (molecular ion peak, acacetin + 2 mol glucose and each 1 mol of rhamnose and acetic acid), m/z 651 [M – rhamnosyl + H]⁺ (fragment ion peak, acacetin + 2 mol glucose and 1 mol acetic acid) and m/z 285 [M – acetylramnosylglucosylglucosyl + H]⁺ (fragment ion peak, acacetin).

Acacetin 7-*O*-ramnosylglucosylglucoside (**3**, Fig. 3–3). HPLC: *t*R (min) 7.70. LC-MS: m/z 755 [M + H]⁺ (molecular ion peak, acacetin + 2 mol glucose and 1 mol rhamnose), m/z 609 [M – rhamnosyl + H]⁺ (fragment ion peak, acacetin + 2 mol glucose) and m/z 285 [M – ramnosylglucosylglucosyl + H]⁺ (fragment ion peak, acacetin).

Apigenin 7-*O*-glucuronide (**4**, Fig. 3–4). HPLC: *t*R (min) 7.21. LC-MS: m/z 447 [M + H]⁺ (molecular ion peak, apigenin + 1 mol glucuronic acid) and m/z 271 [M – glucuronyl + H]⁺ (fragment ion peak, apigenin).

Results and Discussion

Four flavonoids were obtained from the leaves of *Chrysanthemum seticuspe* f. *seticuspe*. Of their flavonoids, two were obtained as a mixture.

Flavonoid **1** was contained as major compound in this plant. Since molecular and fragment ion peaks, m/z 593 [M + H]⁺, m/z 447 [M – rhamnosyl + H]⁺ and m/z 285 [M – rhamnosylhexosyl + H]⁺, were obtained by LC-MS, it was shown that **1** is dihydroxy-monomethoxyflavone rhamnosylhexoside. By UV spectral survey in addition to various shift reagents according to Mabry *et al.* (1970), it was shown that methoxyl and rhamnosylhexosyl groups are attached to 4'- and 7-positions of aglycone. Finally, **1** was identified as acacetin 7-*O*-rutinoside by direct HPLC comparison with authentic sample, which was isolated from the leaves of Japanese *Chrysanthemum* spp. (Asteraceae) (Uehara *et al.*, 2012). Acacetin 7-*O*-rutinoside has been reported from the leaves and flowers of *C. seticuspe* f. *boreale* (Shin *et al.*, 1995; Uehara *et al.*, 2012) and the whole plants of *C. lavandulifolium* (Shen *et al.*, 1997).

It was shown by LC-MS that **2** is dihydroxy-monomethoxyflavone which attached 2 mol hexose and each 1 mol of rhamnose and acetic acid. UV spectral survey indicated the presence of free 5-hydroxyl group, showing the attachment of methoxyl and acetylramnosylhexosylhexosyl groups to 4'- and 7-positions of aglycone. Finally, **2** was characterized as acacetin 7-*O*-acetylramnosylglucosylglucoside by HPLC comparison with authentic sample, which was isolated from the leaves of *Chrysanthemum arisaense* Hayata (Uehara *et al.*, unpublished data).

Flavonoids **3** and **4** were obtained as a mixture. Since LC-MS survey of the mixture showed the molecular ion peaks, m/z 755 [M + H]⁺ (**3**) and m/z 447 [M + H]⁺ (**4**), they were presumed as acacetin rhamnosylhexosylhexoside (**3**) and apigenin monoglucuronide (**4**). Flavonoid **4** was finally identified as apigenin 7-*O*-glucuronide by direct HPLC comparison with authentic sample, which isolated from the leaves of Japanese *Chry-*

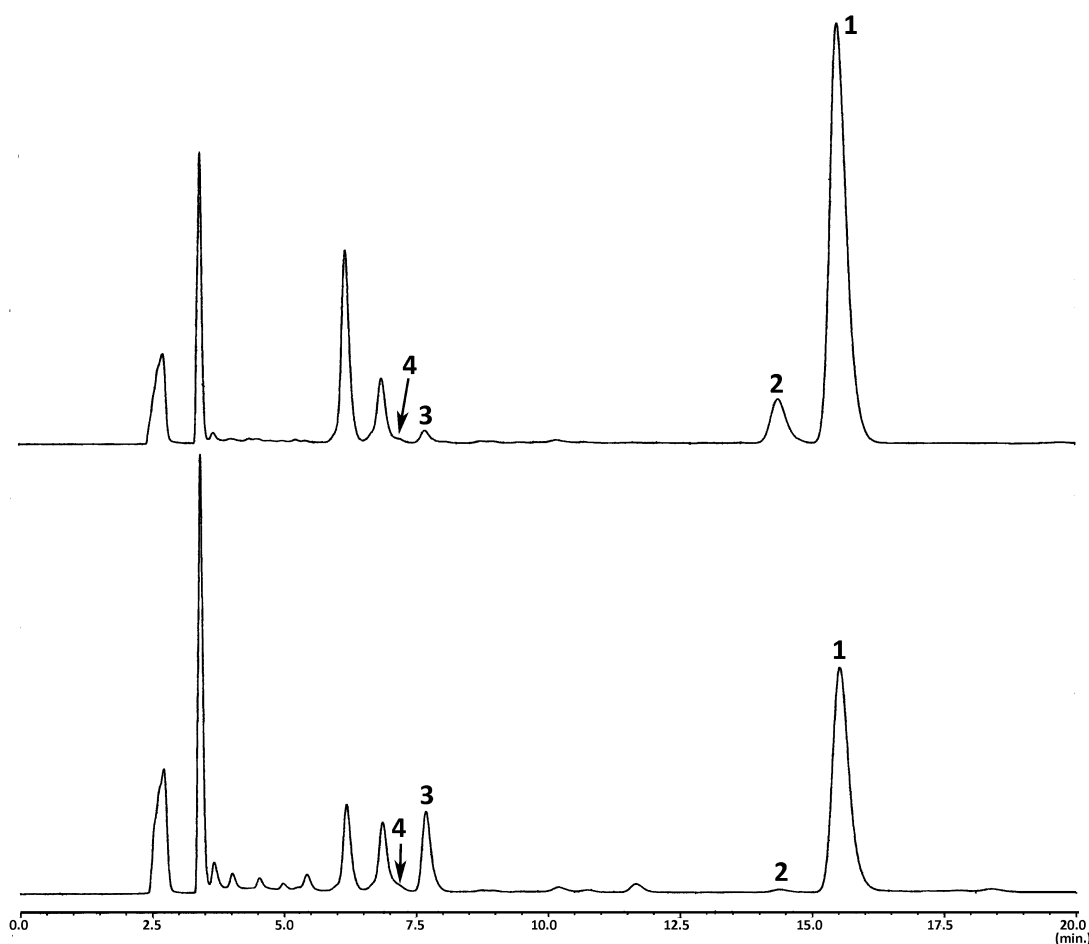
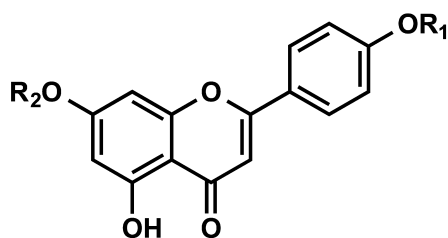


Fig. 2. HPLC chromatogram of foliar flavonoids of *Chrysanthemum seticuspe* f. *seticuspe* (upper), and *C. seticuspe* f. *boreale* (lower). **1** = acacetin 7-*O*-rutinoside, **2** = acacetin 7-*O*-acetylramnosylglucosylglucoside, **3** = acacetin 7-*O*-ramnosylglucosylglucoside and **4** = apigenin 7-*O*-glucuronide. Other peaks are cinnamoyl derivatives.

santhemum spp. (Uehara *et al.*, 2012). This compound has been found from many *Chrysanthemum* species (Harborne *et al.*, 1970; Lee *et al.*, 2003; Uehara *et al.*, 2012). On the other hand, **3** was characterized as acacetin 7-*O*-ramnosylglucosylglucoside.

Flavonoid composition of *C. seticuspe* f. *seticuspe* was compared with those of related taxa, *C. seticuspe* f. *boreale* and *C. lavandulifolium* var. *lavandulifolium*, by HPLC. As the results, flavonoid composition of *C. seticuspe* f. *seticuspe* and f. *boreale* was the same with each other (Fig. 2). Though acacetin 7-*O*-rutinoside

(**1**) and apigenin 7-*O*-glucuronide (**4**) have been reported from *C. seticuspe* f. *boreale* (Shin *et al.*, 1995; Uehara *et al.*, 2012), **2** and **3** were found in this taxa for the first time. Uehara *et al.* (2012) have reported some flavonoid aglycones as external flavonoids from the leaves of *C. seticuspe* f. *boreale*. However, flavonoid aglycones of *C. seticuspe* f. *seticuspe* could not be isolated for small amounts, in this survey. We also compared flavonoid composition of the leaves of *C. seticuspe* f. *seticuspe* with that of *C. lavandulifolium* var. *lavandulifolium* in China. As the results, flavonoid patterns of their taxa were not agreed



- 1: $R_1 = \text{CH}_3$, $R_2 = \text{rutosyl}$
 2: $R_1 = \text{CH}_3$, $R_2 = \text{acetylramnoglucoyl}$
 3: $R_1 = \text{CH}_3$, $R_2 = \text{ramnoglucoyl}$
 4: $R_1 = \text{H}$, $R_2 = \text{glucuronyl}$

Fig. 3. Chemical structures of the flavonoids from the leaves of *Chrysanthemum seticuspe* f. *seticuspe*.

(Uehara *et al.*, unpublished data). From the results described above, it was supported that *C. seticuspe* f. *seticuspe* and f. *boreale* are very related taxa, and are forma in *C. seticuspe* (Kitamura, 1987; Ohashi and Yonekura, 2004) or completely the same taxa. On the other hand, it did not support by this survey that *C. seticuspe* f. *seticuspe* and f. *boreale* are included in *C. lavandulifolium* var. *lavandulifolium* (Lin *et al.*, 2011). More recently, their taxa were surveyed using the molecular biology, and it was shown that *C. seticuspe* f. *seticuspe* and f. *boreale* are included in the sister group and different group with *C. lavandulifolium* var. *lavandulifolium* (Taniguchi *et al.*, 2014). Shen *et al.* (1997) reported acacetin 7-*O*-rutinoside from the whole plants of *C. lavandulifolium*, together with other three flavonoids. Of flavonoids which were isolated from *C. seticuspe* f. *seticuspe* in this survey, **2** may be the same compound with acacetin 7-*O*-ramnosyl-(1→6)-[(2"-acetylglucosyl)-(1→2)-glucoside] from *C. lavandulifolium* (Shen *et al.*, 1997). Since this species widely distributes in China, they may have the geographic variation or some chemotypes. The chemotaxonomical characterization of *C. lavandulifolium* need further survey.

In conclusion, we described the existence of four flavonoid glycosides (**1–4**) in *C. seticuspe* f.

seticuspe. The flavonoid character of this taxa was the same with that of *C. seticuspe* f. *boreale*. Thus, their taxa are very related to each other, and were considered as two forma in *C. seticuspe* or completely the same taxa.

Acknowledgements

The authors thanks Dr. Masashi Nakata, Botanic Gardens of Toyama, Toyama, Japan for plant material collection and for enormous help. We also thank Dr. Liang-Sheng Wang, Beijing Botanical Garden, Institute of Botany, The Chinese Academy of Sciences.

References

- Harborne, J. B., V. H. Heywood and N. A. M. Saleh, 1970. Chemosystematics of the Compositae: flavonoid patterns in the *Chrysanthemum* complex of the tribe Anthemideae. *Phytochemistry*, **9**: 2011–2017.
- Kitamura, S., 1940. Compositae Japonicae Pars Secunda. *Memoirs of the College of Science Kyoto Imperial University, Series B*, **15**: 285–446.
- Kitamura, S., 1967. Report on the distribution of the wild chrysanthemums of Japan. *Acta Phytotaxonomica et Geobotanica*, **22**: 109–137 (in Japanese).
- Kitamura, S., 1987. On three species of Sympetaleae. *Acta Phytotaxonomica et Geobotanica*, **38**: 380–382 (in Japanese).
- Lee, J. S., H. J. Kim and Y. S. Lee, 2003. A new anti-HIV flavonoid glucuronide from *Chrysanthemum morifolium*. *Planta Medica*, **69**: 859–861.
- Lin, Y. R., Z. Shi, C. J. Humphries and M. G. Gilbert, 2011. Anthemideae. In: Wu, Z. Y., P. H. Raven and D. Y. Hong (eds.), *Flora of China*, **20–21**: 653–773. Science Press, Beijing and Missouri Botanical Garden, St. Louis.
- Mabry, T. J., K. R. Markham and M. B. Thomas, 1970. *The Systematic Identification of Flavonoids*. Springer, Berlin.
- Ohashi, H. and K. Yonekura, 2004. New combinations in *Chrysanthemum* (Compositae-Anthemideae) of Asia with a list of Japanese species. *The Journal of Japanese Botany*, **79**: 186–195.
- Sankei Shinbun Shakaibu, 2007. *Flowers of the Imperial Palace*. p. 114. Sankei Shinbun Syuppan, Tokyo (in Japanese).
- Shen, Y. X., L. H. Quan, L. Guan and J. M. Chen, 1997. Studies on the flavonoids from *Dendranthema lavandulifolium*. *Acta Pharmaceutica Sinica*, **32**: 451–

454.
 Shin, K. H., S. S. Kang, E. A. Seo and S. W. Shin, 1995. Isolation of aldose reductase inhibitors from the flowers of *Chrysanthemum boreale*. *Archives of Pharmacal Research*, **18**: 65–68.
- Taniguchi, K., K. Motohara and M. Kusaba, 2014. Phylogenetic relationships between *Chrysanthemum seticuspe* f. *seticuspe* and *C. seticuspe* f. *boreale* based on *NCED3a* gene. *Memoirs of the National Museum of Nature and Science*, (49): 17–22 (in Japanese with English summary).
- Uehara, A., M. Nakata, J. Kitajima and T. Iwashina, 2012. Internal and external flavonoids from the leaves of Japanese *Chrysanthemum* species (Asteraceae). *Biochemical Systematics and Ecology*, **41**: 142–149.

皇居にのみ生育するカモメギクとキクタニギク（キク科）のフラボノイド組成の比較

上 原 歩・門 田 裕 一・岩 科 司

カモメギクは明治時代に登録された園芸品種であり、その後絶滅したとされていたものが近年になって皇居にのみ生育するのが確認された貴重な植物である。このキクに関しては生育地が限られることから、これまで基礎的な研究が行われていない。本研究ではこの植物のフラボノイド組成を明らかにし、近縁と考えられているキクタニギクのものと比較した。各種クロマトグラフィーによる分離、HPLC、UV スペクトルおよびLC-MS による分析から4種類のフラボノイド配糖体が分離された。このうち acacetin 7-*O*-rutinoside (1) と apigenin 7-*O*-glucuronide (4) を同定、他の acacetin 7-*O*-acetylramnosylglucosylglucoside (2) と acacetin 7-*O*-ramnosylglucosylglucoside (3) を定性した。今回分析されたカモメギクのフラボノイド組成はキクタニギクのものと同じであったことから、両種は極めて近縁であり、品種の関係、もしくは同一の種と考えられた。