Bull. Natl. Mus. Nat. Sci., Ser. B, 46(4), pp. 195–214, November 20, 2020

Further Identification of Flavonoids Deposited in the National Museum of Nature and Science in Japan — Flavonoids Isolated from *Cirsium* Taxa and *Carduus nutans* (Asteraceae) —

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(Received 6 July 2020; accepted 23 September 2020)

Abstract Chemical structures of 59 flavonoid powder and crystal samples which are deposited in Department of Botany, National Museum of Nature and Science, Japan, were determined or confirmed by UV spectral survey, LC-MS, NMR and/or HPLC comparisons with authentic samples. They were isolated from 37 *Cirsium* taxa and *Carduus nutans* (Asteraceae) in 1980–2001 and donated to National Museum of Nature and Science. Many crystal samples were identified as pectolinarigenin 7-*O*-rutinoside with minor acacetin 7-*O*-rutinoside. They are popular flavonoids in the genus *Cirsium*. As other flavonoids, hispidulin 7-*O*-neohesperidoside, cirsimaritin 4′ -*O*-glucoside, and luteolin 7-*O*-glucoside, 5-*O*-glucoside, 3′ -*O*-glucoside, 4′ -*O*-glucoside and 7-*O*-vicianoside were identified. Of their flavonoids, luteolin 7-*O*-vicianoside which was obtained from *C. buergeri* was reported from the genus *Cirsium* for the first time. Hispidulin 7-*O*-neohesperidoside was newly found in Japanese *Cirsium* species. In this survey, flavonoids of 20 *Cirsium* taxa, e.g. *C. boninense*, *C. hanamakiense*, *C. alpicola*, *C. aidzuense* and *C. ishizuchiense*, were reported for the first time.

Key words: *Carduus nutans*, *Cirsium* species, crystal samples, luteolin 7-*O*-vicianoside, pectolinaigenin 7-*O*-rutinoside.

Introduction

In Department of Botany, National Museum of Nature and Science, Tsukuba, many flavonoid and related compounds such as aromatic acids, xanthones and tannins are deposited as crystals and powders. Many samples were isolated and characterized by one of the authors (T. Iwashina) and Dr. Kozo Hayashi (former Professor of Tokyo Kyoiku University). The compounds which were recently obtained were identified by acid hydrolysis, UV spectral survey according to Mabry *et al.* (1970), LC-MS and NMR spectra. However, some compounds which were isolated in the early times are insufficiently characterized without LC-MS and NMR. In this survey, we performed complete characterization of the flavonoids which are deposited in National Museum of Nature and Science. As a series of further identification of insufficiently characterized flavonoids, we performed the identification of the flavonoids which were obtained from *Cirsium* taxa and related *Carduus nutans* (Asteraceae) in 1980–2001, by LC-MS and NMR, and/or direct HPLC comparisons with authentic samples.

Materials and Methods

Materials

Of their flavonoids which were characterized in this survey, some compounds have been published as some papers (Iwashina *et al.*, 1988, 1989, 1995, 1999; Iwashina and Ootani, 1998). Other samples were isolated from *Cirsium* species which were collected by Dr. Yuichi Kadota (Curator Emeritus, National Museum of Nature and Science, Japan). Their flavonoids were not published as the papers until now. Moreover, some powders and crystals were isolated from *Cirsium* species by Mr. Tsutomu Ito, published as graduation thesis of Faculty of Agriculture, Tokyo University of Agriculture, and presented to National Museum of Nature and Science in 1986 and 1989. However, they were incompletely characterized. We examined 59 flavonoid powder and crystal samples from 37 *Cirsium* taxa and *Carduus nutans* in this study.

General

Analytical high performance liquid chromatography (HPLC) was performed with Shimadzu HPLC systems using Inertsil ODS-4 (I.D. 6.0 × 150 mm, Chemicals Evaluation and Research Institute, Tokyo) at a flow-rate of 1.0 ml min^{-1}. Detection wavelength was 350 nm. Eluent was $MeCN/H_2O/H_3PO_4$ (20 : 80 : 0.2). Liquid chromatograph-mass spectra (LC-MS) was performed with Shimadzu LC-MS systems using Inertsil ODS-4 (I.D. 2.1 × 100 mm) at flow-rate of 0.2 ml min^{-1}, ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C. Eluents were $MeCN/H_2O$/HCOOH (20 : 75 : 5 or 40 : 55 : 5). Acid hydrolysis was performed in 12%HCl, 100°C, 30 min. After shaking with diethyl ether, aglycones were migrated to the organic layer, and sugars were left in aqueous layer. Aglycones were identified by HPLC comparisons with authentic samples. On the other hand, sugars were identified by paper chromatographic comparisons with authentic sugars, glucose, galactose, glucuronic acid, rhamnose, xylose and arabinose, using solvent systems, BBPW (*n*-BuOH/benzene/pyridine/H_2O = 5 : 1 : 3 : 3) and BTPW (*n*-BuOH/toluene/pyridine/H_2O = 5 : 1 : 3 : 3). Sugar spots were visualized by spraying 1% methanolic aniline hydrochloride on the chromatograms and heating. ^1H and ^{13}C NMR were recorded on a Brucker AV-600 in pyridine-d_5 at 600 MHz (^1H NMR) and 150 MHz (^{13}C NMR).

Results and Discussion

1. Flavonoids from Cirsium aidzuense *Nakai ex Kitam.*

Pectolinarigenin 7-*O*-rutinoside (Pectolinarin, **136-13**). UV: λmax (nm) MeOH 275, 328; + NaOMe 296, 377 (dec.); + $AlCl_3$ 286sh, 299, 353, 387sh; + $AlCl_3$/HCl 286, 299, 346, 386sh; + NaOAc 280, 324; + NaOAc/H_3BO_3 276, 331. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$, 475 $[M-146-H]^-$ (fragment ion peaks, pectolinarigenin + 1 mol glucose), and *m/z* 315 $[M-308+H]^+$ (fragment ion peak, pectolinarigenin).

The sample was obtained as pale yellow needle (ca. 20 mg) from *C. aidzuense* leaves which were collected by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside (Fig. 1) by UV, LC-MS and HPLC comparison with **136-10**. Acacetin

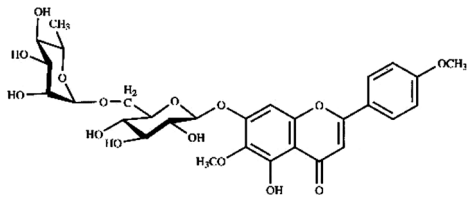

Fig. 1. Pectolinarigenin 7-*O*-rutinoside.

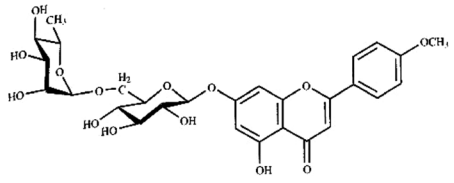

Fig. 2. Acacetin 7-*O*-rutinoside.

7-*O*-rutinoside (Linarin, Fig. 2) was also contained as minor compound (11.7%). Flavonoids of *C. aidzuense* were reported for the first time.

2. Flavonoids from Cirsium alpicola *Nakai*

Pectolinarigenin 7-*O*-rutinoside (**136-29**). UV: λmax (nm) MeOH 273, 329; +NaOMe 296, 380 (dec.); +$AlCl_3$ 283sh, 300, 352, 386sh; +$AlCl_3$/HCl 284, 298, 347, 386sh; +NaOAc 279, 325; +NaOAc/H_3BO_3 274, 332. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained as pale yellow needle from *C. alpicola* leaves which was collected by Dr. Yuichi Kadota in 2000 (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with authentic sample (**136-10**) from *C. brevicaule*. Acacetin 7-*O*-rutinoside was contained in this sample as minor compound (19.8%). Flavonoids of *C. alpicola* were found for the first time.

3. Flavonoids from Cirsium bitchuense *Nakai*

Pectolinarigenin 7-*O*-rutinoside (**136-15**). UV: λmax (nm) MeOH 275, 328; +NaOMe 297, 377 (dec.); +$AlCl_3$ 287sh, 299, 353, 387sh; +$AlCl_3$/HCl 283, 298, 347, 386sh; +NaOAc 278, 324; +NaOAc/H_3BO_3 274, 332. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 313 $[M-308-H]^-$ (fragment ion peak, pectolinarigenin).

The sample was obtained as pale yellow needle (ca. 8 mg) from the leaves of *C. bitchuense* which was collected in 1993 by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Although pectolinarin has been reported from this species (Iwashina *et al.*, 1995), it was proved by this survey that acacetin 7-*O*-rutinoside is also contained as minor flavonoid (14.2%).

4. Flavonoids from Cirsium boninense *Koidz.*

Pectolinarigenin 7-*O*-rutinoside (**136-23**). UV: λmax (nm) MeOH 275, 329; +NaOMe 296, 376 (dec.); +$AlCl_3$ 286sh, 299, 354, 387sh; +$AlCl_3$/HCl 285sh, 297, 345, 386sh; +NaOAc 280, 320; +NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$ (fragment ion peak, pectolinarigenin + 1 mol glucose).

The sample was obtained as pale yellow needle (ca. 2 mg) from the leaves of *C. boninense* which was collected in 1999 in Ogasawara Islands, Japan by Dr. Yuichi Kadota. Molecular weight and UV absorption properties were agreed with those of pectolinarin. Finally, it was identified as pectolinarigenin 7-*O*-rutinoside by HPLC comparison with **136-10**. The flavonoids of *C. boninense* were reported for the first time. Acacetin 7-*O*-rutinoside was mixed in the sample as minor compound (8.9%).

5. Flavonoids from Cirsium borealinipponense *Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**136-18**). UV: λmax (nm) MeOH 275, 328; +NaOMe 295, 378 (dec.); +$AlCl_3$ 283sh, 300, 350, 386sh; +$AlCl_3$/HCl 283, 299, 346, 385sh; +NaOAc 281, 321; +NaOAc/H_3BO_3 273, 333. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated as pale yellow needle (ca. 40 mg) from the leaves of *C. borealinipponense* which was collected by Dr. Yuichi Kadota in 2001 (collection site is unknown), and identified as pectolinarigenin 7-*O*-rutinoside by LC-MS, UV spectra, and HPLC comparison with **136-10** from *C. brevicaule*. Acacetin 7-*O*-rutinoside was contained in the sample as minor flavonoid (25.5%). Flavonoids of *C. borealinipponense* were reported for the first time.

6. Flavonoids from Cirsium brevicaule *A. Gray*

Pectolinarigenin 7-*O*-rutinoside (**136-10**, **136-**

32). UV: λmax (nm) MeOH 276, 329; +NaOMe 295, 377 (dec.); +$AlCl_3$ 283sh, 299, 355, 385sh; +$AlCl_3$/HCl 284, 298, 346, 386sh; +NaOAc 278, 323; +NaOAc/H_3BO_3 274, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$ (fragment ion peak, pectolinarigenin + 1 mol glucose), and *m/z* 313 $[M-308-H]^-$ (fragment ion peak, pectolinarigenin). ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 8.16 (2H, *d*, *J* = 8.9 Hz, H-2′,6′), 7.36 (2H, *d*, *J* = 8.9 Hz, H-3′,5′), 7.31 (1H, *s*, H-8), 6.97 (1H, *s*, H-3), 5.78 (1H, *d*, *J* = 7.7 Hz, glucosyl H-1), 5.45 (1H, *d*, *J* = 1.0 Hz, rhamnosyl H-1), 4.70 (1H, *brd*, *J* = 10.2 Hz, glucosyl H-6a), 4.64 (1H, *dd*, *J* = 1.5 and 13.4 Hz, rhamnosyl H-2), 4.55 (1H, *dd*, *J* = 3.4 and 9.2 Hz, rhamnosyl H-3), 4.48 (1H, *t*, *J* = 9.1 Hz, glucosyl H-5), 4.40 (1H, *dd*, *J* = 7.8 and 9.1 Hz, glucosyl H-2), 4.35 (1H, *m*, glucosyl H-3), 4.29 (1H, *dd*, *J* = 6.1 and 9.4 Hz, rhamnosyl H-5), 4.24 (1H, *t*, *J* = 9.3 Hz, rhamnosyl H-4), 4.15 (1H, *brd*, *J* = 9.5 Hz, glucosyl H-6b), 4.12 (1H, *brd*, *J* = 9.3 Hz, glucosyl H-4), 4.12 (3H, *s*, 6-OCH_3), 3.88 (3H, *s*, 4′-OCH_3), 1.57 (3H, *d*, *J* = 6.1 Hz, rhamnosyl CH_3). ^{13}C NMR (150 MHz, pyridine-d_5): (pectolinarigenin) δ_C 153.5 (C-2), 104.2 (C-3), 183.2 (C-4), 165.1 (C-5), 133.7 (C-6), 157.5 (C-7), 95.2 (C-8), 153.2 (C-9), 107.0 (C-10), 123.7 (C-1′), 129.0 (C-2′,6′), 115.4 (C-3′,5′), 163.2 (C-4′), 61.1 (6-OCH_3), 55.8 (4′-OCH_3); (glucose) δ_C 102.1 (C-1), 74.4 (C-2), 77.3 (C-3), 71.0 (C-4), 77.7 (C-5), 67.4 (C-6); (rhamnose) δ_C 102.0 (C-1), 71.8 (C-2), 72.4 (C-3), 73.7 (C-4), 69.7 (C-5), 18.3 (C-6).

The flavonoid was obtained as pale yellow needle (ca. 60 mg **136-10**, small amount **136-32**). Molecular ion peak, *m/z* 623 $[M+H]^+$, and two fragment ion peaks, *m/z* 477 $[M-146+H]^+$ and 313 $[M-308-H]^-$, appeared on LC-MS, showing the attachment of each 1 mol of hexose and rhamnose to dihydroxy-dimethoxyflavone. The presence of free 5-hydroxyl and substituted 7- and 4′-hydroxyl groups was shown by UV spectral survey according to Mabry *et al.* (1970). Moreover, the additional presence of substituted hydroxyl group to 6- or 8-position was presumed by Band II value of absorption maxima in MeOH solution. Practically, pectolinarigenin (5,7-dihydroxy-6,4′-dimethoxyflavone), glucose and rhamnose were liberated by acid hydrolysis. In NMR, proton and carbon signals were assigned by HSQC, HMBC, COSY and NOESY. Four aromatic proton signals, δ_H 8.16 (2H), 7.36 (2H), 6.97 (1H) and 7.31 (1H) according to H-2′,6′, H-3′,5′, H-3 and H-8, appeared, together with glucosyl and rhamnosyl anomeric protons, δ_H 5.78 (*d*, *J* = 7.7 Hz) and 5.45 (*d*, *J* = 1.0 Hz), and two methoxyl proton signals, δ_H 4.12 and 3.88. Of their proton signals, two methoxyl proton signals correlated with the carbon signals at δ_C 133.7 and 163.2 corresponding to C-6 and C-4′ by HMBC, respectively. On the other hand, sugar anomeric proton signals, δ_H 5.78 and 5.45 were correlated with the carbon signals at δ_C 157.5 and 67.4 corresponding to C-7 of pectolinarigenin and C-6 of glucose. From their results, the flavonoid was identified as pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (pectolinarin, Fig. 1). Pectolinarin has already been reported from some *Cirsium* species (e.g. Iwashina *et al.*, 1988, 1995). In *C. brevicaule*, it has been isolated from the leaves (Morita *et al.*, 1964). However, it was proved by HPLC comparison with authentic sample that acacetin 7-*O*-rutinoside (linarin, Fig. 2) is also contained in their samples as minor compound (10.3% in **136-10** and 8.4% in **136-32**). Linarin was found in *C. brevicaule* for the first time.

7. Flavonoids from Cirsium buergeri *Miq.*

Luteolin 7-*O*-glucoside (**304-1**, **304-11**, **306-1**, **306-2**). UV: λmax (nm) MeOH 255, 266sh, 349; +NaOMe 270, 397 (inc.); +$AlCl_3$ 273, 425; +$AlCl_3$/HCl 270, 294sh, 358, 384sh; +NaOAc 259, 404; +NaOAc/H_3BO_3 260, 371. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose), *m/z* 287 $[M-162+H]^+$, 285 $[M-162-H]^-$ (fragment ion peaks, luteolin).

The samples were isolated by Mr. Tsutomu Ito

(the student of Tokyo University of Agriculture of the day) from the leaves of *C. buergeri* (ca.10 mg, **304-1**, small amount, **304-11**, ca. 40 mg, **306-1** and ca. 20 mg, **306-2**) and confirmed as luteolin 7-*O*-glucoside (Fig. 3) by UV, LC-MS and HPLC comparison with authentic sample from Carl Roth (Germany) in this survey.

Luteolin 7-*O*-vicianoside (**304-2**, **304-5**). UV: λmax (nm) MeOH 256, 266sh, 347; +NaOMe 270, 394 (inc.); +$AlCl_3$ 273, 425; +$AlCl_3$/HCl 271, 294sh, 357, 383; +NaOAc 262, 400; +NaOAc/H_3BO_3 260, 371. LC-MS: *m/z* 581 $[M+H]^+$, 579 $[M-H]^-$ (molecular ion peaks, luteolin + each 1 mol of glucose and arabinose), *m/z* 287 $[M-294+H]^+$ (fragment ion peak, luteolin). ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 13.66 (1H, *s*, 5-OH), 8.04 (1H, *d*, *J* = 2.3 Hz, H-2′), 7.59 (1H, *dd*, *J* = 2.3 and 8.3 Hz, H-6′), 7.37 (1H, *d*, *J* = 8.3 Hz, H-5′), 7.03 (1H, *d*, *J* = 2.2 Hz, H-8), 6.90 (1H, *s*, H-3), 6.83 (1H, *d*, *J* = 2.1 Hz, H-6), 5.68 (1H, *d*, *J* = 7.7 Hz, glucosyl H-1), 4.95 (1H, *d*, *J* = 6.5 Hz, arabinosyl H-1), 4.84 (1H, *brd*, *J* = 9.3 Hz, glucosyl H-6a), 4.55 (1H, *dd*, *J* = 6.5 and 8.3 Hz, arabinosyl H-2), 4.37 (1H, *m*, glucosyl H-6b), 4.36 (1H, *m*, glucosyl H-5), 4.33 (1H, *m*, glucosyl H-3), 4.32 (1H, *m*, arabinosyl H-4), 4.31 (1H, *m*, arabinosyl H-5a), 4.27 (1H, *m*, glucosyl H-2), 4.25 (1H, *m*, glucosyl H-4), 4.22 (1H, *dd*, *J* = 3.5 and 8.4 Hz, arabinosyl H-3), 3.74 (1H, *dd*, *J* = 1.8 and 11.9 Hz, arabinosyl H-5b). ^{13}C NMR (150 MHz, pyridine-d_5): δ_C 165.5 (C-2), 104.1 (C-3), 182.8 (C-4), 162.5 (C-5), 95.4 (C-6), 164.1 (C-7), 100.9 (C-8), 158.0 (C-9), 106.8 (C-10), 122.7 (C-1′), 114.8 (C-2′), 147.7 (C-3′), 151.9 (C-4′), 117.0 (C-5′), 120.0 (C-6′); (glucose) δ_C 102.1 (C-1), 74.7 (C-2), 77.6 (C-3), 71.2 (C-4), 79.2 (C-5), 69.4 (C-6); (arabinose) δ_C 105.3 (C-1), 71.3 (C-2), 74.3 (C-3), 69.0 (C-4), 66.2 (C-5).

Luteolin (hydrolysate of **304-2**). UV: λmax (nm) MeOH 256, 266, 348; +NaOMe 273, 320, 404 (inc.); +$AlCl_3$ 274, 422; +$AlCl_3$/HCl 263sh, 275, 294sh, 356, 385sh; +NaOAc 270, 394; +NaOAc/H_3BO_3 263, 372, 427sh.

Their samples were isolated from the leaves of *C. buergeri* (ca. 10 mg **304-2** and ca. 20 mg

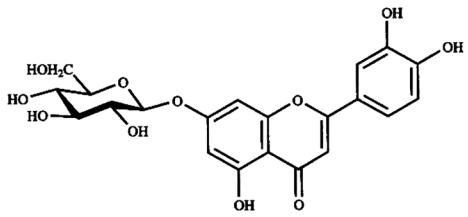

Fig. 3. Luteolin 7-*O*-glucoside.

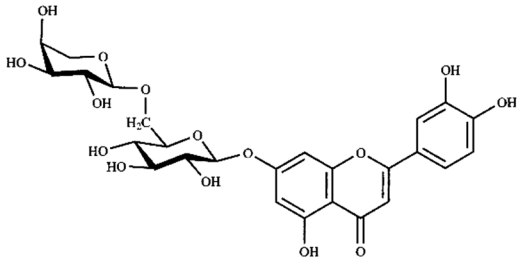

Fig. 4. Luteolin 7-*O*-vicianoside.

304-5) and characterized as luteolin 7-*O*-arabinosylglucoside by Mr. Tsutomu Ito (graduation thesis of Tokyo University of Agriculture in 1986). In the present survey, luteolin, glucose and arabinose were liberated from the original glycoside by acid hydrolysis. Attachment of each 1 mol of glucose and arabinose to 7-position of luteolin was confirmed by UV spectral survey according to Mabry *et al.* (1970) and LC-MS. Final identification of the original glycoside was performed by NMR. Six aromatic proton signals, δ_H 8.04, 7.59, 7.37, 7.03, 6.90 and 6.83 corresponding to H-2′, H-6′, H-5′, H-8, H-3 and H-6, which were determined by COSY, NOESY, HMQC and HMBC, appeared on ^{1}H NMR. Moreover, two sugar anomeric proton signals, δ_H 5.68 (glucosyl H-1) and 4.95 (arabinosyl H-1) also appeared. Attachment of glucose to 7-position of luteolin was confirmed by HMBC correlation of glucosyl anomeric proton signal at δ_H 5.68 with δ_C 164.1 corresponding to C-7. On the other hand, attachment of arabinose to 6-position of glucose was shown by HMBC correlation of arabinosyl anomeric proton signal at δ_H 4.95 with δ_C 69.4 corresponding to C-6 of glucose. Since coupling constants of glucosyl and arabinosyl anomeric proton signals were *J* = 7.7 and 6.5 Hz, respec-

tively, they were determined as *β*-glucopyranose and *α*-arabinopyranose. Thus, the flavonoid was identified as luteolin 7-*O*-*α*-arabinopyranosyl-(1→6)-*β*-glucopyranoside (luteolin 7-*O*-vicianoside, Fig. 4). The plants which their flavonoids were isolated were collected in Mt. Ibuki, Shiga Pref., Japan by Mr. Tatsuya Ueno in 1986. Flavonoids were reported from *C. buergeri* for the first time. Although luteolin 7-*O*-vicianoside has been found in *Dacrydium* spp. (Podocarpaceae) (Markham *et al.*, 1987), it was newly reported from *Cirsium* species in this survey.

8. Flavonoids from Cirsium confertissimum *Nakai*

Luteolin 7-*O*-glucoside (**138-10**, **138-11**, **278-2**, **304-7**, **304-9**, **304-10**). UV: *λ*max (nm) MeOH 255, 265sh, 348; +NaOMe 269, 392 (inc.); +$AlCl_3$ 274, 427; +$AlCl_3$/HCl 273, 294sh, 357, 386; +NaOAc 260, 402; +NaOAc/H_3BO_3 259, 372. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose), *m/z* 287 $[M-162+H]^+$, 285 $[M-162-H]^-$ (fragment ion peaks, luteolin).

The flavonoid was isolated from the leaves of *C. confertissimum* which was collected in Mt. Ibuki, Shiga Prefecture by Mr. Tatsuya Ueno in 1986 and crystalized by Mr. Tsutomu Ito as pale yellow needle (ca. 3 mg **138-10**, 7 mg **138-11**, 50 mg **278-2**, 10 mg **304-7**, 40 mg **304-9** and 15 mg **304-10**). Luteolin and glucose were liberated by acid hydrolysis. Attachment of 1 mol glucose to 7-position of luteolin was determined by LC-MS and UV spectral survey according to Mabry *et al.* (1970). Finally, the flavonoid was identified as luteolin 7-*O*-glucoside by HPLC comparison with authentic sample from Carl Roth. Although luteolin 7-*O*-glucoside has been reported from some *Cirsium* species, e.g. *C. suffultum*, *C. yakushimense* and *C. chikushiense* (Iwashina *et al.*, 1995), it was found in *C. confertissimum* for the first time.

Luteolin 4′-*O*-glucoside (**141-1**). UV: *λ*max (nm) MeOH 246, 269, 335; +NaOMe 267, 295sh, 374 (dec.); +$AlCl_3$ 259sh, 278, 292sh, 350, 380; +$AlCl_3$/HCl 258, 279, 292sh, 342, 379sh; +NaOAc 270, 376; +NaOAc/H_3BO_3 270, 337. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

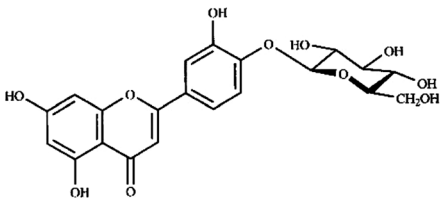

Fig. 5. Luteolin 4′-*O*-glucoside.

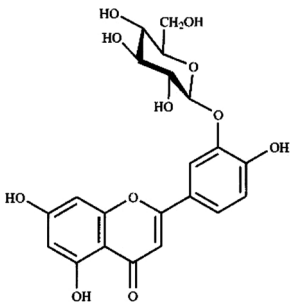

Fig. 6. Luteolin 3′-*O*-glucoside.

The sample was obtained from *C. confertissimum* leaves as pale yellow powder (ca. 3 mg). Luteolin and glucose were produced by acid hydrolysis. Attachment of 1 mol glucose to luteolin was determined by LC-MS. Since bathochromic shift, and decrease of absorbance of Band I occurred in addition of NaOMe to MeOH solution in UV spectral survey, the presence of substituted 4′-hydroxyl group was shown. Finally, the compound was identified as luteolin 4′-*O*-glucoside (Fig. 5) by HPLC comparison with authentic sample from Extrasynthese (France). Luteolin 4′-*O*-glucoside was found in *Cirsium* species for the first time.

Luteolin 3′-*O*-glucoside (**148**). UV: *λ*max (nm) MeOH 239sh, 269, 338; +NaOMe 275, 329, 397 (inc.); +$AlCl_3$ 258, 276, 294sh, 353, 380; +$AlCl_3$/HCl 254, 277, 293sh, 346, 377sh; +NaOAc 274, 331, 396; +NaOAc/H_3BO_3 268, 348. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

The sample was obtained by Mr. Tsutomu Ito

as pale yellow powder (ca. 1 mg) in 1986. The original glycoside produced luteolin and glucose by acid hydrolysis. In UV spectral survey, the presence of free 5-, 7- and 4′-hydroxyl groups was proved by addition of NaOMe, $AlCl_3$, $AlCl_3$/HCl or NaOAc, showing the attachment of glucose to 3′-position of luteolin. Thus, the flavonoid was characterized as luteolin 3′-*O*-glucoside (Fig. 6). Luteolin 3′-*O*-glucoside was newly reported from *Cirsium* species. Flavonoids of *C. confertissimum* were reported for the first time.

9. Flavonoids from Cirsium esculentum *(Sieb.) C.A.Mey*

Pectolinarigenin 7-*O*-rutinoside (**136-22**). UV: λmax (nm) MeOH 273, 329; +NaOMe 296, 375 (dec.); +$AlCl_3$ 282sh, 299, 352, 386sh; +$AlCl_3$/HCl 284, 299, 346, 387sh; +NaOAc 284, 316sh; +NaOAc/H_3BO_3 274, 331. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained from the leaves of *C. esculentum* (TNS9522069) which was collected between Semipalatinsk and Ayaguz, Kazakhstan in 18 July 1995 by T. Iwashina, as pale yellow needle (ca. 40 mg). The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Although two flavonol glycosides, kaempferol 3-*O*-rhamnosylglucoside and quercetin 3-*O*-rutinoside, have been reported (Kozyra *et al.*, 2010; Kasterova *et al.*, 2019), pectolinarigenin 7-*O*-rutinoside newly found in this survey. Acacetin 7-*O*-rutinoside (24.9%) was accompanied with pectolinarin as minor flavonoid.

10. Flavonoids from Cirsium grayanum *(Maxim.) Nakai*

Pectolinarigenin 7-*O*-rutinoside (**136-28**). UV: λmax (nm) MeOH 274, 328; +NaOMe 296, 377 (dec.); +$AlCl_3$ 284sh, 300, 353, 388sh; +$AlCl_3$/HCl 284sh, 298, 345, 386sh; +NaOAc 280, 322; +NaOAc/H_3BO_3 274, 331. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 315 $[M-308+H]^+$, 313 $[M-308-H]^-$ (fragment ion peaks, pectolinarigenin).

The sample was isolated as pale yellow needle from *C. grayanum* leaves, which were collected in 2000 by Dr. Yuichi Kadota (collection site is unknown), and identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Flavonoids of *C. grayanum* were reported for the first time. Minor acacetin 7-*O*-rutinoside (19.9%) was contained in the sample.

11. Flavonoids from Cirsium hanamakiense *Koidz.*

Pectolinarigenin 7-*O*-rutinoside (**136-17**). UV: λmax (nm) MeOH 273, 328; +NaOMe 296, 375 (dec.); +$AlCl_3$ 281sh, 299, 351, 387sh; +$AlCl_3$/HCl 282sh, 299, 343, 386sh; +NaOAc 278, 325; +NaOAc/H_3BO_3 273, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$ (fragment ion peak, pectolinarigenin + 1 mol glucose), and *m/z* 315 $[M-308+H]^+$, 313 $[M-308-H]^-$ (fragment ion peaks, pectolinarigenin).

The sample was isolated as pale yellow needle (ca. 30 mg) from the leaves of *C. hanamakiense* which was collected by Dr. Yuichi Kadota in 1996 (collection site is unknown). It was identified as pectolinarigenin 7-*O*-rutinoside by UV properties, LC-MS and HPLC comparison with the sample **136-10** from *C. brevicaule*. The flavonoids of *C. hanamakiense* were reported for the first time. It was shown by HPLC that linarin is contained in the sample as minor flavonoid (27.6%).

12. Flavonoids from Cirsium happoense *Kadota*

Acacetin 7-*O*-rutinoside (**280-1**). UV: λmax (nm) MeOH 269, 327; +NaOMe 293, 371 (dec.); +$AlCl_3$ 277, 300, 345, 377sh; +$AlCl_3$/HCl 278, 298, 339, 380sh; +NaOAc 272, 316; +NaOAc/H_3BO_3 270, 330. LC-MS: *m/z* 593 $[M+H]^+$ (molecular ion peak, acacetin + each 1 mol of glucose and rhamnose), *m/z* 447 [M-146

$+H]^+$ (fragment ion peak, acacetin + 1 mol glucose), *m/z* 285 $[M-308+H]^+$ (fragment ion peak, acacetin).

The sample (ca. 20 mg) was isolated from the leaves of *C. happoense* which was collected in Nagano Pref., Japan by Dr. Yuichi Kadota in 1994. The flavonoid was identified as acacetin 7-*O*-rutinoside by UV, LC-MS, acid hydrolysis and HPLC comparison with authentic sample (Carl Roth). Pectolinarigenin 7-*O*-rutinoside was accompanied as minor flavonoid (38.4%). Although the plant was collected as *C. happoense*, the species name is not recorded in "Flora of Japan" (Kadota, 1995).

13. Flavonoids from Cirsium hidakamontanum *Kadota*

Pectolinarigenin 7-*O*-rutinoside (**282-6**). UV: λmax (nm) MeOH 275, 327; + NaOMe 297, 376 (dec.); + $AlCl_3$ 285sh, 300, 355, 387sh; + $AlCl_3$/HCl 284sh, 298, 344, 386sh; + NaOAc 280, 323; + NaOAc/H_3BO_3 273, 330. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated as pale yellow needle from the leaves of *C. hidakamontanum* which was collected in Mt. Kamui-Ekuuchikaushi, Hokkaido, Japan by Dr. Yuichi Kadota in 1998. *C. hidakamontanum* was recorded as a new *Cirsium* species by Kadota (1999). The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was accompanied as minor compound (30.5%). Flavonoids were reported from *C. hidakamontanum* for the first time.

14. Flavonoids from Cirsium inundatum *Makino*

Pectolinarigenin 7-*O*-rutinoside (**136-12**). UV: λmax (nm) MeOH 274, 328; + NaOMe 296, 375 (dec.); + $AlCl_3$ 282sh, 299, 352, 386sh; + $AlCl_3$/HCl 283, 299, 344, 385sh; + NaOAc 282, 321; + NaOAc/H_3BO_3 273, 333. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained as pale yellow needle (ca. 20 mg) from *C. inundatum* leaves which were collected in 1995 by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was characterized as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Pectolinarigenin 7-*O*-rutinoside has already been reported from the species (Nakaoki and Morita, 1960). In this survey, acacetin 7-*O*-rutinoside (31.8%) was newly found as minor compound in the sample.

15. Flavonoids from Cirsium irumtiense *Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**136-4**). UV: λmax (nm) MeOH 276, 329; + NaOMe 297, 378 (dec.); + $AlCl_3$ 287sh, 300, 354, 387sh; + $AlCl_3$/HCl 284sh, 299, 347, 386sh; + NaOAc 283, 319; + NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$ (fragment ion peak, pectolinarigenin + 1 mol glucose), and *m/z* 315 $[M-308+H]^+$, 313 $[M-308-H]^-$ (fragment ion peaks, pectolinarigenin).

The sample was isolated as pale yellow needle (ca. 30 mg) from *C. irumtiense* leaves which were collected by Dr. Yuichi Kadota in 1994 (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS, and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside (12.4%) was accompanied as minor flavonoid. Flavonoids were reported from *C. irumtiense* for the first time. Kadota (1995) described that the species is the same with *C. brevicaule*. It was shown by this survey that flavonoid composition of the both species was the same with each other.

16. Flavonoids from Cirsium ishizuchiense *(Kitam.) Kadota*

Pectolinarigenin 7-*O*-rutinoside (**136-1**). UV: λmax (nm) MeOH 275, 328; + NaOMe 296, 378 (dec.); + $AlCl_3$ 286sh, 300, 353, 387sh; + $AlCl_3$/HCl 284sh, 298, 346, 386sh; + NaOAc 280, 325; + NaOAc/H_3BO_3 275, 332. LC-MS: *m/z* 623

$[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample (ca. 40 mg) was obtained from *C. ishizuchiense* leaves which were collected by Dr. Yuichi Kadota in 1996 (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was contained in the sample as minor compound (13.3%). Flavonoids were reported from *C. ishizuchiense* for the first time. *C. ishizuchiense* was independent as a species from a variety, *C. tenue* Kitam. var. *ishizuchiense* Kitam. by Kadota (2000).

17. Flavonoids from Cirsium japonicum *Ledeb. ex DC.*

Pectolinarigenin 7-*O*-rutinoside (**136-30**, **290-3**). UV: λmax (nm) MeOH 273, 330; + NaOMe 296, 379 (dec.); + $AlCl_3$ 281, 299, 352, 387sh; + $AlCl_3$/HCl 280sh, 298, 345, 386sh; + NaOAc 284, 314; + NaOAc/H_3BO_3 272, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

Their samples were obtained from *C. japonicum* leaves which were collected by Mr. Tatsuya Ueno (**136-30**) (collection site is unknown) and Mr. Tsutomu Ito (collection site, Atsugi City, Kanagawa Prefecture, Japan, **290-3**) in 1985 and 1987 as pale yellow needle. The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside (linarin) was accompanied with pectolinarin as minor compound (24.8% in **136-30** and 6.0% in **290-3**). Pectolinarin and linarin have already been reported from the species (Nakaoki and Morita, 1959; Iwashina *et al.*, 1988, 1995).

Cirsimaritin 4′-*O*-glucoside (**290-2**). UV: λmax (nm) MeOH 276, 328; + NaOMe 294, 359 (dec.); + $AlCl_3$ 262sh, 288sh, 299, 356; + $AlCl_3$/HCl 259sh, 287sh, 298, 346; + NaOAc 277, 326, 385; + NaOAc/H_3BO_3 276, 330. LC-MS: *m/z* 477 $[M+H]^+$ (molecular ion peak, cirsimaritin + 1 mol glucose), *m/z* 315 $[M-162+H]^+$, 313 $[M-162-H]^-$ (fragment ion peaks, cirsimaritin). ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 13.55 (1H, *s*, 5-OH), 7.92 (2H, *d*, J = 8.9 Hz, H-2′,6′), 7.49 (2H, *d*, J = 8.9 Hz, H-3′,5′), 6.95 (1H, *s*, H-3), 6.83 (1H, *s*, H-8), 5.80 (1H, *d*, J = 7.2 Hz, glucosyl H-1), 4.65 (1H, *dd*, J = 2.1 and 12.1 Hz, glucosyl H-6a), 4.43 (1H, *m*, glucosyl H-3), 4.42 (1H, *m*, glucosyl H-2), 4.40 (1H, *m*, glucosyl H-6b), 4.35 (1H, *t*, J = 8.6 Hz, glucosyl H-4), 4.24 (1H, *m*, glucosyl H-5), 4.02 (3H, *s*, 6-OCH_3), 3.94 (3H, *s*, 7-OCH_3). ^{13}C NMR (150 MHz, pyridine-d_5): (cirsimaritin) δ_C 153.5 (C-2), 104.7 (C-3), 183.2 (C-4), 164.1 (C-5), 133.2 (C-6), 159.5 (C-7), 91.7 (C-8), 153.7 (C-9), 106.4 (C-10), 125.0 (C-1′), 129.0 (C-2′,6′), 117.0 (C-3′,5′), 161.4 (C-4′), 60.6 (7-OCH_3), 56.5 (6-OCH_3); (glucose) δ_C 101.8 (C-1), 74.9 (C-2), 78.6 (C-3), 71.4 (C-4), 79.3 (C-5), 62.5 (C-6).

Cirsimaritin (hydrolysate of **290-2**). UV: λmax (nm) MeOH 275, 333; + NaOMe 278, 302, 357 (inc.); + $AlCl_3$ 285sh, 301, 358, 387sh; + $AlCl_3$/HCl 286sh, 300, 351, 386sh; + NaOAc 272, 389; + NaOAc/H_3BO_3 275, 336.

The sample was obtained as pale yellow powder (ca. 40 mg) from the leaves of *C. japonicum* which was collected in Atsugi City by Mr. Tsutomu Ito in 1986. The presence of free 5-hydroxyl, and substituted 7- and 4′-hydroxyl groups was shown by UV spectral properties. An aglycone and glucose were liberated by acid hydrolysis of the original glycoside. Attachment of 1 mol glucose to dihydroxy-dimethoxyflavone was proved by appearance of the molecular ion peak, *m/z* 477 $[M+H]^+$ and fragment ion peaks, *m/z* 315 $[M-162+H]^+$ and 313 $[M-162-H]^-$. In ^{1}H NMR, four aromatic proton signals, δ_H 7.92 (H-2′,6′), 7.49 (H-3′,5′), 6.95 (H-3) and 6.83 (H-8) appeared, together with glucosyl anomeric proton signal, δ_H 5.80 (*d*, J = 7.2 Hz) and two methoxyl proton signals, δ_H 4.02 and 3.94. Attachment of glucose to 7-position of flavone was shown by HMBC correlation of anomeric proton signal δ_H 5.80 with δ_C 161.4 corresponding to C-4′. On the other hand, two methoxyl

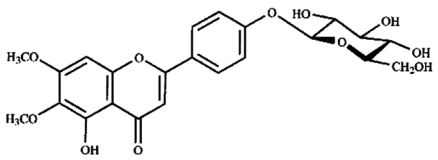

Fig. 7. Cirsimaritin 4′-*O*-glucoside.

proton signals, δ_H 4.02 and 3.94 correlated with δ_C 133.2 and 159.5 corresponding to C-6 and C-7. Thus, this was identified as 5,4′-dihydroxy-6,7-dimethoxyflavone 4′-*O*-*β*-D-glucopyranoside (cirsimaritin 4′-*O*-glucoside, Fig. 7). Cirsimaritin 4′-*O*-glucoside has been reported in *Cirsium maritimum* (Morita and Shimizu, 1963) and *C. kamtschaticum* (Iwashina *et al.*, 1988). It was found in *C. japonicum* for the first time.

18. Flavonoids from Cirsium japonicum *Fisch. ex DC. var.* diabolicum *(Kitam.) Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**136-31**). UV: λmax (nm) MeOH 274, 329; +NaOMe 296, 375 (dec.); +$AlCl_3$ 282, 300, 352, 386sh; +$AlCl_3$/HCl 282, 298, 346, 386sh; +NaOAc 280, 319; +NaOAc/H_3BO_3 272, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained as pale yellow needle from *C. japonicum* var. *diabolicum* which was collected in Toyama Prefecture, Japan by Dr. Yuichi Kadota in 1994. The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with the sample **136-10**. Acacetin 7-*O*-rutinoside (27.8%) was accompanied in the sample as minor compound. Flavonoids of *C. japonicum* var. *diabolicum* were reported for the first time.

19. Flavonoids from Cirsium kagamontanum *Nakai*

Pectolinarigenin 7-*O*-rutinoside (**279-5**). UV: λmax (nm) MeOH 276, 329; +NaOMe 296, 377 (dec.); +$AlCl_3$ 286sh, 300, 353, 387sh; +$AlCl_3$/HCl 285sh, 298, 345, 386sh; +NaOAc 286, 320; +NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$, 645 $[M+H+Na]^+$,

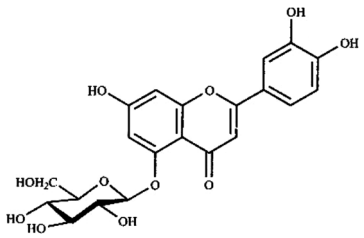

Fig. 8. Luteolin 5-*O*-glucoside.

(molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 315 [M-308 + H]$^+$ (fragment ion peak, pectolinarigenin).

The flavonoid was obtained as pale yellow needle (ca. 80 mg) from the leaves of *C. kagamontanum* which was collected by Dr. Yuichi Kadota (collection site is unknown). It was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was contained as minor flavonoid (18.0%).

Luteolin 5-*O*-glucoside (**279-8**, **279-9**). UV: λmax (nm) MeOH 240, 247sh, 258sh, 342; +NaOMe 260, 312, 402 (inc.); +$AlCl_3$ 265, 390; +$AlCl_3$/HCl 261, 350; +NaOAc 260, 276sh, 319, 388; +NaOAc/H_3BO_3 252, 260sh, 305, 363. LC-MS: *m/z* 447 $[M-H]^-$, 471 $[M+H+Na]^+$ (molecular ion peaks, luteolin + 1 mol glucose).

Their samples were isolated by Mr. Tsutomu Ito from *C. kagamontanum* leaves which were collected in Mts. Hira, Shiga Pref., Japan by Mr. Tatsuya Ueno, and identified as luteolin 5-*O*-glucoside (galuteolin, Fig. 8) by UV, LC-MS, acid hydrolysis and HPLC comparison with **308-2**. Pectolinarigenin 7-*O*-rutinoside has been reported from the species (Nakaoki and Morita, 1960). Acacetin 7-*O*-rutinoside was found in the species for the first time. In this survey, since luteolin 5-*O*-glucoside, and pectolinarigenin and acacetin 7-*O*-rutinosides were obtained from two individuals, respectively, the occurrence of geographic flavonoid variation was presumed in the species.

20. Flavonoids from Cirsium kamtschaticum *Ledeb. ex DC.*

Pectolinarigenin 7-*O*-rutinoside (**317-2**). UV: λmax (nm) MeOH 273, 329; +NaOMe 296, 376 (dec.); +$AlCl_3$ 280sh, 300, 351, 387sh; +$AlCl_3$/HCl 282sh, 298, 344, 385sh; +NaOAc 282, 321; +NaOAc/H_3BO_3 273, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 313 $[M-308-H]^-$ (fragment ion peak, pectolinarigenin).

The flavonoid was isolated as pale yellow needle (ca. 20 mg) from the leaves of *C. kamtschaticum* and identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was accompanied as minor flavonoid (31.3%). Pectolinaigenin and acacetin 7-*O*-rutinosides have been reported from the species (Morita *et al.*, 1973). Additionally, cirsimaritin 4′-*O*-glucoside has also been found in *C. kamtschaticum* (Iwashina *et al.*, 1988).

21. Flavonoid from Cirsium longe-pedunculatum *Kitam.*

Luteolin 5-*O*-glucoside (**308-2**). UV: λmax (nm) MeOH 240, 248sh, 258sh, 343; +NaOMe 260, 311, 406 (inc.); +$AlCl_3$ 264, 396; +$AlCl_3$/HCl 261, 297, 351; +NaOAc 260, 317, 387; +NaOAc/H_3BO_3 251sh, 259sh, 305, 364. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose), *m/z* 287 $[M-162+H]^+$ (fragment ion peak, luteolin). ^1H NMR (600 MHz, pyridine-d_5): δ_H 7.90 (1H, *d*, *J* = 1.7 Hz, H-2′), 7.52 (1H, *dd*, *J* = 2.2 and 8.3 Hz, H-6′), 7.49 (1H, *d*, *J* = 2.2 Hz, H-6), 7.30 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.96 (1H, *d*, *J* = 2.0 Hz, H-8), 6.90 (1H, *s*, H-3), 5.38 (1H, *d*, *J* = 7.7 Hz, glucosyl H-1), 4.44 (1H, *m*, glucosyl H-2), 4.41 (1H, *m*, glucosyl H-6a), 4.38 (1H, *m*, glucosyl H-3), 4.36 (1H, *m*, glucosyl H-6b), 4.35 (1H, *m*, glucosyl H-4), 4.04 (1H, *m*, glucosyl H-5). ^{13}C NMR (150 MHz, pyridine-d_5): (luteolin) δ_C 162.5 (C-2), 107.0 (C-3), 182.8 (C-4), 160.2 (C-5), 104.0 (C-6), 164.3 (C-7), 99.5 (C-8), 158.5 (C-9), 109.6 (C-10), 123.0 (C-1′), 114.4 (C-2′), 147.7 (C-3′), 151.3 (C-4′), 116.9 (C-5′), 119.6 (C-6′); (glucose) δ_C 106.7 (C-1), 75.4 (C-2), 77.6 (C-3), 71.2 (C-4), 79.3 (C-5), 62.5 (C-6).

Luteolin and glucose were liberated by acid hydrolysis of the original glycoside. Attachment of 1 mol glucose to luteolin was shown by LC-MS (appearance of molecular ion peaks, *m/z* 449 $[M+H]^+$ and 447 $[M-H]^-$, and fragment ion peak, *m/z* 287 $[M-162+H]^+$). In ^1H NMR, six aromatic proton signals, δ_H 7.90 (H-2′), 7.52 (H-6′), 7.49 (H-6), 7.30 (H-5′), 6.96 (H-8) and 6.90 (H-3), appeared together with glucosyl anomeric proton signal δ_H 5.38 (*d*, *J* = 7.7 Hz). Attachment of glucose to 5-position of luteolin was shown by HMBC correlation of anomeric proton signal δ_H 5.38 with C-5 of luteolin at δ_C 160.2. Thus, the glycoside was identified as luteolin 5-*O*-*β*-D-glucopyranoside, and has been reported from *C. longe-pedunculatum* (Iwashina *et al.*, 1989).

22. Flavonoid from Cirsium nambuense *Nakai*

Pectolinarigenin 7-*O*-rutinoside (**287**). UV: λmax (nm) MeOH 276, 328; +NaOMe 296, 376 (dec.); +$AlCl_3$ 284sh, 300, 353, 387sh; +$AlCl_3$/HCl 283sh, 298, 346, 387sh; +NaOAc 282, 320; +NaOAc/H_3BO_3 274, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained as pale yellow needle (ca. 3 mg) from the leaves of *C. nambuense*. The plants were collected by Dr. Yuichi Kadota (collection site is unknown). The flavonoid was characterized as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and comparison with **136-10** by HPLC. Minor flavonoid was accompanied with pectolinarin and identified as acacetin 7-*O*-rutinoside (30.2%) by HPLC comparison with authentic sample. Flavonoids of *C. nambuense* were reported in this survey for the first time.

23. Flavonoid from Cirsium norikurense *Nakai*

Acacetin 7-*O*-rutinoside (**310-1**, **310-2**). UV: λmax (nm) MeOH 268, 327; +NaOMe 288, 371 (dec.); +$AlCl_3$ 276, 300, 343, 382; +$AlCl_3$/HCl

277, 299, 337, 381; +NaOAc 269, 318; +NaOAc/H_3BO_3 268, 333. LC-MS: *m/z* 593 $[M+H]^+$ (molecular ion peak, acacetin + each 1 mol of glucose and rhamnose), *m/z* 447 [M-146 $+H]^+$ (fragment ion peak, acacetin + 1 mol glucose), *m/z* 285 [M-308 + H]$^+$, 283 [M-308-H]$^-$ (fragment ion peak, acacetin).

The flavonoids (ca. 40 mg **310-1** and ca. 1 mg **310-2**) were isolated from *C. norikurense* by Mr. Tsutomu Ito. The leaves of the species were collected in Izumi Village, Fukui Pref., Japan by Mr. Tatsuya Ueno. The flavonoid was identified as acacetin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with authentic sample (Carl Roth). Flavonoid of *C. norikurense* was found for the first time in this survey.

24. Flavonoid from Cirsium occidentalinipponense *Kadota*

Luteolin 7-*O*-glucoside (**303**). UV: λmax (nm) MeOH 255, 266sh, 346; +NaOMe 270, 394 (inc.); +$AlCl_3$ 274, 425; +$AlCl_3$/HCl 272, 295sh, 355, 383; +NaOAc 260, 402; +NaOAc/H_3BO_3 256, 372. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose).

The sample was obtained from the leaves of *C. occidentalinipponense* which was collected in Mt. San'nomine, Fukui Pref., Japan by Dr. Yuichi Kadota in 1996. The species was recorded as a new *Cirsium* species by Kadota (1997). The flavonoid was identified as luteolin 7-*O*-glucoside by UV, LC-MS and comparison with authentic sample (Carl Roth) by HPLC. Flavonoid was reported from the species for the first time.

25. Flavonoids from Cirsium oligophyllum *(Franch. et Sav.) Matsum.*

Pectolinarigenin 7-*O*-rutinoside (**136-3**). UV: λmax (nm) MeOH 273, 329; +NaOMe 296, 375 (dec.); +$AlCl_3$ 281sh, 299, 353, 387sh; +$AlCl_3$/HCl 285, 299, 343, 386sh; +NaOAc 277, 324; +NaOAc/H_3BO_3 275, 332. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated as pale yellow needle (ca. 10 mg) from *C. oligophyllum* which was collected in 1993 by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was contained in the sample as minor compound (26.5%). Nepetin and hispidulin 4′-*O*-glucosides have been reported from the species (Iwashina *et al.*, 1999). However, pectolinaigenin 7-*O*-rutinoside was newly isolated.

26. Flavonoids from Cirsium otayae *Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**281-4**). UV: λmax (nm) MeOH 275, 328; +NaOMe 296, 377 (dec.); +$AlCl_3$ 285sh, 300, 353, 390sh; +$AlCl_3$/HCl 284sh, 298, 346, 387sh; +NaOAc 277, 325; +NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated by Mr. Tsutomu Ito from the leaves of *C. otayae* which was collected in Mt. Norikura, Nagano Pref., Japan by Mr. Tatsuya Ueno. The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was contained as minor flavonoid (11.8%). The flavonoids of *C. otayae* were reported in this survey for the first time.

27. Flavonoid from Cirsium pectinellum *A. Gray*

Cirsimaritin 4′-*O*-glucoside (**134**). UV: λmax (nm) MeOH 277, 325; +NaOMe 294, 374 (dec.); +$AlCl_3$ 262, 291sh, 297, 351, 389sh; +$AlCl_3$/HCl 261, 288sh, 295, 343, 387sh; +NaOAc 278, 324; +NaOAc/H_3BO_3 276, 327. LC-MS: *m/z* 477 $[M+H]^+$ (molecular ion peak, cirsimaritin + 1 mol glucose), *m/z* 315 [M-162 $+H]^+$, 313 [M-162-H]$^-$ (fragment ion peaks, cirsimaritin).

The flavonoid was isolated by Mr. Tsutomu Ito from *C. pectinellum* leaves which were collected in Rikubetsu Town, Hokkaido, Japan by one of the authors (T. Iwashina) in 1985. The compound was identified as cirsimaritin 4′-*O*-glucoside by

UV, LC-MS and HPLC comparison with **290-2**. Although pectolinarigenin 7-*O*-rutinoside and acacetin 7-*O*-rutinoside have been reported from *C. pectinellum* (Morita *et al.*, 1973; Iwashina *et al.*, 1988), cirsimaritin 4′-*O*-glucoside was found for the first time in this survey.

28. Flavonoids from Cirsium pendulum *Fisch. ex DC.*

Pectolinarigenin 7-*O*-rutinoside (**288**, **298-1**). UV: λmax (nm) MeOH 275, 327; +NaOMe 297, 378 (dec.); +$AlCl_3$ 286sh, 300, 354, 392sh; +$AlCl_3$/HCl 285sh, 298, 344, 387sh; +NaOAc 280, 324; +NaOAc/H_3BO_3 275, 330. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

Of two samples which are deposited in the National Museum of Nature and Science, **288** was isolated as pale yellow needle (ca. 5 mg) from the leaves of *C. pendulum*. The plants were collected by Dr. Yuichi Kadota in 1998 (collection site is unknown). On the other hand, origin of **298-1**was unknown (may be from Dr. Kozo Hayashi). Both samples were identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and comparison with **136-10** by HPLC. Acacetin 7-*O*-rutinoside was accompanied in their samples (31.7% in **288** and 23.7% in **298-1**). Flavonoids were reported from *C. pendulum* for the first time.

29. Flavonoid from Cirsium purpuratum *(Maxim.) Matsum.*

Acacetin 7-*O*-rutinoside (**305**). UV: λmax (nm) MeOH 268, 326; +NaOMe 286, 375 (dec.); +$AlCl_3$ 275, 299, 345, 375sh; +$AlCl_3$/HCl 278, 299, 336, 378sh; +NaOAc 269, 322; +NaOAc/H_3BO_3 266, 341. LC-MS: *m/z* 593 $[M+H]^+$ (molecular ion peak, acacetin + each 1 mol of glucose and rhamnose), *m/z* 285 [M-308 + H]$^+$ (fragment ion peak, acacetin).

The sample was isolated from the leaves of *C. purpuratum* as powder (ca. 30 mg) by Mr. Tsutomu Ito. The plants were collected in Izumi Village, Fukui Pref. by Mr. Tatsuya Ueno in 1986. The flavonoid was identified as acacetin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with authentic sample (Carl Roth). Acacetin 7-*O*-rutinoside has already been reported from the species (Nakaoki and Morita, 1959).

30. Flavonoids from Cirsium spinosum *Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**136-24**). UV: λmax (nm) MeOH 275, 329; +NaOMe 297, 380 (dec.); +$AlCl_3$ 284sh, 300, 353, 387sh; +$AlCl_3$/HCl 285, 299, 346, 386sh; +NaOAc 280, 326; +NaOAc/H_3BO_3 274, 332. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated from *C. spinosum* leaves which were collected in 1999 by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Flavonoid of acacetin 7-*O*-rutinoside type has been reported from *C. spinosum* (Morita *et al.*, 1964). However, acacetin 7-*O*-rutinoside was shown to be minor flavonoid (17.0%) by HPLC survey. Instead, pectolinarigenin 7-*O*-rutinoside was found as major compound in this survey.

31. Flavonoids from Cirsium suffultum *Nakai*

Pectolinarigenin 7-*O*-rutinoside (**136-21**). UV: λmax (nm) MeOH 276, 328; +NaOMe 297, 378 (dec.); +$AlCl_3$ 287sh, 299, 355, 387sh; +$AlCl_3$/HCl 286sh, 298, 346, 386sh; +NaOAc 285, 317sh; +NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was obtained as pale yellow needle (ca. 40 mg) from the leaves of *C. suffultum* which was collected in 1996 by Dr. Yuichi Kadota (collection site is unknown). Flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside (15.4%) was accompanied with pectolinarin as minor flavonoid. Although luteolin 7-*O*-glucoside has been

reported from the species (Iwashina *et al.*, 1995), pectolinarigenin 7-*O*-rutinoside was found in this survey for the first time.

32. Flavonoid from Cirsium suzukaense *Kitam.*

Luteolin 7-*O*-glucoside (**309-3**, **309-4**, **309-5**). UV: λmax (nm) MeOH 255, 267sh, 350; +NaOMe 271, 397 (inc.); +$AlCl_3$ 273, 424; +$AlCl_3$/HCl 270, 294sh, 358, 382; +NaOAc 260, 405; +NaOAc/H_3BO_3 259, 372. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, luteolin + 1 mol glucose), *m/z* 287 $[M-162+H]^+$ (fragment ion peak, luteolin).

Luteolin (hydrolysate of **309-3**, **309-5**) (**309-1**, **309-2**). UV: λmax (nm) MeOH 254, 266, 349; +NaOMe 272, 409 (inc.); +$AlCl_3$ 273, 422; +$AlCl_3$/HCl 261sh, 273, 294sh, 355, 384sh; +NaOAc 267, 396; +NaOAc/H_3BO_3 260, 373, 427sh. LC-MS: *m/z* 287 $[M+H]^+$, 285 $[M-H]^-$ (molecular ion peaks, tetrahydroxyflavone).

Their samples (**309-3**, **309-4**, **309-5**) were obtained as pale yellow needles from the leaves of three individuals of *C. suzukaense* which was collected in Mt. Ryozen, Shiga Pref., Japan by Mr. Tatsuya Ueno in 1986. Luteolin (**309-1**, **309-2**) and glucose were liberated by acid hydrolysis. The original glycoside was identified as luteolin 7-*O*-glucoside by acid hydrolysis, UV, LC-MS and comparison with authentic sample (Carl Roth) by HPLC. Flavonoid was reported from *C. suzukaense* for the first time.

33. Flavonoids from Cirsium tashiroi *Kitam.*

Pectolinarigenin 7-*O*-rutinoside (**136-2**). UV: λmax (nm) MeOH 275, 328; +NaOMe 297, 377 (dec.); +$AlCl_3$ 287sh, 299, 354, 387sh; +$AlCl_3$/HCl 284, 297, 345, 386sh; +NaOAc 280, 319; +NaOAc/H_3BO_3 274, 332. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated as pale yellow needle (ca. 8 mg) from *C. tashiroi* leaves which were collected in Mt. Ibuki, Shiga Prefecture by Mr. Tatsuya Ueno in 1991. The compound was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Acacetin 7-*O*-rutinoside was accompanied as minor flavonoid (17.9%).

Hispidulin 7-*O*-neohesperidoside (**122**). UV: λmax (nm) MeOH 275, 332; +NaOMe 278, 357 (inc.); +$AlCl_3$ 284, 300, 359, 386sh; +$AlCl_3$/HCl 286, 298, 351, 384sh; +NaOAc 272, 390; +NaOAc/H_3BO_3 275, 336. LC-MS: *m/z* 631 $[M+H+Na]^+$, 607 $[M-H]^-$ (molecular ion peaks, hispidulin + each 1 mol of glucose and rhamnose), *m/z* 301 $[M-308+H]^+$ (fragment ion peak, hispidulin). ^{1}H NMR (600 MHz, pyridine-d_5): δ_H 7.89 (2H, *d*, *J* = 8.8 Hz, H-2′,6′), 7.26 (1H, *s*, H-8), 7.21 (2H, *d*, *J* = 8.8 Hz, H-3′,5′), 6.90 (1H, *s*, H-3), 6.57 (1H, *d*, *J* = 1.1 Hz, rhamnosyl H-1), 5.91 (1H, *d*, *J* = 7.7 Hz, glucosyl H-1), 4.83 (1H, *dd*, *J* = 5.7 and 9.4 Hz, rhamnosyl H-5), 4.83 (1H, *dd*, *J* = 6.5 and 9.4 Hz, rhamnosyl H-3), 4.64 (1H, *dd*, *J* = 7.8 and 8.9 Hz, glucosyl H-2), 4.60 (1H, *dd*, *J* = 3.4 and 9.4 Hz, rhamnosyl H-2), 4.55 (1H, *dd*, *J* = 2.0 and 12.2 Hz, glucosyl H-6a), 4.44 (1H, *t*, *J* = 8.9 Hz, glucosyl H-3), 4.34 (1H, *t*, *J* = 9.4 Hz, rhamnosyl H-4), 4.31 (1H, *dd*, *J* = 5.6 and 11.7 Hz, glucosyl H-6b), 4.23 (1H, *t*, *J* = 8.9 Hz, glucosyl H-4), 4.20 (3H, *s*, 6-OCH_3), 4.18 (1H, *m*, glucosyl H-5), 1.78 (3H, *d*, *J* = 6.1 Hz, rhamnosyl CH_3). ^{13}C NMR (150 MHz, pyridine-d_5): (hispidulin) δ_C 154.0 (C-2), 103.5 (C-3), 183.1 (C-4), 165.0 (C-5), 134.1 (C-6), 156.7 (C-7), 95.2 (C-8), 152.8 (C-9), 107.0 (C-10), 122.1 (C-1′), 128.9 (C-2′,6′), 116.9 (C-3′,5′), 162.8 (C-4′), 61.0 (6-OCH_3); (glucose) δ_C 100.0 (C-1), 77.1 (C-2), 79.5 (C-3), 71.3 (C-4), 79.2 (C-5), 62.3 (C-6); (rhamnose) δ_C 101.8 (C-1), 72.5 (C-2), 72.6 (C-3), 74.1 (C-4), 70.1 (C-5), 19.0 (C-6).

Hispidulin (hydrolysate of **122**) (**128-1**). UV: λmax (nm) MeOH 274, 333; +NaOMe 276, 324, 394 (inc.); +$AlCl_3$ 283sh, 302, 358, 387sh; +$AlCl_3$/HCl 287sh, 299, 350, 387sh; +NaOAc 275, 311, 331, 389; +NaOAc/H_3BO_3 276, 339. LC-MS: *m/z* 301 $[M+H]^+$, 299 $[M-H]^-$ (molecular ion peaks, trihydroxy-monomethoxyflavone).

The sample was obtained as pale yellow powder (ca. 5 mg) from *C. tashiroi* leaves which were

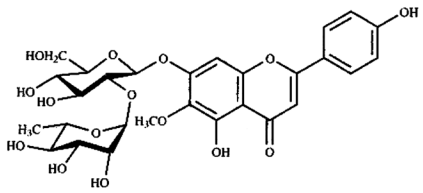

Fig. 9. Hispidulin 7-*O*-neohesperidoside.

collected in another population of Mt. Ibuki by Mr. Tatsuya Ueno in 1992. Attachment of each 1 mol of hexose and rhamnose to trihydroxy-monomethoxyflavone was shown by LC-MS. An aglycone, glucose and rhamnose were liberated by acid hydrolysis of the original glycoside. The presence of free 5- and 4′-hydroxyl groups was proved by UV spectral survey according to Mabry *et al.* (1970). In ^{1}H NMR, four aromatic proton signals, δ_H 7.89 (H-2′,6′), 7.21 (H-3′,5′), 7.26 (H-8) and 6.90 (H-3) appeared, together with two anomeric proton signals, δ_H 6.57 (*d*, J = 1.1 Hz) and 5.91 (*d*, J = 7.7 Hz) corresponding to rhamnosyl and glucosyl H-1, and a methoxyl proton signal δ_H 4.20. Attachment of methoxyl group to 6-position was shown by HMBC correlation of δ_H 4.20 with the carbon signal at δ_C 134.1 corresponding to C-6. On the other hand, attachment of glucose to 7-position of hispidulin was proved by HMBC correlation of anomeric proton signal (δ_H 5.91) with the carbon signal at δ_C 156.7 corresponding to C-7. Moreover, rhamnosyl anomeric proton signal (δ_H 6.57) correlated with carbon signal at δ_C 77.1 corresponding to C-2 of glucose. Thus, the flavonoid was identified as 5,7,4′-trihydroxy-6-methoxyflavone 7-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (hispidulin 7-*O*-neohesperidoside, Fig. 9). Hispidulin 7-*O*-neohesperidoside has been reported from the flowers of *Ipomoea purpurea* Roth (Convolvulaceae) for the first time (Ragunathan and Sulochana, 1994). Although the glycoside has been reported from the whole plants of *Cirsium japonicum* var. *ussuriense* (Park *et al.*, 1995), it was newly found in Japanese *Cirsium* species. Flavonoids of *C. tashiroi* were reported for the first time. Since two plant materials, which pectolinarigenin 7-*O*-rutinoside and hispidulin 7-*O*-neohesperidoside were isolated, respectively, were collected from different populations, the occurrence of chemical geographic variation was presumed.

34. Flavonoids from Cirsium magofukui × C. microspicatum *var.* kiotoense

Pectolinarigenin 7-*O*-rutinoside (**136-5**). UV: *λ*max (nm) MeOH 276, 329; + NaOMe 297, 376 (dec.); + $AlCl_3$ 285sh, 299, 353, 387sh; + $AlCl_3$/HCl 284sh, 298, 347, 387sh; + NaOAc 281, 324; + NaOAc/H_3BO_3 274, 331. LC-MS: *m/z* 623 $[M+H]^+$, 645 $[M+H+Na]^+$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose).

The sample was isolated from the leaves of *C. magofukui* × *C. microspicatum* var. *kiotoense* as pale yellow needle (ca. 90 mg). The plants were collected in 1992 and identified by Dr. Yuichi Kadota (collection site is unknown). The flavonoid was characterized as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**. Trace of acacetin 7-*O*-rutinoside was accompanied as minor compound (3.3%).

35. Flavonoid from Cirsium microspicatum × C. norikurense

Pectolinarigenin 7-*O*-rutinoside (**284-12**). UV: *λ*max (nm) MeOH 276, 328; + NaOMe 296, 377 (dec.); + $AlCl_3$ 286sh, 299, 354, 390sh; + $AlCl_3$/HCl 286sh, 299, 345, 387sh; + NaOAc 280, 324; + NaOAc/H_3BO_3 276, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The flavonoid was isolated from the leaves of *C. microspicatum* × *C. norikurense* as pale yellow needle (ca. 15 mg). The plants were collected and identified by Dr. Yuichi Kadota. The flavonoid was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with **136-10**.

36. Flavonoids from Cirsium yezoense × C. magofukui

Pectolinarigenin 7-*O*-rutinoside (**285-2**). UV: λmax (nm) MeOH 276, 328; + NaOMe 296, 376 (dec.); + $AlCl_3$ 286sh, 300, 354, 389sh; + $AlCl_3$/HCl 284sh, 298, 345, 387sh; + NaOAc 281, 323; + NaOAc/H_3BO_3 275, 331. LC-MS: *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The flavonoid was isolated from *C. yezoense* × *C. magofukui* leaves. The species was collected and identified by Dr. Yuichi Kadota (collection site is unknown). The sample was identified as pectolinarigenin 7-*O*-rutinoside by UV, LC-MS and comparison with **136-10** by HPLC. Acacetin 7-*O*-rutinoside was contained as minor flavonoid (12.9%).

37. Flavonoids from Cirsium *sp.*

Pectolinarigenin 7-*O*-rutinoside and acacetin 7-*O*-rutinoside (**278-1**). UV: λmax (nm) MeOH 272, 327; + NaOMe 294, 373 (dec.); + $AlCl_3$ 279, 300, 352, 387sh; + $AlCl_3$/HCl 279sh, 298, 342, 384sh; + NaOAc 274, 324; + NaOAc/H_3BO_3 272, 330. LC-MS: (major flavonoid) *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (molecular ion peaks, pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 313 $[M-308-H]^-$ (fragment ion peak, pectolinarigenin). LC-MS: (minor flavonoid) *m/z* 593 $[M+H]^+$ (molecular ion peak, acacetin + each 1 mol of glucose and rhamnose), *m/z* 285 $[M-308-H]^-$ (fragment ion peak, acacetin)

The flavonoids were isolated as pale yellow needle from the leaves of *Cirsium* sp. which was collected in Rikubetsu Town, Hokkaido, Japan by one of the authors (T. Iwashina). The crystal was the mixture of two flavonoids and identified as pectolinarigenin 7-*O*-rutinoside (62.8%) and acacetin 7-*O*-rutinoside (37.2%), by UV, LC-MS and HPLC comparisons with authentic samples.

38. Flavonoids from Carduus nutans *L.*

Acacetin 7-*O*-rutinoside and pectolinarigenin 7-*O*-rutinoside (**300**). UV: λmax (nm) MeOH 269, 327; + NaOMe 292, 371 (dec.); + $AlCl_3$ 277, 299, 347, 377sh; + $AlCl_3$/HCl 278, 299, 339, 380sh; + NaOAc 270, 323; + NaOAc/H_3BO_3 269, 330. LC-MS: (major flavonoid) *m/z* 593 $[M+H]^+$ (molecular ion peak, acacetin + each 1 mol of glucose and rhamnose). LC-MS: (minor flavonoid) *m/z* 623 $[M+H]^+$ (molecular ion peak, pectolinarigenin + each 1 mol of glucose and rhamnose).

The flavonoids were isolated from the leaves of *Carduus nutans* as pale yellow needle (ca. 90 mg). The plant (TNS9522025) was collected in near Suusanmyr, Kyrgyz Republic in 1995 by T. Iwashina. It was shown by HPLC survey that the crystal was the mixture of two flavonoids. Of their flavonoids, major compound (69.9%) was identified as acacetin 7-*O*-rutinoside by UV, LC-MS and HPLC comparison with authentic sample (Carl Roth). On the other hand, minor flavonoid (37.1%) was characterized as pectolinarigenin 7-*O*-rutinoside by LC-MS and comparison with **136-10** by HPLC. Flavones, apigenin and its 7-*O*-glucoside and 7-*O*-neohesperidoside, and luteolin and its 7-*O*-glucoside, 7-*O*-galactoside, 7-*O*-rutinoside, 7-*O*-diglucoside and 7-*O*-digalactoside, have been reported from the leaves of the species (Bain and Desrochers, 1988; Warwick *et al.*, 1989; Jordon-Thaden and Louda, 2003). Flavonols, kaempferol and its 3-*O*-rhamnoside, 3-*O*-glucoside-7-*O*-rhamnoside, isorhamnetin, and quercetin 3-*O*-rutinoside, have also been found in the species (Kaloshina *et al.*, 1975; Kaloshina and Mazulin, 1988; Jordon-Thaden and Louda, 2003). Although acacetin 7-*O*-glucoside has been isolated from *C. nutans* (Kaloshina *et al.*, 1975), acacetin 7-*O*-rutioside and pectolinarigenin 7-*O*-rutinoside were found for the first time. Since *C. nutans* is widely distributed in Eurasia and North America, chemical geographic variation may be occurred.

In this survey, the chemical structures of 48 flavonoid powders and crystals, which are deposited in Department of Botany, National Museum of Nature and Science, Japan, from 38 *Cirsium* taxa and *Carduus nutans*, were newly determined or reconfirmed (Table 1). Of their flavonoids,

Table 1. List of the flavonoids isolated from *Cirsium* taxa and related *Carduus nutans* deposited in the National Museum of Nature and Science, Japan

Accession number	Origin	References	Remarks
Acacetin 7-*O*-rutinoside			
113-1	*Cirsium senjoense*	Iwashina *et al.* (1995)	
113-3	*C. babanum*	Iwashina *et al.* (1995)	Mixed with trace of pectolinarigenin 7-*O*-rutinoside
280-1	*C. happoense*	Present paper	Mixed with pectolinaigein 7-*O*-rutinoside (38.4%)
298-4	*C.* sp.	Present paper	Mixed with pectolinaigein 7-*O*-rutinoside (34.1%)
305	*C. purpuratum*	Nakaoki and Morita (1959)	
310-1, **310-2**	*C. norikurense*	Present paper	
300	*Carduus nutans*	Present paper	Mixed with pectolinaigein 7-*O*-rutinoside (37.1%)
Hispidulin 7-*O*-neohesperidoside			
122	*C. tashiroi*	Present paper	
Hispidulin			
128-1	*C. tashiroi*	Present paper	Hydrolysate of hispidulin 7-*O*-neohesperidoside
128-2	*C. oligophyllum*	Iwashina *et al.* (1999)	Hydrolysate of hispidulin 4′-*O*-glucoside
Hispidulin 4′-*O*-glucoside			
129	*C. oligophyllum*	Iwashina *et al.* (1999)	
Cirsimaritin 4′-*O*-glucoside			
134	*C. pectinellum*	Present paper	
290-1, **290-2**, **290-4**	*C. japonicum*	Present paper	Mixed with cirsimaritin (**290-1**)
307	*C.* sp.	Present paper	
311-1, **311-2**, **311-3**	*C. maritimum*	Morita and Shimizu (1963)	
Pectolinarigenin 7-*O*-rutinoside			
136-1	*C. ishidzuchiense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (13.3%)
136-2	*C. tashiroi*	Present paper	Mixed with acacetin 7-*O*-rutinoside (17.9%)
136-3	*C. oligophyllum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (26.5%)
136-4	*C. irumtiense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (12.4%)
136-5	*C. magofukui* × *C. microspicatum* var. *kiotoense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (3.3%)
136-6	*C. senjoense*	Iwashina *et al.* (1995)	
136-7	*C. spicatum*	Iwashina *et al.* (1995)	Mixed with acacetin 7-*O*-rutinoside (11.2%)
136-8	*C. gratiosum*	Iwashina *et al.* (1995)	
136-9	*C. babanum*	Iwashina *et al.* (1995)	Mixed with acacetin 7-*O*-rutinoside (28.7%)
136-10	*C. brevicaule*	Present paper	Mixed with acacetin 7-*O*-rutinoside (10.3%)
136-12	*C. inundatum*	Nakaoki and Morita (1960)	Mixed with acacetin 7-*O*-rutinoside (31.8%)
136-13	*C. aidzuense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (11.7%)
136-15	*C. bitchuense*	Iwashina *et al.* (1995)	Mixed with acacetin 7-*O*-rutinoside (14.2%)
136-17	*C. hanamakiense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (27.6%)
136-18	*C. borealinipponense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (25.5%)
136-21	*C. suffultum*	Morita *et al.* (1973) Iwashina *et al.* (1995)	Mixed with acacetin 7-*O*-rutinoside (15.4%)
136-22	*C. esculentum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (24.9%)
136-23	*C. boninense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (8.9%)
136-24	*C. spinosum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (17.0%)
136-26	*C. yezoense*	Iwashina *et al.* (1988)	Mixed with acacetin 7-*O*-rutinoside (11.3%)
136-28	*C. grayanum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (19.9%)
136-29	*C. alpicola*	Present paper	Mixed with acacetin 7-*O*-rutinoside (19.8%)
136-30, **290-3**	*C. japonicum*	Nakaoki and Morita (1959) Iwashina *et al.* (1988) Ganzera *et al.* (2005) Liu *et al.* (2007) Peng (2011)	Mixed with acacetin 7-*O*-rutinoside (24.8%, **136-30**, and 36.3%, **294-3**)
136-31	*C. diabolicum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (27.8%)
136-32	*C. brevicaule*	Present paper	Mixed with acacetin 7-*O*-rutinoside (8.4%)

Table 1. List of the flavonoids isolated from *Cirsium* taxa and related *Carduus nutans* deposited in the National Museum of Nature and Science, Japan (continued)

Accession number	Origin	References	Remarks
278-1	*C.* sp.	Present paper	Mixed with acacetin 7-*O*-rutinoside (37.2%)
279-5	*C. kagamontanum*	Nakaoki and Morita (1960)	Mixed with acacetin 7-*O*-rutinoside (18.0%)
281-4	*C. otayae*	Present paper	Mixed with acacetin 7-*O*-rutinoside (11.8%)
282-6	*C. hidakamontanum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (30.5%)
283-4	*C. ugoense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (24.4%)
284-1	*C. microspicatum*	Nakaoki and Morita (1959) Iwashina *et al.* (1988)	Mixed with acacetin 7-*O*-rutinoside (17.5%)
284-12	*C. microspicatum* × *C. norikurense*	Present paper	
285-2	*C. yezoense* × *C. magofukui*	Present paper	Mixed with acacetin 7-*O*-rutinoside (12.9%)
286-1, **286-7**, **286-10**, **289-2**	*C. kiotoense*	Iwashina *et al.* (1988)	
287	*C. nambuense*	Present paper	Mixed with acacetin 7-*O*-rutinoside (30.2%)
288, **298-1**	*C. pendulum*	Present paper	Mixed with acacetin 7-*O*-rutinoside (31.7%, **288** and 23.7%, **298-1**)
290-3	*C. japonicum*	Nakaoki and Moria (1959) Iwashima *et al.* (1988, 1995)	Mixed with acacetin 7-*O*-rutinoside (36.0%)
317-2	*C. kamtschaticum*	Morita *et al.* (1973)	Mixed with acacetin 7-*O*-rutinoside (31.3%)
Luteolin 7-*O*-glucoside			
138-1	*C. chikushiense*	Iwashina *et al.* (1995)	
138-5	*C. hachijoense*	Iwashina and Ootani (1998)	
138-10, **138-11**, **278-2**, **297**, **304-7**, **304-9**, **304-10**	*C. confertissimum*	Present paper	
303	*C. occidentalinipponense*	Present paper	
304-1, **304-11**, **306-1**, **306-2**	*C. buergeri*	Present paper	
Luteolin 4′-*O*-glucoside			
141-1	*C. confertissimum*	Present paper	
Luteolin 3′-*O*-glucoside			
148	*C. confertissimum*	Present paper	
Nepetin 4′-*O*-glucoside			
149	*C. oligophyllum*	Iwashina *et al.* (1999)	
Nepetin			
157	*C. oligophyllum*	Iwashina *et al.* (1999)	Hydrolysate of nepetin 4′-*O*-glucoside
Luteolin 7-*O*-vicianoside			
304-2, **304-5**	*C. buergeri*	Present paper	
Luteolin 5-*O*-glucoside			
279-8, **279-9**	*C. kagamontanum*	Iwashina *et al.* (1989)	
308-1	*C. sieboldii*	Iwashina *et al.* (1989)	
308-2	*C. longe-pedunculatum*	Iwashina *et al.* (1989)	
309-3, **309-4**, **309-5**	*C. suzukaense*	Present paper	
312-1, **312-2**	*C. magofukui*	Iwashina *et al.* (1989)	
Luteolin			
309-1, **309-2**	*C. suzukaense*	Present paper	

pectolinarigenin 7-*O*-rutinoside was frequently found (22 samples), and acacetin 7-*O*-rutinoside was accompanied in almost *Cirsium* taxa and *Carduus nutans*. As other flavonoids, hispidulin 7-*O*-neohesperidoside, cirsimaritin 4′-*O*-glucoside, luteolin 7-*O*-glucoside, luteolin 4′-*O*-glucoside, luteolin 3′-*O*-glucoside, luteolin 7-*O*-vicianoside and luteolin 5-*O*-glucoside were found in

some *Cirsium* species. Of their flavonoids, luteolin 7-*O*-vicianoside which was isolated from *C. buergeri* was reported in *Cirsium* species for the first time. Hispidulin 7-*O*-neohesperidoside which was newly isolated from *C. tashiroi* was found in Japanese *Cirsium* species.

Acknowledgments

Authors thank to Mr. Tsutomu Ito for donation of flavonoid crystals and powders, and also to Dr. Yuichi Kadota and Mr. Tatsuya Ueno for offer of plant materials.

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