

Saussurea Species from the Altai Mountains and Adjacent Area, and Their Flavonoid Diversity

Tsukasa Iwashina^{1,*}, Sergey V. Smirnov², Oyunchimeg Damdinsuren³
and Katsuhiko Kondo⁴

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

²Department of Plant Systematics, Altai State University, Barnaul, Altai, 656099 Russia

³Biology Division, Hovd State University, Hovd, Hovd Province, 213500 Mongolia

⁴Laboratory of Plant Genetics and Breeding Science, Department of Agriculture, Faculty of Agriculture,
Tokyo University of Agriculture, Funako 1737, Atsugi, Kanagawa, 243–0034 Japan

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

(Received 15 Aug. 2010; accepted 22 Sept. 2010)

Abstract Seven *Saussurea* species, i.e., *S. alpina*, *S. daurica*, *S. laciniata*, *S. pricei*, *S. pseudo-alpina*, *S. salicifolia* and *S. salsa*, were collected in the Altai Mountains in Russia and Mongolia. They were surveyed for flavonoid characters. Thirteen flavonoids belonging to flavones and flavonols, i.e., apigenin 7-*O*-glucuronide, apigenin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, luteolin 7-*O*-glucoside, chrysoeriol 7-*O*-glucuronide, chrysoeriol 7-*O*-glucoside, chrysoeriol 7-*O*-rhamnoside, jaceosidin, nepetin, hispidulin (flavones), quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside and isorhamnetin 3-*O*-rutinoside (flavonols), were found in various combination from their species. Of their species, *S. daurica*, *S. laciniata* and *S. salsa* synthesized flavone glycosides alone. On the other hand, *S. pricei*, *S. pseudo-alpina* and *S. alpina* were occurred flavonol glycosides. The former species was accompanied with free flavones. *S. salicifolia* synthesized only one flavone aglycone, hispidulin. Thus, it was clear that the genus *Saussurea* is not only morphologically and geographically but also chemically diversified.

Key words: Altai Mountains, flavones, flavonoid diversity, flavonols, *Saussurea*.

Introduction

The genus *Saussurea* DC. (Asteraceae) consists of some 300 species, which are mainly distributed in east Asia and widely adapted from lowlands to high alpine zones (Mabberley, 1997). Some of the plants occur in the Himalayas, the Tien Shan Mountains and the Altai Mountains. *S. medusa* Maxim. is characterized by highly pubescent bracts of its inflorescences, which are considered to be an adaptive morphological trait, allowing the plant to flowers at low temperatures. Therefore, the plant is also named ‘sweater plant’ (Tsukaya *et al.*, 2002). Another *Saussurea* species, *S. involucrata* Karel. & Kir. with large translucent cream-colored bracts concealing the

huge inflorescence, are named ‘glasshouse plant’ (Ohba, 1988).

Flavonoids have been reported from some *Saussurea* species which are mainly native to China. Flavones, apigenin and its 7-*O*-glucoside and 7-*O*-neohesperidoside, chrysoeriol 7-*O*-glucoside and a flavonol, quercetin were isolated from *S. gossypiphora* D. Don (Lin-gang and Min, 1989; Zheng *et al.*, 1991). Quercetin and quercetin 3-*O*-rutinoside, and hispidulin and pectolinarigenin were found in *S. controversa* DC. (Syrchyna *et al.*, 1993) and in *S. elegans* Ledeb. (Sham’yanov *et al.*, 1983), respectively. Two flavonol glycosides, quercetin 3-*O*-rutinoside and 3-*O*-rhamnoside, and two free flavones, jaceosidin and hispidulin were isolated from *S. involu-*

crata (Jia *et al.*, 1983, 1988; Yi *et al.*, 2005). Apigenin and its 7-*O*-neohesperidoside, luteolin and its 7-*O*-neohesperidoside, and quercetin and its 3-*O*-glucoside were reported from *S. medusa* (Jia *et al.*, 1989; Fan and Yue, 2003). From *S. pulchella* Fisch. ex Colla (Basargin and Tsiklauri, 1990) and *S. tridactyla* Sch.Bip. ex Hook.f. (Zhang *et al.*, 2003), common flavonoids, apigenin, luteolin, kaempferol and quercetin and their glycosides were reported. A flavone glycoside, acacetin 7-*O*-rutinoside was also isolated from *S. tridactyla* (Ren *et al.*, 2001). An anthocyanin, cyanidin 3-*O*-glucoside, which was found in *Saussurea* species for the first time, was isolated from *S. pulchella* (Basargin and Tsiklauri, 1990). Comparatively rare polymethoxyflavonol, penduletin was reported from the whole plants of *S. parviflora* DC. (Yang *et al.*, 2003). New two crotonylated flavonoids, kaempferol and quercetin 3-*O*-(6"-crotonyl)-glucosides were found in *S. japonica* (Thunb.) DC. in China (Shi *et al.*, 1991). Dihydrochalcone, asebogenin 2'-*O*-glucoside was first reported from *S. mongolica* Franchet, together with flavonol glycosides, rhamnocitrin 3-*O*-rhamnoside, and kaempferol and quercetin 3-*O*-glucosides and 3-*O*-rhamnosides (Jiang *et al.*, 2004). Rare flavonol glycosides, quercetin and tamarixetin 5-*O*-glucosides, and 6-hydroxykaempferol 3-methyl ether 6-*O*-glucoside were isolated from *S. stella* Maxim., together with apigenin and acacetin and their 7-*O*-glucosides, luteolin, chrysoeriol 7-*O*-glucoside, and kaempferol and quercetin 3-*O*-rhamnosides (Shen *et al.*, 2004; Zheng *et al.*, 1997).

Recently, we surveyed nine *Saussurea* species, *S. alberti* Regel & Winkler, *S. elegans*, *S. gnaphalodes* Ostenf., *S. involucrata*, *S. konuroba* Saposchn., *S. kuschakewiczii* C. Winkl., *S. leucophylla* Schrenk, *S. schanginiana* Fisch. & Herd., and *S. sordida* Kar. & Kir. from the Tien Shan Mountains in Kazakhstan and Kirgыз for flavonoid compounds (Kusano *et al.*, 2007). Ten flavonol glycosides, kaempferol 3-*O*-rutinoside, 5-*O*-glucoside and 7-*O*-glucoside, quercetin 3-*O*-rutinoside, 3-*O*-glucoside, 3-*O*-galactoside, 5-*O*-glucoside and 7-*O*-glucoside, and isorhamnetin

3-*O*-rutinoside and 5-*O*-glucoside, eight flavone aglycones, luteolin, luteolin 7-methyl ether, hispidulin, nepetin, selagin, selagin 7-methyl ether, velutin and jaceosidin, and thirteen flavone glycosides, apigenin 7-*O*-rutinoside and 7-*O*-glucoside, luteolin 7-*O*-rutinoside, 7-*O*-glucoside, 7-*O*-galactoside, 7-*O*-glucuronide and 5-*O*-glucoside, hispidulin and nepetin 7-*O*-glucosides, isovitexin, isoorientin, apigenin 6,8-*C*-glycoside and luteolin 8-*C*-glycoside, were reported (Kusano *et al.*, 2007). Their *Saussurea* species variously differed in their flavonoid composition. It has been shown that *Saussurea* species is not only morphologically but also chemically diversified.

In this paper, as a series of flavonoid survey in the genus *Saussurea*, flavonoid compounds of the species from the Altai Mountains were investigated.

Materials and Methods

Plant materials

Seven *Saussurea* species were collected in Russia and Mongolia during the Altai Mountains and adjacent area Botanical Expedition in September 2007. Voucher specimens have been deposited in the herbarium of National Museum of Nature and Science, Japan (TNS). Collection sites and dates of plant materials are as follows.

Saussurea alpina DC. High mountain meadow, Bayan Gol, 2450 m alt., Erdeneburen Sum, Hovd Prov., Mongolia. 48°38'69"N, 091°07'74"E, 16 Sept. 2007 (TNS9531921).

Saussurea daurica Adams. (Fig. 1). Lake side of Eli-Hur, 2070 m alt., Bayan-Ulgii-Aimak, Bayan-Ulgii Prov., Mongolia. 48°35'52"N, 090°00'24"E, 14 Sept. 2007 (TNS9531904). Modon Ovou, 1555 m alt., South of Hovd City, Hovd Prov., Mongolia. 47°56'88"N, 091°46'67"E, 15 Sept. 2007.

Saussurea laciniata Ledeb. River side, Ongostnii Ulaan Mountain. Northeast of Hovd City, Hovd Prov., Mongolia. 48°16'39"N, 091°53'08"E, 15 Sept. 2007.

Saussurea pricei N.D. Simpson (Fig. 2). Dry steppe of Bayan Gol, 2018 m alt., Tsambagarav

Fig. 1. *Saussurea daurica*.Fig. 2. *Saussurea pricei*.Fig. 3. *Saussurea salsa*.

Mountain, Erdeneburen Sum, Hovd Prov., Mongolia. 48°38'88"N, 091°11'62"E, 16 Sept. 2007 (TNS9531920).

Saussurea pseudo-alpina N.D. Simpson. High mountain meadow, 2450 m alt., Bayan Gol, Erdeneburen Sum, Hovd Prov., Mongolia. 48°38'69"N, 091°07'74"E, 16 Sept. 2007.

Saussurea salicifolia DC. The place which Chuya River joins with Katum River, 780 m alt., Onguday District, Altai Republic, Russia. 50°23'77"N, 086°40'41"E, 21 Sept. 2007 (TNS9531929).

Saussurea salsa Spreng. (Fig. 3). Chavtsaliin Gol, 1260 m alt., Erdeneburen Sum, Hovd Prov., Mongolia. 48°40'23"N, 091°19'94"E, 17 Sept. 2007.

General

UV spectra were recorded on a Shimadzu

MPS-2000 Multi purpose recording spectrophotometer according to Mabry *et al.* (1970). LC-MS were measured using a Inertsil ODS-4 column [I.D. 2.1×100 mm (GL Sciences Inc.)], at a flow-rate of 0.1 ml min⁻¹, eluting with MeCN/H₂O/HCOOH (20:75:5) or (30:65:5), ESI⁺ 4.5 kV and ESI⁻ 3.5 kV, 250°C. HPLC survey of the isolated flavonoids and crude extracts was performed with a Shimadzu HPLC systems using a Senshu Pak PEGASIL ODS column (I.D. 6.0×150 mm, Senshu Scientific Co. Ltd.), at a flow-rate of 1.0 ml min⁻¹. Detection was 350 nm and eluents were MeCN/H₂O/H₃PO₄ (20:80:0.2) for glycosides and (35:65:0.2) for aglycones.

Extraction and separation

Dried leaves of *S. alpina* (0.4 g), *S. daurica* (62.6 g), *S. pricei* (16.5 g), *S. pseudo-alpina* (0.5 g) and *S. salsa* (27.1 g), and dried aerial parts of *S. laciniata* (15.4 g) and *S. salicifolia* (9.1 g) were extracted with MeOH, respectively. After concentration, the extracts were separated by preparative paper chromatography using solvent systems: BAW (*n*-BuOH/HOAc/H₂O=4:1:5, upper phase), 15% HOAc and then BEW (*n*-BuOH/EtOH/H₂O=4:1:2.2). Isolated flavonoids

were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

Identification

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

Apigenin 7-*O*-glucuronide (**1**). TLC: Rf 0.61 (BAW), 0.23 (BEW), 0.15 (15%HOAc); UV – dark purple, UV/NH₃ – dark greenish yellow. UV: λ_{\max} (nm) MeOH 268, 333; +NaOMe 273, 379 (inc.); +AlCl₃ 275, 298, 347, 376; +AlCl₃/HCl 275, 299, 340, 373; +NaOAc 256sh, 267, 388; +NaOAc/H₃BO₃ 268, 340. LC-MS: m/z 447 [M+H]⁺, 445 [M-H]⁻ (apigenin+1 mol glucuronic acid); m/z 271 [M-176+H]⁺, 269 [M-176-H]⁻ (apigenin).

Apigenin 7-*O*-glucoside (cosmosiin, **2**). TLC: Rf 0.66 (BAW), 0.66 (BEW), 0.13 (15%HOAc); UV – dark purple, UV/NH₃ – dark greenish yellow. UV: λ_{\max} (nm) MeOH 268, 333; +NaOMe 273, 379 (inc.); +AlCl₃ 276, 299, 347, 377; +AlCl₃/HCl 276, 298, 339, 376; +NaOAc 257, 267, 389; +NaOAc/H₃BO₃ 268, 341. LC-MS: m/z 433 [M+H]⁺, 431 [M-H]⁻ (apigenin+1 mol glucose); m/z 271 [M-162+H]⁺ (apigenin).

Luteolin 7-*O*-glucuronide (**3**). TLC: Rf 0.34 (BAW), 0.19 (BEW), 0.08 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 256, 268, 348; +NaOMe 269, 392 (inc.); +AlCl₃ 273, 422; +AlCl₃/HCl 267, 273sh, 294, 358, 378sh; +NaOAc 260, 402; +NaOAc/H₃BO₃ 260, 369. LC-MS: m/z 463 [M+H]⁺, 461 [M-H]⁻ (luteolin+1 mol glucuronic acid); m/z 287 [M-176+H]⁺, 285 [M-176-H]⁻ (luteolin).

Luteolin 7-*O*-glucoside (**4**). TLC: Rf 0.36 (BAW), 0.38 (BEW), 0.06 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 255, 266sh, 348; +NaOMe 267, 391 (inc.); +AlCl₃ 273, 427; +AlCl₃/HCl 264sh, 273, 295, 359, 383; +NaOAc 260, 405; +NaOAc/H₃BO₃ 259, 373. LC-MS: m/z 449 [M+H]⁺, 447 [M-H]⁻ (luteolin+1 mol glu-

cose); m/z 287 [M-162+H]⁺, 285 [M-162-H]⁻ (luteolin).

Chrysoeriol 7-*O*-glucuronide (**5**). TLC: Rf 0.41 (BAW), 0.19 (BEW), 0.08 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 253, 268, 345; +NaOMe 266, 391 (inc.); +AlCl₃ 264sh, 273, 296sh, 364sh, 387; +AlCl₃/HCl 262, 273sh, 294sh, 357, 384sh; +NaOAc 259, 406; +NaOAc/H₃BO₃ 255sh, 265sh, 354. LC-MS: m/z 477 [M+H]⁺, 475 [M-H]⁻ (chrysoeriol+1 mol glucuronic acid).

Chrysoeriol 7-*O*-glucoside (**6**). TLC: Rf 0.49 (BAW), 0.44 (BEW), 0.08 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 253, 268, 345; +NaOMe 266, 391 (inc.); +AlCl₃ 264sh, 273, 296sh, 364sh, 387; +AlCl₃/HCl 262, 273sh, 294sh, 357, 384sh; +NaOAc 259, 406; +NaOAc/H₃BO₃ 255, 265sh, 354. LC-MS: m/z 463 [M+H]⁺, 461 [M-H]⁻ (chrysoeriol + 1 mol glucose), m/z 301 [M-162+H]⁺ (chrysoeriol).

Chrysoeriol 7-*O*-rhamnoside (**7**). LC-MS: m/z 447 [M+H]⁺ (chrysoeriol+1 mol rhamnose), m/z 299 [M-146-H]⁻ (chrysoeriol).

Quercetin 3-*O*-rutinoside (rutin, **8**). TLC: Rf 0.49 (BAW), 0.52 (BEW), 0.46 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 257, 266sh, 356; +NaOMe 275, 323, 405 (inc.); +AlCl₃ 273, 424; +AlCl₃/HCl 274, 303, 361, 405sh; +NaOAc 272, 328, 400; +NaOAc/H₃BO₃ 264, 373. LC-MS: m/z 611 [M+H]⁺, 609 [M-H]⁻ (quercetin+each 1 mol glucose and rhamnose); m/z 303 [M-308+H]⁺ (quercetin).

Isorhamnetin 3-*O*-rutinoside (narcissin, **9**). TLC: Rf 0.55 (BAW), 0.61 (BEW), 0.48 (15%HOAc); UV – dark purple, UV/NH₃ – yellow. UV: λ_{\max} (nm) MeOH 255, 265sh, 357; +NaOMe 273, 330, 411 (inc.); +AlCl₃ 269, 303, 367sh, 403; +AlCl₃/HCl 268, 300, 359, 395; +NaOAc 272, 325, 408; +NaOAc/H₃BO₃ 256, 267sh, 361. LC-MS: m/z 625 [M+H]⁺, 623 [M-H]⁻ (isorhamnetin+each 1 mol glucose and rhamnose); m/z 479 [M-146+H]⁺ (isorhamnetin+1 mol glucose); m/z 317 [M-308+H]⁺, 315 [M-308-H]⁻ (isorhamnetin).

Hispidulin (5,7,4'-Trihydroxy-6-methoxyflavone, **11**). UV: λ_{\max} (nm) MeOH 275, 335; +NaOMe 276, 324, 393 (inc.); +AlCl₃ 263sh, 282sh, 302, 360, 387sh; +AlCl₃/HCl 287sh, 300, 354, 387sh; +NaOAc 275, 312, 330, 391; +NaOAc/H₃BO₃ 277, 340. LC-MS: m/z 301 [M+H]⁺, 299 [M-H]⁻ (trihydroxymonomethoxyflavone).

Nepetin (5,7,3',4'-Tetrahydroxy-6-methoxyflavone, **12**). UV: λ_{\max} (nm) MeOH 256, 272, 345; +NaOMe 272, 336, 405 (inc.); +AlCl₃ 274, 303sh, 422; +AlCl₃/HCl 259, 283, 294sh, 363, 393sh; +NaOAc 273, 326sh, 397; +NaOAc/H₃BO₃ 266, 376, 427sh. LC-MS: m/z 317 [M+H]⁺, 315 [M-H]⁻ (tetrahydroxymonomethoxyflavone).

Jaceosidin (5,7,4'-Trihydroxy-6,3'-dimethoxyflavone, **13**). UV: λ_{\max} (nm) MeOH 274, 343; +NaOMe 274, 330, 405 (inc.); +AlCl₃ 256sh, 273sh, 282sh, 366, 387sh; +AlCl₃/HCl 275sh, 285sh, 359, 387sh; +NaOAc 266sh, 275, 323sh, 402; +NaOAc/H₃BO₃ 275, 348. LC-MS: m/z 331 [M+H]⁺, 329 [M-H]⁻ (trihydroxydimethoxyflavone).

Results and Discussion

Identification of flavonoids

Flavonoid **1** was obtained as pale yellow powder (ca. 5 mg) from *S. daurica* and *S. laciniata*. UV spectral properties of the compound showed the presence of free 5- and 4'-hydroxyl and substituted 7-hydroxyl groups of flavone nucleus. Its acid hydrolysis liberated apigenin and glucuronic acid. The attachment of 1 mol glucuronic acid to apigenin was shown by LC-MS, i.e., appearance of a molecular ion peak, m/z 447 [M+H]⁺. Original glycoside was finally identified as apigenin 7-*O*-glucuronide (Fig. 4) by direct TLC and HPLC comparison with authentic sample from the leaves of *Uncarina grandidieri* (Baill.) Stapf. (Pedaliaceae) (Yamazaki *et al.*, 2007).

UV spectral properties of flavonoid **2** was essentially the same with those of apigenin 7-*O*-glucuronide, showing that the compound is apigenin 7-*O*-glucoside. LC-MS survey indicated

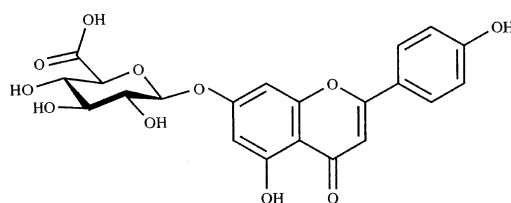


Fig. 4. Apigenin 7-*O*-glucuronide (**1**).

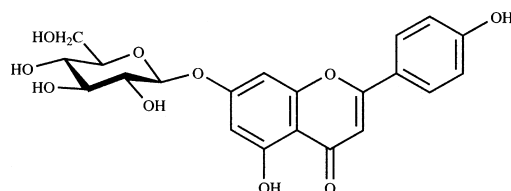


Fig. 5. Apigenin 7-*O*-glucoside (**2**).

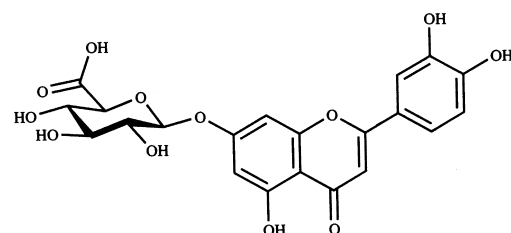


Fig. 6. Luteolin 7-*O*-glucuronide (**3**).

that 1 mol hexose is attached to apigenin. Direct TLC and HPLC comparison of flavonoid **2** with authentic cosmosiin from the leaves of *Helwingia japonica* (Thunb.) F.G. Dietrich (Cornaceae) (Iwashina *et al.*, 1997) showed that the flavonoid is apigenin 7-*O*-glucoside (Fig. 5).

Flavonoids **3** and **4** (ca. 10 mg and 5 mg) were obtained as pale yellow powders from *S. daurica* and *S. salsa*, respectively, and shown by UV spectral survey to be flavones having free hydroxyl groups to 5-, 3'- and 4'-positions and a glycosyl group to 7-position. Luteolin and glucuronic acid (**3**) and luteolin and glucose (**4**) were liberated by their acid hydrolysis. Molecular ion peaks, m/z 463 [M+H]⁺ (**3**) and m/z 449 [M+H]⁺ (**4**) appeared on the chromatograms of LC-MS, showing the attachment of 1 mol glucuronic acid and glucose to luteolin, respectively. Finally, flavonoids **3** and **4** were identified as luteolin 7-*O*-glucuronide (Fig. 6) and luteolin 7-*O*-

glucoside (Fig. 7) by direct TLC and HPLC comparisons with authentic samples from *Uncaria grandidiaria* (Yamazaki *et al.*, 2007) and *Schmalhausenia nidulans* Petrak (Asteraceae) (Iwashina and Kadota, 1999).

Flavonoid **5** produced chrysoeriol and glucuronic acid by acid hydrolysis. The attachment of 1 mol glucuronic acid to chrysoeriol was shown by UV spectral and LC-MS survey. Original glycoside was compared its R_f values and retention time with those of authentic chrysoeriol 7-*O*-glucuronide (Fig. 8) from the leaves of *Chrysanthemum japonense* Nakai var. *ashizuriense* Kitam. (Uehara *et al.*, unpublished data), by TLC and HPLC, and they completely agreed with each other. Flavonoids **6** and **7** were also characterized as chrysoeriol 7-*O*-glucoside (Fig. 9) and chrysoeriol 7-*O*-rhamnoside (Fig. 10) by UV spectral and LC-MS survey.

UV spectral properties of flavonoid **8** were

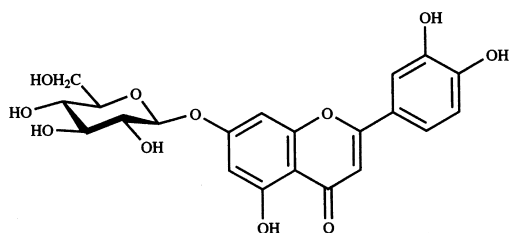


Fig. 7. Luteolin 7-*O*-glucoside (**4**).

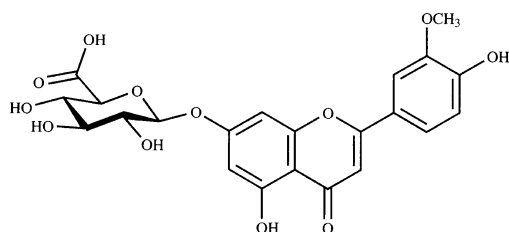


Fig. 8. Chrysoeriol 7-*O*-glucuronide (**5**).

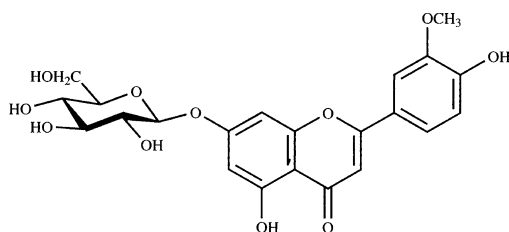


Fig. 9. Chrysoeriol 7-*O*-glucoside (**5**).

those of flavonol having free 5-, 7-, 3'- and 4'-hydroxyl and 3-*O*-glycosyl groups. By acid hydrolysis, quercetin, glucose and rhamnose were liberated. The presence of each 1 mol glucose and rhamnose was shown by LC-MS survey. Finally, flavonoid **8** was identified as quercetin 3-*O*-rutinoside (Fig. 11) by TLC and HPLC comparison with authentic rutin from the leaves of *Saruma henryi* Oliver (Aristolochiaceae) (Iwashina *et al.*, 2002).

Flavonoid **9** was obtained as pale yellow powder (ca. 15 mg) from *S. pricei* and was flavonol having 5-, 7- and 4'-hydroxyl, and 3- and 3'-substituted hydroxyl groups, which was shown by UV spectral survey. Since isorhamnetin (3,5,7,4'-tetrahydroxy-3'-methoxyflavone) was liberated by acid hydrolysis, it was shown that 3-hydroxyl group was glycosylated. As glycosidic sugars, glucose and rhamnose were produced. R_f values of TLC and retention time of HPLC of flavonoid **9** agreed with those of authentic isorhamnetin 3-*O*-rutinoside (Fig. 12) from the leaves of *Asarum caulescens* Maxim. (Aristolochiaceae)

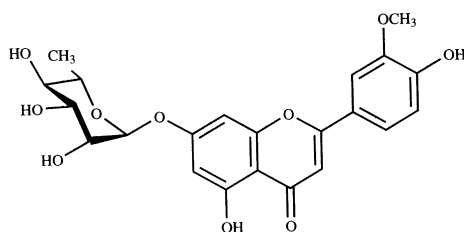


Fig. 10. Chrysoeriol 7-*O*-rhamnoside (**7**).

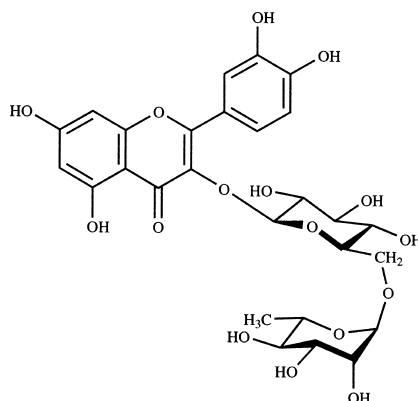


Fig. 11. Quercetin 3-*O*-rutinoside (rutin, **8**).

(Iwashina *et al.*, 2005).

LC-MS of three flavone aglycones, **11**, **12** and **13** indicated molecular ion peaks, m/z 301 $[M+H]^+$ (trihydroxy-monomethoxyflavone), 317 $[M+H]^+$ (tetrahydroxy-monomethoxyflavone) and 331 $[M+H]^+$ (trihydroxy-dimethoxyflavone), respectively. By UV spectral survey, they were shown to be 5,7,4'-trihydroxy-6 or 8-substituted flavone, 5,7,3',4'-tetrahydroxy-6 or 8-substituted flavone and 5,7,4'-trihydroxy-6,3' or 8,3'-substituted flavone. Finally, they were identified as hispidulin (**11**, Fig. 13), nepetin (**12**, Fig. 14) and jaceosidin (**13**, Fig. 15) by direct HPLC comparisons with authentic standards from the leaves of *Cirsium oligophyllum* (Franch. & Savat.) Matsum. as hydrolysates of 4'-*O*-glucosides (hispidulin and nepetin) (Iwashina *et al.*, 1999) and the leaves of *Chrysanthemum japonense* var. *ashizuriense* (jaceosidin) (Uehara *et al.*, unpublished data).

Though flavonoid **10** could not be isolated, it was characterized as quercetin 3-*O*-glucoside (Fig. 16) by HPLC comparison of peak retention time of crude extract from *S. pseudo-alpina* and

authentic isoquercitrin from the leaves of *Phytolacca americana* L. (Phytolaccaceae) (Iwashina and Kitajima, 2009).

Flavonoid composition of seven *Saussurea* species in the Altai Mountains

Seven flavone glycosides, apigenin 7-*O*-glucuronide (**1**), apigenin 7-*O*-glucoside (**2**), luteolin 7-*O*-glucuronide (**3**), luteolin 7-*O*-glucoside (**4**), chrysoeriol 7-*O*-glucuronide (**5**), chrysoeriol 7-*O*-glucoside (**6**) and chrysoeriol 7-*O*-rhamnoside (**7**), three flavonol glycosides, quercetin 3-*O*-rutinoside (**8**), isorhamnetin 3-*O*-rutinoside (**9**) and quercetin 3-*O*-glucoside (**10**), and three flavone aglycones, hispidulin (**11**), nepetin (**12**) and jaceosidin (**13**) were found in seven *Saussurea* species used as plant materials. Of flavone glycosides found in this experiment, **2** has been reported from *S. involucrata* (Kusano *et al.*, 2007), *S. gossypiphora* (Lin-gang and Min, 1989; Zheng *et al.*, 1991), *S. tridactyla* (Zheng *et al.*, 2003) and *S. stella* (Shen *et al.*, 2004; Zheng *et al.*, 1997), **3** from *S. gnaphalodes* (Kusano *et al.*, 2007), **4** from *S. involucrata*, *S. gnaphalodes* (Kusano *et al.*, 2007) and *S. tridactyla* (Zhang *et al.* 2003), and **6** from *S. gossypiphora* (Zheng *et al.*, 1991) and *S. stella* (Zheng *et al.*, 1997; Shen *et al.*, 2004) (Table 1). However, **1**, **5** and **7** were found

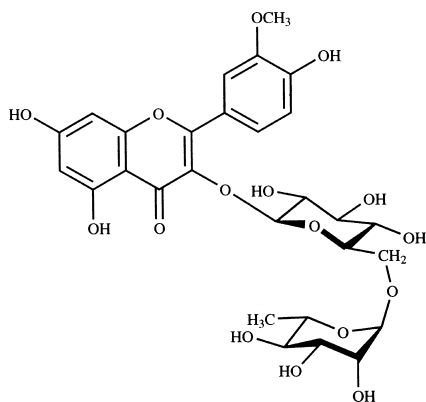


Fig. 12. Isorhamnetin 3-*O*-rutinoside (narsissin, **9**).

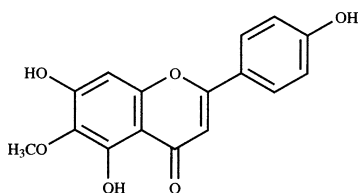


Fig. 13. Hispidulin (**11**).

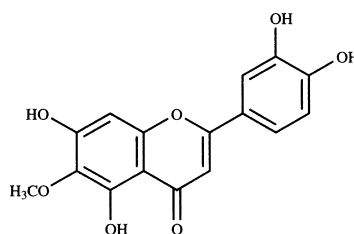


Fig. 14. Nepetin (**12**).

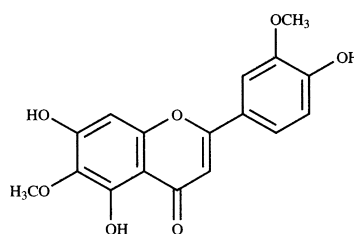


Fig. 15. Jaceosidin (**13**).

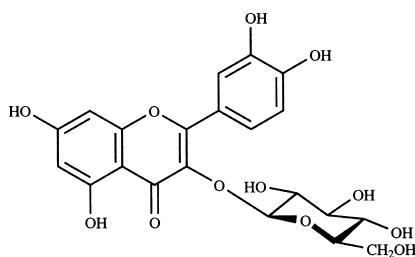
Table 1. Reports of flavonoids from *Saussurea* species

Species	Flavone aglycones	Flavone glycosides	Flavonol aglycones	Flavonol glycosides	Others	References
<i>S. gossypiphora</i>	Apigenin	Apigenin 7-glucoside Apigenin 7-neohesperidoside Chrysoeriol 7-glucoside	Quercetin			Lin-gang and Min (1989) Zheng <i>et al.</i> (1991)
<i>S. elegans</i>	Hispidulin Pectolinarigenin			Kaempferol 3-rutinoside Isorhamnetin 3-rutinoside Quercetin 3-rutinoside		Sham'yanov <i>et al.</i> (1983) Kusano <i>et al.</i> (2007)
<i>S. controversa</i>			Quercetin	Quercetin 3-rutinoside		Syrchina <i>et al.</i> (1993)
<i>S. involucreata</i>	Hispidulin Jaceosidin Luteolin Nepetin	Apigenin 7-glucoside Hispidulin 7-glucoside Luteolin 7-g-glucoside Nepetin 7-g-glucoside	Quercetin	Quercetin 3-rhamnoside Quercetin 3-rutinoside		Jia <i>et al.</i> (1983) Jia <i>et al.</i> (1988) Yi <i>et al.</i> (2005) Kusano <i>et al.</i> (2007)
<i>S. medusa</i>	Apigenin Luteolin	Apigenin 7-neohesperidoside Apigenin 7-rutinoside Luteolin 7-neohesperidoside	Quercetin	Quercetin 3-glucoside		Jia <i>et al.</i> (1989) Fan and Yue (2003)
<i>S. pulchella</i>		Apigenin 7-glucorhamnoside Luteolin 7-glucorhamnoside	Quercetin 3-rhamnoside Quercetin 3-rutinoside	Quercetin 3-glucoside Quercetin 3-rutinoside (Anthocyanin)		Basargin and Tsiklauri (1990)
<i>S. tridactyla</i>	Luteolin	Acacetin 7-rutinoside Apigenin 7-glucoside Apigenin 7-rutinoside Luteolin 7-g-glucoside	Kaempferol 3-glucoside Quercetin 3-rutinoside			Ren <i>et al.</i> (2001) Zhang <i>et al.</i> (2003)
<i>S. parviflora</i>			Quercetin Penduletin			Yang <i>et al.</i> (2003)

<i>S. japonica</i>				Kaempferol 3-(6''-crotonyl)-glucoside Kaempferol 3-glucoside Quercetin 3-(6''-crotonyl)-glucoside Quercetin 3-glucoside	Shi <i>et al.</i> (1991)
<i>S. mongolica</i>				Kaempferol 3-glucoside Kaempferol 3-rhamnoside Quercetin 3-glucoside Quercetin 3-rhamnoside Rhamnocitrin 3-rhamnoside	Jiang <i>et al.</i> (2004)
<i>S. stella</i>	Acacetin Apigenin Luteolin	Acacetin 7-glucoside Apigenin 7-glucoside Chrysoeriol 7-glucoside	Kaempferol	Isorhamnetin 5-glucoside Kaempferol 3-rhamnoside Quercetin 5-glucoside Quercetin 3-rhamnoside 6-Hydroxykaempferol 3-methyl ether 6-glucoside	Zheng <i>et al.</i> (1997) Shen <i>et al.</i> (2004)
<i>S. alberti</i>				Quercetin 3-rutinoside	Kusano <i>et al.</i> (2007)
<i>S. komuloba</i>				Quercetin 3-rutinoside	Kusano <i>et al.</i> (2007)
<i>S. kuschakewiczii</i>				Quercetin 3-glucoside Quercetin 3-rutinoside	Kusano <i>et al.</i> (2007)
<i>S. sortida</i>		Luteolin 5-glucoside		Isorhamnetin 5-glucoside Kaempferol 5-glucoside Kaempferol 7-glucoside Quercetin 5-glucoside Quercetin 7-glucoside	Kusano <i>et al.</i> (2007)
<i>S. leucophylla</i>	Hispidulin Luteolin 7-methyl ether Nepetin Velutin			Isorhamnetin 3-rutinoside Quercetin 3-rutinoside	Kusano <i>et al.</i> (2007)

Table 1. (Continued)

Species	Flavone aglycones	Flavone glycosides	Flavonol aglycones	Flavonol glycosides	Others	References
<i>S. gnaphalodes</i>	Luteolin Selagin 7-methyl ether	Apigenin 7-rutinoside Luteolin 7-glucoside Luteolin 7-glucuronide Luteolin 7-rutinoside				Kusano <i>et al.</i> (2007)
<i>S. schanginiana</i>		Apigenin 6,8-C-glycoside Isoorientin Isovitexin Luteolin 7-galactoside Luteolin 8-C-glycoside		Quercetin 3-galactoside Quercetin 3-glucoside Quercetin 3-rutinoside		Kusano <i>et al.</i> (2007)

Fig. 16. Quercetin 3-*O*-glucoside (isoquercitrin, **10**).

in *Saussurea* species for the first time.

Of three flavonol glycosides, quercetin 3-*O*-rutinoside (**8**) has been reported from some species, i.e., *S. alberti*, *S. konuloba*, *S. kuschakewiczii*, *S. elegans*, *S. leucophylla*, *S. involucrata*, *S. schanginiana* (Kusano *et al.*, 2007), *S. controversa* (Syrchina *et al.*, 1993), *S. pulchella* (Basargin and Tsiklauri, 1990) and *S. tridactyla* (Zhang *et al.*, 2003). Isorhamnetin 3-*O*-rutinoside (**9**) and quercetin 3-*O*-glucoside (**10**) have been found in *S. elegans* and *S. leucophylla* (Kusano *et al.*, 2007), and *S. kuschakewiczii*, *S. schanginiana* (Kusano *et al.*, 2007), *S. medusa* (Jia *et al.*, 1989), *S. japonica* (Shi *et al.*, 1991) and *S. mongolica* (Jiang *et al.*, 2004).

Of three flavone aglycones found in this experiment, hispidulin (**11**) has been reported from *S. elegans*, *S. leucophylla* and *S. involucrata*, nepetin (**12**) from *S. gnaphalodes* and *S. involucrata*, and jaceosidin (**13**) from *S. involucrata* (Kusano *et al.*, 2007).

Flavonoid patterns of the species used as plant materials in this experiment were apparently different with each other (Table 2). Their species were roughly divided into four types, i.e., 1) flavone glycoside alone in *S. daurica* (Fig. 17), *S. laciniata* (Fig. 18) and *S. salsa* (Fig. 19), 2) flavonol glycosides alone in *S. alpina* (Fig. 20) and *S. pseudo-alpina* (Fig. 21), 3) coexistence of flavonol glycosides and flavone aglycones in *S. pricei* (Fig. 22), and 4) flavone aglycone alone in *S. salicifolia* (Fig. 23). We have already surveyed nine *Saussurea* species native to the Tien Shan Mountains for flavonoids (Kusano *et al.*, 2007). We have shown that flavonoid diversity of

Table 2. Flavonoid composition of seven *Saussurea* species in the Altai Mountains and adjacent area

Species	Flavone glycosides							Flavonol glycosides			Flavone aglycones		
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>S. daurica</i>	+	+	+	+	+		+						
<i>S. laciniata</i>	+	+	+		+								
<i>S. salsa</i>		+	+	+	+	+							
<i>S. pricei</i>								+	+		+	+	+
<i>S. pseudo-alpina</i>								+	+	+			
<i>S. alpina</i>								+	+				
<i>S. salicifolia</i>											+		

1=apigenin 7-glucuronide, 2=apigenin 7-glucoside, 3=luteolin 7-glucuronide, 4=luteolin 7-glucoside, 5=chrysoeriol 7-glucuronide, 6=chrysoeriol 7-glucoside, 7=chrysoeriol 7-rhamnoside, 8=quercetin 3-rutinoside, 9=isorhamnetin 3-rutinoside, 10=quercetin 3-glucoside, 11=hispidulin, 12=nepetin and 13=jaceosidin.

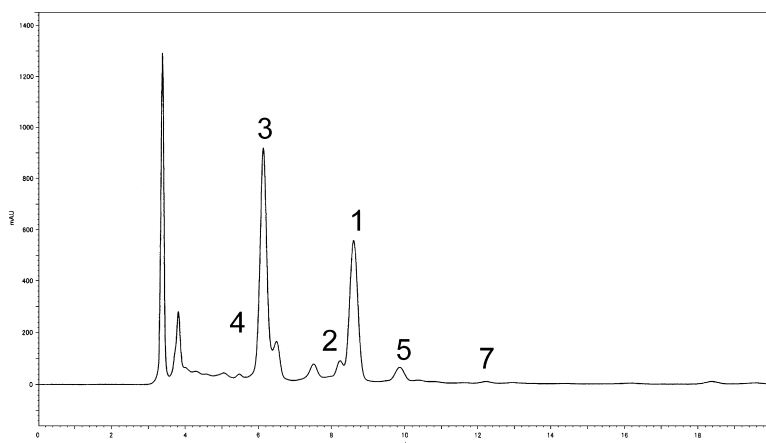


Fig. 17. HPLC chromatogram of flavonoid glycosides from *Saussurea daurica*. 1=apigenin 7-glucuronide, 2=apigenin 7-glucoside, 3=luteolin 7-glucuronide, 4=luteolin 7-glucoside, 5=chrysoeriol 7-glucuronide and 7=chrysoeriol 7-rhamnoside. Other peaks were organic acids and so on.

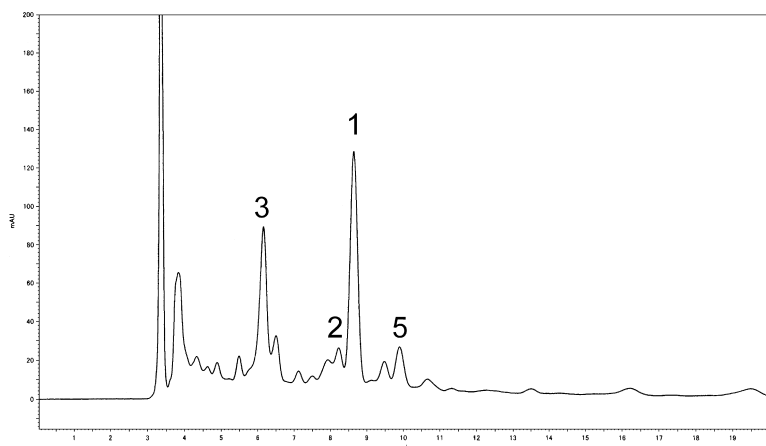


Fig. 18. HPLC chromatogram of flavonoid glycosides from *Saussurea laciniata*. 1=apigenin 7-glucuronide, 2=apigenin 7-glucoside, 3=luteolin 7-glucuronide and 5=chrysoeriol 7-glucuronide. Other peaks were organic acids and so on.

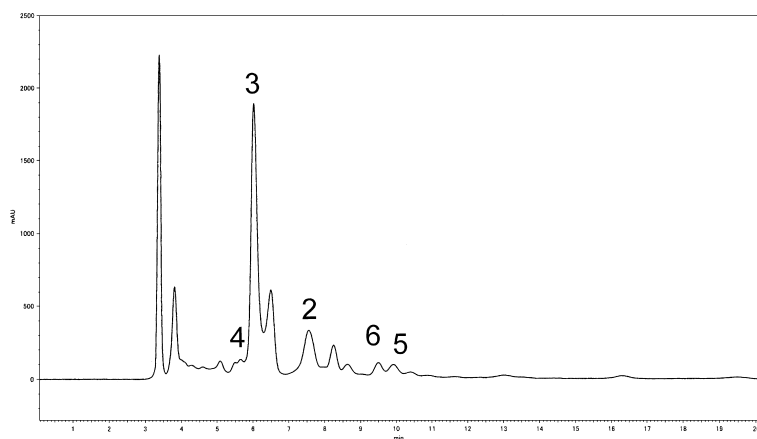


Fig. 19. HPLC chromatogram of flavonoid glycosides from *Saussurea salsa*. 2=apigenin 7-glucoside, 3=luteolin 7-glucuronide, 4=luteolin 7-glucoside, 5=chrysoeriol 7-glucuronide and 6=chrysoeriol 7-glucoside. Other peaks were organic acids and so on.

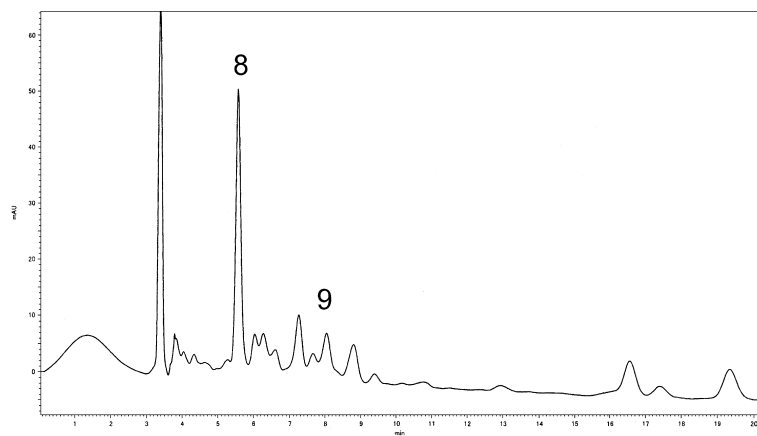


Fig. 20. HPLC chromatogram of flavonoid glycosides from *Saussurea alpina*. 8=quercetin 3-rutinoside and 9=isorhamnetin 3-rutinoside. Other peaks were organic acids and so on.

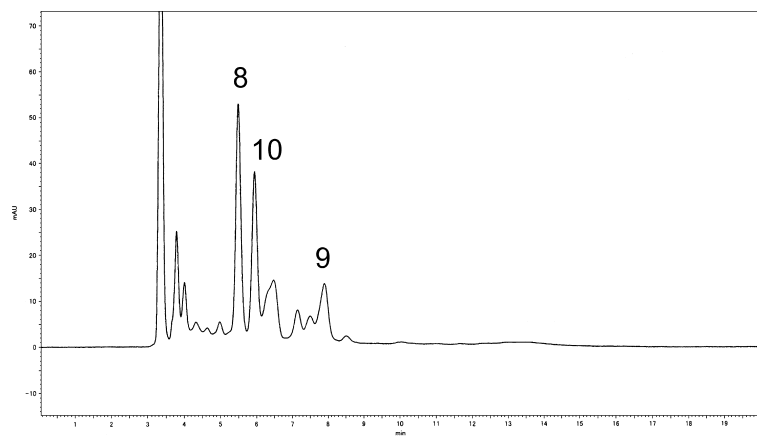


Fig. 21. HPLC chromatogram of flavonoid glycosides from *Saussurea pseudo-alpina*. 8=quercetin 3-rutinoside, 9=isorhamnetin 3-rutinoside and 10=quercetin 3-glucoside. Other peaks were organic acids and so on.

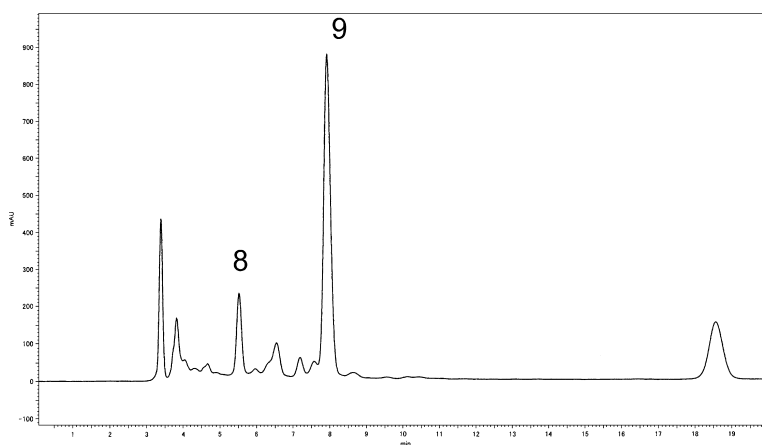


Fig. 22. HPLC chromatogram of flavonoid glycosides from *Saussurea pricei*. 8=quercetin 3-rutinoside and 9=isorhamnetin 3-rutinoside. Other peaks were organic acids and so on.

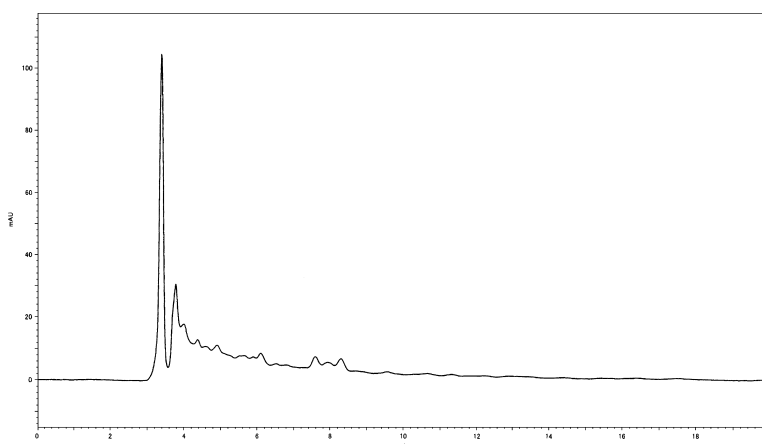


Fig. 23. HPLC chromatogram of flavonoid glycosides from *Saussurea salicifolia*. Peak of flavonoid glycoside is absent on the chromatogram.

Saussurea species is also comparatively high in the Altai Mountains, and various flavonoid patterns appeared. Thus, it was shown that the genus *Saussurea* is not only morphologically and geographically but also chemically diversified.

Acknowledgements

The collections of plant materials from the Altai Mountains and adjacent area in 2007 were supported by a Grant-in-Aid for the Scientific Programs (A) (no. 19255004; Representative, Katsuhiko Kondo) from the Ministry of Educa-

tion, Culture, Sports, Science and Technology, Japan.

References

- Basargin, D.D. and Tsiklauri, G.C. 1990. Phenolic compounds of *Saussurea pulchella* (Fisch.) Fisch. *Rastitel'nye Resursy* 26: 68–71.
- Fan, C.-Q. and Yue, J.M. 2003. Biologically active phenols from *Saussurea medusa*. *Bioorganic and Medicinal Chemistry* 11: 703–708.
- Iwashina, T., Kamenosono, K. and Hatta, H. 1997. Flavonoid glycosides from leaves of *Aucuba japonica* and *Helwingia japonica* (Cornaceae): phytochemical

- relationship with the genus *Cornus*. The Journal of Japanese Botany 72: 337–346.
- Iwashina, T. and Kadota, Y. 1999. Flavonoids from *Schmalhausenia nidulans* (Compositae): a taxon endemic to the Tien Sian Mountains. Biochemical Systematics and Ecology 27: 97–98.
- Iwashina, T., Kamenosono, K. and Ueno, T. 1999. Hispidulin and nepetin 4'-glucosides from *Cirsium oligophyllum*. Phytochemistry 51: 1109–1111.
- Iwashina, T., Marubashi, W. and Suzuki, T. 2002. Chalcones and flavonols from the Chinese species, *Saruma henryi* (Aristolochiaceae). Biochemical Systematics and Ecology 30: 1101–1103.
- Iwashina, T., Kitajima, J., Shiuchi, T. and Itou, Y. 2005. Chalcones and other flavonoids from *Asarum* sensu lato (Aristolochiaceae). Biochemical Systematics and Ecology 33: 571–584.
- Iwashina, T. and Kitajima, J. 2009. Flavonoids from the leaves of betalain-containing species, *Phytolacca americana* (Phytolaccaceae). Bulletin of the National Museum of Nature and Science, Series B 35: 99–104.
- Jia, Z., Li, Y., Du, M. and Chu, T.T. 1983. Studies on the constituents of *Saussurea involucrata* Kar. et Kin. (I). Chemical Journal of Chinese Universities 4: 581–584.
- Jia, Z., He, K., Du, M., Li, Y. and Chu, T.T. 1988. Studies on the constituents of *Saussurea involucrata* Kar. et Kin. (IV). Chemical Journal of Chinese Universities 9: 198–200.
- Jia, Z., Gong, N. and Du, M. 1989. Studies on the chemical constituents of *Saussurea medusa* Maxim. (II). Chemical Journal of Chinese Universities 11: 202–204.
- Jiang, T.F., Li, Y. and Shi, Y.P. 2004. Determination of six major flavonoid glycosides in *Saussurea mongolica* by capillary electrophoresis. Planta Medica 70: 282–284.
- Kusano, K., Iwashina, T., Kitajima, J. and Mishio, T. 2007. Flavonoid diversity of *Saussurea* and *Serratula* species in Tien Shan Mountains. Natural Product Communications 2: 1121–1128.
- Lin-gang, Q. and Min, L. 1989. Studies on the components of *Saussurea gossypiphora* D. Don. Acta Botanica Sinica 31: 398–401.
- Mabberley, D.J. 1997. The Plant-Book, 2nd ed. p. 642. Cambridge University Press, Cambridge.
- Mabry, T.J., Markham, K.R. and Thomas, M.B. 1970. The Systematic Identification of Flavonoids. Springer, Berlin.
- Ohba, H. 1988. The alpine flora of the Nepal Himalayas: an introductory note. In: Ohba, H. (ed.), The Himalayan Plants, 1. pp. 19–46.
- Ren, Y., Yang, J. and Chen, J. 2001. Study on chemical constituents of *Saussurea tridactyla* III. Chinese Pharmaceutical Journal 36: 732–734.
- Sham'yanov, I.D., Batirov, E.K., Yuldashev, M.P. and Malabaev, A. 1983. Components of *Saussurea elegans*. Chemistry of Natural Compounds 19: 763–764.
- Shen, J., Liang, J., Peng, S. and Ding, L. 2004. Chemical constituents from *Saussurea stella*. Natural Product Research and Development 16: 391–394.
- Shi, J.G., Jia, Z.J. and Li, Y. 1991. Studies on the chemical constituents of *Saussurea japonica* (Thunb.) DC. Chemical Journal of Chinese Universities 12: 906–909.
- Syrchina, A.I., Chernousova, A.V., Vereshchagin, A.L. and Semenov, A.A. 1993. The chemical composition of the extractive substances of *Saussurea controversa*. Chemistry of Natural Compounds 29: 686–687.
- Tsukaya, H., Fujikawa, K. and Wu, S.-G. 2002. Thermal insulation and accumulation of heat in the downy inflorescences of *Saussurea medusa* (Asteraceae) at high elevation in Yunnan, China. Journal of Plant Research 115: 263–268.
- Yamazaki, K., Iwashina, T., Kitajima, J., Gamou, Y., Yoshida, A. and Tannowa, T. 2007. External and internal flavonoids from Madagascarian *Uncarina* species (Pedaliaceae). Biochemical Systematics and Ecology 35: 743–749.
- Yang, Z.D., Gao, K. and Jia, Z.J. 2003. Endesmane derivatives and other constituents from *Saussurea parviflora*. Phytochemistry 62: 1195–1199.
- Yi, H., Qian, Z., Ming, Y., Fang, X., Airong, K., Huan, Y., Lijun, H., Xintang, W. and Jie, Z. 2005. Study of the content of flavonoids of different parts in *Saussurea involucrata* and their HPLC fingerprint chromatogram. Chinese Traditional Herb Drugs 28: 980–982.
- Zhang, Y., Yu, M. and Chen, J. 2003. Simultaneous determination of seven compounds in snow lotus herb using high-performance liquid chromatography. Journal of Chromatographic Science 41: 241–244.
- Zheng, S.-Z., Yu, J.H. and Shen, Y.-W. 1991. Studies on the chemical constituents of *Saussurea gossypiphora* D. Don. Chemical Journal of Chinese Universities 12: 1613–1616.
- Zheng, R.L., Xing, G.X., Ju, Y. and Jia, Z.J. 1997. Flavonoids from *Saussurea stella* Maxim. as superoxide scavengers and antioxidants. Indian Journal of Chemistry 36B: 1201–1203.