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Flavonols from the Aerial Parts of two *Aztekium* and three *Ariocarpus* Species (Cactaceae)

Tsukasa IWASHINA*

Tsukuba Botanical Garden, National Science Museum, 4–1–1 Amakubo, Tsukuba, Ibaraki, 305-0005 Japan

Abstracts. Four and three flavonoids were isolated from the aerial parts of two Cactaceous species, *Aztekium ritteri* and *Ariocarpus kotschoubeyanus* var. *macdowellii*, respectively. They were identified as kaempferol, isokaempferide, quercetin 3-methyl ether and quercetin 3-methyl ether 7-*O*-arabinosyl-galactoside from the former species, and isorhamnetin 3-*O*-rutinoside, isorhamnetin 3-*O*-rhamnosyl-galactoside and isorhamnetin 3-*O*-digalactoside from the latter one. Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside was found in nature for the first time, and also detected by PC and HPLC survey from the aerial parts of another *Aztekium* species, *A. hintonii* which was recently found as a new species. Isorhamnetin 3-*O*-rutinoside and 3-*O*-rhamnosylgalactoside were also found in two *Ariocarpus* species, *A. agavioides* and *A. scapharostrus* by PC and HPLC.

Introduction

The genus *Aztekium* (Cactaceae) has consisted of only one species, *A. ritteri* (Böd.) Böd., which is endemic to Nuevo León, Mexico (Backeberg 1979). However, second species, *A. hintonii* Glass & Fitz Maurice, was recently found on steep gypsum hills and cliffs in Nuevo León, Mexico, as a new species. (Glass and Fitz Maurice 1992). Thus, the genus *Aztekium* now consists of two species, *A. ritteri* and *A. hintonii*. On the other hand, the genus *Ariocarpus* contains ca. 6 species and distributes in Texas, USA and Mexico (Britton and Rose 1963).

The flower flavonoids of Cactaceous species have been reported by Iwashina and co-workers. They have reported 15 flavonol aglycones and glycosides based on quercetin, quercetin 3-methyl ether, kaempferol and isorhamnetin from 269 species belonging to the subfamily Cereoideae (Iwashina *et al.* 1986, Iwashina and Ootani 1986). Of their flavonoids, a new flavonol glycoside has been isolated from the flowers of *Neochilenia*, *Neoporteria* and *Parodia* species, identified as quercetin 3-methyl ether 4′-*O*-glucoside and named as neochilenin (Iwashina *et al.* 1984). The flower flavonoids were also surveyed for some *Echinocereus* species and estimated as some flavonol glycosides based on kaempferol, quercetin and isorhamnetin, flavones; apigenin and luteolin 7-*O*-glycosides, flavanones; naringenin and eriodictyol 7-*O*-glycosides, and dihydroflavonols; taxifolin, aromadendrin and ampelopsin 7-*O*-glycosides (Miller and Bohm 1982, Leuck II and Miller 1982, Miller 1988). Isorhamnetin 3-*O*-galactoside and 3-*O*-rutinoside, and several quercetin, kaempferol and isorhamnetin 3-*O*-glycosides were isolated from the flowers of *Cereus grandiflora* Mill. (Hörhammer *et al.* 1966) and some *Opuntia* species (Shabbir and Zaman 1968, Rösler *et al.* 1966, Clark and Parfitt 1980, Clark *et al.* 1980).

Flavonoids of the aerial parts except the flowers were also reported by some authors. The leaves and thornes of 22 Cactaceous species, including *Pereskia*, *Rhodocactus*, *Pereskiopsis*, *Austrocylindropuntia*,

*Corresponding author. Tel: +81-29-851-5159; Fax: +81-29-853-8998. *E-mail address*: iwashina@kahaku.go.jp

Opuntia, *Rhipsalis*, *Cleistocactus*, *Oreocereus*, *Trichocereus*, *Chamaecereus*, *Neobuxbaumia*, *Mirtillocactus*, *Cereus*, *Pilosocereus* and *Mamillaria* species, were surveyed and it was shown that the several classes of flavonoid aglycones, e.g., flavonols; quercetin, kaempferol, isorhamnetin, quercetin 3-methyl ether, rhamnocitrin, isokaempferide, kumatakenin and annulatin, flavones; apigenin and luteolin, flavanones; naringenin and naringenin 7,4´-dimethyl ether, and dihydroflavonols; taxifolin and aromadendrin are present (Burret *et al.* 1982). The presence of proanthocyanidins, which closely correlate with anthocyanin biosynthesis but not in cacti, have been reported from the seeds of *Pereskia nemorosa* Rojas Acosta, *Opuntia stricta* (Haw.) Haw., *Rhipsalis baccifera* (J.S. Miller) Stearn and *Coryphantha macromeris* (Engelm.) Orcutt (Bittrich and Amaral 1991). An aurone, cephalocerone, was isolated from liquid suspension culture of *Cephalocereus senilis* Pfeiff. (old-man-cactus) together with 6,7-dihydroxy-5-methoxyflavone 7-*O*-glucoside, baicalein, baicalein 7-*O*-glucoside, baicalein 7-*O*-(6´´-malonylglucoside), baicalein 6-*O*-glucoside, chrysin 7-*O*-glucoside, (2*S*)-6,7-dihydroxy-5-methoxyflavanone 7-*O*-glucoside and (2*S*)-5,6,7-trihydroxyflavanone 7-*O*-glucoside, and showed antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Pare *et al.* 1991, Liu *et al.* 1993a, 1993b). A new flavonol glycoside, kaempferol 3-*O*-glucosyl-(1→2)-*O*-[rhamnosyl-(1→6)]-galactoside-7-*O*-rhamnoside, was also isolated from the fresh plant material of this species together with two known flavonoids, kaempferol 7-*O*-rhamnoside and kaempferol 3-*O*-rhamnosyl-(1→6)-galactoside-7-*O*-rhamnoside (Liu *et al.* 1994).

In the genus *Ariocarpus*, a polymethoxylated flavonol, retusine (5-hydroxy-3,7,3´,4´-tetramethoxyflavone) was isolated from the whole plant of *A. retusus* Schw. (Domínguez *et al.* 1969). However, *Aztekium* species are not analyzed for flavonoid compound.

In this paper, I describe the flavonoid isolation and identification from the aerial parts of *Aztekium ritteri* and *Ariocarpus kotschoubeyanus* var. *macdowellii*. Their flavonoid composition was compared with those of *Aztekium hintonii*, and *Ariocarpus agavioides* and *A. scapharostrus* by PC and HPLC, respectively.

Materials and Methods

Plant materials

Two *Aztekium* species, *A. ritteri* (Böd.) Böd. and *A. hintonii* Glass & Fitz Maurice, and three *Ariocarpus* species, *A. kotschoubeyanus* (Lemaire) Schumann var. *macdowellii* (Backeb.) Krainz (= *Roseocactus kotschoubeyanus* (Lem.) Berg. var. *macdowellii* Backeb.), *A. agavioides* (Castañ.) And. (= *Neogomesia agavioides* Cast.) and *A. scapharostrus* Böd., were used as plant materials. Living plants are grown in Tsukuba Botanical Garden, National Science Museum, Tsukuba, Japan.

Flavonoid extraction and isolation from Aztekium ritteri *and* Ariocarpus kotschoubeyanus *var.* macdowellii

The fresh aerial parts of *Aztekium ritteri* (166 g) and *Ariocarpus kotschobeyanus* var. *macdowellii* (57 g) were extracted with MeOH, respectively. The flavonoids were isolated by preparative paper chromatography using solvent systems: BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase), 15% HOAc and then BEW (*n*-BuOH/EtOH/H_2O = 4:1:2.2). Isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

HPLC and PC survey of crude extracts from Aztekium hintonii, Ariocarpus agavioides *and* A. scapharostrus

The MeOH extracts from the aerial parts of *A. hintonii* (ca. 140 g), *A. agavioides* (ca. 200 g) and *A. scapharostrus* (ca. 242 g) were surveyed for flavonoid composition by HPLC. Flavonoid composition of the cacti was surveyed by HPLC using Shimpack CLC-ODS (I. D. 6.0 × 150 mm, Shimadzu), at flowe-rate: 1.0 ml/min, injection: 10 µl, detection: 190 – 400 nm, and eluents: MeCN:H_2O:H_3PO_4 (22:78:0.2) for glycosides (Sol. I) and (35:65:0.2) for aglycones (Sol. II). Two-dimensional paper chromatography (2D-PC) was performed using solvent systems: BAW (1st) and 15%HOAc (2nd).

LC-MS of flavonoids

LC-MS was syurveyed with Symmetry C_{18} column [I. D. 2.1 × 150 mm (Waters), at a flow-rate of 0.18 ml/min, eluting with 15% MeCN rising to 45% MeCN (30 min), ESI^+ 3.0 kV, cone voltage 30 V, ESI^- 3.0 kV, cone voltage 30 V, 400°C, ion energy 1.0 V.

Identification of flavonoids

Isolated flavonoids were identified by UV spectra according to Mabry *et al.* (1970), LC-MS, acid hydrolysis of glycosides (in 12% aq.HCl, 100°C, 30 min), and direct PC and HPLC comparisons with authentic specimens. Their PC, HPLC, UV and LC-MS data were as follows.

Isokaempferide (Kaempferol 3-methyl ether, **1**). HPLC: retention time (Rt) 21.66 min (Sol. II). UV λ max nm: MeOH 267, 350; +NaOMe 275, 323, 397 (inc.); +$AlCl_3$ 275, 305, 354, 393; +$AlCl_3$/HCl 276, 303, 350, 393sh; +NaOAc 275, 313, 389; +NaOAc/H_3BO_3 268, 352. LC-MS: *m/z* 301 $[M + H]^+$.

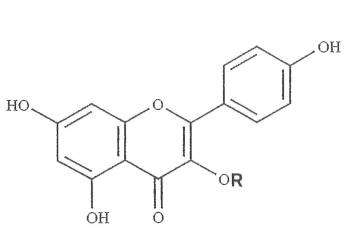
Kaempferol (**2**). HPLC: Rt 15.59 min (Sol. II). UV λ max nm: MeOH 266, 366; +NaOMe decomp.; +$AlCl_3$ 269, 306, 357, 422; +$AlCl_3$/HCl 269, 304, 354, 422; +NaOAc 275, 310sh, 396; +NaOAc/H_3BO_3 267, 370. LC-MS: *m/z* 286 $[M + H]^+$.

Quercetin 3-methyl ether (**3**). HPLC: Rt 11.76 min (Sol. II). UV λ max nm: MeOH 256, 266sh, 357; +NaOMe 272, 324, 411 (inc.); +$AlCl_3$ 275, 436; +$AlCl_3$/HCl 266, 299, 360, 394; +NaOAc 273, 322, 398; +NaOAc/H_3BO_3 261, 378. LC-MS: *m/z* 317 $[M + H]^+$.

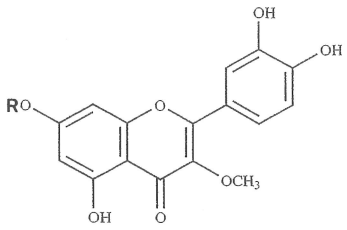
Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside (**4**). PC: Rf 0.32 (BAW), 0.38 (BEW), 0.34 (15%HOAc); UV - dark purple, UV/NH_3 - yellow. HPLC: Rt 7.58 min (Sol. I). UV λ max nm: MeOH 256, 266sh, 359; +NaOMe 272, 398 (inc.); +$AlCl_3$ 274, 442; +$AlCl_3$/HCl 268, 299sh, 363, 400sh; +NaOAc 263, 404; +NaOAc/H_3BO_3 261, 381. LC-MS: *m/z* 633 $[M + Na]^+$, 479 $[M$ - arabinosyl $+ H]^+$ (quercetin 3-methyl ether 7-*O*-galactoside), 317 $[M$ – glycosyl $+ H]^+$ (quercetin 3-methyl ether).

Isorhamnetin 3-*O*-rutinoside (**5**) and isorhamnetin 3-*O*-rhamnosylgalactoside (**6**). PC: Rf 0.49 (BAW), 0.51 (BEW), 0.47 (15%HOAc); UV - dark purple, UV/NH_3 - yellow. HPLC: Rt 9.60 min (**5**) and 9.33 min (**6**) (Sol. I). UV λmax nm: MeOH 255, 266sh, 358; +NaOMe 273, 330, 411 (inc.); +$AlCl_3$ 269, 302, 367, 398sh; +$AlCl_3$/HCl 268, 301, 361, 397sh; +NaOAc 274, 323, 409; +NaOAc/H_3BO_3 256, 266sh, 359.

Isorhamnetin 3-*O*-digalactoside (**7**). PC: Rf 0.28 (BAW), 0.36 (BEW), 0.67 (15%HOAc); UV - dark purple, UV/NH_3 - yellow. HPLC: Rt 4.75 min (Sol. I). UV λ max nm: MeOH 256, 265sh, 356; +NaOMe 266, 400 (inc.); +$AlCl_3$ 267, 300sh, 367sh, 403; +$AlCl_3$/HCl 267, 300sh, 363, 397; +NaOAc 264, 419; +NaOAc/H_3BO_3 256, 360.



R = CH_3: Isokaempferide (**1**)
R = H: Kaempferol (**2**)



R = H: Quercetin 3-methyl ether (**3**)
R = arabinogalactosyl: Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside (**4**)

Fig. 1. The chemical structures of the flavonols isolated from the aerial part of *Aztekium ritteri*.

Results and Discussion

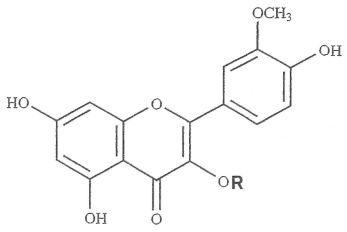
Flavonoids from Aztekium ritteri

Four flavonoids (**1** – **4**) were isolated from the aerial part of *A. ritteri*. Since flavonoids **1** – **3** except **4** were soluble in diethyl ether and could not be hydrolysed by hot HCl, they were shown to be aglycones. LC-MS data (*m/z* 301) of flavonoid **1** showed that the compound is trihydroxy-monomethoxyflavone. UV spectral properties of flavonoid **1** showed that the compound is flavonol (Mabry *et al.* 1970). In addition of NaOMe, Band I was bathochromically schifted with increase in intensity relative to the peak of MeOH solution, showing the presence of free 4´-hydroxyl and substituted 3-hydroxyl groups. The presence of *ortho*-dihydroxyl group in B-ring and free 7-hydroxyl group was shown by addition of $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3BO_3 to MeOH solution. From the results described above, flavonoid **1** was identified as isokaempferide, i.e., kaempferol 3-methyl ether (Fig. 1).

Flavonoids **2** and **3** were estimated as kaempferol (**2**) and quercetin 3-methyl ether (**3**) (Fig. 1) by UV spectroscopy, LC-MS and direct HPLC comparisons with authentic specimens which were obtained *Neochilenia* and *Astrophytum* species (Cactaceae) (Iwashina *et al.* 1984, 1988).

Acid hydrolysis of flavonoid **4** liberated quercetin 3-methyl ether, arabinose and galactose. It was indicated by UV spectral survey that the presence of free 5-, 3´- and 4´-hydroxyl and substituted 3- and 7-hydroxyl groups, showing that both arabinose and galactose are attached to 7-position. Appearance of molecular ion, *m/z* 633 $[M + H]^+$, in LC-MS showed that each 1 mol arabinose and galactose are attached to quercetin 3-methyl ether. Since fragment ion, *m/z* 479, also appeared, it was shown that galactose is directly attached to aglycone but not arabinose. Thus, flavonoid **4** was characterized as quercetin 3-methyl ether 7-*O*-arabinosylgalactoside (Fig. 1).

Of four flavonoids which were isolated from the aerial part of *Aztekium ritteri*, quercetin 3-methyl ether has been reported from the flowers of *Neochilenia*, *Neoporteria* and *Parodia* species (Iwashina *et al.* 1984) and the thornes of *Opuntia* spp. and *Pereskiopsis diguetii* Br. & R. as free state in Cactaceae (Burret *et al.* 1982). Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside was found in nature for the first time, though related quercetin 3-methyl ether 7-*O*-arabinosylglucoside has been reported from the whole plant of a fern, *Lepisorus ussuriensis* (Regel & Maack.) Ching as 7-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (Choi *et al.* 1996).



R = rutinosyl: Isorhamnetin 3-*O*-rutinoside (**5**)
R = rhamnogalactosyl: Isorhamnetin 3-*O*-rhamnosylgalactoside (**6**)
R = digalactosyl: Isorhamnetin 3-*O*-digalactoside (**7**)

Fig. 2. The chemical structures of the flavonols isolated from the aerial part of *Ariocarpus kotschoubeyanus* var. *macdowellii*.

Flavonoid composition of another species, *A. hintonii*, belonging to the genus *Aztekium* was surveyed by HPLC and the presence of quercetin 3-methyl ether 7-*O*-arabinosylgalactoside was recongnized, but other three flavonoids were not detected.

Flavonoids from Ariocarpus kotschoubeyanus *var.* macdowellii

Three flavonoids were isolated from the aerial part of the plant. They were characterized as isorhamnetin 3-*O*-glycosides by acid hydrolysis and UV spectral survey. The mixture of flavonoids **5** and **6** liberated glucose, galactose and rhamnose as glycosidic sugars by acid hydrolysis. Since flavonoid **5** was identified as isorhamnetin 3-*O*-rutinoside by direct HPLC and PC comparisons with authentic narcissin, another **6** was estimated as isorhamnetin 3-*O*-rhamnosylgalactoside (Fig. 2). UV spectral survey of flavonoid **7** showed the presence of free 5-, 7- and 4´-hydroxyl and substituted 3- and 3´-hydroxyl groups. Isorhamnetin and galactose were liberated by acid hydrolysis of the original glycoside, showing the attachment of the sugar to 3-position of flavonoid nucleus. Since Rf values, especially 15% HOAc, of flavonoid **7** is 0.67 (cf. authentic isorhamnetin 3-*O*-glucoside: 0.33), it was presumed that 2 mol galactose are attached. Thus, flavonoid **7** was characterized as isorhamnetin 3-*O*-digalactoside (Fig. 2).

Of their flavonoids isolated from *A. kotschoubeyanus* var. *macdowellii*, isorhamnetin 3-*O*-rutinoside and 3-*O*-rhamnosylgalactoside have been reported from many Cactaceous species (e.g., Iwashina *et al.* 1982, 1986, Iwashina and Ootani 1986, Rösler *et al.* 1966, Shabbir and Zaman 1968, Clark and Parfitt 1980). On the other hand, another isorhamnetin 3-*O*-digalactoside has been isolated from the leaves of *Villarsia calthifolia* F. Muell. (Menyanthaceae) (Bohm *et al.* 1986) and *Barbacenia rubro-virens* Mart. (Velloziaceae) (Williams *et al.* 1994) in plant kingdom.

The MeOH extracts from the aerial parts of *Ariocarpus agavioides* and *A. scapharostrus* were surveyed for flavonoids, and the presence of isorhamnetin 3-*O*-rutinoside and isorhamnetin 3-*O*-rhamnosylgalactoside was recognized by PC and HPLC. Though a flavonol aglycone, retusine, has been reported from *Ariocarpus retusus* (Domínguez *et al.* 1969), it could not be found in three *Ariocarpus* species which were used as plant materials in this experiment.

摘　要

メキシコ原産の *Aztekium* 属および *Ariocarpus* 属の 5 種のサボテン科植物、*Aztekium ritteri*, *Aztekium hintonii*, *Ariocarpus kotschoubeyanus* var. *macdowellii*, *Ariocarpus agavioides* および *Ariocarpus scapharostrus* の地上部に含まれるフラボノイドの分析が行われた。フラボノイドの分離は *A. ritteri* と *A. kotschoubeyanus* var. *macdowellii* で行われ、合計 7 種類が得られた。このうち、*A. ritteri* から得られたフラボノイドは UV スペクトル、加水分解、LC-MS による分子量の測定などからそれぞれ、Isokaempferide (Kaempferol 3-methyl ether, **1**), Kaempferol (**2**), Quercetin 3-methyl ether (**3**)および Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside (**4**)と同定された。これらのフラボノイドのうち、後者（**4**）はこれまで自然界で報告のない新規の化合物であった。またわずか 2 種から構成されている *Aztekium* 属のもう一つの種、*A. hinotnii* のメタノール抽出物をペーパークロマトグラフィー（PC）および高速液体クロマトグラフィー（HPLC）で分析したところ、Quercetin 3-methyl ether 7-*O*-arabinosylgalactoside のみが検出された。

一方、*Ariocarpus kotschoubeyanus* var. *macdowellii* からは 3 種類の Isorhamnetin を基本骨格とする配糖体が分離され、それぞれ 3-*O*-rutinoside, 3-*O*-rhamnosylgalactoside および 3-*O*-digalactoside と定性された。また、*Ariocarpus* 属の他の 2 種、*A. agavioides* と *A. scapharostrus* の地上部のメタノール粗抽出物を PC および HPLC で分析したところ、上記配糖体のうちの前 2 者の存在が認められた。

なお、*Aztekium* 属植物に含まれるフラボノイドの報告はこれまでなかったが、*Ariocarpus* 属植物については、*A. retusus* からまれなポリメトキシル化フラボノールである Retusine (5-Hydroxy-3,7,3′,4′-tetramethoxyflavone)が報告されていたが、今回分析を行なった同属植物からは検出できなかった。

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