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Flavonoids from *Reaumuria soongarica* (Tamaricaceae) in Mongolia

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Abstract Ten flavonoids were isolated from the aerial parts of *Reaumuria soongarica* which is growing in the deserts of Mongolia. They were identified as kaempferol 7-*O*-diglucoside, quercetin 7-*O*-arabinoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, quercetin 7-*O*-rhamnoside, quercetin 3-*O*-rutinoside, quercetin 3-methyl ether, quercetin 3-methyl ether 7-*O*-glucoside, quercetin 3-methyl ether 4′-*O*-glucoside and isorhamnetin 7-*O*-rhamnoside by UV spectra, acid hydrolysis, LC-MS, and direct TLC and HPLC comparisons with authentic samples. Though the flavonoids of the genus *Reaumuria* have been found in another species, *R. mucronata*, those of *R. soongarica* were reported for the first time.

Key words: flavonoids, isorhamnetin, kaempferol, quercetin, quercetin 3-methyl ether, *Reaumuria soongarica*, Tamaricaceae.

Introduction

The genus *Reaumuria* consists of 12 species, belongs to the family Tamaricaceae and is distributed in the deserts of northern Africa, Asia and southern Europe (Yang and Gaskin, 2006). *Reaumuria soongarica* (Pall.) Maxim. is shrubs and 10–30 cm tall, and growing in the deserts and margins of lowlands in Mongolia. A flavonoid of the *Reaumuria* has been isolated from the leaves of *R. mucronata* Jaub. & Spach and identified as sulphated flavonol, kaempferol 3,7-disulphate (Nawwar *et al.*, 1977). However, the flavonoids of other *Reaumuria* species including *R. soongarica* were never reported. The family Tamaricaceae including *Reaumuria* was recently incorporated into the order Caryophyllales by Angiosperm Phylogeny Group (APG), together with the Plumbaginaceae, Polygonaceae, Droseraceae and Nepenthaceae. Eight families of the Carylphyllales, Aizoaceae, Amaranthaceae including Chenopodiaceae, Basellaceae, Cactaceae, Didiereaceae, Nyctaginaceae, Portulacaceae and Phytolaccaceae, synthesize the betalain pigments, instead of the anthocyanins (Clement *et al.*, 1994; Piattelli and Minale, 1964). In the Tamaricaceae, anthocyanins have been reported from three *Tamarix* species, *T. parviflora* DC., *T.* sp. and *T. tetrandra* Pall. ex Bieb., and characterized as cyanidin 3-*O*-glycoside, cyanidin and delphinidin glycosides (Forsyth and Simmonds, 1954), and cyanidin 3-*O*-glucoside and 3,5-di-*O*-glucoside (Scogin, 1977), respectively. Flavonols, kaempferol and quercetin, and their glycosides are major flavonoids, and also methylated flavonols, rhamnazin, rhamnetin, rhamnocitrin, kaempferide, tamarixetin, kaempferol 7,4′-dimethyl ether and dillenetin, and their glycosides are found in some

species of the family (e.g., Chumbalov *et al.*, 1975; La *et al.*, 2011; Chakrabarty *et al.*, 1965; Nawwar *et al.*, 1975, 1984; El Sissi *et al.*, 1973; Birkbulatova and Korul'kina, 2001; Wang *et al.*, 2009; Umbetova *et al.*, 2005). Flavonols frequently occur as sulphates in the Tamaricaceae (Harborne, 1975; Tomás-Barberán *et al.*, 1990; Saleh *et al.*, 1975; El Ansari *et al.*, 1976). Flavones, chrysoeriol, apigenin 7,4′-dimethyl ether, luteolin 5-methyl ether were found in *Myricaria bracteata* (Royle) Franchet. (Zhou *et al.*, 2006) and three *Tamarix* species, *T. chinensis* Lour. (Wang *et al.*, 2009), *T. elongata* Redeb. and *T. laxa* Willd. (Umbetova *et al.*, 2004, 2005). Polymethylated flavones, gardenins A–C and E, nevadensin, tamadone and tamaridone were also isolated from *Tamarix dioica* Roxb. ex Roth (Parmar *et al.*, 1994). In this paper, we describe the identification of flavonoids and their chemical properties from the aerial parts of *Reaumuria soongarica* growing in Mongolia.

Materials and Methods

Plant materials

Reaumuria soongarica (Pall.) Maxim. was collected between Hovd and Erdeneburen Sum, 2350 m alt., Hovd Prov., Mongolia in 16 Sept. 2007, during the Altai Mountains and adjacent area Botanical Expedition in September 2007. Voucher specimen was deposited in the herbarium of National Museum of Nature and Science, Japan (TNS).

General

UV spectra were recorded on a Shimadzu MPS-2000 Multi purpose recording spectrophotometer according to Mabry *et al.* (1970). LC-MS were measured on a Shimadzu LC-MS systems using a Inertsil ODS-4 column [I.D. 2.1 × 100 mm (GL Sciences Inc., Japan)], at a flow-rate of 0.1 ml min^{-1} eluting with MeCN/H_2O/HCOOH (20 : 75 : 5), ESI^+ 4.5 kV and ESI^- 3.5 kV, 250ºC. 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., Japan), at a flow-rate of 1.0 ml min^{-1}. Detection was 350 nm and eluent was used MeCN/H_2O/H_3PO_4 (20 : 80 : 0.2). Acid hydrolysis of the flavonol glycosides was performed in 12% aq. HCl, 100°C, 30 min. Flavonol aglycones and sugars were identified with HPLC and PC, respectively, in comparisons with authentic specimens. The solvent systems of TLC (Merck) and preparative PC (Advantec) are as follows; BAW (*n*-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), 15% HOAc and BEW (*n*-BuOH/EtOH/H_2O = 4 : 1 : 2.2) in room temperature.

Extraction and separation

Dryed aerial parts (23.3 g) of *R. soongarica* were extracted with MeOH. After concentration, crude extracts were applied to preparative paper chromatography using solvent systems, BAW, 15% HOAc and then BEW. The obtained flavonoids were purified with Sephadex LH-20 column chromatography using solvent system, 70% MeOH.

Identification

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

Kaempferol 7-*O*-diglucoside (**1**). TLC (Rf): 0.83 (BAW), 0.81 (BEW), 0.07 (15%HOAc); color UV (365 nm)—yellow, UV/NH_3—bright yellow. HPLC (tR): 13.89 min. UV λmax (nm): MeOH 256, 369; +NaOMe decomposition; +$AlCl_3$ 267, 306sh, 360, 434; +$AlCl_3$/HCl 265, 303sh, 360, 422; +NaOAc 257, 409; +NaOAc/H_3BO_3 255, 375. LC-MS: *m/z* 609 $[M-H]^-$ (molecular ion peak, kaempferol + 2 mol glucose), *m/z* 287 $[M-324+H]^+$ (fragment ion peak, kaempferol).

Quercetin 7-*O*-arabinoside (**2**). TLC (Rf): 0.28 (BAW), 0.31 (BEW), 0.07 (15%HOAc); color UV (365 nm) and UV/NH_3—yellow. HPLC (tR): 5.16 min. UV λmax (nm): MeOH 256, 372;

+NaOMe decomposition; +$AlCl_3$ 271, 457; +$AlCl_3$/HCl 265, 299sh, 362, 425; +NaOAc 263, 416; +NaOAc/H_3BO_3 260, 390. LC-MS: *m/z* 435 $[M+H]^+$, 433 $[M-H]^-$ (molecular ion peaks, quercetin + 1 mol arabinose).

Quercetin 3-*O*-glucoside (isoquercitrin, **3**). TLC (Rf): 0.68 (BAW), 0.77 (BEW), 0.20 (15%HOAc); color UV (365 nm)—dark purple, UV/NH_3—dark yellow. HPLC (tR): 4.82 min. UV λmax (nm): MeOH 258, 263sh, 360; +NaOMe 272, 330, 408 (inc.); +$AlCl_3$ 275, 436; +$AlCl_3$/HCl 269, 296sh, 362, 402; +NaOAc 273, 325, 390; +NaOAc/H_3BO_3 262, 381. LC-MS: *m/z* 465 $[M+H]^+$, 463 $[M-H]^-$ (molecular ion peaks, quercetin + 1 mol glucose), *m/z* 303 $[M-162+H]^+$ (fragment ion peak, quercetin).

Quercetin 7-*O*-rhamnoside (vincetoxicoside B, **4**). TLC (Rf): 0.67 (BAW), 0.69 (BEW), 0.07 (15%HOAc); color UV (365 nm)—yellow, UV/NH_3—bright yellow. HPLC (tR): 8.53 min. UV λmax (nm): MeOH 256, 269sh, 374; +NaOMe decomposition; +$AlCl_3$ 256, 272, 459; +$AlCl_3$/HCl 265, 298sh, 361, 427; +NaOAc 263, 413; +NaOAc/H_3BO_3 260, 389. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (molecular ion peaks, quercetin + 1 mol rhamnose), *m/z* 303 $[M-146+H]^+$, 301 $[M-146-H]^-$ (fragment ion peaks, quercetin).

Quercetin 3-*O*-rutinoside (rutin, **5**). TLC (Rf): 0.51 (BAW), 0.51 (BEW), 0.58 (15%HOAc); color UV (365 nm)—dark purple, UV/NH_3—dark yellow. HPLC (tR): 4.53 min. UV λmax (nm): MeOH 257, 264sh, 358; +NaOMe 273, 323, 411 (inc.); +$AlCl_3$ 274, 432; +$AlCl_3$/HCl 268, 299, 362, 396; +NaOAc 273, 327, 403; +NaOAc/H_3BO_3 262, 378. LC-MS: *m/z* 611 $[M+H]^+$, 609 $[M-H]^-$ (molecular ion peaks, quercetin + each 1 mol glucose and rhamnose), *m/z* 303 $[M-308+H]^+$ (fragment ion peak, quercetin).

Quercetin 3-*O*-glucuronide (miquelianin, **6**). TLC (Rf): 0.49 (BAW), 0.44 (BEW), 0.30 (15%HOAc); color UV (365 nm)—dark purple, UV/NH_3—dark yellow. HPLC (tR): 5.02 min. UV λmax (nm): MeOH 257, 266sh, 356; +NaOMe 275, 323, 405 (inc.); +$AlCl_3$ 273, 424; +$AlCl_3$/HCl 274, 303, 361, 405sh; +NaOAc 272, 328, 400; +NaOAc/H_3BO_3 264, 373. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (molecular ion peaks, quercetin + 1 mol glucuronic acid), *m/z* 303 $[M-176+H]^+$ (fragment ion peak, quercetin).

Quercetin 3-methyl ether (**7**). TLC (Rf): 0.89 (BAW), 0.89 (BEW), 0.07 (15%HOAc); color UV (365 nm)—dark purple, UV/NH_3—dark yellow. HPLC (tR): 12.17 min. UV λmax (nm): MeOH 257, 265sh, 358; +NaOMe 274, 323, 410 (inc.); +$AlCl_3$ 275, 435; +$AlCl_3$/HCl 264, 301, 359, 396sh; +NaOAc 273, 327, 398; +NaOAc/H_3BO_3 262, 379. LC-MS: *m/z* 317 $[M+H]^+$, 315 $[M-H]^-$ (molecular ion peak, tetrahydroxy-monomethoxyflavone).

Quercetin 3-methyl ether 7-*O*-glucoside (transilin, **8**). TLC (Rf): 0.66 (BAW), 0.75 (BEW), 0.29 (15%HOAc); color UV (365 nm)—dark purple, UV/NH_3—dark yellow. HPLC (tR): 5.17 min. UV λmax (nm): MeOH 257, 268sh, 360; +NaOMe 270, 395 (inc.); +$AlCl_3$ 276, 440; +$AlCl_3$/HCl 270, 298sh, 364, 403; +NaOAc 261, 371; +NaOAc/H_3BO_3 261, 370. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (molecular ion peaks, quercetin 3-methyl ether + 1 mol glucose), *m/z* 317 $[M-162+H]^+$, 315 $[M-162-H]^-$ (fragment ion peaks, quercetin 3-methyl ether).

Quercetin 3-methyl ether 4′-*O*-glucoside (neochilenin, **9**). TLC (Rf): 0.62 (BAW), 0.59 (BEW), 0.23 (15%HOAc); color UV (365 nm) and UV/NH_3—dark yellow. HPLC (tR): 6.74 min. UV λmax (nm): MeOH 256sh, 269, 349; +NaOMe 273, 380 (dec.); +$AlCl_3$ 268sh, 277, 298sh, 354, 400; +$AlCl_3$/HCl 260sh, 278, 295sh, 348, 398; +NaOAc 275, 377; +NaOAc/H_3BO_3 257sh, 269, 352. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (molecular ion peaks, quercetin 3-methyl ether + 1 mol glucose).

Isorhamnetin 7-*O*-rhamnoside (**10**). TLC (Rf): 0.76 (BAW), 0.77 (BEW), 0.07 (15%HOAc); color UV (365 nm)—yellow, UV/NH_3—bright yellow. HPLC (tR): 16.60 min. UV λmax (nm): MeOH 255, 267sh, 373; +NaOMe decomposi-

tion; + $AlCl_3$ 265, 320sh, 364, 429; + $AlCl_3$/HCl 264, 319sh, 362, 427; + NaOAc 256, 415; + NaOAc/H_3BO_3 255, 374. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (molecular ion peaks, isorhamnetin + 1 mol rhamnose), *m/z* 317 $[M-146+H]^+$, 315 $[M-146-H]^-$ (fragment ion peaks, isorhamnetin).

Results and Discussion

Ten flavonoids (**1**–**10**) were isolated from the aerial parts of *Reaumuria soongarica*. Flavonoid **1** produced kaempferol and glucose by acid hydrolysis. UV spectral properties in addition to various shift reagents (NaOMe, $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3BO_3) according to Mabry *et al.* (1970) showed that **1** is 7-substituted kaempferol. The attachment of 2 mol glucose to kaempferol was shown by LC-MS survey, i.e., appearance of the molecular ion peak, *m/z* 609 $[M-H]^-$ and fragment ion peak, *m/z* 287 $[M-324+H]^+$. From the results described above, **1** was characterized as kaempferol 7-*O*-diglucoside.

Acid hydrolysis of **2** liberated quercetin and arabinose. UV spectral properties showed that this glycoside is 7-substituted quercetin. Since molecular ion peak, *m/z* 435 $[M+H]^+$, appeared on LC-MS, it was shown that 1 mol arabinose is attached to quercetin. Thus, **2** was identified as quercetin 7-*O*-arabinoside (Fig. 1).

UV spectral data of **3** and **5** showed that they are 3-substituted quercetin. Quercetin and glucose, and quercetin, glucose and rhamnose were produced by acid hydrolysis of **3** and **5**, respectively. Finally, **3** and **5** were identified as quercetin 3-*O*-glucoside (isoquercitrin, Fig. 2) and quercetin 3-*O*-rutinoside (rutin, Fig. 4) by direct TLC and HPLC comparisons with authentic samples from the leaves of *Corylopsis* spp. (Hamamelidaceae) (Iwashina *et al.*, 2012).

Flavonoid **4** showed yellow under UV light (365 nm), which shows that the compound is a flavonol having free 3- and 4′-hydroxyl groups, as well as **1** and **2**. Quercetin and rhamnose were liberated by acid hydrolysis of **4**. It was shown by UV spectral survey and LC-MS that 1 mol rhamnose is attached to 7-position of quercetin.

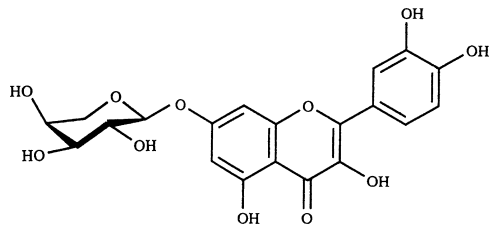

Fig. 1. Quercetin 7-*O*-arabinoside (**2**).

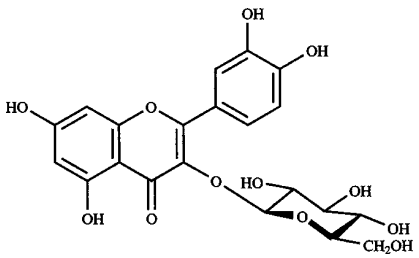

Fig. 2. Quercetin 3-*O*-glucoside (Isoquercitrin, **3**).

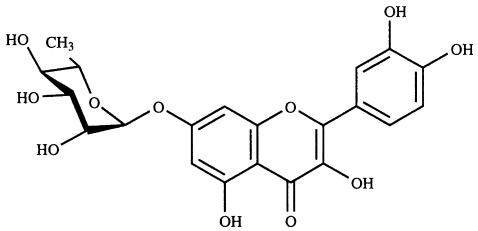

Fig. 3. Quercetin 7-*O*-rhamnoside (Vincetoxicoside B, **4**).

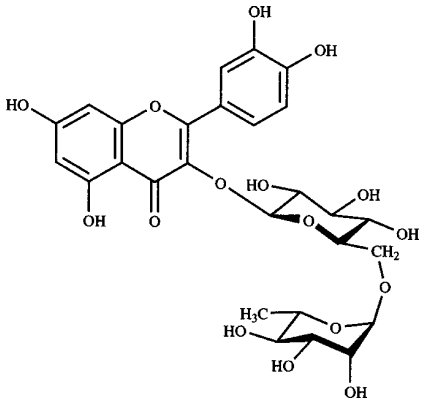

Fig. 4. Quercetin 3-*O*-rutinoside (Rutin, **5**).

Thus, **4** was identified as quercetin 7-*O*-rhamnoside (Fig. 3).

Flavonoid **6** produced quercetin and glucuronic acid by acid hydrolysis. LC-MS survey showed the attachment of 1 mol glucuronic acid to quercetin. UV spectral properties of original glycoside indicated that **6** is 3-substituted quercetin. Finally, **6** was identified as quercetin 3-*O*-glucuronide (miquelianin, Fig. 5) by direct TLC and HPLC comparison with authentic sample from the fronds of *Adiantum capillus-veneris* L. (Adiantaceae) (Iwashina *et al.*, 1995).

LC-MS survey of **7** showed that the compound is tetrahydroxy-monomethoxyflavone. It was determined by UV spectral survey that a methoxyl group is attached to 3-position of flavonol. Finally, Rf values of TLC and retention time of HPLC of the compound completely agreed with those of authentic quercetin 3-methyl ether from the flowers of *Neoporteria* spp. (Cactaceae) (Iwashina *et al.*, 1984). Thus, **7** was identified as an aglycone, quercetin 3-methyl ether (Fig. 6).

It was shown by acid hydrolysis that **8** and **9** are quercetin 3-methyl ether glycosides. Glucose was liberated as a glycosidic sugar from the both glycosides. The attachment of 1 mol glucose to 7-position and 4′-position of quercetin 3-methyl ether was determined by UV spectral and LC-MS survey of **8** and **9**, respectively. Finally, **8** and **9** were identified as quercetin 3-methyl ether 7-*O*-glucoside (transilin, Fig. 7) and quercetin 3-methyl ether 4′-*O*-glucoside (neochilenin, Fig. 8) by direct TLC and HPLC comparisons with authentic samples from the flowers of *Parodia sanguiniflora* Frič ex Backbg. and *Neochilenia* spp. (Cactaceae) (Iwashina *et al.*, 1984).

Isorhamnetin and rhamnose were produced by acid hydrolysis of **10**. It was shown by UV spectral and LC-MS survey that 1 mol rhamnose is attached to 7-position of isorhamnetin. From the results described above, **10** was determined as isorhamnetin 7-*O*-rhamnoside (Fig. 9).

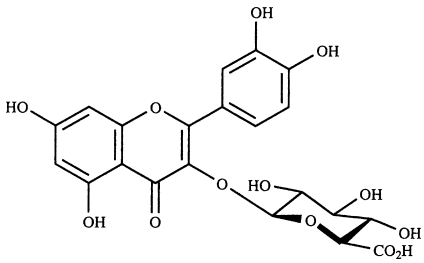

Fig. 5. Quercetin 3-*O*-glucuronide (Miquelianin, **6**).

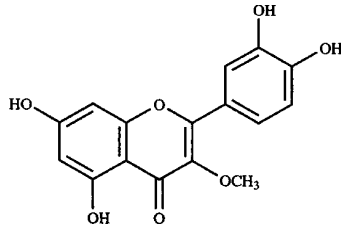

Fig. 6. Quercetin 3-methyl ether (**7**).

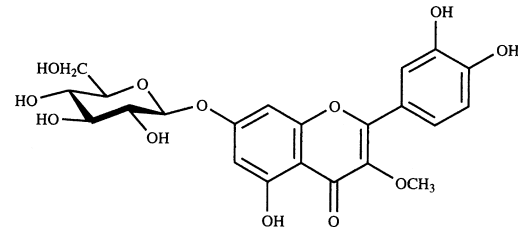

Fig. 7. Quercetin 3-methyl ether 7-*O*-glucoside (Transilin, **8**).

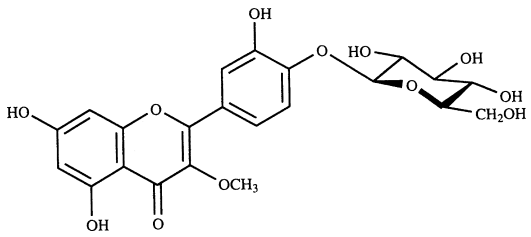

Fig. 8. Quercetin 3-methyl ether 4′-*O*-glucoside (Neochilenin, **9**).

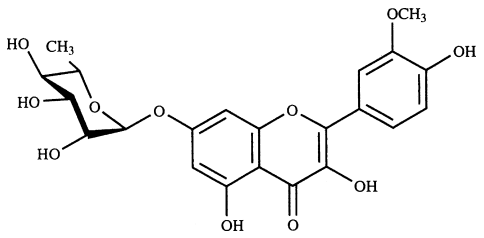

Fig. 9. Isorhamnetin 7-*O*-rhamnoside (**10**).

Since kaempferol 3,7-disulphate alone was reported from the genus *Reaumuria* (Nawwar *et al.*, 1977), ten flavonols, which were isolated from *R. soongarica* in this survey, were reported from the genus for the first time. Moreover, though quercetin 3-*O*-glucuronide (**6**) and quercetin 3-*O*-glucoside (**3**) have been found in other Tamaricaceous species, *Myricaria germanica* (L.) Desv. (La *et al.*, 2011) and *Tamarix nilotica* (Ehrenb.) Bunge (Nawwar *et al.*, 1984), and some *Myricaria* and *Tamarix* species (La *et al.*, 2011; Chakrabarty *et al.*, 1965; Ishak *et al.*, 1972; Umbetova *et al.*, 2005; Nawwar *et al.*, 1984), respectively, other eight flavonols, kaempferol 7-*O*-diglucoside (**1**), quercetin 7-*O*-arabinoside (**2**), quercetin 7-*O*-rhamnoside (**4**), quercetin 3-*O*-rutinoside (**5**), quercetin 3-methyl ether (**7**), quercetin 3-methyl ether 7-*O*-glucoside (**8**), quercetin 3-methyl ether 4′-*O*-glucoside (**9**) and isorhamnetin 7-*O*-rhamnoside (**10**), were found in the Tamaricaceae for the first time. Flavonol sulphates have been reported from some Tamaricaceous species (Harborne, 1975; Tomás-Barberán *et al.*, 1990). However, they were not recognized in *R. soongarica*.

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