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Flavonoid sulfates from the Convolvulaceae☆

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Abstract

A novel flavonoid, quercetin 7-methyl ether-3,3′-disulfate (**5**), was obtained from the roots of *Argyreia mollis*, together with the known compounds quercetin 7-methyl ether-3-sulfate (**4**) and kaempferol 7-methyl ether-3-sulfate (**2**). Two further new flavonoid sulfates, quercetin 3,7-dimethyl ether-4′-sulfate (**6**) and quercetin 3′,4′,7-trimethyl ether-3-sulfate (**8**), were isolated from the aerial vegetative parts of *Ipomoea regnellii*, together with the known compound kaempferol 4′,7-dimethyl ether-3-sulfate (**3**). This is the first report on sulfated flavonoids in the Convolvulaceae family and, furthermore, the presence of flavonoids in *A. capitata*, *A. mollis*, *I. reticulata* and *I. regnellii* is reported for the first time.

Keywords: *Argyreia capitata*; *Argyreia mollis*; *Argyreia nervosa*; *Ipomoea alba*; *Ipomoea muricata*; *Ipomoea regnellii*; *Ipomoea reticulata*; *Ipomoea turbinata*; *Merremia medium*; *Merremia umbellata*; Convolvulaceae; Flavonoid sulfates; Quercetin 7-methyl ether-3,3′-disulfate; Quercetin 3,7-dimethyl ether-4′-sulfate; Quercetin 3′,4′,7-trimethyl ether-3-sulfate

1. Introduction

The Convolvulaceae to date appear to be characterized by the widespread occurrence of the flavonols kaempferol and quercetin, their *O*-methylated derivatives and their glycosides (Geetha, Daniel, & Sabnis, 1986; Ahmad, Jain, Shafiullah, Khan, & Ilyas, 1993; Khan, Kamil, Shaffiullah, & Ilyas, 1992; Hegnauer, 1989). The presence of peonidin, cyanidin and pelargonidin 3-glycosides, 3,5-diglycosides and their acylated derivatives, e.g. in the genus *Ipomoea*, as well as the occurrence of one delphinidin derivative in an *Evolvulus* species is also a well-documented fact (Saito, Lu, Akaizawa, Yokoi, Shigihara, & Honda, 1994; Imbert, 1969; Toki, Saito, Kawano, Lu, Shigihara, & Honda, 1994; Pomilio & Sproviero, 1972). Reports on the presence of flavones, flavone glycosides and glycosylflavones are less frequent (Geetha et al., 1986; Ahmad et al., 1993; Khan et al., 1992; Hegnauer, 1989).

The present study comprises a phytochemical investigation on flavonoids of five convolvulaceous species: *Argyreia capitata* (VAHL) CHOISY, *A. mollis* (BURM. f.) CHOISY, *A. nervosa* (BURM. f.) BOJ., *Ipomoea regnellii* MEISN. and *I. reticulata* O'DONELL. From the leaves of *A. nervosa* two new flavone glycosides, a kaempferol glycoside, kaempferol and quercetin have previously been isolated (Ahmad et al., 1993; Khan et al., 1992). However, there are neither reports on the presence of flavonoids in the other two *Argyreia* species nor in the two *Ipomoea* species.

2. Results and discussion

The flavonoids (**1**–**8**) described in this paper were isolated from the *n*-butanolic extracts of the investigated species (Fig. 1), according to their occurrence as given in Table 1. Their structural elucidation was carried out by UV, EIMS, HRMS and FAB mass spectrometry, ^{1}H NMR, ^{13}C NMR and COSY as well as NOE experiments.

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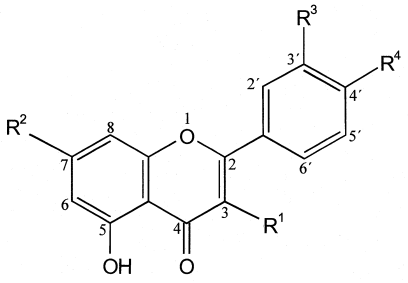

	R^1	R^2	R^3	R^4
1	SO_3Na	OH	H	OH
2	SO_3Na	OCH_3	H	OH
3	SO_3Na	OCH_3	H	OCH_3
4	SO_3Na	OCH_3	OH	OH
5	SO_3Na	OCH_3	OSO_3Na	OH
6	CH_3	OCH_3	OH	OSO_3Na
7	SO_3Na	OCH_3	OCH_3	OH
8	SO_3Na	OCH_3	OCH_3	OCH_3

Fig. 1. Sulfated flavonoids isolated from the investigated species (for details see Table 1).

Compounds **1**–**5**, **7** and **8** reacted positively to the Tauböck test (Tauböck, 1942), whereas the reaction of compound **6** was negative. Each FABMS spectrum obtained in the negative mode gave a quasi-molecular ion together with a sodium adduct. The ^{1}H NMR spectra of all compounds revealed the presence of a chelated hydroxy signal at δ 12.67–13.08.

Compounds **1**–**4** and **7** were characterized as flavonoid sulfates, which have only been found in a few species of other plant families so far. Compound **1** was first found in *Tetracera*, *Schumacheria* and *Dillenia* species (Dilleniaceae). Compounds **2** and **4** were first isolated from *Ammi visnaga* (Apiaceae) and compound **7** from *Polygonum hydropiper* (Polygonaceae) (Gurni & Kubitzki, 1981; Harborne & King, 1976; El Ansari, Nawwar, El Dein, El Sherbeiny, & El Sissi, 1976; Hörhammer & Hänsel, 1953). The hitherto unreported ^{1}H NMR data of **2**, **4** and **7** are presented in Table 2.

Compound **5** was isolated from an *n*-butanolic root extract of *Argyreia mollis*. The EIMS exhibited an ion peak at *m/z* 316 [M-160], and its formula was determined as $C_{16}H_{12}O_7$. The FABMS (negative mode) spectrum showed characteristic ion peaks at *m/z* 519 $[(M–3H) + 2Na]^-$, *m/z* 497 $[(M–2H) + Na]^-$, *m/z* 417 $[(M–2H-80) + Na]^-$ and *m/z* 315 $[M–H-160]^-$. This suggested the presence of two sulfate groups in this

Table 1
Occurrence of sulfated flavonoids in the investigated species

Compound	*A. capitata* (roots)	*A. mollis* (roots)	*A. nervosa* (roots)	*I. regnellii* (aerial vegetative parts)	*I. reticulata* (roots)
1, Kaempferol 3-sulfate	–	–	–	n.d.	+
2, Kaempferol 7-OMe-3-sulfate	+	+ [a]	+	n.d.	+
3, Kaempferol 4′,7-diOMe-3-sulfate	+	–	–	+	–
4, Quercetin 7-OMe-3-sulfate	–	+	–	n.d.	–
5, Quercetin 7-OMe-3,3′-disulfate	–	+	–	n.d.	–
6, Quercetin 3,7-diOMe-4′-sulfate	–	–	–	+	–
7, Quercetin 3′,7-diOMe-3-sulfate	–	–	+	n.d.	–
8, Quercetin 3′,4′,7-triOMe-3-sulfate	–	–	–	+	–

All compounds, except one, were identified by isolation and structural elucidation.
[a]Detection was achieved by co-TLC with an authentic reference compound.n.d. = not determined.

Table 2
^{1}H NMR data for compounds **2** and **4**–**8** [400 MHz, DMSO-d_6, δ (ppm), (*J* (Hz))]

Protons	**2**	**4**	**5**	**6**	**7**	**8**
H-6	6.36, d, (2.1)	6.35, d, (2.0)	6.36, d, (2.1)	6.36, d, (2.0)	6.36, d, (2.1)	6.37, d, (1.9)
H-8	6.73, d, (2.1)	6.67, d, (2.0)	6.73, d, (2.1)	6.69, d, (2.0)	6.76, d, (2.1)	6.77, d, (1.9)
H-2′	8.14, d, (8.8)	7.67, d, (2.0)	7.91, d, (1.8)	7.62, d, (2.0)	8.09, d, (2.0)	8.05, d, (2.0)
H-3′	6.87, d, (8.8)					
H-5′	6.87, d, (8.8)	6.81, d, (8.5)	6.92, d, (8.7)	7.02, d, (9.0)	6.89, d, (8.5)	7.09, d, (8.8)
H-6′	8.14, d, (8.8)	7.62, dd, (2.0, 8.5)	8.12, dd, (1.8, 8.7)	7.78, dd, (2.0, 9.0)	7.69, dd, (2.0, 8.5)	7.80, dd, (2.0, 8.8)
OCH_3	3.86, s	3.86, s	3.88, s	3.85, s, 3.87,s	3.86, s, 3.87, s	3.84, s, 3.85, s, 3.87, s
C_5-OH	12.89	12.91	12.67	13.08	12.70	13.05

molecule and sodium is the counter ion. Acid hydrolysis of **5** yielded quercetin 7-methyl ether and sulfate. The UV spectral data obtained with standard shift reagents (see Section 3) indicated substitutions of the flavonoid at C-7, C-3 and C-3′ (Mabry, Markham, & Thomas, 1970). After addition of hydrochloric acid to a methanolic solution of **5**, a bathochromic shift of 25 nm (band I) was observed, therefore the positions 3 and 3′ had to be sulfated (Barron & Ibrahim, 1988a).

The ^{1}H NMR spectrum of compound **5** showed signals for a pair of *meta* coupled protons at δ 6.36 (1 H, d, $J = 2.1$ Hz, H-6) and δ 6.73 (1 H, d, $J = 2.1$ Hz, H-8), a one proton doublet at δ 6.92 ($J = 8.7$ Hz, H-5′), a one proton doublet at δ 7.91 ($J = 1.8$ Hz, H-2′), a one proton double doublet at δ 8.12 ($J = 1.8$, 8.7 Hz, H-6′), a methoxy group at δ 3.88 (s) and a one proton singlet at δ 12.67. The position of this methoxy group was established by NOE difference experiments. Irradiation of the methoxy singlet at δ 3.88 led to enhancement of the H-6 (δ 6.36) and H-8 (δ 6.73) signals, indicative of the position of the methoxy group at C-7. A comparison with the corresponding quercetin 7-methyl ether spectrum showed that the signals of H-2′ and H-6′ of flavonoid **5** were significantly shifted downfield (Barbera, Sanz, Sanchez-Parareda, & Marco, 1986). Correspondingly, in the ^{13}C NMR spectrum the carbon signals of C-2′ at δ 124.4 and C-6′ at δ 128.2, as well as the signal of C-4 at δ 178.7 were significantly shifted downfield, when compared to the corresponding carbon signals of quercetin 7-methyl ether (Markham & Chari, 1982; Barron & Ibrahim, 1988b; Barron & Ibrahim, 1987). Consequently **5** is quercetin 7-methyl ether-3,3′-disulfate.

The compounds **6** and **8** were obtained from an *n*-butanolic extract of the aerial vegetative parts of *I. regnellii*. The EIMS of compound **6** exhibited a base peak at *m/z* 330 [M-80] and its formula was determined as $C_{17}H_{14}O_7$ by HRMS. The FABMS (negative mode) gave a quasi-molecular ion peak at *m/z* 409 $[M–H]^-$ and significant peaks at *m/z* 431 $[(M–2H) + Na]^-$ as well as *m/z* 329 $[M–H-80]^-$, indicative of the loss of one molecule of SO_3. The UV spectral data indicated substitutions of the hydroxy groups at C-3, C-7 and C-4′ (Mabry et al., 1970). The ^{1}H NMR spectrum of compound **6** exhibited singlets from a chelated hydroxy group (δ 13.08) and two methoxy groups (δ 3.85 and 3.87) as well as two *meta* coupled aromatic proton signals at δ 6.36 (1 H, d, $J = 2.0$ Hz, H-6) and 6.69 (1 H, d, $J = 2.0$ Hz, H-8), an aromatic proton doublet at δ 7.02 ($J = 9.0$ Hz, H-5′), a one proton doublet at 7.62 ($J = 2.0$ Hz, H-2′) and an aromatic proton double doublet at 7.78 ($J = 2.0$; 9.0 Hz, H-6′). Irradiation of the methoxy resonance at δ 3.85 gave an enhancement of the doublet at δ 6.36 (H-6) and the doublet at 6.69 (H-8) indicating the positioning at C-7. On irradiation of the methoxy signal at δ 3.87 no measurable signal enhancement of any proton was observable, supporting the placement of this methoxy group at C-3. Finally the position of the sulfate moiety was determined by methylation of **6**, followed by hydrolysis with hydrochloric acid. The product of the hydrolysis, a trimethylated quercetin derivative, exhibited one additional methoxy singlet at δ 3.98 in the ^{1}H NMR spectrum. Irradiation at δ 7.76 (H-2′) showed an NOE enhancement of the methoxy signal at δ 3.98. On the basis of these data compound **6** was shown to be quercetin 3,7-dimethyl ether-4′-sulfate.

The EIMS of compound **8** showed a base peak at *m/z* 344 $[M - 80]$, the formula of which was determined as $C_{18}H_{16}O_7$. The FABMS showed a quasi-molecular ion peak at *m/z* 423 $[M–H]^-$ as well as peaks at *m/z* 445 $[(M–2H) + Na]$ and at *m/z* 343 $[M–H-80]^-$. The UV spectral data indicated, as in compound **6**, substituted hydroxy groups at C-3, C-7 and C-4′ (Mabry et al., 1970). Addition of hydrochloric acid to a methanolic solution of **8** led to a distinctive bathochromic shift (band I), indicating an attachment of the sulfate moiety to C-3 (Barron & Ibrahim, 1988a).

The ^{1}H NMR spectrum of **8** (Table 2) showed the signals of a trimethylated quercetin derivative. Irradiation at δ 3.84, δ 3.85 and δ 3.87 led to NOE enhancements at the signals of H-6 and H-8, H-2′ and H-5′, respectively, showing that the methoxy groups were attached to C-7, C-3′ and C-4′. Compound **8** is therefore quercetin 3′,4′,7-trimethyl ether-3-sulfate.

Prior to this work, no sulfated flavonoids have been reported in convolvulaceous species at all. Such compounds have also neither been described in the Solanaceae nor in any other family closely related to the Convolvulaceae so far. The ability to synthesize flavonoid sulfates is not a universal feature for the convolvulaceous species, though. This is evident from the fact that no such compounds could be detected in the following species: *Ipomoea alba* L., *I. muricata* (L.) JACQ. (syn. *I. turbinata* LAG.), *Merremia medium* (L.) HALL. and *M. umbellata* (L.) HALL.

3. Experimental

3.1. Spectroscopic methods

EIMS, HRMS and FABMS spectra were obtained using MAT-711 and CH_5-DF Finnigan spectrometers. All ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker AC-400 spectrometer, using TMS as internal standard.

3.2. Plant material

Roots and aerial vegetative parts were obtained from plants cultivated in our greenhouse from seeds,

which were collected in the following areas: Java (*Argyreia capitata*, *A. nervosa*), Madura Island near Surabaya/Java (*A. mollis*), Ecuador (*Ipomoea alba*, *I. regnellii*, *I. reticulata*, *I. muricata*, *Merremia umbellata*) and Madagascar (*M. medium*). Voucher specimens are deposited at the Institut für Pharmazie II (Pharmazeutische Biologie), Freie Universität Berlin, Germany.

3.3. Extraction

Ground dried material was extracted with MeOH and 80% MeOH at room temperature. After evaporation the residue was dissolved in water and extracted with petrol, CH_2Cl_2, EtOAc and *n*-BuOH, successively.

3.4. Isolation of **1**–**5** and **7**

Roots of *A. capitata* (180 g), *A. mollis* (100 g), *A. nervosa* (150 g) and *I. reticulata* (95 g) were used for the isolation. Each dried *n*-BuOH-soluble fraction was chromatographed over a silica gel column (27×3 cm) using EtOAc–MeOH–H_2O (79:11:10) as eluent. Compound **3** eluted after 200–250 ml, compounds **1**, **2** and **7** after 270–400 ml and compounds **4** and **5** after 330–690 ml. These subfractions were further chromatographed on Sephadex LH-20 by elution with a stepwise H_2O–MeOH gradient. The isolation was monitored by TLC on silica gel 60 F_{254} with EtOAc–MeCOEt–HCO_2H–H_2O (5:3:1:1) as solvent system (system I). The TLC plates were examined in UV light (254 nm), then sprayed with 2-aminoethyl diphenylborinate and checked for fluorescent spots (UV light, 366 nm).

3.5. Isolation of **3**, **6** and **8**

Aerial vegetative parts of *I. regnellii* (100 g) were extracted as described in Section 3.3. The *n*-butanolic extract was evaporated to dryness and extracted with acetone. After evaporation, the flavonoids were isolated from the residue by prep. TLC on silica gel 60 F_{254} using EtOAc–HCO_2H–H_2O (10:1:4, upper layer) as a solvent system (system II). The corresponding areas quenching UV light at 254 nm were cut out and extracted five times with 100 ml 80% MeOH. The combined filtered solutions were evaporated in vacuo.

3.6. Acid hydrolysis

1 mg of each isolated flavonoid was dissolved in 2 ml MeOH and heated with 5 ml 3% HCl under reflux for 15 min. After evaporation of MeOH the resultant aglycones were extracted with EtOAc and analysed by TLC on silica gel with CH_2Cl_2–Me_2CO–HCO_2H (76:16:8). Sulfate was detected in the water layer as a white precipitate with $BaCl_2$.

3.7. Methylation

Flavonoid **6** (15 mg) was dissolved in 10 ml acetone; 25 mg K_2CO_3 and crown ether (18-crown-6) were added and the mixture was stirred for 20 min at room temperature. Then 0.5 ml dimethyl sulfate were added under stirring. After 1 h the reaction was finished by addition of 30 ml 10% aqueous Na_2CO_3-solution and the mixture was extracted with EtOAc (Imperato, 1979). After evaporation of the organic layer the methylated derivative was purified by preparative TLC and hydrolysed with hydrochloric acid. The product of the hydrolysis was analysed with NOE experiments.

3.8. Quercetin 7-methyl ether-3,3′-disulfate (**5**)

10 mg. R_f 0.39 (I). UV λ_{max}^{MeOH} nm: 268, 344; +NaOAc: 268, 358, 399 (sh); +NaOAc + H_3BO_3: 268, 348; +$AlCl_3$: 277, 308 (sh), 363, 400; +$AlCl_3$ + HCl: 277, 302 (sh), 353, 396; +NaOMe: 267, 396; +HCl: 256, 369 nm. EIMS 70 eV, *m/z* (rel. int.): 316 $[M\text{-}160]^+$ (100), 167 (10), 137 (13). HRMS 80 eV, *m/z*: 316.0582 ($C_{16}H_{12}O_7$, calc. 316.0583). FABMS *m/z*: 519 $[(M–3H) + 2Na]^-$, 497 $[(M–2H) + Na]^-$, 417 $[(M–2H\text{-}80) + Na]^-$, 315 $[M–H\text{-}160]^-$.

3.9. Quercetin 3,7-dimethyl ether-4′-sulfate (**6**)

19 mg. R_f 0.48 (II). UV λ_{max}^{MeOH} nm: 255, 270 (sh), 349; +NaOMe 270, 377; +NaOAc 256, 348; +$AlCl_3$ 270, 296 (sh), 367 (sh), 395; +$AlCl_3$/HCl 270, 296 (sh), 367 (sh), 395; +HCl 256, 270 (sh), 351. EIMS 70 eV, *m/z* (rel. int.): 330 $[M\text{-}80]^+$ (100), 315 (30), 167 (5), 151 (6), 149 (12), 123 (7). HRMS 80 eV, *m/z*: 330.07368 ($C_{17}H_{14}O_7$, calc. 330.07396), 315.05068 ($C_{16}H_{11}O_7$, calc. 315.05048), 301.07052 ($C_{16}H_{13}O_6$ calc. 301.07122). FABMS *m/z*: 431 $[(M–2H) + Na]^-$, 409 $[M–H]^-$, 329 $[M–H\text{-}80]^-$.

3.10. Quercetin 3′,4′,7-trimethyl ether-3-sulfate (**8**)

3 mg. R_f 0.55 (II). UV λ_{max}^{MeOH} nm: 254, 268 (sh), 346; +NaOMe 283, 368; +NaOAc 254, 349; +$AlCl_3$ 277, 300 (sh), 361 (sh), 394; +$AlCl_3$/HCl 277, 300 (sh), 361 (sh), 394; + HCl 255, 269, 368. EIMS 70 eV, *m/z* (rel. int.): 344 $[M\text{-}80]^+$ (100), 329 (19), 314 (40), 301 (23), 271 (9), 243 (5), 172 (9), 135 (10). HRMS 80 eV, *m/z*: 344.08931 ($C_{18}H_{16}O_7$, calc. 344.08961), 329.06602 ($C_{17}H_{13}O_7$, calc. 329.06613), 314.07924 ($C_{17}H_{14}O_6$ calc. 314.07904), 301.07023 ($C_{16}H_{13}O_6$, calc. 301.07122), 299.05510 ($C_{16}H_{11}O_6$, calc. 299.05557), 135.04465

($C_8H_7O_2$, calc. 135.04461). FABMS *m/z*: 445 [(M–2H) + Na]$^-$, 423 [M–H]$^-$, 343 [M–H-80]$^-$.

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