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FLAVONOL GLYCOSIDES FROM *SECURIDACA DIVERSIFOLIA*

M. HAMBURGER, M. GUPTA* and K. HOSTETTMANN

Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie de l'Université de Lausanne, 2 rue Vuillermet, CH-1005 Lausanne, Switzerland; *Laboratorio Especializado de Analysis, Facultad de Ciencias Naturalis y Farmacia, Universidad de Panama, Panama, Republic of Panama

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Key Word Index—*Securidaca diversifolia*; Polygalaceae; flavonol glycosides; apiosides; DCCC; ^{13}C NMR; FAB MS; D/CI MS.

Abstract—Twelve flavonol glycosides have been isolated from the leaves of *Securidaca diversifolia*. The separation of ten quercetin 3-glycosides and two kaempferol 3-glycosides was achieved by droplet counter-current chromatography (DCCC), preparative reversed-phase chromatography and gel chromatography. The structures were established on the basis of partial and total acid hydrolysis and spectral data (UV, ^{13}C NMR, FAB MS, D/CI MS). The four apiosides: quercetin 3-(2″-β-D-apiofuranosyl-β-D-glucopyranoside), 3-(2″-β-D-apiofuranosyl-β-D-galactoside), 3-(2″-β-D-apiofuranosyl-α-L-arabinopyranoside) and 3-(2″-β-D-apiofuranosyl-β-D-xylopyranoside) are new natural products. The structure of kaempferol 3-(2″-β-D-apiofuranosyl-β-D-glucopyranoside), previously isolated from *Cicer arietinum*, is confirmed.

INTRODUCTION

Very little is known about the secondary plant constituents of the genus *Securidaca* from the Polygalaceae, although species such as *S. longepedunculata* Fres. are widely used in African folk medicine, as well as fish poisons [1–3]. Saponins (derived from presenegenin) and methyl salicylate in the fresh roots have been previously reported from some *Securidaca* species [2–5]. Within the scope of an investigation of different Polygalaceae for biologically-active compounds [6–8], we therefore studied *S. diversifolia* S. F. Blake. This shrub grows in the subtropical regions of the American continent. No previous phytochemical work on this species has been published. We now report on the isolation and structure elucidation of glycosidic constituents from the leaves of the plant.

RESULTS

A thin-layer chromatographic screening of the methanolic leaf extract of *S. diversifolia* showed a complex pattern in UV light. The deep purple colour of the spots under UV 366 nm light changed to a yellow fluorescence after spraying with aluminium chloride, suggesting the presence of flavonoids. Acid hydrolysis of the crude methanol extract with 2 N HCl, followed by the usual workup gave quercetin and kaempferol.

The methanol extract was submitted to DCCC using $CHCl_3$–MeOH–*n*-BuOH–H_2O (10:10:1:6) as solvent system in the ascending mode and 9 fractions (I–IX) were collected. The fractions VI, VIII and IX consisted of the pure flavonoid monoglycosides **12**, **9** and **11**, respectively. Fractions I, III, IV, V and VII were further separated by chromatography on Sephadex LH 20 with methanol as eluent. Fraction I yielded compound **1**; fractions III and IV afforded pure diglycosides **4** and **6**, and **4** and **5**, respectively. V and VII contained further flavonoid monoglycosides. From fraction V, compounds **7** and **8** were obtained; VII could be separated into **10** and **12**. The two quercetin derivatives **2** and **3** were isolated from fraction II by preparative reversed-phase chromatography with MeOH–H_2O–HCOOH (25:73:2).

Acid hydrolysis of **4** and **11** afforded kaempferol as aglycone, whilst all the other compounds yielded quercetin. The sugars of the monoglycosides **7**–**12** were identified by TLC. The position of attachment of the sugar moieties was determined by the UV spectra measured in methanol and after addition of the usual shift reagents [9]. The glycosidation site was found to be in each case at C-3 of the aglycone. Compounds **7**–**12** were therefore identified as quercetin 3-galactoside (hyperoside) **7** [10], quercetin 3-glucoside (isoquercitrin) **8** [11], quercetin 3-xyloside (reynoutrin) **10** [12], quercetin 3-rhamnoside (quercitrine) **12** [13], quercetin 3-α-L-arabinopyranoside (guaijaverin) **9** [14] and kaempferol 3-α-L-arabinopyranoside **11**. For the latter two compounds, the pyranoside form of the sugar was established on the basis of ^{13}C NMR spectral data [15].

The sugars, obtained after hydrolysis of the diglycosides with 2 N HCl were identified by TLC as glucose for **1**, **3** and **4**, galactose for **2**, arabinose for **5** and xylose for **6**. Mild acid treatment of compounds **2**–**6** with 0.1 N H_2SO_4 according to Shoji [personal communication] afforded apiose in addition to the above mentioned sugars.

The desorption chemical ionization MS (D/CI MS) [16] of **1** showed a quasimolecular ion at m/z 627 ($[M+H]^+$). The fragment peaks at m/z 465 ($[(M+H)-162]^+$) and 303 ($[(M+H)-324]^+$) were due to the consecutive loss of two glucosyl units. Confirmation of these results was obtained by fast atom bombardment (FAB MS) [17], measured in the negative ion mode, where signals at m/z 625 ($[M-H]^-$) and 301 ($[M-H)-324]^-$) could be observed. On the basis of the ^{13}C spectral data,

glycoside **1** was identified as the already known quercetin 3-sophoroside [18].

The mass spectra of both **2** and **3** showed identical fragmentation patterns. A weak quasimolecular ion at *m/z* 597 ($[M+H]^+$) was obtained by the D/CI technique. Fragment peaks at *m/z* 465 ($[M+H)-132]^+$) and 303 ($[M+H)-294]^+$) indicated the successive cleavage of the terminal apiosyl and the inner galactosyl or glucosyl moiety. In the FAB spectrum very intense quasimolecular ions at *m/z* 595 ($[M-H]^-$) appeared, together with a signal at *m/z* 301 ($[M+H)-294]^-$). The position of attachment of the sugar moiety and the interglycosidic linkage were deduced from ^{13}C NMR spectral data (Table 1). For both compounds, the C-2 and C-3 signals of the aglycone appeared at 155.6 and 133.2 ppm, respectively. Thus, the site of glycosidation is at C-3 [15]. The chemical shifts of the C-atoms of the glucose moiety of **3** indicated that the apiose was attached at position 2. The C-2 was shifted downfield by 2.9 ppm in comparison with quercetin 3-*β*-D-glucopyranoside [15], and appeared at 77.2 ppm. On the other hand the anomeric carbon was observed upfield at 98.6 ppm, whereas the other signals were not affected. The chemical shift values of the sugar moiety were in good agreement with reported data for apigenin 7-(2″-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside) (apiin) [15]. However, due to the different glycosidation site, the signal for C-1″ of **3** appeared at 1.1 ppm higher field than the corresponding signal for apiin. Thus, **3** was established as the previously unknown quercetin 3-*O*-(2″-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside).

In the ^{13}C NMR spectrum of **2** (Table 1), the signals attributable to the galactosyl moiety indicated a linkage of the terminal apiose at C-2″. The signal for this C atom was shifted downfield by 3.7 ppm with regard to quercetin 3-*O*-*β*-D-galactopyranoside and appeared at 75.0 ppm, while the adjacent anomeric carbon underwent an upfield shift of 3.3 ppm. The other signals remained unchanged. **2** was therefore identified as quercetin 3-*O*-(2″-*O*-*β*-D-apiofuranosyl-*β*-D-galactopyranoside), a new flavonoid glycoside.

The sugar sequence of **4** was established by D/CI MS. A quasimolecular peak at *m/z* 581 ($[M+H]^+$), together with fragment ions at *m/z* 449 ($[(M+H)-132]^+$) and 287 ($[M+H)-294]^+$) proved that apiose was the terminal sugar. A similar fragmentation pattern was observed in the FAB spectrum with ions at *m/z* 579 ($[M-H]^-$), 447 ($[(M-H)-132]^-$) and 285 ($[(M-H)-294]^-$). In the ^{13}C NMR spectrum the signals attributable to C-2 and C-3 of the aglycone were at 155.6 and 133.0 ppm. Thus, the sugar chain is attached at C-3. As the chemical shifts of the sugar signals were virtually identical with **3** [Table 1], the structure of **4** was identified as kaempferol 3-*O*-(2″-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside), which has probably been isolated from *Cicer arietinum* [20].

Similar D/CI MS were obtained for **5** and **6**. A quasimolecular ion at *m/z* 567 ($[M+H]^+$) was followed by successive elimination of two pentose units, leading to fragment peaks at *m/z* 435 ($[M+H)-132]^+$) and 303 ($[(M+H)-264]^+$). The glycosidation site on the aglycone was at C-3 for both **5** and **6**, as could be deduced from the ^{13}C NMR signals of C-2 and C-3, which appeared at 156 and 133 ppm, respectively. The sugar sequence of both compounds was established by sequential hydrolysis of the sugar chains. Selective cleavage of the terminal apiose with 0.1 N methanolic HCl at room temperature yielded **9** and **10**, which were further hydrolysed to yield arabinose and xylose respectively as the inner sugars. The interglycosidic linkage was established on the basis of the ^{13}C NMR data. In comparison with **9** [15], the signal attributable to the C-2″ of the arabinopyranosyl moiety of **5** was shifted downfield by 3.6 ppm and appeared at 75.2 ppm, whereas the signal of the adjacent anomeric carbon atom appeared upfield at 99.3 ppm. Thus, the apiose was attached at C-2″ of the arabinosyl moiety. For compound **6**, a similar behavior could be observed for the signals of the xylose unit. **5** and **6** were therefore identified as the two new flavonol apiosides quercetin 3-*O*-(2″-*O*-*β*-D-apiofuranosyl-*α*-L-arabinopyranoside) and quercetin 3-*O*-(2″-*O*-*β*-apiofuranosyl-*β*-D-xylopyranoside), respectively.

Table 1. ^{13}C NMR chemical shifts of **2–6** (sugar moieties) in d_6-DMSO

	2†	3†	4†	5†	6‡
C-1″	99.0	98.6	98.7	99.3	99.5
C-2″	75.0	77.2*	77.3*	75.2	76.6*
C-3″	73.7	77.0	77.0	69.8	76.1*
C-4″	68.3	70.3	70.3	65.2	69.4
C-5″	75.6	77.3*	77.3*	62.5	65.7
C-6″	60.1	60.9	60.8		
C-1‴	108.8	108.6	108.7	108.6	108.5
C-2‴	76.3	76.2	76.2	76.1	76.1*
C-3‴	79.1	79.1	79.1	78.8	79.0
C-4‴	73.9	73.9	73.9	73.7	73.8
C-5‴	64.4	64.3	64.2	63.8	64.2

*Assignments interchangeable.
†50.29 MHz.
‡100.54 MHz.

DISCUSSION

The application of droplet counter-current chromatography (DCCC) to the separation of flavonoids has been reviewed recently [19]. For the isolation of closely related flavonol glycosides, the combination of DCCC with preparative reversed-phase chromatography and gel chromatography proved to be very efficient, due to the different selectivities of these chromatographic techniques. Thus, each of the twelve glycosides could be separated in just two steps.

Among the isolated compounds, the apiosides **2**, **3**, **5** and **6** are new natural products. The kaempferol 3-*O*-(2″-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside) **4** is probably identical with a kaempferol 3-*O*-apiosylglucoside isolated from *Cicer arietinum* L. [20]. However, the interglycosidic linkage of the latter compound was not established with certainty. Flavones with apiose as a sugar moiety are known [21], but very few flavonol glycosides containing that sugar have been reported.

Apiose seems to occur frequently in glycosides of the Polygalaceae, and this fact might be of chemotaxonomic interest. Apart from the above described flavonoids, this rare pentose has also been found as a sugar moiety in several *Polygala* saponins [23, 24]. Very little is actually known about the flavonoids of the Polygalaceae, since

only afzelin, myricitrin and rutin have been reported from *Polygala chinensis* L. [25], and no work on the flavonoids of the genus *Securidaca* has been published before.

EXPERIMENTAL

General procedures. Mps are uncorr. TLC was carried out on silica gel precoated A1 sheets (Merck) or on RP-8 nanoplates (Merck). DCCC was carried out on a Büchi DCCC 760 apparatus (288 tubes; i.d. 2.7 mm), equipped with a LKB Uvicord II detector (detection at 275 nm). The flow rate was 30 ml/hr. For gel chromatography, a Sephadex K 26 column (70 × 2.5 cm) was used together with a Duramat-80 pump and a LKB Uvicord II detector using 120 g Sephadex LH 20 in MeOH. Prep. reversed-phase chromatography was achieved on a Lobar Lichroprep RP-8 column (40–63 μm, 27 × 2.5 cm, Merck), flow rate 7 ml/min. UV spectra were recorded in MeOH and after addition of usual shift reagents [9]. ^{13}C NMR spectra were measured with a Bruker WP 200 at 50.29 MHz and with a Varian XL 400 at 100.54 MHz in d_6-DMSO and with TMS as int. standard. Desorption/chemical ionization (D/CI) MS were obtained on a Ribermag R10-10B quadrupole with NH_3 as reactant gas. FAB MS were recorded on a ZAB-1S spectrometer. The samples were suspended in thioglycerol and the target was bombarded with 5 keV Xe-atoms.

Plant material. S. diversifolia S. F. Blake was collected in Panama and a voucher specimen deposited at the Herbarium of the University of Panama.

Extraction and isolation. Dried leaves (58 g) were extracted at room temp. successively with petrol, $CHCl_3$, MeOH and MeOH–H_2O 1:1. The MeOH extract was further investigated. 5 g (2 × 2 g, 1 × 1 g) of the extract were submitted to DCCC with $CHCl_3$–MeOH–*n*-BuOH–H_2O (10:10:1:6) in the ascending mode. Nine fractions were collected. Fractions VI, VIII and IX, containing a compound each, were purified on a Sephadex LH 20 column to yield **12** (8 mg), **9** (27 mg) and **11** (14 mg), respectively. Chromatography of fraction VII (92 mg) on a Sephadex LH 20 column gave pure **10** (7 mg) and **12** (45 mg). Fraction III–V were purified by the same method. V (329 mg) yielded **7** (35 mg) and **8** (34 mg), IV (91 mg) gave **4** (14 mg) and **5** (5 mg); from III (94 mg), compound **5** (16 mg) and **6** (21 mg) were obtained. **2** and **3** were separated by prep. reversed-phase chromatography with MeOH–H_2O–HCOOH (25:73:2). The MeOH was removed from the eluates after neutralization with NH_3. The residue, obtained after lyophilization of the aq. soln, was partitioned between BuOH and H_2O and the organic layer evaporated. Purification of the residues on Sephadex LH 20 yielded **2** (35 mg) and **3** (25 mg). Fraction I (581 mg) afforded **1** (16 mg) after gel chromatography on Sephadex LH 20.

Hydrolysis of **1–12** *with* 2 N HCl. Each glycoside (1 mg) was refluxed in 2 N HCl (5 ml) for 2 hr. The aglycone was extracted with EtOAc and identified by co-TLC with authentic samples on silica gel with $CHCl_3$–MeOH–H_2O (45:5:1) and on RP-8 with MeOH–H_2O (13:7). The aq. layer was adjusted with $NaHCO_3$ to pH 6. After lyophilization, the sugars were extracted with pyridine from the residue and analyzed on silica gel with EtOAc–MeOH–H_2O–HOAc (13:3:3:4); detection with *p*-anisidine phthalate.

Hydrolysis of **2–5** *with* 0.1 N H_2SO_4. Each compound (1 mg) was refluxed in 0.1 N H_2SO_4 (2 ml) for 20 min. Extraction and TLC were carried out as above. Apiose was detected with naphthoresorcin as spray reagent.

Sequential hydrolysis of **4** *and* **5**. The compounds (1 mg) were kept in 0.1 N methanolic HCl (2 ml) at room temp. for 2 hr (**4**) and 10 hr (**5**), respectively, the soln diluted with H_2O and extracted with BuOH. The monoglycosides were identified by co-TLC with authentic samples. After evaporation of the BuOH, the compounds were refluxed with 2 N HCl (5 ml) for 2 hr, and the sugars identified as above.

Quercetin 3-*O*-(2″-*O*-β-D-*apiofuranosyl*-β-D-*galactopyranoside*) (**2**). Amorphous, yellow powder; mp 183–188°; TLC [SiO_2, EtOAc–MeCOEt–HCOOH–H_2O, 5:3:1:1) (System A)]: R_f 0.40; UV λ_{max} nm: (MeOH) 254, 262 sh, 300 sh, 353; (NaOMe) 268, 323, 401; (NaOAc) 270, 321, 389; ($AlCl_3$) 272, 298 sh, 430; ($AlCl_3$ + HCl) 267, 296 sh, 359 sh, 398; ^{13}C NMR (50.29 MHz, DMSO-d_6): δ177.3 (C-4), 164.3 (C-7), 161.3 (C-5), 156.2 (C-9 or C-2), 155.6 (C-2 or C-9), 148.4 (C-4′), 144.9 (C-3′), 133.2 (C-3), 122.3 (C-6′), 121.2 (C-1′), 115.6 (C-5′ or C-2′), 115.2 (C-2′ or C-5′), 103.8 (C-10), 98.6 (C-6), 93.4 (C-8); sugar signals: see Table 1; D/CI MS (NH_3, positive ions) *m/z*: 597 ($[M+H]^+$), 465 ($[(M+H)-132]^+$), 303 ($[(M+H)-294]^+$); FAB MS (negative ions) *m/z*: 653 ($[MNaCl]^-$), 631 ($[MHCl]^-$), 595 ($[M-H]^-$), 301 ($[(M-H)-294]^-$).

Quercetin 3-*O*-(2″-*O*-β-D-*apiofuranosyl*-β-D-*glucopyranoside*) (**3**). Amorphous, yellow powder; mp 171–175°; TLC (System A): R_f 0.45; UV λ_{max} nm: identical with **2**; ^{13}C NMR (50.29 MHz, DMSO-d_6): δs of the aglycone identical with **2**; sugar signals: see Table 1; D/CI MS (NH_3, positive ions) *m/z*: 597 ($[M+H]^+$), 465 ($[(M+H)-132]^+$), 303 ($[(M+H)-294]^+$); FAB MS (negative ions) *m/z*: 631 ($[MHCl]^-$), 595 ($[M-H]^-$), 301 ($[(M-H)-294]^-$).

Kaempferol 3-O-(2″-O-β-D-*apiofuranosyl*-β-D-*glucopyranoside*) (**4**). Amorphous, yellowish powder; mp 165–170°; TLC (System A): R_f 0.53; UV λ_{max} nm: (MeOH) 263, 300 sh, 346; (NaOMe) 271, 320, 393; (NaOAc) 272, 308, 385; ($AlCl_3$) 272, 301, 349, 397; ($AlCl_3$ + HCl) 271, 295 sh, 342, 394; ^{13}C NMR (50.29 MHz, DMSO-d_6): δ177.3 (C-4), 164.3 (C-7), 161.2 (C-5), 159.8 (C-4′), 156.3 (C-9 or C-2), 155.6 (C-2 or C-9), 133.0 (C-3), 130.7 (C-2′ and C-6′), 121.0 (C-1′), 115.1 (C-3′ and C-5′), 103.9 (C-10), 98.5 (C-6), 93.6 (C-8); sugar signals: see Table 1; D/CI MS (NH_3, positive ions) *m/z*: 581 ($[M+H]^+$), 449 ($[(M+H)-132]^+$), 287 ($[(M+H)-294]^+$); FAB (negative ions) *m/z*: 579 ($[M-H]^+$), 447 ($[(M-H)-132]^-$), 285 ($[(M-H)-294]^-$).

Quercetin 3-O-(2″-O-β-D-*apiofuranosyl*-α-L-*arabinopyranoside*) (**5**). Amorphous, yellow powder; mp 162–166°; TLC (System A): R_f 0.54; UV λ_{max} nm: identical with **2**; ^{13}C NMR (50.29 MHz, DMSO-d_6): δs of the aglycone identical with **2**; sugar signals: see Table 1; D/CI MS (NH_3, positive ions) *m/z*: 567 ($[M+H]^+$), 435 ($[(M+H)-132]^+$, 303 ($[(M+H-264]^+$); FAB (negative ions) *m/z*: 601 ($[MHCl]^-$), 565 ($[M-H]^-$), 433 ($[(M-H)-132]^-$), 301 ($[(M-H)-264]^-$).

Quercetin 3-O-(2″-O-β-D-*apiofuranosyl*-β-D-*xylopyranoside*) (**6**). Amorphous, yellow powder; mp 164–168°; TLC (System A): R_f 0.57; UV λ_{max} nm: identical with **2**; ^{13}C NMR (100.29 MHz, DMSO-d_6): δs of the aglycone identical with **2**; sugar signals: see Table 1; D/CI MS (NH_3, positive ions) *m/z*: 567 ($[(M+H]^+$), 435 ($[M+H)-132]^+$), 303 ($[(M+H)-264]^+$); FAB (negative ions) *m/z*: 601 ($[MHCl]^-$), 565 ($[M-H]^-$), 301 ($[(M-H)-264]^-$).

The structures of quercetin 3-sophoroside **1** [18], hyperoside **7** [10], isoquercitrin **8** [11], guaijaverin **9** [14], reynoutrin **10** [12], kaempferol-3-α-L-arabinopyranoside **11** and quercitrin **12** [13] were established by comparison with authentic samples and with reported data.

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