FLAVONOIDS OF *SULLIVANTIA*

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ABSTRACT.—Species of the genus *Sullivantia* contain quercetin 3-β-D-galactoside, pedalitin and its 6-β-D-glucoside as well as the new glycosides quercetin 3-galactosyl-glucuronoside and pedalitin 6-galactosylglucoside. A pedalitin 6-galactosyl compound was also found. An additional compound was identified as quercetin 3-x"-(galactosyl)-x"-(glucuronosyl)-glucuronoside. Six species were examined.

Sullivantia (Saxifragaceae), a small, little studied genus of herbaceous perennials restricted to the central and western United States, consists of six disjunct species (1): *S. oregana* from Oregon and Washington; *S. purpusii* from Colorado; *S. hapemanii* from Wyoming; *S. halmicola* from Wyoming and Montana; *S. renifolia* from Iowa, Illinois, Wisconsin, Minnesota and Missouri; and *S. sullivantii* from Indiana and Ohio. As part of a biosystematic study of *Sullivantia* we report here the major flavonoids of these rare and narrowly endemic taxa (see table 1).

TABLE 1. Distribution of flavonoids in *Sullivantia* species.

Species	Flavonoid compounds						
	Pedalitin	Pedalitin 6-glucoside	Pedalitin 6-di-(?)-galactosyl derivative	Pedalitin 6-galactosyl-glucoside	Quercetin 3-galactoside	Quercetin 3-galactosyl-glucuronoside	Quercetin 3-triglycoside
S. oregana	+	+	+	+	+	−	+
S. purpusii	+	+	+	−	+	+	+
S. hapemanii	+	+	+	−	+	−	+
S. halmicola	+	+	+	−	+	−	+
S. renifolia	+	+	+	+	+	+	+
S. sullivantii	+	+	+	+	+	−	+

The presence or absence of the different flavonoid compounds is indicated as follows: − not detected, + compound present.

All *Sullivantia* species accumulate small amounts of pedalitin (6-hydroxyluteolin 7-methyl ether), a flavone previously known from *Eupatorium inulaefolium* (Compositae) (2), as well as its 6-glucoside and a 6-galactoside derivative (possibly a digalactoside). Pedalitin is known from *Sesamum indicum* in the Pedaliaceae (3, 4). All six species also contain quercetin 3-galactoside and a quercetin 3-triglycoside. *Sullivantia oregana, S. sullivantii* and *S. renifolia* also contain pedalitin 6-galactosylglucoside. *Sullivantia purpusii* and some populations of *S. renifolia* are distinguished by the presence of quercetin 3-galactosylglucuronoside. The flavonoid chemistry differentiates *Sullivantia* from other genera in the Saxifragaceae

for which flavonoid data are available and also indicates that the genus is closely allied with *Boykinia* (5). The pattern of flavonoid variation found in *Sullivantia* species parallels the pattern of variation documented in gross morphology, a matter that will be discussed as part of a revision of the genus in a later publication.

EXPERIMENTAL

PLANT MATERIAL.—Leaves from the following plants were used in this study: *Sullivantia oregana* (collected from Hood River and Multnomah Counties, Oregon); *S. purpusii* (collected from Garfield and Gunnison Counties, Colorado); *S. hapemanii* (collected from Johnson County, Wyoming); *S. halmicola* (collected from Sheridan and Big Horn Counties, Wyoming, and Carbon County, Montana); *S. renifolia* (collected from Fayette, Allamakee and Jones Counties, Iowa, and Jo Daviess County, Illinois, Richland, Vernon and Iowa Counties, Wisconsin, Winona County, Minnesota, Warren and Franklin Counties, Missouri); and *S. sullivantii* (collected from Clark, Jefferson and Jennings Counties, Indiana, Hocking and Highland Counties, Ohio). Voucher specimens[1] of all plants are deposited in the Indiana University Herbarium, Bloomington, Indiana. All plant material was air-dried prior to extraction.

GENERAL TECHNIQUES.—Column chromatography was carried out with Sephadex LH-20 (Pharmacia); Whatman 3MM paper was used for pc, and tlc was performed on precoated cellulose (Merck) and polyamide (Macharey-Nagel) plates. Tlc solvents were BPMM (benzene-petrol (65–110°)-methyl ethyl ketone-methanol, 60:26:7:7); BMM (benzene-methyl ethyl ketone-methanol, 4:3:3); TBA (*t*-butanol-acetic acid-water, 3:1:1); 15% acetic acid (glacial acetic acid-water, 15:85); 40% acetic acid (glacial acetic acid-water, 40:60) and *n*-BAW (upper layer, *n*-butanol-acetic acid-water, 4:1:5). The flavonoids were visualized by uv light with exposure to NH_3 and by spraying with NA (Naturstoffreagenz-A, Carl Roth, Germany) in methanol. Hydrolyses were carried out with 2N HCl or 0.1N TFA on a steam cone for 1 hr or with β-glucosidase, β-galactosidase, and β-glucuronidase (Sigma). Sugars were identified by cochromatography on cellulose plates run in pyridine-ethyl acetate-acetic acid-water, 36:36:7:21. After drying, plates were sprayed with aniline phthalate (Merck) and heated (115°, 10 min).

Spectra were recorded with the following instruments: uv, Beckman DB spectrophotometer; ^{1}H nmr, Varian HA-100 and Varian 390; ms, Dupont 21-491.

EXTRACTION AND PURIFICATION.—Ground leaf material was extracted sequentially with chloroform and methanol. The methanol extracts were concentrated and subjected to two-dimensional chromatography on Whatman 3MM paper, developed first in TBA (3:1:1) and then in 15% acetic acid. Compounds were isolated by elution from the two-dimensional paper chromatograms with aqueous methanol and purified on Sephadex LH-20 with methanol. Because of the small amounts of the plant material available, insufficient quantities of the flavonoids were obtained to characterize fully the glycosyl moieties in the di- and tri-saccharides.

IDENTIFICATION OF THE FLAVONOIDS: PEDALITIN.—Pedalitin (6-hydroxyluteolin 7-methyl ether) exhibited uv and ^{1}H nmr spectral data corresponding to those previously recorded [2,3]. The ms of the PDM ether of pedalitin showed an $M^{+\cdot}$ at *m/e* 384 (38%); in addition, an ion at *m/e* 366 (M–18, 100%), characteristic for the loss of $-CD_3$ from the oxygen function at C-6, and an ion for B_1 at *m/e* 168 (13%) were also observed.

PEDALITIN 6-β-D-GLUCOSIDE.—Hydrolysis of this compound in 0.1N TFA and with β-glucosidase yielded pedalitin and glucose. Uv spectral data for the glucoside (Band I in $AlCl_3$ relative to Band I in MeOH) supported the 3',4'-ortho dihydroxyl system (Δ+78 nm). In addition, comparison of Band I in the $AlCl_3$/HCl spectrum with Band I in the spectrum in MeOH (Δ+20 nm) indicated a substituted oxygen function at the C-6 position [6]. The presence of a methoxyl group at the 7 position was supported by the NaOMe spectrum (no Band III [7]) and by the NaOAc spectrum. Uv spectral data: λ max MeOH (nm), 254sh, 272, 346; NaOMe, 268, 392; $AlCl_3$ 271, 302sh, 334, 424; $AlCl_3$/HCl 259, 280, 294sh, 366; NaOAc 266, 378; NaOAc/H_3BO_3 262, 373. ^{1}H nmr in DMSO: H-6': δ 7.65 (dd, *J*=2.5 and 8 Hz); H-2': 7.35 (d, *J*=2.5 Hz); H-5': 6.9 (d *J*=8 Hz); H-8: 6.5 (s); H-3: 6.4 (s); H-1': 4.8 (s); 6 sugar protons δ 3.5–4.1 (br·m); 7-OMe: 3.9 (s).

PEDALITIN 6-DI-(?)GALACTOSYL DERIVATIVE.—Although insufficient material was available to characterize fully this compound, acid hydrolysis did afford galactose and pedalitin. The uv spectral data for the pedalitin 6-di-(?)galactosyl derivative were similar to those obtained for pedalitin 6-glucoside indicating the same substitution pattern. However, the R_f values

[1]The voucher numbers for collections are as follows: *S. oregana, Soltis & Hammond-Soltis 1034, 1038, 1039; S. purpusii, Soltis & Hammond-Soltis 1040, 1042, 1044; S. hapemanii, Soltis & Hammond-Soltis 1024; S. halmicola, Soltis & Hammond-Soltis 1027, 1028, 1029, 1033; S. renifolia, Soltis & Doyle 1048, 1049, 1050, 1051, 1056, Soltis & Haufler 988, 997, 998, 1006; S. sullivantii, Soltis & Doyle 975, Soltis & Haufler 979, Soltis & Hammond-Soltis 1009, 1012, 1022, 1023.*

for the former compound (0.47 in TBA and 0.31 in 15% acetic acid) were sufficiently different from those obtained for pedalitin 6-glucoside (0.34 TBA and 0.23 in 15% acetic acid) to suggest a di-or acylated galactosyl moiety. This pedalitin 6-di(?)galactosyl derivative will have to be reisolated for any additional studies. Unfortunately, the plants which contain it are rare endemics and difficult to obtain in bulk quantities.

PEDALITIN 6-GALACTOSYLGLUCOSIDE.—R_f values (0.22 TBA and 0.39 15% acetic acid) favored a diglycoside, and hydrolysis of this new glycoside yielded glucose and galactose in a 1:1 ratio (determined on tlc with standard sugars) as well as pedalitin. Hydrolysis with β-galactosidase yielded the monoglycoside pedalitin 6-β-D-glucoside (uv, tlc comparison with standard).

QUERCETIN 3-β-D-GALACTOSIDE.—Upon acid and β-galactosidase hydrolysis, this compound yielded galactose and quercetin. Uv data and R_f values established a 3-monogalactosyl substituent; cochromatography with an authentic sample confirmed the structure.

QUERCETIN 3-GALACTOSYLGLUCURONOSIDE.—The uv data and R_f values (0.70 TBA and 0.48 15% acetic acid) of this glycoside suggested a quercetin skeleton substituted only at the 3 position with a glycosyl moiety. The compound yielded galactose and glucuronic acid along with quercetin when hydrolyzed with 2N HCl. Since hydrolysis with β-galactosidase yielded a monoglycoside, the natural product is quercetin 3-galactosylglucuronoside. The quantities available did not permit further characterization.

QUERCETIN 3-X"-(GALACTOSYL)-X"-(GLUCURONOSYL)-GLUCURONOSIDE.—Uv data and R_f values of the glycoside (0.49 TBA and 0.71 15% acetic acid) suggested a quercetin structure with at least a 3-*O*-trisaccharide substituent. The compound yielded galactose and glucuronic acid when hydrolyzed with 2N HCl. Enzymatic hydrolyses with either β-galactosidase or β-glucuronidase gave different quercetin 3-*O*-disaccharides, establishing a branched tri-glycoside. Successive hydrolysis with β-glucuronidase and β-galactosidase gave quercetin 3-glucuronoside identical with the compound obtained by the β-galactosidase hydrolysis of quercetin 3-galactosylglucuronoside. These data establish the compound as quercetin 3-x"-(galactosyl)-x"-(glucuronosyl)-glucuronoside. The small amount of material available did not permit ms or nmr analyses.

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