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EIGHT FLAVONOL GLYCOSIDES IN *PYROLA* (PYROLACEAE)

JOHN E. AVERETT and BRUCE A. BOHM*

Department of Biology, University of Missouri-St. Louis, St. Louis, MO 63121, U. S. A.;* Department of Botany, University of British Columbia, Vancouver, V6T 2B1, Canada

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Key Word Index—*Pyrola*; Pyrolaceae; flavonoids; flavonols; glycosides.

Abstract—As part of a general survey of the flavonoids of Pyrolaceae, the flavonoids of *Pyrola virens* and *P. chlorantha* were investigated. Eight flavonol glycosides based upon kaempferol, quercetin and rhamnetin were identified from each of the two species. Two of the glycosides, rhamnetin 3,3',4'-tri-*O*-glucoside and rhamnetin 3-*O*-arabinoside-3',4'-di-*O*-glucoside are previously unreported and further, represent an unusual pattern of glycosylation. The similarity of flavonoids and the presence of the unusual substitution pattern supports a conspecific status for the two taxa.

INTRODUCTION

The Pyrolaceae is a segregate family of a formerly broad Ericaceae. Cronquist [1] recognizes four genera: *Chimaphila*, *Pyrola*, *Moneses* and *Orthilia*. The latter two are monotypic and are often included in *Pyrola*. The genera, like those of the achlorophyllous Monotropaceae, are characterized by strong mycotrophic associations.

Compared to the amount of information in the literature on members of the Ericaceae, little is known about the flavonoids of Pyrolaceae. As part of a detailed study of the flavonoids of the family, the North American species have been investigated. The results from analyses of two species, *P. virens* and *P. chlorantha*, are herein reported. The two species are of particular interest because an unusual array of flavonol glycosides is produced by each. Further, there is some question as to whether or not *P. virens* and *P. chlorantha* should be treated as separate species.

RESULTS AND DISCUSSION

Whole plant, methanolic extracts of *P. virens* and *P. chlorantha* yielded eight flavonol glycosides: kaempferol 3-*O*-arabinoside (**1**), kaempferol 3-*O*-glucoside (**2**), quercetin 3-*O*-arabinoside (**3**), quercetin 3-*O*-glucoside (**4**), rhamnetin 3-*O*-arabinoside (**5**), rhamnetin 3-*O*-glucoside (**6**), rhamnetin 3-*O*-arabinoside-3',4'-di-*O*-glucoside (**7**) and rhamnetin 3,3',4'-tri-*O*-glucoside (**8**). All compounds were present in both taxa and no additional compounds were detected. The triglycosides are new compounds and represent the first report of this pattern of glycosylation.

Haber [2] reported six 3-*O*-monoglycosides of kaempferol and quercetin from *Pyrola asarifolia*. In an initial survey of the genus, we confirmed the presence of those flavonoids, along with additional kaempferol monoglycosides, in *P. asarifolia* and detected similar compounds in other North American species. All of the compounds have not yet been identified in the latter taxa, but the triglycosides clearly are not present in any of the other species of *Pyrola* nor were they present in *Chimaphila*, *Moneses* or *Orthilia*. Thus, the overall similarity in the flavonoid pattern of *P. virens* and *P. chlorantha* and the presence of the novel glycosides strongly support a conspecific status for the two taxa.

EXPERIMENTAL

Flavonoid extraction, isolation and identification. Plant material was extracted with MeOH and the extracts worked up according to described procedures [3, 4]. Standard methods of UV spectral analysis were employed [5, 6]. Commonly encountered compounds were compared chromatographically with known compounds using solvent systems described by Wilkins and Bohm [3]. Structures of the unusual glycosides were determined as follows.

The unknown compounds exhibited a combination of chromatographic and UV spectral characteristics that were unfamiliar to us. R_f values in four systems are given in Table 1; values for rutin are given for comparison. Compound **7** exhibited UV absorption maxima at 267 and 342 nm, compound **8** at 268 and 346 nm. These maxima were not significantly altered by addition

Table 1. R_f Values for compounds **7** and **8** compared to rutin

	Chromatographic system*			
	A	B	C	D
Rutin	0.68	0.56	0.31	0.74
Compound **7**	0.66	0.58	0.50	0.73
Compound **8**	0.72	0.65	0.35	0.60

*A = 15% HOAc on MN 300 Cellulose (0.25 mm homemade); B = H_2O–*n*-BuOH–Me_2CO–dioxane (70:15:10:5) on Polyamid 6.6 (0.25 mm homemade); C = C_6H_6–MeOH–MeCOEt–H_2O (55:22:20:3) on Polyamid 6.6 (0.25 mm homemade); D = *n*-BuOH–HOAc–H_2O (4:1:5) on MN 300 (commercial sheet).

of NaOAc. NaOMe gave maxima of 266 and about 365 nm (broad peaks) for both compounds. Spectra for both compounds in the presence of $AlCl_3$ were essentially identical: 240 sh, 272, 300 sh, 350 and 393 nm. Addition of HCl had no significant effect. These observations indicate the presence of an unsubstituted hydroxyl group only at C-5. The compounds were subjected to hydrolysis using trifluoroacetic acid at 100° for *ca* 1 hr. Both compounds gave the same phenolic hydrolysis product as judged by chromatography, colour reaction with β-aminoethyl diphenylborate and UV spectral data. Compound **7** gave glucose and arabinose after hydrolysis, compound **8** only glucose. The phenolic hydrolysis product was shown by spectral means to have free hydroxyl groups at C-3 and C-4′ (Band I shift with NaOAc). Chromatography of the phenolic hydrolysis product in the C_6H_6–MeOH–MeCOEt–H_2O system (see Table 1) showed it to have an R_f value less than that of 7-*O*-methylkaempferol which suggested to us that the original acid hydrolysis had not been complete. The hydrolysis product was treated with β-D-glucosidase which gave 7-*O*-methylquercetin (rhamnetin) whose identity was confirmed by MS analysis [6] [*m/z* = 316 (63.5%), 137 (43.1%), 167 (11.5%)]. Compound **8** is thus rhamnetin 3,3′,4′-tri-*O*-glucoside. Compound **7** was treated with β-D-glucosidase which yielded rhamnetin 3-*O*-arabinoside whose structure was established by usual means. The structure of compound **7** must then be rhamnetin 3-*O*-arabinoside-3′,4′-di-*O*-glucoside. Selective hydrolysis of the unknowns with TFA was, it appears, a fortuitous observation. Hydrolysis of the unknowns with 2 N HCl at 100° for *ca* 1 hr resulted in cleavage of all glycosidic bonds in both compounds.

Plant material. Pyrola virens Schweigg. Canada: British Columbia, Manning Park, Lightening Lake, *Bohm* 1803; U. S. A.: Colorado, Larimer Co., *Bohm* 1805. *Pyrola chlorantha* Swartz. Canada: British Columbia, Mable Lake, *Bohm* 1820. Voucher specimens are deposited at UBC.

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