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FLAVONOIDS FROM *PYROLA ELLIPTICA*

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Key Word Index—*Pyrola elliptica*; Pyrolaceae; dihydroflavonols; flavonoids; phenols; iridoid; dihydrorhamnetin 3-*O*-arabinoside.

Abstract—A new flavonoid, dihydrorhamnetin 3-*O*-β-D-arabinopyranoside, was isolated from *Pyrola elliptica*, together with quercetin 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-α-L-arabinofuranoside, quercetin 3-*O*-β-D-galactopyranoside, rhamnetin 3-*O*-β-D-galactopyranoside, taxifolin 3-*O*-β-D-arabinopyranoside, taxifolin 3-*O*-β-D-xylopyranoside, homoarbutin, isohomoarbutin and monotropein.

INTRODUCTION

As a part of our phytochemical investigation of species used by North American Indians [1, 2], we have investigated *Pyrola elliptica* Nutt. This small herbaceous plant grows in dry woods and is recognisable by its basal rosette of leaves and the fragrance of its flowers [3]. The Mohegan Indians used decoctions of *P. elliptica* as a gargle to cure sores or cankers in the mouth [4]. To our knowledge, this species has never been studied phytochemically. However, phenols, phenolic acids, quinones, flavonoids, an iridoid, tannins and terpenoids have been found in the genus *Pyrola* [5–9]. We report here the isolation and structure elucidation of a novel flavonoid along with six known flavonoids, two phenols and an iridoid.

RESULTS AND DISCUSSION

The ground whole plant of *P. elliptica* was extracted successively with dichloromethane and methanol. The methanol extract was filtered on Sephadex LH-20 gel and further fractionated on silica gel to give compounds **1**–**10**. Analysis by HPLC-UV allowed the identification of the principal classes of compounds from their UV spectra [10]: dihydroflavonols (**1**–**3**), flavonols (**4**–**7**), phenols (**8**, **9**) and an iridoid (**10**).

Compounds **2**–**9** were identified as taxifolin 3-*O*-β-D-arabinopyranoside (**2**), taxifolin 3-*O*-β-D-xylopyranoside (**3**), quercetin 3-*O*-β-D-glucopyranoside (**4**), quercetin 3-*O*-α-L-arabinofuranoside (**5**), quercetin 3-*O*-β-D-galactopyranoside (**6**), rhamnetin 3-*O*-β-D-galactopyranoside (**7**), homoarbutin (**8**), isohomoarbutin (**9**) by comparison of their ^{1}H and ^{13}C NMR data with reported data [11, 12].

The UV spectrum of compound **10** gave a maximum at 240 nm, typical for an iridoid. However, the ^{1}H and ^{13}C NMR chemical shifts of **10** differed from literature data of monotropein at carbons 3, 4, 5 and 11 (Table 1). This compound was therefore obtained as a salt and required passage over ion exchange resin to give the free acid. The corresponding NMR spectral data were then identical with literature values (Table 1) [13].

The UV spectrum of **1** showed a maximum at 292 nm and a shoulder at 330 nm which was typical for flavonones and dihydroflavonols [10]. The thermospray mass spectrum gave a pseudomolecular ion at m/z 451 ($[M+H]^+$). Loss of 132 amu suggested the presence of a pentosyl moiety. In the ^{13}C NMR and DEPT spectra, signals indicative of 8 quaternary carbons, 11 CH, 1 CH_2 and 1 CH_3 were present, consistent with a molecular formula of $C_{21}H_{22}O_{11}$. The chemical shifts of the C-2 (81.3 ppm) and C-3 (75.2 ppm) carbon atoms, were characteristic of dihydroflavonols. The substitution of the A ring was determined by the use of UV shift reagents as well as from the ^{1}H NMR data. The 5-OH was identified by the bathochromic shift (23 nm) observed after addition of $AlCl_3$/HCl to a methanolic solution of **1**. In the ^{1}H NMR spectrum, two doublets at δ 6.09 and 6.08 with a coupling constant of 2.2 Hz, typical of two *meta* coupled protons, were assigned to H-8 and H-6 respec-

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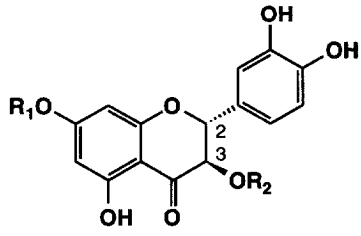
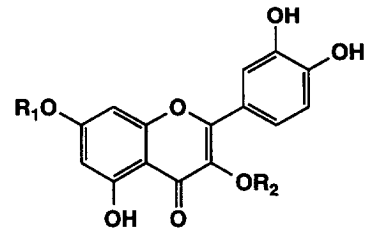
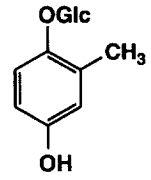




	R_1	R_2
1	CH_3	Ara
2	H	Ara
3	H	Xyl

	R_1	R_2
4	H	Glc
5	H	Ara
6	H	Gal
7	CH_3	Gal



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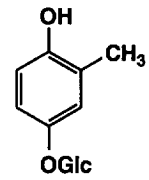

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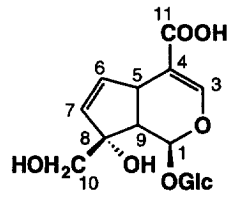

tively. The signals at δ 145.9 and 145.2 ppm in the ^{13}C NMR spectrum were indicative of two oxygenated protons in *ortho* positions on ring **B**. Comparison of ^{13}C NMR data (Table 2) of **1** with taxifolin 3-*O*-arabinoside (**2**) showed that **1** and **2** were similar except for the presence of a methyl group in **1**. The position of the methyl group was determined by comparison of ^{13}C NMR data of these two compounds. A downfield shift (1.1 ppm) of carbon 10 and upfield

Table 1. Comparison of ^{13}C NMR spectral data of compound **10** and monotropein before and after ion exchange on Amberlyst 15 (50 MHz in D_2O, δ ppm)

C	**10**	After ion exchange	Monotropein*
1	94.7	95.2	95.2
3	147.3	152.5	152.4
4	116.2	111.1	111.0
5	39.2	37.9	37.9
6	138.8	138.1	138.0
7	132.1	132.8	132.8
8	85.8	85.7	85.6
9	45.0	44.9	44.8
10	67.5	67.3	67.4
11	175.6	171.3	171.3
1′	98.9	99.1	99.1
2′	73.5	73.5	73.5
3′	76.4	76.5	76.5
4′	70.4	70.4	70.4
5′	77.1	77.2	77.1
6′	61.5	61.6	61.5

Values from Ref. [13].

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (50 MHz in DMSO-d_6O, δ ppm)

C	**1**	**2**
C-2	81.2	81.0
C-3	75.1	74.9
C-4	194.0	193.1
C-5	163.0	163.3
C-6	94.7	96.0
C-7	167.8	167.7
C-8	93.8	95.2
C-9	161.8	161.9
C-10	101.8***	100.8
C-1′	126.4	126.6
C-2′	115.3**	114.5
C-3′	145.8*	145.8*
C-4′	145.1*	145.1*
C-5′	114.5**	115.4
C-6′	118.6	118.6
C-1″	100.5***	100.3
C-2″	71.5	71.5
C-3″	69.8	69.8
C-4″	65.1	65.0
C-5″	62.3	62.0
OMe	55.9	

*, **, *** Signals interchangeable.

Table 3. CD data for compounds **1–3**.

Compound	λ nm ($\Delta\varepsilon$)
1	337 (+1.81), 295 (−6.29), 251 (+1.03), 223 (+3.95), 210 (−2.52)
2	340 (+0.76), 298 (−2.92), 255 (+0.80), 231 (+2.24), 214 (−0.76)
3	338 (+2.09), 295 (−6.34), 253 (+0.87), 223 (+3.69), 210 (−4.20)

shifts of carbons 6 (1.2 ppm) and 8 (1.3 ppm) suggested a methylation of the C-7 hydroxyl moiety. Irradiation of the methoxyl group signal at 3.79 ppm in the ^{1}H NMR spectrum gave a NOE effect on protons 6 and 8 which confirmed the hypothesis of a 7-methoxylated carbon. The sugar was identified from ^{13}C NMR data as an arabinopyranoside [14]. The coupling constant (3.4 Hz) of the anomeric proton at δ 3.98 in the ^{1}H NMR spectrum indicated that it was a β-arabinopyranose [15]. The sugar was positioned on carbon 3, as this carbon was upfield shifted by 4 ppm as compared with the aglycone. Thus **1** is dihydrorhamnetin 3-*O*-β-D-arabinopyranoside.

The CD data of **1–3** are shown in Table 3. Each compound showed five Cotton effects in the order (+), (−), (+), (+), (−) from 360–205 nm, typical for 3-hydroxyflavonones of 2*R*,3*R* configuration [16]. The band at longest wavelength (337–340 nm) has been unequivocally assigned as the n→π* transition of the acetophenone chromophore of **1–3**, and according to Snatzke's helicity rule [17], their heterocyclic ring must adopt a half-chair conformation. Since the 3,4-dihydroxyphenyl group at C-2 and the glycosyl moiety at C-3 were oriented equatorially, the 2*R*,3*R* absolute configuration was deduced for **1–3**.

Chemotaxonomic significance

While dihydroflavonols have not been reported before in the genus *Pyrola*, many flavonols have already been isolated. Rhamnetin 3-*O*-arabinoside and rhamnetin 3-*O*-glucoside have been isolated from *P. virens* and *P. chlorantha* [18] but this is the first time, however, that rhamnetin-3-*O*-glactoside has been found in *Pyrola*. Quercetin 3-*O*-glucoside is known in *P. virens*, *P. chlorantha*, *P.morrisonensis*, *P. decorata* [18, 19] and *P. asarifolia* [20]. Quercetin 3-*O*-galactoside has been obtained from *P. morrisonensis*, *P. incarnata* [19] and *P. asarifolia* [20]. Additional flavonols have been identified in *P. asarifolia* [20] and *P. picta* [21]. Homoarbutin has been isolated from *P. alpina*, *P. incarnata*, *P. nephrophylla* and *P. rotundifolia*, and isohomoarbutin from *P. incarnata*, *P. nephrophylla* and *P. rotundifolia* [22–25]. Monotropein has been reported to occur in all investigated species (*P. media*, *P. renifolia*, *P. incarnata*, *P. japonica*, *P. nephrophylla*, *P. alpina* and *P. secunda*) [26, 27].

EXPERIMENTAL

General

TLC: Silica gel 60F$_{254}$ (Merck), detection UV (254, 366 nm) and Godin reagent. HPLC: Hewlett Packard 1050. UV: Shimadzu UV-106A spectrophotometer. CD: Jobin-Yvon Dichrograph-VI in CH_3CN (conc. 0.76–0.94 mmol l^{-1}; cell length 0.05 cm) at 20°C. ^{1}H and ^{13}C NMR: Varian VXR 200 at 200.06 and 50.03 MHz, respectively, in DMSO-d_6, D_2O; TMS as int. standard for ^{1}H and ^{13}C; multiplicities of ^{13}C were obtained by DEPT experiments. FAB-MS and TSP-MS: Finnigan MAT-TSQ-700 triple stage quadrupole instrument. $[\alpha]_D$: Perkin-Elmer-241 polarimeter.

Plant material

The roots of *P. elliptica* were collected in Saint-Nicolas, Québec (Canada), in October 1995. A voucher specimen is deposited at the Herbier Louis-Marie, Laval University, Québec, Canada.

Extraction and isolation

The dried whole plant (280 g) was ground and extracted at room temp. successively with dichloromethane (1.5l) and MeOH (1.5l) to yield 5.6 g of dichloromethane and 26.5 g of methanol extract.

The methanol extract (15 g) was fractionated by Sephadex LH-20 gel filtration (MeOH–H_2O, 1:1) into 17 fractions (I–XVII). Silica gel CC of fractions II($CHCl_3$–MeOH–H_2O; 9:12:8, using lower phase), V (EtOAc–petrol ether; 1:1), VIII ($CHCl_3$–MeOH–H_2O; 9:12:8, lower phase), IX ($CHCl_3$–MeOH–H_2O; 65:35:5), XI ($CHCl_3$–MeOH–H_2O; 9:12:8, lower phase), XIV ($CHCl_3$–MeOH–H_2O; 65:35:5), XVI ($CHCl_3$–MeOH–H_2O; 9:12:8, lower phase), and XVII ($CHCl_3$–MeOH–H_2O; 65:35:5) gave **10** (100 mg), **8** and **9** (20 mg), **1** (138 mg), **2** (38 mg), **3** (30 mg) and **7** (38 mg), **6** (54 mg), **5** (54 mg) and **4** (5 mg), respectively. Compound **10** was treated by ion exchange chromatography on Amberlyst 15 (Fluka). The purity of the compounds was checked by HPLC on a Nucleosil RP-18 column (7 μm; 250×4 mm i.d., Macherey-Nagel; MeCN–H_2O; 0:100→40:60 +0.05% TFA in 30 min.).

(2R,3R)-Dihydrorhamnetin 3-O-β-D-arabinopyranoside (**1**). $C_{21}H_{22}O_{11}$. UV λ_{max}^{MeOH} nm (log ε): 207 (4.19), 292 (4.48), 330 (sh.); +NaOMe: 210, 291, 348; +NaOAc: 220, 294, 340 (sh.); +AcOHa/H_3BO_3: 220, 293, 340 (sh.); +$AlCl_3$: 209, 316, 387; +$AlCl_3$/HCl: 206, 223, 314, 387; TSP-MS (+ve ion mode) *m/z*: 451 $[M+H]^+$, 319 $[M+H-132]^+$, 301 $[M+H-132-H_2O]^+$; $[\alpha]_D$ −11.7° (MeOH, *c* 1.5); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.85-6.72 (3H, *m*, H-2′, 5′, 6′), 6.09 (1H, *d*, *J* = 2.2 Hz, H-8), 6.08 (1H, *d*, *J* = 2.2 Hz, H-6), 5.39 (1H, *d*, *J* = 8.3 Hz, H-2), 4.71 (1H, *d*, *J* = 8.3 Hz, H-3), 3.98 (1H, *d*, *J* = 3.4 Hz, H-1″), 3.79 (3H, *s*, OMe), 3.69-3.07 (5H, *m*, H-2″–5″);

^{13}C NMR (50 MHz, DMSO-d_6): δ 194.1 (C-4), 167.9 (C-7), 163.1 (C-5), 161.9 (C-9), 145.9 (C-3′*), 145.2 (C-4′*), 126.5 (C-1′), 118.8 (C-6′), 115.4 (C-2′**), 114.6 (C-5′**), 101.9 (C-10), 100.6 (C-1″), 94.8 (C-6), 93.9 (C-8), 81.3 (C-2), 75.2 (C-3), 71.6 (C-2″), 69.9 (C-3″), 65.2 (C-4″), 62.4 (C-5″), 55.9 (OMe).
*, ** assignments interchangeable

Monotropein (**10**). $C_{16}H_{22}O_{11}$. FAB-MS (Glycerol, +ve ion mode) m/z: 413 $[M+Na]^+$; $[\alpha]_D$ −26.0° (H_2O, c 1.10); ^{1}H NMR (200 MHz, DMSO-d_6; salt form): δ 7.12 (1H, d, J = 1.2 Hz, H-3), 6.25 (1H, dd, J = 5.7, 2.7 Hz, H-6), 5.71 (1H, dd, J = 1.7, 5.7 Hz, H-7), 5.60 (1H, d, J = 1.9 Hz, H-1), 3.98-3.27 (9H, m, H-1′-6′, 5, 10), 2.70 (1H, dd, J = 8.7, 2.1 Hz, H-9); ^{1}H NMR (200 MHz, D_2O; free acid) δ 7.40 (1H, s, H-3), 6.21 (1H, dd, J = 5.6, 2.5 Hz, H-6), 5.67 (1H, d, J = 5.8 Hz, H-7), 5.60 (1H, d, J = 1.7 Hz, H-1), 4.14-3.18 (9H, m, H-1′-6′, 5, 10), 2.67 (1H, d, J = 10.5 Hz, H-9); ^{13}C NMR (50 MHz, D_2O): Table 1.

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