# Flavonoids from the Leaves of *Rhododendron brachycarpum*

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*(Received November 25)*

**Abstract** ☐ The flavonoids isolated from the leaves of *Rhododendron brachycarpum* were identified as quercetin, avicularin, quercitrin and hyperin.

**Keywords** ☐ *Rhododendron brachycarpum*, Ericaceae, Flavonoid, Quercetin, Avicularin, Quercitrin, Hyperin, $^{13}$C-NMR.

*Rhododendron brachycarpum* (Ericaceae) is an indeciduous shrub, which has been used in traditional Chinese medicine as antirheumatic agent and diuretic. Since its chemistry has not yet been investigated, we have examined the leaves and here report the isolation of flavonoid components of this plant.

Recrystallization of the ethylacetate fraction from the methanol extract of the leaves afforded the major flavonoid hyperin (4) as yellow needles. The mother liquor was subjected to chromatography using $SiO_2$ and Sephadex LH-20 to yield compounds 1, 2, 3, and 4 in the order of increasing polarity.

Compound 1, mp 315-6°, showed positive $FeCl_3$, Zn+HCl and Mg+HCl tests and identified as quercetin by direct comparisons with an authentic sample (mp, TLC, UV and MS).

Compound 2, mp 220-1°, compound 3, mp 178-180° and compound 4, mp 253-4°, showed positive Mg+HCl, Zn+HCl and Molisch tests. Acid hydrolysis of each compound afforded as the aglycone, quercetin, mp 315-6° and as the sugar, L-arabinose from compound 2, L-rhamnose from compound 3 and D-galactose from compound 4. The $^1$H-NMR spectra of each compound and its acetate showed only one anomeric proton signal, indicating the presence of one mole of sugar in each compound.

The UV spectrum of each compound, exhibiting band I peak at 355-360 nm, was very similar to those reported for a number of 3-hydroxyl substituted flavonols (1). A bathochromic shift of band I in the presence of $AlCl_3$ or $AlCl_3$+HCl and of band II in the presence of NaOAc indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. And also a bathochromic shift with NaOMe, without a decrease in intensity, showed the presence of a free 4′-hydroxyl group. It was, thus, suggested that the sugar might be attached to 3-hydroxyl group.

This suggestion was supported by permethylation of each compound according to Brimacombe's method (2) followed by methanolysis, which afforded 5, 7, 3′, 4′-tetra-O-methyl quercetin, mp 193-195°. The $^{13}$C-NMR spectrum (Table I) of each compound confirmed this suggestion. The configuration and conformation of sugar moiety was determined not only by the J value of the anomeric proton signal (Experimental) but also by application of the method of comparing molecular rotation described by Kovalev and Litvinenko (3) (Table II).

Compound 2, 3 and 4 were, therefore, identified as quercetin 3-O-$\alpha$-L-arabinofuranoside (avicularin), quercetin 3-O-$\alpha$-L-rhamnopyranoside (quercitrin) and quercetin 3-O-$\beta$-D-galactopyranoside (hyperin), respectively.

## EXPERIMENTAL METHODS

The mps were taken on a Thomas Hoover

**Table I.** $^{13}$C-NMR chemical shift of compound 2, 3 and 4.

| Carbon No. | 2 | 3 | 4 |
|---|---|---|---|
| 2 | 156.8 | 157.2 | 156.3 |
| 3 | 133.3 | 134.2 | 133.5 |
| 4 | 177.7 | 177.7 | 177.5 |
| 5 | 161.2 | 161.3 | 161.2 |
| 6 | 98.5 | 98.6 | 98.6 |
| 7 | 164.1 | 164.1 | 164.1 |
| 8 | 93.4 | 93.5 | 93.4 |
| 9 | 156.2 | 156.4 | 156.3 |
| 10 | 103.9 | 103.9 | 104.1 |
| 1′ | 120.9 | 120.7 | 121.1 |
| 2′ | 115.4 | 115.4 | 115.1 |
| 3′ | 145.0 | 145.1 | 144.7 |
| 4′ | 148.3 | 148.3 | 148.4 |
| 5′ | 115.4 | 115.6 | 115.9 |
| 6′ | 121.6 | 121.0 | 121.9 |
| 1″ | 107.8 | 101.7 | 101.9 |
| 2″ | 82.0 | 70.0[a] | 71.2 |
| 3″ | 76.9 | 70.3[a] | 73.4 |
| 4″ | 85.9 | 71.2 | 67.9 |
| 5″ | 60.6 | 70.5[a] | 75.7 |
| 6″ | | 17.4 | 60.2 |

a; assignments may be reversed

6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Varian Techtron Model 635 spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. The $^1$H-NMR (80 MHz) was recorded with a Varian FT-80A in DMSO-$d_6$ (free) and $CDCl_3$ (acetate) containing TMS as an internal standard and chemical shifts are given as $\delta$ (ppm). $^{13}$C-NMR (100 MHz) was recorded with a Bruker WH-400 instrument in the same solvent using the same internal standard and chemical shift are given as $\delta$ (ppm). Mass spectra were taken a Hewlett-Packard 5985B GC/MS spectrometer operating at 70eV. Optical rotations were measured on Rudolph Autopol® III automatic polarimeter.

***Isolation of flavonoids***

Powdered leaves of *R. brachycarpum* (1.4 kg) was refluxed with MeOH. The MeOH extract (130 g) was partitioned with hexane, $CHCl_3$, ethylacetate and butanol successively. The ethylacetate extract (31g) was recrystallized from MeOH to give hyperin (4) as yellow needles. The mother liquor was subjected to chromatography using $SiO_2$ ($CHCl_3$ : MeOH: 7% HAC=25 : 9 : 5, lower phase) and Sephadex LH-20(MeOH) columns to yield quercetin (1), avicularin (2), quercitrin (3) and hyperin (4) in the order of elution.

***Quercetin*** (1)

mp 315-6°, UV $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$); 258(3.88), 305(sh, 3.50), 375(3.86); $\lambda_{max}^{MeOH+NaOMe}$ 248(sh, 3.66), 335(3.84), 420(dec, 3.10); $\lambda_{max}^{MeOH+AlCl_3}$ 275(3.88), 340(sh, 3.10), 460(3.97); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270(3.86), 307(sh, 3.28), 365(3.44), 435(3.88); $\lambda_{max}^{MeOH+NaOAc}$ 260(sh, 3.76), 278(3.77), 328(3.55), 388(3.79); $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 243(3.90), 285(sh, 3.33), 372(3.89) IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400-3200(OH), 1670(C=O), 1610,1500(C=C); MS, m/z(rel. int.); 302($M^+$, 100), 153(RDA fragment with A ring+H, 22.6), 137(RDA fragment with B ring, 30.9).

***Avicularin*** (2)

mp 220-1°, $[\alpha]_D^{25}$ −150° (c=0.1, MeOH); UV $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$); 258(4.16), 268(sh, 4.09), 296(sh, 3.76), 358(4.07); $\lambda_{max}^{MeOH+NaOMe}$ 272(4.23), 331(3.81), 405(4.17); $\lambda_{max}^{MeOH+AlCl_3}$ 272(4.21), 305(sh, 3.72), 440(4.22); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270(4.22), 302(3.86), 365(4.04), 401(4.04); $\lambda_{max}^{MeOH+NaOAc}$ 258(sh, 4.11), 270(4.16), 325(3.87), 372(4.01); $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 261(4.27), 293(3.74), 380(4.12); $^1$H-NMR(DMSO-$d_6$, TMS) $\delta$ 12.6(1H, brs, $C_5$-OH), 7.55(1H, dd, J=2 and 8,

**Tabel II. $[M]_D$ of flavonoid glycosides and related substances(3).**

| Compound | $[M]_D$ | $K_D$ | $[M]_D \cdot K_D$ |
|---|---|---|---|
| 2 | −651.0 | 0.52 | −338.5 |
| 3 | −483.4 | 0.54 | −261.3 |
| 4 | −324.8 | 0.55 | −178.6 |
| phenyl α-L-arabinopyranoside | + 13.5 | 1.00 | + 13.5 |
| phenyl β-L-arabinopyranoside | +540.0 | 1.00 | +540.0 |
| phenyl α-L-arabinofuranoside | −359.0 | 1.00 | −359.0 |
| phenyl β-L-arabinofuranoside | − 59.0 | 1.00 | − 59.0 |
| phenyl α-L-rhamnopyranoside | −254.0 | 1.00 | −254.0 |
| phenyl β-L-rhamnopyranoside | +210.0 | 1.00 | +210.0 |
| phenyl α-L-rhamnofuranoside | −410.0 | 1.00 | −410.0 |
| phenyl β-L-rhamnofuranoside | − 90.0 | 1.00 | − 90.0 |
| phenyl α-D-galactopyranoside | +555.0 | 1.00 | +555.0 |
| phenyl β-D-galactopyranoside | −110.0 | 1.00 | −110.0 |
| phenyl α-D-galactofuranoside | +408.0 | 1.00 | +408.0 |
| phenyl β-D-galactofuranoside | −379.0 | 1.00 | −379.0 |

H-6′), 7.49(1H, d, J=2, H-2′), 6.85(1H, d, J=8, H-5′), 6.40(1H, d, J=2, H-8), 6.20(1H, d, J=2, H-6), 5.58(1H, d, J=1, anomeric); IR $\nu_{max}^{KBr}$ ; $cm^{-1}$ 3300-3200(OH), 1642(C=O), 1600, 1493 (C=C), 1354, 1190, 1105, 1060, 1038, 995, 937, 918, 818, 792.

***Acid hydrolysis of 2***

Twenty mg of 2 was refluxed with 5% $H_2SO_4$ (50m*l*) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to afford quercetin as yellow needles, mp 315-6° . It was confirmed by direct comparisons with an authentic sample (TLC, mmp and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated. L-arabinose was identified by TLC (precoated cellulose, pyridine: ethylacetate: HOAc: $H_2O$=36 : 36 : 7 : 21 : , Rf 0.55).

***Permethylation of 2, 3, and 4 followed by acid hydrolysis***

Fifty mg of samples was permethylated using Brimacombe's method (2) and followed by the usual work up. Acid hydrolysis of the crude permethylether with 5% $H_2SO_4$ in 50% dioxane under reflux for 3 hr was followed by the usual work up. Crystallization of the aglycone from MeOH gave 5, 7, 3′, 4′-tetra-O-methyl quercetin, mp 193-5° , which was confirmed by direct comparisons with an authentic sample (TLC, mmp and UV).

***Acetylation of 2***

A sample (30 mg) in pyridine and $Ac_2O$ (1m*l* each) was allowed to stand at room temperature overnight. The reaction mixture was poured into crushed ice and filtered. The precipitate was crystallized from $CHCl_3$-hexane to give amorphous white powder (29 mg), mp 83-5° .

IR $\nu_{max}^{KBr}$ $cm^{-1}$ ; 1775, 1740, 1200(acetate); $^1$H-NMR($CDCl_3$, TMS) $\delta$ ; 7.84(1H, dd, J=2 and 7.5, H-6′), 7.76(1H, d, J=2, H-2′), 7.34(1H, d, J=7.5, H-5′), 7.30(1H, d, J=2, H-8), 6.84(1H, d, J=2, H-6), 5.78(1H, s, H-1″), 5.47(1H, d, J=2, H-2″), 4.97(1H, dd, J=2 and 5.5, H-3″), 4.10(2H, t-like, H-5″), 3.65—3.85(1H, m, H-4″), 2.42(3H, s, -OAc), 2.31(9H, s, 3×-OAc), 2.10(3H, s, -OAc), 2.09(3H, s, -OAc), 2.01(3H, s, -OAc).

***Quercitrin (3)***

mp 178-180° , $[\alpha]_D^{25}$ -108° (c=0.1, MeOH); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$); 260(4.15), 305(sh, 3.80), 355(4.02) ; $\lambda_{max}^{MeOH+NaOMe}$ 275(4.24), 332(3.86), 400(4.11) ; $\lambda_{max}^{MeOH+AlCl_3}$ 278(4.24), 305(sh, 3.71), 440(4.16) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 275(4.16), 305 (sh, 3.75), 360(3.88), 405(3.91) ; $\lambda_{max}^{MeOH+NaOAc}$ 275(4.17), 325(sh, 3.88), 370(3.95) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 265(4.24), 300(sh, 3.77), 370(4.06) ; $^1$H-NMR(DMSO-$d_6$, TMS) $\delta$ ; 12.6(1H, brs, $C_5$-OH), 7.15(1H, d, J=2, H-2′), 7.11(1H, dd, J=2 and 8.5, H-6′), 6.72(1H, d, J=8.5, H-5′), 6.23(1H, d, J=2, H-8), 6.05(1H, d, J=2, H-6), 5.11(1H, s, anomeric), 0.80(3H, d, J=6, Me of rhamnose).

***Acid hydrolysis of 3***

Ten mg of 3 was refluxed with 5% $H_2SO_4$ (30 m*l*) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to afford quercetin as yellow needles, mp 315-6° . It was confirmed by direct comparisons with an authentic sample (TLC, mmp and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated.

L-rhamnose was identified by TLC (precoated cellulose, pyridine: ethylacetate: HOAc: $H_2O$=36 : 36 : 7 : 21, Rf 0.65).

***Hyperin (4)***

mp 253-4° , $[\alpha]_D^{25}$ -70° (c=0.1, MeOH), IR $\nu_{max}^{KBr}$ $cm^{-1}$ ; 3200(OH), 1650(C=O), 1600, 1540, 1500 (C=C), 1075, 1050(C-O), 1015, 990, 928, 880, 855, 818, 785 ; UV $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$); 256(4.10), 268(sh, 4.00), 295(sh, 3.69), 360(4.02) ; $\lambda_{max}^{MeOH+NaOMe}$ 272(4.15), 330(3.73), 410(4.11) ; $\lambda_{max}^{MeOH+AlCl_3}$ 272(4.16), 302(3.64), 333(3.50), 431(4.14) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 268(4.14), 300(3.76), 360(3.94), 398(3.96) ; $\lambda_{max}^{MeOH+NaOAc}$ 272(4.10), 325(3.81), 372(3.95) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 260(4.16), 296(3.60), 380(4.06) ; $^1$H-NMR(DMSO-$d_6$, TMS) $\delta$ ; 12.6(1H, brs, OH), 7.66(1H, dd, J=2 and 8.5, H-6′), 7.53(1H, d, J=2, H-2′), 6.81(1H, d, J=8.5, H-5′), 6.40(1H, d, J=2, H-8), 6.19(1H, d, J=2, H-6), 5.36(1H, d, J=7, anomeric).

***Acid hydrolysis of 4***

Twenty mg of 4 was refluxed with 5% $H_2SO_4$ (50m*l*) for 3 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to give quercetin as yellow needles, mp 315-6° . It was confirmed by direct comparisons with authentic sample (TLC, mmp and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated *in vacuo*. D-galactose was identified by TLC (precoated cellulose, pyridine: ethylacetate: HOAc: $H_2O$=36 : 36 : 7 : 21, Rf=0.45).

***Acetylation of 4***

A sample (50 mg) in pyridine and $Ac_2O$ (1m*l* each) was allowed to stand at room temperature overnight. The reaction mixture was poured into crushed ice and filtered. The precipitate was crystallized from MeOH-$H_2O$ to give amorphous white powder (55 mg).

IR $\nu_{max}^{KBr}$ $cm^{-1}$ ; 1740, 1200(acetate); $^1$H

-NMR($CDCl_3$, TMS) $\delta$: 7.88(1H, dd, J=2 and 8, H-6′), 7.27(1H, d, J=2, H-2′), 7.25(1H, d, J=8, H-5′), 6.61(1H, d, J=2, H-8), 6.46(1H, d, J=2, H-6), 5.38(1H, d, J=8, anomeric), 2.41(3H, s, -OAc), 2.32(9H, s, 3×-OAc), 2.11(6H, s, 2×-OAc), 1.99(3H, s, -OAc), 1.89(3H, s, -OAc).

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