Natural Product Sciences
5(1) : 60~63 (1999)

Phenolic Compounds from the Leaves of *Vaccinium koreanum*

Wha Kyun Joo, Sang Hack Doh, Soon Sung Lim[1], Kuk Hyun Shin[1]* and Won Sick Woo[1]

College of Pharmacy, Dongduk Women's University, Seoul 136-714, Korea
[1]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Abstract – From the leaves of *Vaccinium koreanum*, isoquercitrin, quercetin, avicularin, 2'-*O*-caffeoylarbutin and arbutin were isolated and their structures were elucidated by spectral data.
Key words – *Vaccinium koreanum*; Ericaceae; isoquercitrin; 2'-*O*-caffeoylarbutin; arbutin; quercetin; avicularin

Introduction

Vaccinium koreanum (Ericaceae) is one of Korean-indigenous small deciduous shrub widely distributed in sunny areas of mountains in Korean peninsula.

The plants of genus *Vaccinium* have been utilized as astringent, tonic and traditional folk medicine for the treatment of diarrhea, dysentery, gastrointestinal inflammations, mouth infections, haemorrhoids and urinary infections in many European countries. They were also used in local applications for inflammation of eyes, mouth and burns, and from the leaves of the genus *Vaccinium* several compounds have been isolated, which have different chemical classes including flavonoids, vitamins, sugars, pectins, organic acids, iridoids, and terpenes (Morazoni *et al.*, 1996).

Despite a lot chemical and pharmacological researches on the genus *Vaccinium* have been reported, almost no research has been performed on *V. koreanum*. In the course of a screening program to evaluate aldose reductase inhibitors from medicinal plants, we found that the methanol extracts from the leaves of *V. koreanum* exhibited a potent inhibition on aldose reductase (Shin *et al.*, 1995). Bioassay guided fractionation of the MeOH extract led to the isolation of three flavonoids and two phenolic compounds.

This study deals with the structure elucidation of these phenolic compounds isolated from this plant.

Experimental

Plant materials

The leaves of *V. koreanum* were collected at Mt. Wall-ak, Chungbuk province around mid-September, 1995 and then dried under shade. The botanical identification was performed by Prof. Cho, Sunhaeng and a voucher specimen has been deposited in the herbarium of Natural Products Research Institute, Seoul National University.

Reagents and Instruments

For column packing materials Kieselgel 60 (70-230 mesh ASTM, Merck, Art 7734), Kieselgel 63 (230-400 mesh ASTM, Merck, Art 9385), and Sephadex LH-20 (bead size 25-100 μm and Pharmacia Fine Chemicals) were used. For TLC, Kieselgel $60F_{254}$ (Precoated, Merck, Art, 5715) and cellulose plate (Precoated, Merck, Art 5552) were employed.

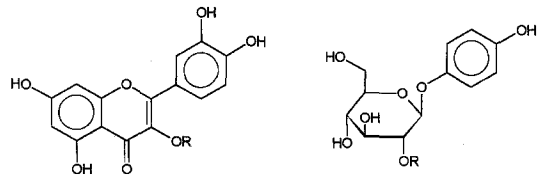
The melting points were taken on a Mitamura-Riken apparatus (uncorrected). UV absorption spectra were measured by Gilford 2600 UV-VIS spectrophotometer, FT-IR by JASCO FT/IR-5300 spectrophotometer. EI-MS and FAB-MS were taken with a JEOL JMS-AX505 WA and JEOL JMS-AX505 WA. ^{1}H- and ^{13}C-NMR were recorded with a Varian Gemini 2000.

Extraction, fractionation and isolation

The air dried leaves of *V. koreanum* (2 kg), were extracted repeatedly 3 times refluxing with MeOH for 3 hours. The total filtrate was concentrated to dryness *in vacuo* at 40°C to obtain MeOH extract (150 g) which was partitioned with hexane (96.5 g), $CHCl_3$ (0.6 g), EtOAc (9.7 g) and *n*-BuOH (10.8 g), successively.

The EtOAc soluble fraction (9 g) was subjected to silica gel column chromatography by eluting with MeOH-CH_2Cl_2 (gradient 5~9%) to obtain 9 subfractions. Subsequently, gel filtration of subfraction 3 on

*Author for correspondence



I R = Glc
III R = H
IV R = Ara

II R = *trans*-Caffeoyl-
V R = H

Fig. 1. Structures of flavonoids and phenolic compounds.

Sephadex LH-20, eluting with MeOH, MeOH-CH_3COCH_3 (1 : 1) led to the isolation of compounds I (50 mg) and II (4.2 mg).

The *n*-BuOH soluble fraction (10 g) was also chromatographed on a silica gel column eluting with EtOAc : MeOH gradient to obtain 8 subfractions. The subfractions 3 and 4 were further chromatographed on a Sephadex LH-20 eluting with MeOH, MeOH-CH_3COCH_3 (3 : 1) to obtain compounds III (25 mg), IV (5 mg) and V (10 mg).

Compound I–Yellow powder (recrystallized from MeOH); m.p. 196~198°C; R_f (TLC): 0.26 ($CHCl_3$: MeOH : H_2O = 65 : 20 : 3); Molish test: Positive; IR ν_{max} cm^{-1} (KBr): 3418 (-OH); 1655 (α,β-unsaturated C=O); 1606, 1508 (aromatic, C=C); 1203, 1084 (glycosidic, C-O). Positive ion FAB-MS [*m/z*]: 465 (M+H)$^+$, 487 (M+Na)$^+$, 303 [M+H-($C_6H_{10}O_5$)]$^+$ (4.1), 273 [M-HCO]$^+$ (6.1), 245 [M-(CO+HCO)]$^+$ (6.9). UV data, see Table 2; ^{1}H-NMR (300MHz, MeOH-d_6) δ: 7.84 (1H, d, *J*=2.1 Hz, H-2'), 7.57 (1H, dd, *J*=2.1, 8.4 Hz, H-6'), 6.88 (1H, d, *J*=8.4 Hz, H-5'), 6.38 (1H, d, *J*=1.8 Hz, H-8), 6.19 (1H, d, *J*=1.8 Hz, H-6), 5.15 (1H, d, *J*=7.5 Hz, anomeric H-1"), 3.71 (1H, dd, *J*=5.1, 12 Hz, Glc-H-6"), 3.41 (1H, dd, *J*=2.4, 11.4 Hz, Glc-H-6"), 3.12~3.34 (4H, m, Glc H-2",3",4",5"). ^{13}C-NMR data, see Table 3.

Compound II–Amorphous powder (recrystallized from MeOH); m.p. 292~295°C; R_f (TLC): 0.42 ($CHCl_3$: MeOH : H_2O = 65 : 20 : 3); IR ν_{max} cm^{-1} (KBr): 3423 (-OH); 1736(ester, -COO-); 1637 (α,β-unsaturated C=O); 1510, 1460 (aromatic, C=C); 1265, 1072 (aromatic, C-O). Positive ion FAB-MS [*m/z*]: 435 [M+H]$^+$. ^{1}H-NMR (300 MHz, MeOH-d_4) δ: 7.61 (1H, d, *J*=15.9 Hz, cinnamoyl H-8"): 7.08 (1H, d, *J*=1.8 Hz, H-2"): 6.95 (1H, dd, *J*=1.8, 8.1 Hz, H-6"); 6.86 (2H, d, *J*=8.7 Hz, H-2,6); 6.77 (1H, d, *J*=8.1 Hz, H-5"); 6.65 (2H, d, *J*=8.7 Hz, H-3,5); 6.33 (1H, d, *J*=15.9 Hz, cinnamoyl H-7"): 5.04 (1H, d,

Table 1. ^{13}C-NMR chemical shifts of compounds II, V, and related compounds

Carbon No.	Compound II (2'-*O*-Caffeoylarbutin)[a]	Compound V (Arbutin)[a]	2'-*O*-*p*-Coumaroyl arbutin[b]	4'-*O*-Caffeoyl arbutin[b]	6'-*O*-*p*-Coumaroyl arbutin[b]
1	153.9	153.8	154.1	153.9	144.0
2	119.4	119.3	119.5	119.5	119.7
3	116.8	116.6	116.8	116.7	116.7
4	152.4	152.4	152.3	153.4	152.4
5	116.8	116.6	116.8	116.7	116.7
6	119.4	119.3	119.5	119.5	119.7
sugar 1'	102.5	103.6	102.2	103.7	103.8
2'	75.1	75.0	75.2	75.2	75.0
3'	76.0	78.0	76.2	76.3	78.0
4'	72.0	71.3	71.6	72.3	71.9
5'	78.0	78.0	78.3	75.3	75.6
6'	62.5	62.5	62.6	62.3	64.7
1"	127.5		127.2	127.7	127.2
2"	115.3		131.3	114.8	131.3
3"	148.0		116.9	149.8	117.0
4"	147.0		161.3	146.9	161.5
5"	116.7		116.9	116.6	117.0
6"	123.0		131.3	123.1	131.3
7"	147.0		147.1	147.7	146.9
8"	115.0		115.1	114.8	115.0
9"	168.0		168.5	168.6	169.0

[a] 75 MHz, CD_3OD
[b] 100 MHz, CD_3OD (Data from Fridrich, H., 1961).

J=8.4 Hz, H-1', anomeric proton); 3.95 (1H, dd, J=1.8, 12 Hz, H-6'b); 3.75 (1H, dd, J=5.3, 12 Hz, H-6'a); 3.60~3.69 (1H, m, H-5'); 3.46~3.52 (3H, m, H-2',3',4'). ^{13}C-NMR data see Table 1.

Compound III–Yellow powder (recrystallized from MeOH); m.p. 314~317°C; R_f (TLC): 0.54 ($CHCl_3$: MeOH : H_2O = 65 : 20 : 3); Molish test: Negative; IR ν_{max} cm^{-1} (KBr): 3342 (-OH); 1658 (α,β-unsaturated C=O); 1616, 1560 (aromatic, C=C); 1246 (aromatic, C-O). UV data, see Table 2; EI-MS (70 eV) *m/z*] (rel. int. %): 302 $[M]^+$ (93.0), 301 $[M-H]^+$ (31.1), 274 $[M-CO]^+$ (10.7), 273 $[M-HCO]^+$ (16.0), 245 $[M-(CO+HCO)]^+$ (27.5), 153 $[A_1+H]^+$ (59.0), 152 $[A_1]$ (10.1), 137 $[B_2]^+$ (4.9), 134 $[B_1]^+$ (18.5), 124 $[A_1-CO]^+$ (15.2), 123 $[A_1-CHO]^+$ (9.0), 109 $[B_2^+-CO]^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.50 (IH, brs, 5-OH): 7.67 (IH, d, J=2.1 Hz, H-2'); 7.52 (IH, dd, J=2.1, 8.4 Hz, H-6'); 6.88 (IH, d, J=8.4 Hz, H-5'); 6.39 (IH, d, J=1.8 Hz, H-8); 6.18 (IH, d, J=1.8 Hz, H-6). ^{13}C-NMR data see Table 3.

Compound IV–Yellow powder (recrystallized from MeOH); m.p. 216~218°C; R_f (TLC): 0.47 ($CHCl_3$: MeOH : H_2O = 65 : 20 : 3); Molish test: Positive; IR ν_{max} cm^{-1} (KBr): 3423 (-OH); 1655 (α,β-unsaturated C=O); 1606, 1508 (aromatic, C=C); 1197, 1114 (glycosidic, C-O). Positive ion FAB-MS [*m/z*]: 435 $(M+H)^+$. UV data, see Table 2; ^{1}H-NMR (300 MHz, MeOH-d_4) δ: 7.53 (1H, d, J=2.1 Hz, H-2'); 7.48 (1H, dd, J=2.1, 8.1 Hz, H-6'); 6.91 (1H, d, J=8.4 Hz H-5'); 6.40 (1H, d, J=2.1 Hz, H-8); 6.21 (1H, d, J=2.1 Hz, H-6) ; 5.47 (1H, brs, anomeric, H-1"); 4.33 (1H, dd, J=0.9, 3.0 Hz, arab. H-2"); 3.91 (1H, dd, J=3.0, 5.1 Hz, arab. H-5"b): 3.85 (1H, dd, J=3.9, 5.1 Hz, arb. H-5"a); 3.45~3.50 (2H, brs, Arab. H-3",4"). ^{13}C-NMR data see Table 3.

Compound V–Needles (recrystallized from MeOH); m.p. 199~200°C; R_f (TLC): 0.31 ($CHCl_3$: MeOH : H_2O = 65 : 20 : 3); IR ν_{max} cm^{-1} (KBr): 3423 (-OH); 1637 (α,β-unsaturated C=O); 1510, 1460 (aromatic, C=C); 1265, 1072 (aromatic, C-O). Positive ion FAB-MS [*m/z*]: 273 $[M+H]^+$. ^{1}H-NMR (300 MHz, MeOH-d_4) δ: 5.04 (1H, d, J=8.4 Hz, H-1', anomeric proton); 3.95 (1H, dd, J=1.8, 12 Hz, H-6'b); 3.75 (1H, dd, J=5.3, 12 Hz, H-6'a); 3.60~3.69 (1H, m, H-5'); 3.46~3.52 (3H, m, H-2',3',4'). ^{13}C-NMR data see Table 1.

Identification of sugar of compounds I, II, IV and V

Compounds I, II, IV and V were applied on silica gel TLC and left in an HCl atmosphere at room temperature for 1 hr. HCl vapor was eliminated under hot ventilation and then authentic sugar samples were applied to the plate. The plate was developed with the solvent system of $CHCl_3$-MeOH-H_2O (26 : 14 : 5) and spots were detected by spraying with aniline hydrogenphthalate reagent followed by heating. Glucose was detected for sugar from compounds I, II and V, whereas arabinose from compound IV (Woo *et al.*, 1992).

Table 2. UV spectral data for compounds I, III and IV (λ_{max} nm, log ε)

Solvent	I	III	IV
MeOH	273(4.52)	253(4.30)	270(4.52)
	304(4.30, sh)	305(3.91, sh)	301(3.60, sh)
	375(3.51)	373(4.30)	350(3.55)
+ NaOH	275(4.83)	253(4.26)	275(4.68)
	310(4.35)	270(4.16, sh)	313(3.67, sh)
	410(3.50)	380(4.17)	375(3.52)
+ NaOAc	279(4.69)	270(4.24)	280(4.78)
	310(4.12)	323(4.01)	301(3.92)
	410(3.52)	388(4.28)	378(3.61)
+ NaOAc	272(4.59)	245(4.53)	275(4.71)
+ H_3BO_3	299(3.85)	334(4.24, sh)	302(3.49, sh)
	410(3.53)	388(4.51)	380(3.85)
+ $AlCl_3$	280(4.80)	253(4.36)	284(4.91)
	301(3.83)	351(3.86, sh)	302(3.55)
	453(3.51)	395(4.01)	430(3.73)
+ $AlCl_3$	278(4.59)	250(4.35)	275(4.70)
+ HCl	301(3.86)	320(3.75, sh)	301(3.52)
	415(3.52)	365(3.95)	405(3.50)
		428(4.38)	

Results and Discussion

Activity-guided fractionation and repeated column chromatography of a MeOH extract from the leaves of *V. koreanum* led to the isolation of five active principles. Compounds I, III, IV and V were identified as isoquercitrin (Agrawal, 1989), quercetin (Harborne, 1975), avicularin (Pachaly and Klein, 1987) and arbutin (Machida *et al.*, 1993), respectively, by comparison of their physical and spectroscopic data in the literature (UV, positive ion FABMS, ^{1}H and ^{13}C-NMR) and confirmed by co-TLC with authentic samples.

Compound II showed the molecular ion peak as a cationized cluster ion $[M+H]^+$ at *m/z* 435 in its positive ion FAB-MS spectrum and displayed hydroxyl (3,423 cm^{-1}), ester (1,736 cm^{-1}), aromatic (1,637, 1,510 cm^{-1}) and glycosidic bond (1,072 cm^{-1}) absorptions in its IR spectrum.

The ^{1}H-NMR spectrum of this compound showed

Table 3. ^{13}C-NMR chemical shifts for compounds I, III and IV[a)]

Carbon No.	I[b)]	III[c)]	IV[b)]
2	148.03	146.92	146.56
3	135.90	135.86	135.09
4	179.74	176.03	180.22
5	163.23	160.87	163.32
6	96.15	98.23	99.98
7	166.66	164.07	166.24
8	94.86	93.39	94.85
9	158.93	156.28	158.78
10	105.32	103.05	105.74
1'	123.04	122.03	123.26
2'	116.20	115.13	116.58
3'	146.00	145.19	146.53
4'	150.14	147.84	148.47
5'	117.87	115.68	116.97
6'	122.09	120.06	123.10
Sugar 1"	100.23		109.67
2"	73.18		83.14
3"	75.11		78.74
4"	70.02		84.41
5"	77.20		62.58
6"	61.89		

[a)]The carbonyl resonances of C-4 absorbed at about 4 ppm lower field in compound I and IV which indicated that glycosylation site was 3-hydroxyl position.
[b)]MeOH-d_4 (75 MHz)
[c)]MeOH-d_4 (75 MHz)

the *ortho*-coupled doublets each of two protons with a *J* value of 8.7 Hz at δ 6.86 and 6.65, indicating the presence of a 1,4-disubstituted benzene ring and an *ortho*-coupled doublet of one proton with a *J* value of 8.1 Hz at 6.77, a *meta*-coupled doublets of one proton with a *J* value of 1.8 Hz at δ 7.08 and a double of doublets of one proton with *J* values of 8.1 and 1.8 Hz centered at δ 6.95, indicating the presence of a 1,2,4-trisubstituted benzene ring. Appearance of two *trans*-olefinic protons with a *J* value of 15.9 Hz at δ 7.61 and 6.33 in ^{1}H-NMR and a carboxylic carbon signal at δ 168.0 in ^{13}C-NMR indicated the presence of caffeoyl group.

Therefore, compound II was suggested to be a caffeoyl ester of arbutin. The attachment point of acyl group in compound II was determined by the ^{13}C-NMR spectral analysis. A comparison of the ^{13}C-NMR spectrum of compound II with that of arbutin (compound V) revealed that signals due to C-1 and C-3 of glucose were shifted by -1.1 and -2.0 ppm, respectively, while other resonances appeared almost unshifted. It was, therefore, suggested that the acyl group was bonded to the C-2 position of glucose. Moreover, in comparison of ^{13}C-NMR data of compound II with those of several reported acyl esters of arbutin (Table 1), the chemical shifts due to the sugar moiety of compound II were superimposable with those of 2'-*O*-*p*-coumaroylarbutin. From these evidences, compound II was identified as 2'-*O*-caffeoylarbutin. The occurrence of 2'-*O*-caffeoylarbutin in *Vaccinium* species has already been reported (Fridrich, 1961), but this is the second report of the natural occurrence of the compound.

All of five compounds were first isolated from this plant.

A detailed biological data on aldose reductase inhibitory activity of the isolates will be reported elsewhere (Jmanuscript in preparation).

References

Agrawal P. K., ^{13}C-NMR of flavonoids. *Elsevier,* 154, (1989).

Ballinger, W. E., Maness, E. P. and Ballington, J. R., Anthocyanin and total flavonol content of *V. stamineum* L. *Fruit. Scientia Horticulture,* **15**, 173-178 (1981).

Fridrich, H., From Leaves of cowberry (*Vaccinium vitis idea* L). *Pharmazie.* **15**, 650 (1960).

Fridrich, H., *Naturwissenschaften,* **48**, 304 (1961).

Harborne, J. B., The flavonoids *Advances in Research.* Chapman & Hall, London, 620 (1994).

Harborne, J. B., The flavonoids *Advances in Research.* Chapman & Hall, London, 452-455 (1994).

Harborne, J. B., Mabry, T. J. and Mabry, H., The flavonoids Part 1. *Academic Press* (1975).

Machida, K. and Kikuchi, M., Studies on the constituents of *Viburnum* species on phenolic glycosides from the leaves of *Viburnum wrightii* MIQ. *Chem. Pharm. Bull.,* **41**, 248-251 (1993).

Morazzoni, P., Bonbrdelli, E., *V. myrtillus* L. *Fitoterapia,* L, 4 (1996).

Pachaly, P. and Klein, M., Inhaltsstoffe von *Andromeda polifolia, Planta Med.,* 442-444 (1987).

Shin, K. H., Lim, S. S., Moon, H. I. and Cho, S. H., The effects of leaves of *V. koreanum* on aldose reductase.: presented at the annual meeting of the Korean Society of Pharmacognosy (1995).

Woo, E. H., Woo, W. S., Chmurny, G. N. and Hilton, B. D., Melandrioside A., A saponin from *Melandrium firmum, J. Nat. Prod.,* **55**(6), 786-794 (1992).

(Accepted March 9, 1999)