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Chemical Study on the Leaf of *Prunus davidiana*

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ABSTRACT

From the leaf of *Prunus davidiana*, naringenin and its glucoside, kaempferol and its glucoside, kaempferide glucoside, quercetin glucoside and d-catechin were isolated.

Key words : *Prunus davidiana, Rosaceae*, flavonoids.

Introduction

The leaf of *Prunus davidiana* has been used as folk medicine to treat for neuritis and rheumatism in Korea. In the course of screening of Korean folk medicine for hypolipemic and antidiabetic activities it was found that the methanol extract from this plant showed the significant activity. We report the isolation of flavonoid components from the leaf of this plant.

Silica gel and Sephadex LH-20 column chromatography of the ethylacetate soluble portion of the methanol extract yielded 7 compounds (1-7) as shown in Chart 1. Compounds 1, 2 and 5 were readily elucidated as naringenin, kaempferol and d-catechin respectively, by comparison of reported spectroscopic data(Mabry 등, 1970; Woo 등, 1983; Son 등, 1989) and finally confirmed by comparison with authentic samples. Compounds 3, 4, 6 and 7 showed positive results in Molisch tests besides flavonoid color reactions and showed absorpthion bands for glycoside linkage(1000-1100cm^{-1}) in their IR spectra. On acid hydrolysis all compounds gave glucose as the sugar and kaempferide from 3, kaempferol from 4, naringenin from 6 and quercetin from 7 as the aglycone.

The ^{1}H-NMR spectrum of each compond showed only one anomeric proton signal, indicating the presence of one mole of glucose in each compound. The band Ⅱ in the UV spectra of compound 3, 4, 6 and 7 was not affected by an addition of NaOAc, indicating that 7-hydroxyl group must be glucosylated. This was further confirmed by the inspection of the ^{13}C-NMR spetra (Table 1).

The configuration and conformation of sugar moiety was determined by the J value of each anomeric protein signal (See Experimental). Thus the structures of 3, 4, 6 and 7 were elucidated as kaempferide 7-O-β-D-glucopyranoside (mumenin), kaempherol 7-O-β-D-glucopyranoside (populnin), naringenin 7-O-β-D-glucopyranoside (prunin) and quercetin 7-O-β-D-glucopyranoside (quercimeritrin), respectively.

Materials and Methods

All melting points were determined on a Thomas Hoover 6406-H apparatus and are uncorrected. The optical rotations were measured with a Rudolph Autopol^{R} Ⅲ automatic polarimeter. The IR spectra were obtained in KBr pellets on a Shimazu IR-400 spectrophotometer and the UV spectra were recorded with a CE 599 universal automatic scanning spectrophotometer. The EI-MS were measured with a Hewlett-Packard 5985 B GC/MS spectrometer operating at 70eV. The ^{1}H- and ^{13}C-NMR spectra were recorded with Varian FT-80A, Brucker AM-200 and Brucker AM-300 spectrometer ; Chemical shifts are given on a δ(ppm) scale with tetramethylsilane as an internal standard. Column chromatography was carried out with Kieselgel 60

(70-230 mesh, Merck) and Sephadex LH-20 (25-100μ, Pharmacia Co., Ltd.). TLC was performedon precoated Kieselgel 60 F_{254} plates (0.2㎜, Merck) using benzene : ether : MeOH = 8 : 2 : 1 as developing solvent for the free compounds and water saturated EtOAc for glycosides and detection was achieved by spraying 50% H_2SO_4 reagent followed by heating, or by irradiating with a UV lamp (254nm).

Results and Discussion

The dried leaf (2Kg) of commercial available *Prunus davidiana* was extracted with MeOH under reflux. The MeOH extract was partitioned with $CHCl_3$ (10g), EtOAc (55g), BuOH (40g) and H_2O (30g) successively. The EtOAc extract (55g) was chromatographed over silica gel (1Kg) using $CHCl_3$-MeOH mixture to give frs. 1-15. Frs. 2-3, 12-13 and 14-15 were further chromatographed to afford compound 1-7 as shown in Chart 1.

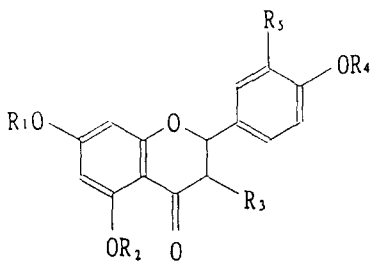
Compound 1 (naringenin)

Colorless needles from MeOH, mp. 254-6°, $[\alpha]_D^{20}$ -19.8° (c=0.116, MeOH). IR $\nu_{max}^{KBr}cm^{-1}$: 3300-3000 (br., -OH), 1590 (br., α, β-unsaturated C=O), 1490, 1460, 1415, 1380, 1335, 1305, 1245, 1175, 1155, 1075, 1060, 827. UV λ_{max}^{MeOH}nm (log ε) : 290 (4.33), 330 (sh., 3.74) ; $\lambda_{max}^{MeOH+NaOMe}$nm : 246 (4.38), 325 (4.57) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 290 (4.34), 330 (sh., 3.91) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 313 (4.51), 380 (3.73) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 310 (4.48), 375 (3.73). MS (m/z, rel. int.) : 272 (M^+, 100), 254 (M^+-H2O, 9.9), 179 (M^+-B ring, 32.4), 153 (RDA+H, A ring, 69), 152 (RDA, A ring, 12.5), 124 (152-CO, 6), 120 (RDA, B ring, 31). ^{1}H-NMR (DMSO-d_6, 300 MHz, TMS) : 12.1 (1H, s, C_5-OH), 7.31 (2H, d, J=8.5 Hz, H-2' and 6'), 6.79 (2H, d, J=8.5 Hz, H-3' and 5'), 5.88 (2H, s, H-6 and 8), 5.43 (1H, dd, J=3.0 & 12.7 Hz, H-2), 3.24 (1H, dd, J=12.9 & 17.2 Hz, H-

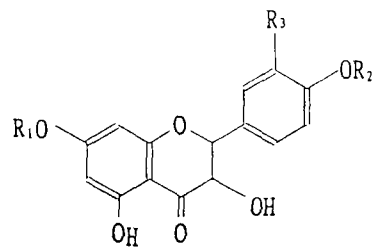
Table 1. ^{13}C-NMR spectral data of 1, 2, 3a, 3, 5, 6, and 7 (DMSO-d_6)

Compound Carbon No.	1[a]	2 (75 MHz)	3a[a]	3 (75 MHz)	5[b] (20 MHz)	6 (50 MHz)	7 (75 MHz)
2	78.4	144.8	146.4	147.2	80.9	78.6	147.9
3	42.0	133.7	135.8	137.0	67.0	42.0	136.1
4	196.2	174.0	175.8	176.7	27.0	197.1	176.0
5	163.6	158.8	160.6	160.5	155.7	162.9	160.4
6	95.9	96.3	98.2	99.2	94.2	96.4	98.8
7	166.7	162.0	163.9	163.1	155.8	165.3	162.7
8	95.0	91.5	93.4	94.9	95.3	95.4	94.3
9	162.9	154.3	156.2	156.1	155.3	162.7	155.8
10	101.8	101.2	103.1	105.2	99.0	103.2	104.7
1'	128.9	119.8	123.2	123.6	130.2	128.6	121.9
2'	128.2	127.5	129.0	129.8	113.8	128.3	115.6
3'	115.2	113.5	114.0	114.3	144.2	115.1	145.1
4'	157.8	157.3	160.6	160.9	144.2	157.7	147.9
5'	115.2	113.5	114.0	114.3	114.7	115.1	115.4
6'	128.2	127.5	129.0	129.8	118.4	128.3	120.1
-OMe			55.3	55.5			
1"				100.4		99.6	100.0
2"				73.3		73.0	73.2
3"				76.6		76.3	76.4
4"				69.8		69.5	69.6
5"				77.5		77.0	77.2
6"				60.7		60.5	60.7

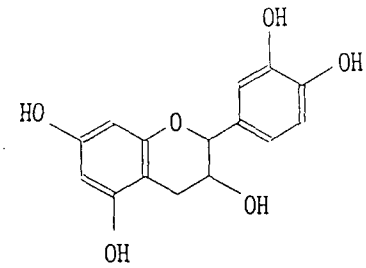
[a]Data taken from ref. 5. [b]DMSO-d6+$CDCl_3$ (5:1)



1: $R_1=R_2=R_3=R_4=R_5=H$
6: $R_1=glc$, $R_2=R_3=R_4=R_5=H$



2: $R_1=R_2=R_3=H$
3: $R_1=glc$, $R_2=CH_3$, $R_3=H$
4: $R_1=glc$, $R_2=R_3=H$
7: $R_1=glc$, $R_2=H$, $R_3=OH$



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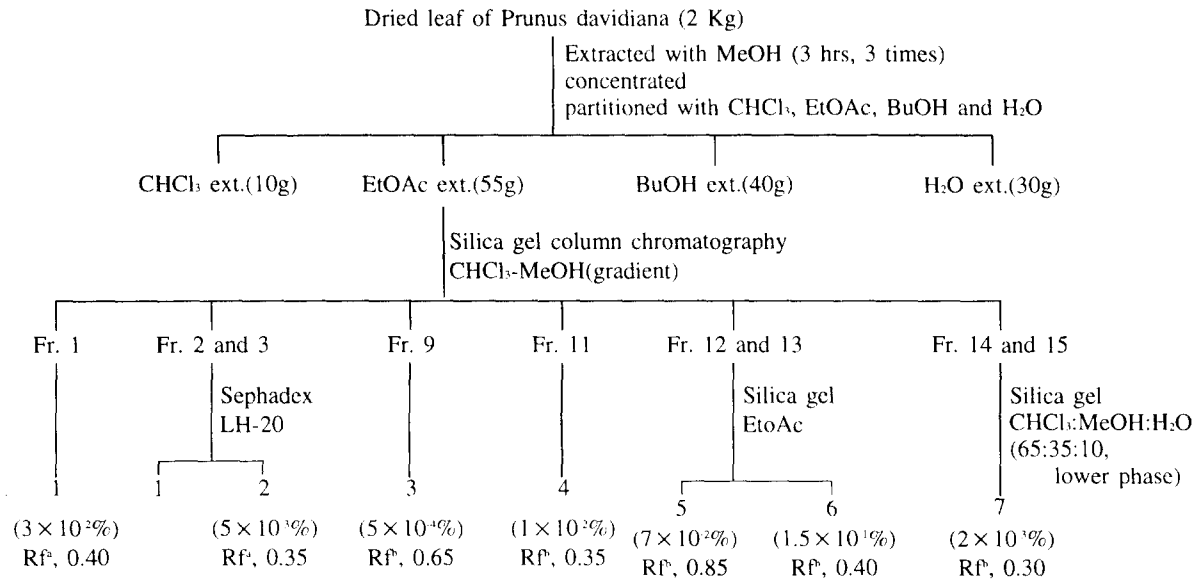

Chart 1. Extraction, fractionation and separation of *Prunus davidiana*

[a]TLC solvent system (Bezene : ether : MeOH = 8 : 2 : 1)

[b]TLC solvent system (Water saturated EtOAc).

3_{trans}), 2.69 (1H, dd, J=3.0 & 17.2 Hz, H-3_{cis}).

Compound 2 (kaempherol)

Yellowish needles from MeOH, mp. 275-8°, IR ν_{max}^{KBr} cm^{-1} : 3350 (OH), 1660 (α, β-unsaturated C=O), 1614, 1570, 1508 (C=C). UV λ_{max}^{MeOH}nm (log ε):257 (sh., 364), 269 (3.72), 300 (sh., 3.47), 330 (3.50), 370 (3.78) ; $\lambda_{max}^{MeOH+NaOMe}$nm: 280 (3.80), 320 (3.54), 420 (3.84) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 270 (3.80), 308 (3.30), 350 (3.42), 428 (3.85) ; $\lambda\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : 258 (sh., 3.72), 270 (3.77), 308 (3.31), 350 (3.47), 427 (3.83) ; $\lambda_{max}^{MeOH+NaOAc}$ nm : 274 (3.79), 310 (3.55), 380 (3.74) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 270 (3.73), 298 (3.46), 324 (3.51), 370 (3.79). MS (m/z, rel. int.) : 286 (M^+, 100), 285 (M^+-H, 30.1), 258 (M^+-CO, 9.8), 257 (M^+-HCO, 9.8), 229 (257-CO, 10.4), 153 (AH^+, 5.9), 121 (B_2^+, 18.6), 93 (B_2^+-CO, 5.8). ^{1}H-NMR (DMSO-d_6, 300 MHz, TMS) δ : 12.45 (1H, brs., H-5), 8.05 (2H, d, J=8.89 Hz, H-2' & 6'), 6.92 (2H, d, J=8.89

Hz, H-3' & 5'), 6.44 (1H, d, J=2 Hz, H-8), 6.19 (1H, d, J=2 Hz, H-6).

Compound 3 (kaempferide 7-O-glucoside)

Yellow amorphous powder, mp. 296-7o. Mg/HCl and Molisch test : positive. IR ν_{max}^{KBr} cm^{-1} : 3360 (br., -OH), 1640 (α, β-unsaturated CO), 1620, 1590, 1500 (C=C), 1080 (C-O). UV λ_{max}^{MeOH} nm (log ε) : 257 (4.39), 273 (4.46), 291 (4.33), 327 (4.24), 370 (4.41) ; $\lambda_{max}^{MeOH+NaOMe}$ nm : 260 (4.47), 278 (4.46), 330 (3.99), 412 (4.38) ; $\lambda_{max}^{MeOH+NaOAc}$ nm : 257 (4.39), 271 (4.45), 290 (4.31), 325 (4.21), 370 (4.37) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 257 (4.39), 273 (4.46), 291 (4.31), 327 (4.24), 370 (4.41) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 258 (sh., 4.47), 272 (4.53), 298 (sh., 4.23), 358 (4.18), 428 (4.50) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : 258 (sh., 4.48), 270 (4.52), 305 (sh., 4.27), 354 (4.24), 428 (4.44). ^{1}H-NMR (DMSO-d_6, 300MHz, TMS) δ : 12.36 (1H, brs., C_5-OH, D_2O exchanged), 8.17 (2H, d, J=9.0 Hz, H-2' & 6'), 7.11 (2H, d, J=9.1 Hz, H-3' & 5'), 6.80 (1H, d, J=2.1 Hz, H-8), 6.44 (1H, d, J=2.1 Hz, H-6), 5.06 (1H, d, J=7.15 Hz, H-1"), 3.85 (3H, s, $-OCH_3$).

Acid hydrolysis of 3

Ten mg of 3 was refluxed with 10% H_2SO_4 (20ml) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to give kaempferide as yellow needles, mp. 226-8°. It was confirmed by direct comparison with an authentic sample (TLC, mmp and MS). The filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. D-glucose was identified by TLC.

Compound 4 (kaempferol 7-O-glucoside, populnin)

Yellowish needles from MeOH. Mg/HCl and Molisch tests : positive, mp. 243-4°. IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1650 (α, β-unsaturated C=O), 1600, 1550, 1493 (C=C), 1070 (C-O). UV λ_{max}^{MeOH} (log ε) : 258 (4.14), 274 (sh., 4.11), 295 (sh., 4.02), 328 (sh., 3.83), 374 (3.94) ; $\lambda_{max}^{MeOH-NaOMe}$ nm : 252 (4.14), 274 (4.21), 440 (4.23) ; $\lambda_{max}^{MeOH+NaOAc}$ nm : 255 (4.17), 274 (sh., 4.08), 295 (sh., 3.98), 328 (sh., 3.87), 372 (4.00) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 260 (4.16), 271 (4.17), 295 (4.01), 325 (sh., 3.89), 372 (4.00) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 272 (4.28), 300 (3.96), 326 (3.86), 350 (3.79), 428 (4.06) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : 270 (4.20), 300 (3.98), 324 (3.83), 355 (3.79), 428 (3.98) ; MS (m/z, rel. int.) ; 286 (M^+-glucose, base peak). ^{1}H-NMR (DMSO-d_6 +D_2O, 300 MHz, TMS) δ : 8.28 (2H, d, J=8.6 Hz, H-2' & 6'), 6.87 (2H, d, J=8.9 Hz, H-3' & 5'), 6.63 (1H, brs., H-8), 6.29 (1H, brs., H-6), 5.01 (1H, d, J=7.25 Hz, H-1"), 3.70-3.10 (6H, m, H-2"-6"). ^{13}C-NMR (DMSO-d_6, 75 MHz, TMS) δ : sugar, 99.9 (C-1"), 77.1 (C-3"), 76.5 (C-5"), 73.1 (C-2"), 69.7 (C-4"), 60.7 (C-6").

Acid hydrolysis of 4

Twenty mg of 4 was refluxed with 10% H_2SO_4 (50ml) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to give kaempferol as yellow needles, mp. 275-7°. It was confirmed by direct comparison with an authentic sample (TLC, mmp and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. D-glucose was identified by TLC.

Compound 5 ((+)-catechin)

Colorless needles from aq.-MeOH, mp. 174-5°, $[\alpha]_D^{20}$+10° (c=0.123, MeOH).

IR ν_{max}^{KBr} cm^{-1} ; 3350, 1630, 1525, 1470, 1290, 1150, 1080, 1030. UV λ_{max}^{MeOH} nm (log ε) : 282 (3.50). MS (m/z, rel. int.) : 290 (M^+, 21.1), 152 (RDA fragment with B ring, 43.4), 139 (RDA fragment with A ring +H, 100), 123 (152-CHO, 69.7), 109 (123-CO, 8.0). ^{1}H-NMR (DMSO-d6, 80 MHz, TMS) δ : 6.74 (1H, d, J=2 Hz, H-2'), 6.68 (1H, d, J=8 Hz, H-5'), 6.62 (1H, dd, J=8 & 2 Hz, H-6'), 5.91 (1H, d, J=2.0 Hz, H-8), 5.72 (1H, d, J=2.0 Hz, H-6), 4.51 (1H, d, J=7 Hz, H-2), 3.85-3.75 (1H, m, H-3), 2.50 (2H, ddd, J=5.4, 8.1 and 16.0, H_2-H).

Compound 6 (naringenin-7-O-glucoside, prunin)

Colorless needles from MeOH, mp. 220-3°, $[\alpha]_D^{20}$ 66.6

(c=0.3, MeOH), Mg/HCl and Molisch tests ; positive. IR ν_{max}^{KBr} cm^{-1} : 3300 (br., -OH), 1615 (α, β-unsaturated C=O), 1570, 1520 (C=C), 1100-1000 (br., glycoside). UV λ_{max}^{MeOH} nm(logε) : 228 (4.61), 285 (4.36), 330 (3.51) ; $\lambda_{max}^{MeOH+NaOMe}$ nm : 246 (4.57), 286 (4.31), 370 (3.91), 420 (3.84) ; $\lambda_{max}^{MeOH+NaOAc}$ nm : 285 (4.42), 330 (3.51) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 285 (4.42), 330 (3.51) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 226 (4.76), 312 (4.55), 360 (3.94) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : 226 (4.72), 309 (4.47), 360 (3.91). ^{1}H-NMR(DMSO-d_6, 200 MHz, TMS) δ : 12.16 (1H, br s, C_5-OH), 7.44 (2H, d, J=8.5 Hz, H-2' & 6'), 6.90 (2H, d, J=8.5 Hz, H-3' & 5'), 6.26 (1H, d, J=2.2 Hz, H-8), 6.23 (1H, d, J=2.2 Hz, H-6), 5.61 (1H, dd, J=2.5 & 12.6 Hz, H-2), 5.06 (1H, d, J=7.0 Hz, H-1"), 3.30 (1H, dd, J=12.6 & 17.2 Hz, H-3B), 2.84 (1H, dd, J=2.9 & 17.2 Hz, H-3_A).

Acid hydrolysis of 6

Fifty mg of 6 was refluxed with 10% H_2SO_4 (50ml) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was caystallized from MeOH to give naringenin as colorless needles, mp. 254-5°. It was confirmed by direct comparison with an authentic sample (TLC, mmp, and MS). The filtrate was neutralized with $BaCO_3$, filrered and concentrated in vacuo. D-glucose was identified by TLC.

Compound 7 (quercetin-7-O-glucoside, quercimeritrin)

Yellowish needles from MeOH, mp. 250° Mg/HCl and Molisch test ; positive. UV λ_{max}^{MeOH} (logε) : 258 (4.77), 263 (sh., 4.56), 375 (4.73) ; $\lambda_{max}^{MeOH+NaOMe}$ nm : 249 (4.71), 268 (4.58), 423 (4.64) ; $\lambda_{max}^{MeOH+NaOAc}$ nm : 259 (4.75), 383 (4.67), 420 (4.34) ; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm : 261 (4.81), 387 (4.76) ; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 258 (sh., 4.70), 272 (4.80), 345 (sh., 3.97), 460(4.82) ; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : 269 (4.80), 360 (sh., 4.29), 430 (4.77). ^{1}H-NMR (DMSO-d_6, 300 MHz, TMS) δ : 12.5 (1H, s, C_5-OH), 7.74 (1H, d, J=2.0 Hz, H-2'), 7.59 (1H, dd, J=8.5 and 2.0 Hz, H-6'), 6.92 (1H, d, J=8.5 Hz, H-5'), 6.78 (1H, d, J=2.2 Hz, H-8), 6.45 (1H, d, J=2.2 Hz, H-6), 5.09 (1H, d, J=7.2 Hz, H-1").

Acid hydrolysis of 7

Ten mg of 7 was refluxed with 10% H_2SO_4 (30ml) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to give quercetin as yellow needles, mp. 314.5°. It was confirmed by direct comparison with an authentic sample (TLC, mmp and MS). The filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. D-glucose was identified by TLC.

적 요

우리나라 민간약으로서 신경통 및 류마치스의 치료에 널리 사용되고 있는 산복사나무(*Prunus davidiana*)의 잎으로부터 naringenin 및 그 배당체, kaempferol 및 그 배당체, kaempferide glucoside, quercetin glucoside 및 d-catechin을 단리하였다.

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