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Further Study on the Flavonoids from the Leaves of *Machilus thunbergii* in Korea[1]

Jong Cheol Park, Byung Woo Kim* and Han Suk Young*
Department of Oriental Medicine Resources, Suncheon National University, Suncheon 540-070,
*College of Pharmacy, Pusan National University, Pusan 609-735, Korea

Abstract—Quercetin, trifolin and quercitrin were isolated from the leaves of *Machilus thunbergii* Sieb. et Zucc. (Lauraceae) and characterized by spectral data. These are reported for the first time from this plant.

Keywords—*Machilus thunbergii* • Lauraceae • flavonoid • quercetin • trifolin • quercitrin • ^{13}C-NMR

Machilus thunbergii Sieb. et Zucc. (Lauraceae) is the evergreen broad-leaved tree which has been widely distributed throughout the islands of west-southern area in Korea.[2] The bark of this plant has been used for abdominal pain and distention in Korea.[3] In our previous paper,[1] we reported the identification of afzelin, guiyaverin and rutin from the leaves of this plant. In a course of continous work on this plant part, additional three compounds were isolated.

Experimental

Silica gel(Merck No. 7734) was used for column chromatography and precoated silica gel (Merck, No. 5735) and cellulose plate (Merck, No. 5577) for TLC. Detection of components was made by the use of a UV lamp.

Mps were determined on a Thomas-Hoover 6404-H apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on Hitachi 270-30 and Bomen MB 100-C14 FT-IR spectrophotometer. UV spectra were run with CE 599 Universal automatic scanning spectrophotometer. ^{1}H- and ^{13}C-NMR spectra recorded with a Bruker AM 200 spectrometer with TMS as an internal standard and chemical shifts are given as δ(ppm).

Plant material and fractionation

This was carried out as described previously.[1]

Isolation

The ethyl acetate phases were combined, concentrated *in vacuo*, and separated by chromatography on silica gel column with $CHCl_3$-$MeOH$-H_2O(25:8:5, lower layer) and $CHCl_3$-$MeOH$-H_2O(7:3:1, lower layer) to afford compounds 1, 2 and 3.

Characterization of compound 1

Mp 311~313°; IRν_{max}^{KBr} cm^{-1} 3350(OH), 1685 (C=O), 1615, 1505(C=C); UV, see Table I; ^{1}H-NMR(DMSO-d_6, 200 MHz) δ : 6.17(1H, d, J=2.0 Hz, H-6), 6.39(1H, d, J=2.0 Hz, H-8), 6.87(1H, d, J=8.5Hz, H-5′), 7.53(1H, dd, J=8.5 and 2.1 Hz, H-6′), 7.66(1H, d, J=2.1 Hz, H-2′), 12.46(1H, brs, C_5-OH)

Characterization of compound 2

Mp 220~224°; IRν_{max}^{KBr} cm^{-1} 3550(OH), 1655 (C=O), 1600, 1490(C=C), 1180, 1090(C—O); UV, see Table I; ^{1}H-NMR(DMSO-d_6, 200 MHz) δ : 5.39(1H, d, J=7.5 Hz, anomeric H), 6.19

(1H, d, J=1.9 Hz, H-6), 6.42(1H, d, J=1.9 Hz, H-8), 6.85(2H, d, J=8.8 Hz, H-3′ and 5′), 8.06(2H, d, J=8.8 Hz, H-2′ & 6′), 12.61(1H, s, C_5-OH); ^{13}C-NMR, see Table II.

Characterization of compound 3

Mp 180~182°; IRν_{max}^{KBr} cm^{-1} 3228(OH), 1655 (C=O), 1605, 1504(C=C), 1200, 1072(C—O); UV, see Table I; ^{1}H-NMR(DMSO-d_6, 200MHz) δ : 0.81(3H, d, J=5.4 Hz, CH_3 of rhamnose), 5.25(1H, s, anomeric H), 6.19(1H, d, J=1.7 Hz, H-6), 6.38(1H, d, J=1.7 Hz, H-8), 6.86 (1H, d, J=8.2 Hz, H-5′), 7.22(1H, d, J=1.9 Hz, H-2′), 7.28(1H, dd, J=8.2 and 1.9 Hz, H-6′), 12.63(1H, s, C_5-OH); ^{13}C-NMR, see Table II

Acid hydrolysis of compound 3

Compound 3(15 mg) was refluxed with 5% H_2SO_4 in MeOH for 5 hr. Work-up in the usual way followed by crystallization from methanol afforded quercetin as yellowish needles.

The filtrate was neutralized and concentrated *in vacuo*. Rhamnose was identified by cellulose TLC (pyridine:ethyl acetate:acetic acid:water= 36:36:7:21).

Methylation and hydrolysis of compound 3

To a solution of compound 3 (15 mg) in MeOH, ethereal CH_2N_2 was added and allowed to stand in cold room for 4 days. The reaction mixture was concentrated, dissolved in 5% H_2SO_4 and then refluxed for 3 hr. The aglycone, after the usual work-up, was identified as 5, 7, 3′, 4′-tetra-O-methyl quercetin, mp 193~195°, by direct comparison with an authentic sample (TLC, mmp and UV).

Results and Discussion

Column chromatography of the ethyl acetate soluble fraction of the methanol extract and crystallization yield three compounds(1~3). All compounds showed positive results in $FeCl_3$, Zn/HCl and Mg/HCl, and UV spectra were characteristic for flavonoids.

Compound 1 was identified as a well known compound, quercentin by comparison of reported IR and ^{1}H-NMR data and the UV spectral response to shift reagents. It was finally confirmed by direct comparison of an authentic sample.

Compounds 2 and 3 gave a positive reaction in Molisch test and showed glycosidic bond in IR spectra.

The UV maxima of compounds 2 and 3, exhibiting band I peak at 357~359 nm(Table I) was very similar to those reported for a number of 3-hydroxyl substituted flavonol.[4] These compounds showed a bathochromic shift with

Table I. UV spectral data for compounds 1, 2 and 3

Solvent	1	2	3
MeOH	256(3.85)	266(4.27)	258(4.31)
	368(3.85)	359(4.15)	300sh(3.89)
			357(4.22)
+NaOMe	244(3.61)	276(4.30)	272(4.33)
	330(3.96)	326(4.02)	330(3.93)
		408(4.16)	406(4.25)
+$AlCl_3$	272(3.96)	274(4.32)	275(4.34)
	458(3.94)	306(3.98)	307sh(3.81)
		356(4.08)	342(3.57)
		410(3.98)	442(4.31)
+$AlCl_3$/HCl	266(3.95)	274(4.22)	270(4.30)
	427(3.52)	304(3.92)	305sh(3.83)
		351(4.05)	366(4.04)
		404(3.71)	408(4.15)
+NaOAc	260(3.79)	274(4.31)	273(4.32)
	383(3.16)	306(4.02)	326(4.01)
		374(4.13)	376(4.14)
+NaOAc/H_3BO_3	260(3.99)	267(4.44)	262(4.37)
	387(3.61)	360(4.35)	298sh(3.80)
			378(4.23)

Figures in parentheses denote log ϵ.

Table II. ^{13}C-NMR chemical shifts of compounds 2 and 3 (50.3 MHz, DMSO-d_6)

Carbon	2	3
2	156.4	157.4
3	133.6	134.3
4	177.5	177.8
5	161.2	161.4
6	98.7	98.8
7	164.2	164.5
8	93.7	93.8
9	156.3	156.5
10	103.9	104.1
1′	120.9	120.8
2′	131.0	115.6
3′	115.0	145.3
4′	159.9	148.6
5′	115.0	115.7
6′	131.0	121.0
1″	101.6	101.9
2″	71.2	70.1*
3″	73.0	70.4*
4″	67.9	71.3
5″	75.7	70.7*
6″	60.2	17.6

*These assignments may be reversed.

$AlCl_3$ and $AlCl_3/HCl$ in band I and with NaOAc in band II which indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. A bathochromic shift of the UV with NaOMe, without a decrease in intensity of band I, indicated the presence of a free 4′-hydroxyl group.

In compound **3** the hypsochromic shift in band I of the $AlCl_3$ on addition of acid results the presence of B-ring ortho-dihydroxyl group. But we could not observe that hypsochromic shift in compound **2**.

The ^1H-NMR spectra of compounds **2** and **3** showed one anomeric proton signals at δ 5.39 (d) and 5.25(s), respectively. In compound **2** two doublets at δ 6.19(1H) and 6.42(1H), and two ortho-coupled doublets(J=8.8 Hz) at δ 6.85 (2H) and 8.08(2H) in the ^1H-NMR spectrum showed the presence of a proton at C-6, C-8 and hydroxyl at C-4′, respectively.

^1H-NMR spectrum of compound **3** showed two meta-coupled doublets at δ 6.19(1H, H-6) and 6.38(1H, H-8), a ortho-coupled doublet at δ 6.86 (1H, H-5′), a meta-coupled doublet at δ 7.22 (1H, H-2′), a double-doublet at δ 7.28(1H, H-6′) and a singlet at δ 12.63(1H, 5-OH).

These data indicated that compounds **2** and **3** were kaempferol and quercetin glycosides, respectively.

As compound **2** was obtained as a very small amount, we could not hydrolyze this compound. Acid hydrolysis of compound **3** yielded quercetin as its genin and rhamnose as the sugar moiety.

UV spectrum suggested that the sugar moiety was attached to 3-hydroxyl group. The methylation followed by acid hydrolysis of compound **3** gave 5, 7, 3′, 4′-tetra-O-methyl quercetin, which supported that rhamnose was attached at C-3 of quercetin. These facts were further evidenced by the ^{13}C-NMR spectrum, which showed glycosylation shift for the carbon signals of C-2, C-3 and C-4, by the comparison with those of the genin reported in the literature.[5]

The configuration and conformation of the sugar moiety was determined to be β-D-galactopyranose and α-L-rhamnopyranose not only the J values of the anomeric proton signals but also the ^{13}C-NMR data.

From the above results, compounds **2** and **3** were characterized as kaempferol 3-O-β-D-galactopyranoside (trifolin) and quercetin 3-O-α-L-rhamnopyranoside (quercitrin), respectively. These are reported for the first time from this plant.

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