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Flavonol Glycosides from the Leaves of *Machilus thunbergii*

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Abstract—From the leaves of *Machilus thunbergii* Sieb. et Zucc. (Lauraceae) afzelin, guaiyaverin and rutin were isolated and identified by chemical and spectral analysis.

Keywords—*Machilus thunbergii* • Lauraceae • flavonol glycosides • afzelin • guaiyaverin • rutin • ^{13}C-NMR

As a part of our chemical investigation of Korean medicinal plants, we have examined the leaves of *Machilus thunbergii* Sieb. es Zucc. (Lauraceae) and here report the isolation of flavonol glycosides of this plant.

The bark of *Machilus thunbergii* has been used for abdominal pain and distention in traditional medicine.[1] But in China the bark of *Magnolia officinalis* (Magnoliaceae) and in Japan *Magnolia abovata* (Magnoliaceae) have been used as same applications.

From the bark of *Machilus thunbergii*, machilin A-I[2,3] were so far identified and from the leaves essential oils[4~8] and mucilage[9~11] have been isolated.

The methanol extract of the leaves of *Machilus thunbergii* was fractionated with hexane, $CHCl_3$, ethylacetate, BuOH and H_2O successively. The ethylacetate extract was subjected to chromatograph using SiO_2 to yield compounds **1** and **2** in order of elution. And the BuOH extract was chromatographed on SiO_2 to give compound **3**.

Compounds **1**, mp 173~8°, **2**, mp 236~8° and **3**, mp 186~8°, showed characteristic flavonol glycosidic color reactions, a positive $FeCl_3$, Zn+HCl, Mg+HCl and Molisch tests. The IR spectra of each compound showed a broad hydroxyl, α,β-unsaturated carbonyl and C-O stretching bands indicating its glycosidic nature. Acid hydrolysis of each compound afforded as the aglycone, kaempferol, mp 272~3°, from compound **1**, quercetin, mp 315~7°, from compounds **2** and **3**. As the sugar, L-rhamnose from compound **1**, L-arabinose from compound **2**, D-glucose and L-rhamnose from compound **3** were identified respectively. The UV spectra of each compound in MeOH showed absorption at 344~361 nm(band I), which indicated a sugar residue at C-3 in flavonol skeleton. The bathochromic shifts of band I with $AlCl_3$/HCl (44~55 nm) were a characteristic feature of a 5-hydroxy-3-O-substituted flavonol. The bathochromic shift of band II (8~17 nm) with NaOAc also indicated the presence of unsubstituted hydroxyl group at C-7. The bathochromic shift of band I (50 nm) with NaOMe suggested that

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free 4′-hydroxyl group existed in B-ring in compound **1**. But in compounds **2** and **3** the hypsochromic shift (36∼37 nm) in band I of the $AlCl_3$ spectrum on additon of acid results the presence of B-ring ortho-dihydroxyl group. These were, thus, suggested that the sugar might be attached to 3-hydroxyl group.[12] This was further supported by the fact that methylation of compounds **2** and **3** with diazomethane followed by acid hydrolysis with 5% H_2SO_4, afforded 5, 7, 3′, 4′-tetra-O-methyl quercetin, mp 193∼5°. In addition, the ^{13}C-NMR spectrum (Table I) of each compound comfirmed this suggestion. The configuraion and conformation of the sugar moiety was determined not only by the *J* value of the anomeric proton signals but also by ^{13}C-NMR spectra. Consequently the structure of compounds **1**, **2** and **3** were elucidated to be kaempferol 3-O-*α*-L-rhamnopyranoside (afzelin), quercetin 3-O-*α*-L-arabinopyranoside (guaijaverin) and quercetin 3-O-*α*-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside (rutin) respectively. These are the first report of the isolation from this plant.

Table I. ^{13}C-NMR spectral data of compounds 1, 2 and 3 in DMSO-d_6

C	1	2	3
2	157.5	156.3	156.6
3	134.4	133.8	133.3
4	177.9	177.6	177.3
5	161.5	161.2	161.2
6	99.0	98.7	98.7
7	164.4	164.3	164.0
8	94.0	93.6	93.6
9	156.7	156.3	156.4
10	104.4	103.9	104.0
1′	120.8	120.9	121.2
2′	130.9	115.8	115.2
3′	115.7	145.0	144.7
4′	160.3	148.6	148.4
5′	115.7	115.4	116.2
6′	130.9	122.1	121.6
1″	101.9	101.5	101.2
2″	70.3*	71.7	74.1
3″	70.6*	70.8	76.4
4″	71.4	66.1	70.5*
5″	70.9*	64.3	75.9
6″	17.7		67.0
1‴			100.7
2‴			70.4*
3‴			70.0*
4‴			71.8
5‴			68.2
6‴			17.7

* These assignments may be reversed in each vertical column.

Experimental

The mps were taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Hitachi 270-30 spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. The NMR spectra was recorded with a Brucker AM-200 spectrometer containing TMS as an internal standard and chemical shifts are given as *δ* (ppm). Optical rotations were measured on JASCO DIP-360 polarimeter.

Plant material

The leaves of *Machilus thunbergii* were collected in August 1988 in Odong-do (Yosu, Chunnam) and a voucher specimen was deposited at the Suncheon Nat'l Univ.

Extraction and isolation

The dried leaves of *Machilus thunbergii* (500 g) were refluxed with MeOH for 3hr (3 times) and concentrated *in vacuo* (86 g). The concentrate was fractionated to yield hexane (30 g), $CHCl_3$ (7.8 g), ethylacetate (3.2 g), n-BuOH (8.6 g) and H_2O(14.6 g) soluble portions successively. The ethylacetate soluble portion was subjected to chromatography using SiO_2 ($CHCl_3$-

MeOH-H_2O=25:8:5, lower phase and $CHCl_3$-MeOH-H_2O=7:3:1, lower phase) column to give compound C(1) and D(2) in order of elution. And the n-BuOH soluble part was chromatographed on SiO_2 using $CHCl_3$-MeOH-H_2O(65:35:10, lower layer) as eluent to yield compound H(3).

Compound 1

Mp 173~8°; $[\alpha]_D^{20}$-152.0 (c=0.35, MeOH); IRν_{max}^{KBr} cm^{-1} 3400(OH), 1660(C=O), 1615, 1510 (C=C), 1210, 1180, 1080(C—O); UVλ_{max} (MeOH) 266 nm (logϵ 4.37), 295(sh, 4.09), 344(4.23); λ_{max} (NaOMe) 274(4.43), 325(4.15), 394 (4.39); λ_{max} ($AlCl_3$) 275(4.37), 303(4.09), 350(4.18), 400(4.16); λ_{max} ($AlCl_3$+HCl) 275(4.36), 303 (4.11), 345(4.19), 399(4.12); λ_{max} (NaOAc) 274(4.44), 302(sh, 4.12), 364(4.17); λ_{max} (NaOAc+H_3BO_3) 265(4.37), 300(sh, 4.09), 347(4.22); ^{1}H-NMR(DMSO-d_6. TMS) δ: 0.79 (3H, d, J=5.2, Me of rhamnose), 5.31(1H, s, anomeric), 6.19(1H, d, J=1.6, H-6), 6.39(1H, d, J=1.6, H-8), 6.90(2H, d, J=8.6, H-3′ and 5′), 7.73(2H, d, J=8.6, H-2′ and 6′), 12.60(1H, s, C_5-OH)

Acid hydrolysis of 1

Twenty mg of 1 was refluxed with 5% H_2SO_4 (50 ml) for 5 hr. After cooling the reaction mixture was filtered. The aglycone was crystallized from MeOH to afford as yellow needles, mp 272~3°. It was confirmed by comparison with an authentic sample (TLC, UV and ^{1}H-NMR). 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, :21, Rf 0.46)

Compound 2

Mp 236~8°; $[\alpha]_D^{20}$ -50° (c=0.5, MeOH); IR$\nu_{max}^{KBr}$$cm^{-1}$ 3370(OH), 1660(C=O), 1610, 1560, 1510(C=C), 1190, 1120, 1060, 1020(C—O), UV λ_{max} (MeOH) 257 nm (logϵ 4.29), 303(4.18), 357(4.22); λ_{max}(NaOMe) 274(4.32), 330(sh, 3.88), 409(4.26); λ_{max}($AlCl_3$) 276(4.37), 303 (sh, 3.87), 438(4.27); λ_{max}($AlCl_3$+HCl) 270 (4.34), 300(4.03), 370(4.09), 402(4.13); λ_{max} (NaOAc) 274(4.30), 326(3.92), 390(4.12); λ_{max}(NaOAc+H_3BO_3) 262(4.34), 287(sh, 3.78), 380(4.23); ^{1}H-NMR(DMSO-d_6, TMS) δ: 5.26 (1H, d, J=5.2, anomeric), 6.19(1H, d, J=2.0, H-6), 6.40(1H, p, J=2.0, H-8), 6.73(1H, d, J=8.4, H-5′), 7.50(1H, d, J=2.1, H-2′), 7.65(1H, dd, J=2.1 and 8.4, H-6′)

Acid hydrolysis of 2

Twenty mg of 2 was refluxed with 5% H_2SO_4 (50 ml) for 5 hr. After cooling the reaction mixture was filtered. The aglycone was crystallized from MeOH to give quercetin as yellow needles, mp 315~7°. It was confirmed by comparison with an authentic sample (TLC, UV and ^{1}H-NMR). 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 0.38)

Methylation of 2 and 3 followed by acid hydrolysis

Thirty mg of compounds 2 and 3, separately, was treated with an ethereal CH_2N_2 sol'n at room temp. for 4 days. Acid hydrolysis of the crude methylether with 5% H_2SO_4 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 194~5°, which was confirmed by direct comparison with an authentic sample (TLC, mmp and UV).

Compound 4

Mp 186~8°; $[\alpha]_D^{20}$ +10.2(c=0.5, MeOH); IRν_{max}^{KBr} 3450(OH), 1660(C=O), 1600, 1510(C=C), 1210, 1070, 1020(C—O); UVλ_{max}(MeOH) 258 nm(logϵ 4.41), 306(sh, 3.96), 359(4.34); λ_{max}(NaOMe) 272(4.48), 332(4.04), 409(4.47); λ_{max}($AlCl_3$) 274(4.50), 308(sh, 3.89), 440(4.47); λ_{max}($AlCl_3$+HCl) 271(4.43), 305(3.96), 368

(sh, 4.20), 403(4.30); λ_{max}(NaOAc) 270(4.43), 328(4.05), 385(4.25); λ_{max}(NaOAc+H_3BO_3) 262(4.48), 300(sh, 3.87), 380(4.36); ^{1}H-NMR (DMSO-d_6, TMS) δ: 0.98(3H, d, J=6.0, Me of rhamnose), 4.45(1H, s, anomeric H of rhamnose), 5.33(1H, d, J=7, anomeric H of glucose), 6.18(1H, d, J=1.9, H-6), 6.37(1H, d, J=1.9, H-8), 6.83(1H, d, J=9.0, H-5′), 7.51(1H, d, J=1.9, H-2′), 7.53(1H, dd, J=1.9 and 9.0, H-6′)

Acid hydrolysis of 3

Compound **3** (20 mg) was treated with 5% H_2SO_4 for 5 hr. Quercetin, mp 315~7°, was identified by direct comparison with an authentic sample. The sugars were identified as D-glucose and L-rhamnose by TLC(precoated cellulose, pyridine-ethylacetate-HOAc-H_2O= 36:36:7:21, Rf 0.24 and 0.46)

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Literature Cited

1. Namba, T.: 1. Namba, T.: Colored Illustrations of Wakan-Yaku, Hoikusha, Osaka, p. 146 (1980).
2. Shimomura, H., Sashida, Y. and Oohara, M.: *Phytochem.* **26**, 1513 (1987).
3. *Idem.*: *ibid.* **27**, 634 (1988).
4. Komae, H. and Hayashi, N.: *ibid.* **11**, 1181 (1972).
5. Nii, H., Furukawa, K., Iwakiri, M. and Kubota, T.: *Nippon Nogei Kagaku Kaishi* **55**, 37(1981).
6. *Idem.*: *ibid.* **55**, 1179(1981).
7. *Idem.*: *ibid.* **57**, 323 (1983).
8. Hiao, J.Y.: *Proc. Natl. Sci. Counc.* **10**, 221 (1986).
9. Kusunose, H. and Oshibuchi, T.: *Nippon Nogei Kagaku Kaishi* **45**, 156 (1971).
10. *Idem.*: *Kochi Daigaku Gakujutsu Kenkyu Hokoku*, **19**, 43 (1971).
11. *Idem.*: *ibid.* **19**, 95 (1971).
12. Mabry, T.J., Markham, K.R. and Thomas, M.B.: The Systematic Identification of Flavonoids, Springer, N.Y. (1970).