Natural Product Sciences
7(1) : 23~26 (2001)

Aromatic Acid and Flavonoids from the Leaves of *Zanthoxylum piperitum*

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Abstract – Five flavonoids and one aromatic acid were isolated from the leaves of *Zanthoxylum piperitum*. The structures of compounds were elucidated as quercetin, afzelin, quercitrin, hyperoside, hesperidin and protocatechuic acid on the basis of spectral evidence.
Key words – *Zanthoxylum piperitum* A.P.DC., Rutaceae, flavonoid, protocatechuic acid

Introduction

Zanthoxylum piperitum A.P.DC. (Rutaceae) have been used for the treatment of stomachache, vomiting, diarrhea, abdominal pain and moist dermal ulcer (Hsu, 1986) and for the aromatic in Korea. Chemical components such as sanshool-I, sanshool-II, sanshoamide, methyl 2,4-dimethoxy-5-hydroxycinnamate and xanthoxylol (Aihara, 1950a; 1950b; 1951; Abe *et al.*, 1973; 1974) have been isolated from *Z. piperitum*. The present paper deals with the structure elucidation of five flavonoids and one aromatic acid isolated from the leaves of *Z. piperitum* on the basis of spectroscopic analysis.

Experimental

Plant material and apparatus – *Z. piperitum* was collected by the author (Dr. J.M. Hur) in Sunchon, Jonnam, Korea on July 25, 1998. The voucher specimen (No. NM0337) is deposited in department of oriental medicine resources, Sunchon National University.

The IR spectra were determined in KBr tablets on a Hitachi 2703 spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. The NMR spectra were recorded with a Brucher AM-200 spectrometer containing TMS as an internal standard and chemical shifts were given as δ (ppm).

Extraction, fractionation and isolation – The dried and powdered leaves (1.6 kg) of *Z. piperitum* was refluxed with MeOH. This extract (250 g) has been partitioned with organic solvents of the different polarities to afford dichloromethane (80 g), ethyl acetate (29 g), n-butanol (37 g) and aqueous (70 g) fractions, respectively. The ethyl acetate fraction (27 g) was subjected to chromatograph using silica gel with $CHCl_3$-MeOH-H_2O (25:7:5, lower layer; 7:3:1, lower layer) as solvents to give subfractions of E1 (tubes 1-20), E2 (tubes 21-80), E3 (tubes 81-120), E4 (tubes 121-159), E5 (tubes 160-175) and E6 (tubes 176-204). Subfr. E2 was rechromatographed on silica gel and Sephadex LH-20 to yield compounds 1 and 6. Subfr. E4 was rechromatographed on silica gel and Sephadex LH-20 to give compounds 2, 3 and 4. And compound 5 was obtained from tubes 91-100 by silica gel column chromatography of n-BuOH soluble fraction (30 g) with the elution of $CHCl_3$-MeOH-H_2O (7:3:1, lower layer; 65:35:10, lower layer; 65:42:10, lower layer).

Compound 1 (quercetin) – IR ν_{max}^{KBr} cm^{-1}: 3310, 1665, 1617, 1656, 1518, 1365, 1323; UV λ_{max}(MeOH)nm : 274, 370; NaOMe 318, 410; $AlCl_3$ 293, 456; $AlCl_3$ + HCl 274, 426; NaOAc 294, 383; NaOAc + H_3BO_3 298, 386; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 12.5 (1H, s, 5-OH), 7.66 (1H, d, J=2.1 Hz, H-2'), 7.53 (1H, dd, J=2.1 & 8.5 Hz, H-6'), 6.87 (1H, d, J=8.5 Hz, H-5'), 6.40 (1H, d, J=1.9 Hz, H-8), 6.17 (1H, d, J=1.9 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) Table 1

Compound 2 (afzelin) – IR ν_{max}^{KBr} cm^{-1} : 3400, 1672, 1615, 1515, 1210, 1184, 1053; UV λ_{max}(MeOH)nm : 266, 295sh, 344; NaOMe 274, 325, 394; $AlCl_3$ 275, 303, 350, 400; $AlCl_3$ + HCl 275, 303, 345, 399; NaOAc 274, 302, 364; NaOAc + H_3BO_3 265, 300; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 12.6 (1H, s, 5-OH), 7.74 (2H, d, J=8.7 Hz, H-2' & 6'), 6.90 (2H, d,

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Table 1. ^{13}C-NMR spectral data for compounds **1-5** in DMSO-d_6

Carbon No.	1	2	3	4	5
2	147.7	157.2	157.3	156.3	78.4
3	135.7	134.2	134.2	133.5	42.0
4	175.8	177.7	177.8	177.5	197.0
5	160.7	161.3	161.3	161.2	163.0
6	98.2	98.7	98.7	98.7	96.3
7	163.9	164.2	164.2	164.2	165.1
8	93.3	93.7	93.6	93.5	95.5
9	156.1	156.5	156.4	156.3	162.5
10	102.9	104.1	104.1	103.9	103.3
1'	121.9	120.5	121.1	121.1	130.9
2'	115.0	130.6	115.5	115.2	114.1
3'	145.0	115.4	145.2	144.8	146.4
4'	146.8	160.0	148.5	148.5	147.9
5'	115.6	115.4	115.7	116.0	112.0
6'	119.9	130.6	120.7	122.0	117.9
1"		101.8	101.6	101.9	99.4
2"		70.0	70.1	71.2	73.0
3"		70.3	70.4	73.2	76.2
4"		71.2	71.2	67.9	70.7
5"		70.6	70.6	75.8	75.5
6"		17.4	17.5	60.2	66.0
1"'					100.6
2"'					70.3
3"'					69.5
4"'					72.0
5"'					68.3
6"'					17.8
OCH_3					55.9

J=8.7 Hz, H-3' & 5'), 6.40 (1H, d, J=1.9 Hz, H-8), 6.20 (1H, d, J=1.9 Hz, H-6), 5.29 (1H, d, J=1.4 Hz, anomeric H), 0.77 (3H, d, J=5.3 Hz, -CH_3); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) Table 1

Compound 3 (quercitrin) – IR ν_{max}^{KBr} cm^{-1}: 3430, 1660, 1628, 1565, 1504, 1130, 1050, 1018; UV λ_{max}(MeOH)nm : 259, 303sh, 352; NaOMe 272, 328, 395; $AlCl_3$ 278, 306sh, 335, 433; $AlCl_3$ + HCl 273, 304sh, 354, 405; NaOAc 275, 324sh, 374; NaOAc + H_3BO_3 263, 303sh, 369; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 12.6 (1H, s, 5-OH), 7.29 (1H, d, J=2.0 Hz, H-2'), 7.24 (1H, dd, J=2.0 and 8.2 Hz, H-6'), 6.65 (1H, d, J=8.2 Hz, H-5'), 6.38 (1H, d, J=1.9 Hz, H-8), 6.20 (1H, d, J=1.9 Hz, H-6), 5.24 (1H, d, J=1.3 Hz, anomeric H), 0.80 (3H, d, J=5.5 Hz, -CH_3); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) Table 1

Compound 4 (hyperoside) – IR ν_{max}^{KBr} cm^{-1}: 3420, 1666, 1608, 1512, 1464, 1449, 1128, 1050, 1080; UV λ_{max}(MeOH)nm : 273, 358; NaOMe 277, 329, 409; $AlCl_3$ 278, 440; $AlCl_3$ + HCl 278, 299, 368, 401; NaOAc 278, 324, 383; NaOAc + H_3BO_3 276, 379; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 7.69 (1H, dd, J=1.9 & 8.5 Hz, H-6'), 7.52 (1H, d, J=1.9 Hz, H-2'), 6.81 (1H, d, J=8.5 Hz, H-5'), 6.68 (1H, d, J=1.8 Hz, H-8), 6.18 (1H, d, J=1.8 Hz, H-6), 5.46 (1H, d, J=7.5 Hz, anomeric H); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) Table 1

Compound 5 (hesperidin) – IR ν_{max}^{KBr} cm^{-1}: 3350, 1672, 1612, 1580, 1534, 1512, 1070; UV λ_{max}(MeOH)nm : 284, 329; NaOMe 242, 286, 360; $AlCl_3$ 308, 382; $AlCl_3$ + HCl 306, 380; NaOAc 286, 328; NaOAc + H_3BO_3 285, 328; ^{1}H-NMR (pyridine-d_5, 200 MHz) δ: 7.52 (1H, d, J=1.9 Hz, H-2'), 7.10 (1H, dd, J=1.9 & 8.3 Hz, H-6'), 6.97 (1H, d, J=8.3 Hz, H-5'), 6.61 (1H, d, J=2.2 Hz, H-8), 6.29 (1H, d, J=2.2 Hz, H-6), 5.63 (1H, d, J=7.1 Hz, anomeric H of glucose), 5.48 (1H, dd, J=3.0 & 12.5 Hz, H-2), 5.47 (1H, s, anomeric H of rhamnose), 3.72 (3H, s, -OCH_3), 3.22 (1H, dd, J=3.0 & 17.1 Hz, H-3a), 2.84 (1H, dd, J=12.5 & 17.1 Hz, H-3b), 1.56 (3H, d, J=5.4 Hz, -CH_3 of rhamnose); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) Table 1.

Acid hydrolysis of 2-5 – 20 mg of each compounds 2-5 was separately refluxed with 10% H_2SO_4 for 4 hr. After cooling, the reaction mixtures were filtered. The aglycones were crystallized from MeOH to give kaempferol from compound 2, quercetin from compounds 3 and 4, and hesperetin from compound 5. They were confirmed by direct comparison with authentic sample (TLC and ^{1}H-NMR).

Compound 6 (protocatechuic acid) – IR ν_{max}^{KBr} cm^{-1}: 3365, 1692, 1612, 1510, 1266, 1055; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 7.32 (1H, d, J=2.0 Hz, H-2), 7.27 (1H, dd, J=2.0 & 8.1 Hz, H-6), 6.77 (1H, d, J=8.1 Hz, H-5); ^{13}C-NMR (DMSO-d_6, 50.3 MHz) δ: 167.3 (C=O), 150.0 (C-4), 144.9 (C-3), 121.9 (C-6), 121.7 (C-1), 116.6 (C-2), 115.2 (C-5)

Results and Discussion

Z. piperitum have been used for the treatment of stomachache, vomiting and diarrhea (Hsu, 1986). In the present study the phytochemical study of *Z. piperitum* leaves was investigated.

The methanol extract of the leaves of *Z. piperitum* was fractionated with dichloromethane, ethyl acetate, n-butanol and water successively. Column chromatography of ethyl acetate and n-butanol soluble fractions afforded six compounds. Compound 1 was identified as a

well known compound, quercetin by comparison of reported ^{1}H- and ^{13}C-NMR data (Markham *et al.*, 1978). It was finally confirmed by direct comparison of an authentic sample. Compounds 2-5 gave a positive reaction in Molisch test and showed absorption bands for glycosidic linkage (1000-1100 cm^{-1}) in their IR spectra. In UV maxima of compounds 2, 3 and 4, the range of 344-358 nm of MeOH spectra was very similar to those reported for a number of 3-hydroxy substituted flavonol (Mabry *et al.*, 1970). These compounds showed a bathochromic shifts (8 nm, 16 nm and 5 nm) of band in the presence of NaOAc which indicated the presence of free 7-hydroxyl groups. A bathochromic shift with NaOMe, without a decrease in intensity of band I, indicated the presence of a free 4'-hydroxyl group. In compounds 3 and 4 the hypsochromic shift in band I of the $AlCl_3$ on addition of acid resulted the presence of ortho-dihydroxyl group of B-ring (Mabry *et al.*, 1970). On acid hydrolysis compounds gave kaempferol from 2, and quercetin from 3 and 4 as their genins, respectively. The ^{1}H-NMR spectra of compounds 2-4 showed one anomeric proton signal at δ5.29 (J=1.4 Hz), δ5.24 (J=1.3 Hz) and δ5.46 (J=7.5 Hz), respectively. The ^{1}H-NMR spectra of compounds 3 and 4 showed two meta-coupled doublets ascribable to H-8 (δ6.38 and δ6.68) and H-6 (δ6.20 and δ6.18) of A-ring, and an ortho-coupled doublet, a meta-coupled doublet and a ortho, meta-coupled doublet-doublets attributable to H-2' (δ7.29 and δ7.52), H-6' (δ7.24 and δ7.69) and H-5' (δ6.65 and δ6.81) of B-ring, respectively. However the ^{1}H-NMR spectra of compound 2 showed two ortho-coupled doublets ascribable to H-2', 6' and H-3', 5' of B-ring in the flavonoid skeleton. These data indicated that compounds 2 and 3-4 were kaempferol and quercetin glycosides, respectively. The UV spectra suggested that the sugar moiety was attached to 3-hydroxyl group. These facts were further evidenced by the ^{13}C-NMR spectra (Table 1), 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 (Markham *et al.*, 1978). The sugar moiety of compounds 2-3 and 4 were determined to be α-$_{L}$-rhamnopyranose and β-$_{D}$-galactopyranose, respectively, by the J values of the anomeric proton signals and the ^{13}C-NMR data. From the above results, compounds 2, 3 and 4 were characterized as afzelin, quercitrin and hyperoside, respectively (Fig. 1).

The ^{1}H-NMR spectrum of compound 5 showed

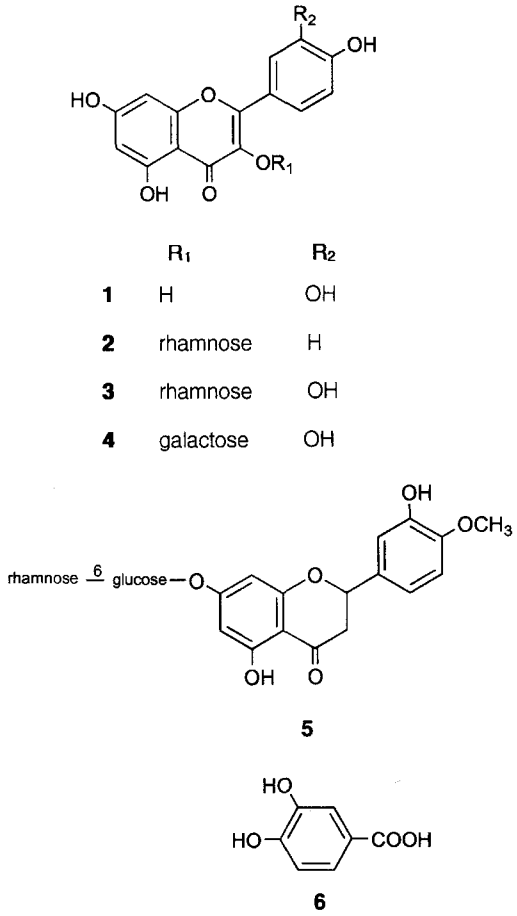

Fig. 1. Chemical structures of compounds from the leaves of *Zanthoxylum piperitum*. 1: quercetin, 2: afzelin, 3: quercitrin, 4: hyperoside, 5: hesperidin, 6: protocatechuic acid.

the meta-coupled doublets of one proton at δ6.61 (H-8) and δ6.29 (H-6), and the signals at δ7.52 (1H, d, J=1.9 Hz), δ7.10 (1H, dd, J=1.9 and 8.3 Hz) and δ6.97 (1H, d, J=8.3 Hz) assignable to the protons of 1, 3, 4-trisubstituted benzene ring, and one methoxy group at δ3.72, and two anomeric protons at δ5.63 (d, J=7.1 Hz) and δ5.47 (s). The singal at δ197.0 in ^{13}C-NMR spectrum and 284 nm with MeOH in UV spectrum suggested the presence of a flavanone moiety (Mabry *et al.*, 1970). Acid hydrolysis of compound 5 yielded hesperetin as its genin. The band II in the UV spectra of compound 5 was not affected by an addition of NaOAc, indicating the absence of a free 7-hydroxyl group in flavanone. These data indicated that compound 5 was hesperetin 7-glycoside. As the sugar-sugar linkage of disaccharide was deduced from its ^{13}C-NMR data, it could be

suggested that compound 5 had a rutinose composed of inner glucose and terminal rhamnose. Therefore, the structure of compound 5 was elucidated as hesperidin (Fig. 1) and identified by comparison of ^{13}C-NMR spectral data with those reported in the literature (Markham *et al.*, 1976).

Compound 6 displayed the presence of hydroxyl (3365 cm^{-1}), carboxyl (1692 cm^{-1}) and double bond (1612 and 1510 cm^{-1}) absorptions in its IR spectrum. The ^{1}H-NMR spectrum of compound 6 indicated the presence of aromatic signals of an ABX type at δ6.77 (J=8.1 Hz), δ7.27 (J=2.0 and 8.1 Hz) and δ7.32 (J=2.0 Hz), respectively assignable to H-5, H-6 and H-2. Its ^{13}C-NMR spectrum also showed the signals of two oxygen-bearing aromatic ring (δ150.0 and 144.9) and a ketone group (δ167.3). These data were expected that compound 6 is protocatechuic acid (Fig. 1). The identity with 3,4-dihydroxybenzoic acid was identified by comparison of ^{1}H- and ^{13}C-NMR spectra with those reported literature (Pouchert *et al.*, 1993). Investigation of further bioactive components is now in progress.

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(Accepted March 26, 2001)