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Pharmacological activities and the constituents of the leaves of *Hedera rhombea* Bean (II): On the constituents of the leaves

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Three phenolic compounds and a dammarane glycoside were isolated from the leaves of *Hedera rhombea* Bean (Araliaceae). Their structures were characterized as rutin, caffeic acid, 3,5-dicaffeoyl quinic acid and Kizuta saponin K_5 by chemical and spectral analysis.

Key words: *Hedera rhombea*, Araliaceae, Rutin, Caffeic acid, 3,5-Dicaffeoyl quinic acid, Dammarane glycoside, Kizuta saponin K_5

INTRODUCTION

Hedera rhombea Bean (Araliaceae) is an evergreen viny plant indigenous to South Korea and Japan. It has been used for treatments of hemorrage, chronic catarrh, jaundice, lithisis and convulsion as a folk medicine.

The constituents of Hedera species including *H. rhombea* have been studied since Shimizu *et al.* reported hederagenin glycoside from the stem and bark of this plant in 1978 (Shimizu *et al.*, 1978). Various dammarane and triterpenoidal saponins were isolated from the stem and bark (Kizu *et al.*, 1985a,b,c,; Tomimori *et al.*, 1987).

Recently, we have reported on the analgesic and anti-inflammatory activities of the leaves (Lee *et al.*, 1990). In this paper, chemical investigation on the leaves has led to the isolation of three phenolic compounds and one dammarane saponin.

MATERIALS AND METHODS

Instruments

Melting point was recorded on a METTLER FP 62 melting point apparatus. UV and IR spectra were measured on a Shimidzu UV visible recording spectro photometer UV-240 Graphicord and a PERKIN-ELMER 1420 Ratio recording spectr ophotometer respectively. ^{13}C-NMR and ^{1}H-NMR spectra were obtained on Brucker AMX-500 spectrometer. Mass spectra were taken by High resolution Mass (Low EI) VG 70 VSEQ. TLC chromatography was performed on precoated Kieselgel 60 F_{254} (Merck, 5715) and cellulose plate.

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Fractionation and Isolation

The leaves of *Hedera rhombea* were collected in October (1991) at Koheung of-southern Korea. They were dried into 2.45 Kg, and were extracted with methanol (90L) (4h, 3 times). The methanol extract was concentrated *in vacuo* (526.7 g) and partitioned successively with diethyl ether (12.92 g), ethylacetate (15.39 g) and then n-butanol (46 g).

The ethylacetate fraction (15.39 g) was subjected to Sephadex LH-20 column chromatography with H_2O-MeOH (gradient 0 to 100%) to afford compound **1** and compound **2**. Compound **3** was isolated by elution with 30% EtOH solution in Sephadex LH-20 and by prepatative TLC, respectively.

The BuOH fraction (46 g) was chromatographed with repeated silica gel column chromatography by elution with $CHCl_3$-MeOH, gradiently, to get compound **4**.

Compound 1 (rutin): Yellow powder(H_2O), TLC; Rf=0.12 (benzene : ethylformate : formic acid=1 : 5 : 2), $FeCl_3$; olive green, Mg-HCl and Zn-HCl; red purple, mp; 195-197℃, IR ν_{max} (cm^{-1}) 3390 (OH), 1650 (C=O), UV λ_{max} (nm); 258, 358, ^{1}H-NMR(DMSO-d_6) δ (ppm); 1.00 (3H, d, J=6 Hz, rha-CH_3), 4. 40 (1H, s, rha-1), 5.40 (1H, d, J=5. 5 Hz, glc-1), 6.25 (1H, d, J=2 Hz, H-6), 6.45 (1H, d, J=2 Hz, H-8), 6.89 (1H, dd, J=8 Hz, H-5), 7.60 (1H, d, J=2 Hz, H-2'), 7.63 (1H, dd, J=8 and 2 Hz, H-6').

Table I. ^{1}H-NMR of compound **2**, caffeic acid, quinic acid moiety from 3,5-dicaffeoyl quinic acid and compound **3**

Compound	Chemical shift(ppm)						
	C-3-H	C-5-H	C-8-H C-9-H	C-2-H	QC-3,5	QC-4	QC-2,6
Compound **2**	7.51(d)	7.14(s)	6.85(d) 6.97(d) 6.76(d)	6.26(d)			
Caffeic acid	7.41(d)	7.02(s)	6.91(d)	6.17(d)			
Quinic acid moiety					5.5(m)	4.2(dd)	2.3(m)
	7.54(d)	7.13(s)	6.83(d, 2H)	6.31(d)			
Compound **3**	7.57(d)	7.15(s)	6.99(d, 2H)	6.40(d)	5.37(m)	4.0(dd)	2.3(m)

Compound 2(caffeic acid): Yellow brown amorphous powder (H_2O), TLC; Rf=0.9 (benzene : ethylformate : formic acid=1 : 7 : 1), $FeCl_3$; yellow green, Pb $(Ac)_2$; yellow ppt., mp; 35-245℃(dec.), IR ν_{max} (cm^{-1}); 3400-2400 (OH, COOH), 1650 (C=O), 1600, 1520, 1120 (C-O). UV λ_{max} (nm): 245, (300), 328, ^{1}H-NMR (Acetone-d_6+D_2O) (ppm) (300 M Hz); see Table I.

Compound 3 (3,5-dicaffeoyl quinic acid): Yellow brown amorphous powder (H_2O), TLC; Rf=0.71 (benzene : ethylformate : formic acid=1 : 7 : 1), $FeCl_3$; yellow green, $Pb(Ac)_2$; yellow ppt., mp; 235-245℃(dec.), IR ν_{max} (cm^{-1}); 3500-2400 (OH, COOH), 1650 (C=O), 1600, 1520, 1120 (C-O), UV λ_{max} (nm); 245, (300), 328, ^{1}H-NMR(Acetone-d_6+D_2O) (ppm) (500 M Hz); see Table I, ^{13}C-NMR(Acetone-d_6+D_2O) (ppm); see Table II.

Alkaline hydrolysis of compound 3: 1N-NaOH (1 ml) was added to compound **3** (2 mg). After stirring for 10 mins, the mixture was neutralized with 1N-HCl (1 ml). TLC (benzene : ethylformate : formic acid : H_2O =1 : 7 : 1 : 0.1) of the hydrolysate was compared with that of a hydrolysate of monocaffeoyl quinic acid standard (chlorogenic acid) in the same condition. Thin layer chromatograms of both of the hydrolysates on silica gel plate were superimposed on each other, and each showed two spots. The one showed the same Rf value (0.9) as caffeic acid standard. The other (Rf= 0.7) which was not detected by $FeCl_3$ reagent but by vanillne-sulfuric acid was a spot of quinic acid.

Compound 4 (Kizuta saponin K_5): Colorless needles, TLC; Rf=0.47 ($CHCl_3$: CH_3OH : H_2O=9 : 3 : 0.3), mp; 133-135℃, Lieberman Burchard reaction; positive, IR ν_{max} (cm^{-1}); 3400 (OH), 1690 (C=O), 1100-1000 (C-O-), ^{1}H-NMR (CH_3OH-d_4) (ppm); 0.95, 1.16, 1.24 (each 3H, s, tert-CH_3×3), 1.33 (3H, s, C_{20}-Me). 1.47, 1.51 (each 3H, s, tert-CH_3+2), 1.89 (3H, s, C_{25}-Me), 4.98 (1H, d, J=7.4 Hz, glc-1), 5.68 (1H, t, C_{24}-H), ^{13}C-NMR (CH_3OH-d_4) (ppm); see Table III, Mass(EI); 456 (40.6), 438 (96), 420 (84), 357 (26), 314 (60.5), 203 (99.5), 126 (100).

Acid hydrolysis of compound 4: Compound **4** (2 mg) in MeOH (1 ml) was hydrolysed with 10% H_2SO_4 (1 ml) for 4 hrs and aglycon residue was removed with chloroform, and then $BaCO_3$ was added to water layer for precipitation of SO_4^{-2}. The spot of water layer in cellulose plate was compared with authentic samples, such as glucose, rhamnose, arabinose, lactose, and fructose using pyridine: EtOAc : HOAc : H_2O=5 : 5 : 1 : 3 as a solvent system. Its Rf value was as the same as that of D-glucose (Rf=0.48).

RESULTS AND DISCUSSION

Compound **1**, yellow needles, mp 195-197℃, was not soluble in $CHCl_3$ but soluble in MeOH. **1** was detected by $FeCl_3$, Mg-HCl and Zn-HCl reagents as a positive spot on TLC plate, suggesting it to be a flavonoid. The two bands of broad hydroxyl absorption at 3390 cm^{-1} and conjugated carbonyl absorption of γ-pyron at 1650cm^{-1} were showed in IR spectrum. UV λ_{max} of **1**, 256 and 358 nm, were appeared in a typical pattern of flavonol.

In the aromatic region of ^{1}H-NMR spectrum, two doublets at 6.25(J=2 Hz) and 6.45 ppm (J=2 Hz) suggested the presence of two meta-coupled protons of H-6 and H-8 on A-ring of the flavonoid respectively. The presence of a doublet at 7.60 ppm (J=2 Hz) and two sets of double doublets at 6.89 ppm (J=8 and 2 Hz) and 7.63 ppm (J=8 and 2 Hz) indicated that **1** had B ring of the flavonoid substituted at 3′ and 4′ carbon positions. In the aliphatic field, a typical doublet by three protons of methyl group from rhamnose was shown at 1.06 ppm (J=6 Hz). And each anomeric proton signal of rhamnose and glucose was revealed at 4.46 (br, s) and 5.40 ppm (d, J=6 Hz).

By the above data, **1** was expected to be quercetin with a β-bound rutinose,rutin. The TLC,IR and UV of **1** was also the same as that of rutin standard. **1** was identified as quercetin-3-O-rutinoside, rutin.

Compound **2**, yellow brown amorphous powder, mp 235-245℃(dec.), was not soluble in acetone, H_2O,

Table II. ^{13}C-NMR of compound **3**, caffeoyl methyl ester and quinic acid moiety from 3,5-dicaffeoyl quinic acid

Carbon No.	Compound **3**	caffeoyl-methyl ester	quinic acid moiety(Q)
1(1)	168.41 (168.63)	172.20	
2(2)	126.85 (127.06)	130.58	
3(3)	145.99 (146.15)	149.68	
4(4)	122.50 (2×C)	126.12	
5(5)	115.46 (2×C)	118.84	
6(6)	145.49 (145.54)	147.72	
7(7)	148.17 (148.31)	150.54	
8(8)	114.94 (2×C)	117.95	
9(9)	116.23 (2×C)	119.81	
QC-1	73.7		74.2
2	39.8		37.5
3	71.9		72.0
4	71.0		70.1
5	72.1		72.2
6	36.7		35.7
7	170.7		175.8

MeOH and $CHCl_3$ but soluble in acetone-H_2O mixture. The blue green spot on TLC plate by spraying of $FeCl_3$ reagent indicated that **2** was a phenolic compound.

In IR spectrum, the absorption bands of hydroxyl and carboxylic acid groups were shown at 3400 and 3500-2400(br), and the peak of α,β-unsaturated carbonyl group was shown at 1650 cm^{-1}. In ^{1}H-NMR spectrum, two doublets at 6.26 (1H, J=16 Hz) and 7.51 ppm (1H, J=16 Hz) indicated two protons of the olefine linked to carbonyl group. Since J-value was 16 Hz, it is clear that the two protons were trans form. A doublet (1H, J=8 Hz) at 6.85 ppm was ortho-coupled with a double doublet (J=8 and 2 Hz) at 6.97 ppm, which was meta-coupled with a doublet (J=2 Hz) at 7.14 ppm, suggesting **2** had a aryl group substituted at 3,4 position. From IR and ^{1}H-NMR spectrum, it was suggested that **2** was the combination of α,β-unsaturated carbonyl group, especially carboxylic group, and a aryl group substituted at 3,4 position. Furthermore, UV spectrum of **2** showed λ_{max} at 245, 328 nm and a shoulder at 300 nm. The shoulder at 300 nmm was the specific character of caffeic acid derivates. So we expected that **2** was a caffeic acid.

By the comparison of TLC and ^{1}H-NMR of **2** with caffeic acid standard (Table I), compound **2** was superimposed on a caffeic acid.

Compound **3**, yellow brown amorphous powder, mp 235-245℃(dec.), was not soluble in acetone, H_2O, MeOH and $CHCl_3$ but soluble in acetone-H_2O mixture. The blue green spot on TLC plate by spraying of $FeCl_3$ reagent indicated that **3** was a phenolic compound. Its UV and IR spectra were resembled with those of caffeic acid (**2**), indicating that **3** was a caffeic acid derivative. In the aromatic region of its ^{1}H-NMR spectrum, several proton signals of were also similar to that of **2**. However, the proton number of **3** was twice as many. So we expected that **3** had two caffeic acid groups. In the aliphatic region of ^{1}H-NMR, three multiple signals were showed. The one at 2.30 ppm indicated two methylene groups in similar environment. The double doublet at 3.98 ppm revealed the presence of methyne group adjacent to hydroxyl group. And another at 5.37 ppm was two methyne proton signals coupled with each two methylene groups. Therefore, **3** was considered to be a polyhydroxyl compound with two caffeic acid moieties. Total carbon number of **3** was 25 as shown in ^{13}C-NMR. Fourteen peaks were shown between 110 and 170ppm. Out of them, 10 were showed like as five doublet at 127, 145.5, 146, 148, 168 ppm due to each two carbon peaks under similar environment. Especially, the two peaks

Table III. ^{13}C-NMR of compound **4** comparing with that of Kizuta Saponin K_5

Carbon No.	Compound **4**[a]	Kizuta Saponin K_5[b]
1	40.9	40.2
2	34.1	33.4
3	223.0	218.6
4	48.5	47.8
5	59.9	59.6
6	68.1	66.9
7	45.9	45.9
8	41.8	41.3
9	50.3	49.6
10	39.4	38.4
11	23.2	22.9
12	25.2	25.3
13	43.4	42.4
14	51.3	50.6
15	32.3	31.6
16	28.7	28.1
17	50.5	50.4
18	18.1	17.7
19	16.1	16.1
20	75.1	74.3
21	25.8	26.0
22	42.1	41.5
23	23.5	23.0
24	130.4	129.4
25	132.7	132.3
26	75.9	75.2
27	14.2	14.2
28	32.5	32.2
29	19.9	20.0
30	16.9	16.8
26-O-glc-1	102.6	103.5
2	75.9	75.2
3	78.1	78.6
4	71.7	72.0
5	77.9	78.2
6	62.8	63.1

[a] solvent: methanol-d_4, [b] solvent: pyridine-d_5

at 168 ppm indicated that **3** had two carbonyl ester groups.

3 was confirmed to be a caffeic acid derivative because comparisons of the chemical shifts from the spectrum of it with that of caffeoyl methyl ester (Table II) indeed showed a close resemblance.

In that comparison, we found that above 14 peaks and 9 aromatic carbon peaks of caffeoyl methyl ester were showed at almost same positions. Therefore, each two peaks at 168, 148, 146, 145.5, and 127 ppm in ^{13}C-NMR were for C_1, C_7, C_3, C_6 and C_2 of two caffeoyl ester groups, and 4 peaks at 122, 116, 115.5, and 114.9 ppm were for C_4, C_9, C_5, C_8 (Table II). The carbons of caffeoyl moieties were numbered from carbonyl carbon. Another seven peaks in ^{13}C-NMR were expected for cyclohexane bearing poly hydroxyl group and one carbonyl group on the basis of ^{1}H-NMR. A signal at 170.7 showed the presence of carboxylic acid group and 4 peaks at 73.7, 72.1, 71.9 and 71.0 ppm showed the carbons linked hydroxyl group. Two peaks at 39.8 and 36.7 were by two methylene group.

Among the natural products, quinic acid was mainly found as a dicaffeoyl compounds. Therefore, **3** was expected to be dicaffeoyl quinic acid. By comparison of ^{1}H-NMR and ^{13}C-NMR of **3** with some data of disubstituted quinic acid(Saizo,1990) 3 was identified as 3,5-dicaffeoyl quinic acid one of the isochlorogenic acid (Table I, II).

Compound **4**, Colorless needles, mp 133-135℃, was not soluble in $CHCl_3$ but soluble in MeOH. Since **4** was not detected by UV and there was no proton peak on 6-8 ppm in ^{1}H-NMR, **4** was not expected to be a aromatic compound. The seven singlets between of 0.95 and 1.89 ppm in ^{1}H-NMR spectrum revealed seven angular methyls. The ^{1}H-NMR spectrum showed multiple signals at 3.8-4.6 ppm and anomeric proton peak at 4.98 ppm attributable to the sugar that was identified as D-glucose through comparison of its acid hydrolysate with authentic sugar samples. And ^{13}C-NMR also revealed six peaks of a glucose shown in Table III. Triplet(1H) at 5.68 ppm on ^{1}H-NMR spectrum indicated olefinic proton adjacent to methylene. That is, a propenyl groups ($C=CH-CH_2$-) is a part of compound **4**. Total carbon number of **4** is 36 as shown in ^{13}C-NMR (Table III). Out of them, six is for glucose, seven for angular methyl radicals and three carbons for propenyl groups. The twenty carbons were expected that they were for tetracyclic triterpenoid since the proton peak at 1.5-2.5 ppm in ^{1}H-NMR and the signal at 75.1 ppm in ^{13}C-NMR exhibited characters of tetracyclic triterpene. Out of angular methyl signals of ^{13}C-NMR, five peaks at 16.1, 16.8, 18.1, 19.9 and 32.5 ppm revealed those linked to the tetracyclic ring and two peaks at 75.1, 75.9 ppm to C_{20} of tetracyclic triterpene and olefinic carbon. The carbonyl absorption band at 1690 cm^{-1} in IR spectrum and the peak at 223 ppm in ^{13}C-NMR suggested that 4 has a tetracyclic triterpene possessing with a carbonyl group. And there was hydroxyl absorption band at 3400 cm^{-1} in IR spectrum.

The glucose was considered to be linked to the propenyl radical by p -osition (the J value of anomeric proton peak in proton NMR is 7-8 Hz). In mass spectrum, the intensive peak of m/z 203 supported the presence of triterpene, and m/z 456, 438, 420 indicated the presence of three hydroxyl groups in the aglycon moiety.

From above inspection and comparison of the spectral data of **4** and a paper reporting constituents of stem and bark of this plant, compound **4** was identified as 3-oxo-20(S)-dammar-24-ene-6α,20,26-triol-26-O-β-D-glucopy-ranoside, that is, Kizuta saponin K_5 (Table III).

From the above results, we found two important facts as followings. The one is that there are some relations between the leaves of *Hedera rhombea* and the roots of Panax ginseng. It is the fact that both of them have dammarane saponins, and especially, the leaves, not medicinal part so far, of *Hedera rhombea* have a lot of them. So pharmacological relationship between the two plants should be studied. The other is that the caffeic acid derivatives, few of which were reported in Araliaceae, was found. These compounds are main components of coffee bean, tabacco leaves and artichoke. Particularly, artichoke (Saengyakhak Yonguhoe, 1992) whose main components are dicaffeoyl quinic acid derivatives, cynarin is famous as a folk medicine with hepatotropic activities. Therefore, the leaves of this plant is also expected to have hepatotropic activities and the study on this point must be subsequent.

Furtheremore, the data of TLC showed that this plant has many other compounds, so the study on the constituents and those pharmacological activities will be processed.

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