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Phenolic Compounds of the Leaves of *Eucommia ulmoides*

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Abstract □ From the leaves of *Eucommia ulmoides*, quercetin, astragalin, isoquercitrin, quercetin 3-*O*-β-D-sambubioside and chlorogenic acid were isolated and characterized by spectral data.

Keywords □ *Eucommia ulmoides*, quercetin, astragalin, isoquercitrin, quercetin 3-*O*-β-D-sambubioside, chlorogenic acid. ^{13}C-NMR.

Dù Zhòng(杜仲), the bark of *Eucommia ulmoides* OLIV. (Eucommiaceae) is a very expensive crude drug in traditional Chinese medicine as an antihypertensive, diuretics, analgesic, sedative and the enhancement of liver activity[1].

Leaves of *Eucommia ulmoides* are used as a source of Dù Zhòng Cha because of sufficiently acceptable qualities[2]. Hattori *et al.* evaluated leaves of *Eucommia ulmoides* pharmacologically and histologically and reported that the water extracts showed a hypotensive effect, diuretic effect, brain and NG 108-15 cell membrane adenylate cyclase suppressing activities and improvement of the function of liver cells[3,4]. Hong *et al.* also reported that the water extracts showed diuretic, bile secretory, anti-fatigue and anti-diabetic effects[5,6].

The isolation of several iridoids, a cyclohexane derivative and some monomeric phenylpropanoids[7-9] from the leaves of *E. ulmoides* was previously reported.

The present paper deals with the isolation and identification of an phenolic compound and flavonol glycosides from this plant part.

EXPERIMENTAL METHODS

The mps were taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Varian Techtron Model 635 spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. 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. The mass spectrum was taken a Hewlett-Packard 5985 B GC/MS spectrometer operating at 70 eV. Optical rotation was measured on Rudolph Autopol® III automatic polarimeter.

Extraction, fractionation and isolation

Commercially available powdered leaves of *E. ulmoides* (0.8 kg) was refluxed with MeOH. The MeOH extract (83 g) was partitioned with $CHCl_3$ (20 g), ethylacetate (6.8 g), *n*-BuOH (35.8 g) and H_2O (20 g), respectively. The ethylacetate extract (6.5 g) was subjected to chromatography using SiO_2 ($CHCl_3$:MeOH:H_2O=65:35:10, lower phase) and Sephadex LH-20 (MeOH) columns to yield **1-5** in the order of elution.

Quercetin (1)

mp. 315-6°C. UV λ_{max}^{MeOH} nm: 258, 305 (sh.), 375; $\lambda_{max}^{MeOH+NaOMe}$ 248 (sh.), 335, 420 (dec.); $\lambda_{max}^{MeOH+AlCl_3}$ 275, 340, 460; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270, 307 (sh.), 365, 435; $\lambda_{max}^{MeOH+NaOAc}$ 260 (sh.), 278, 328, 388; λ_{max}^{MeOH+

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$^{NaOAc+H_3BO_3}$ 243, 285 (sh.), 372, IR ν_{max}^{KBr} cm^{-1}; 3400-3200 (OH), 1670 (C=O), 1610, 1500 (C=C).

Astragalin (2)

mp. 224-5℃, UV λ_{max}^{MeOH} nm; 266, 300 (sh.), 357; $\lambda_{max}^{MeOH+NaOMe}$ 277, 329, 403; $\lambda_{max}^{MeOH+AlCl_3}$ 278, 303, 352, 400; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 278, 302, 348, 394; $\lambda_{max}^{MeOH+NaOAc}$ 273, 308, 361; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 268, 297, 355; IR ν_{max}^{KBr} cm^{-1}; 3400-3200 (OH), 1650 (α, β-unsat. ketone), 1605, 1575, 1505 (aromatic), 1350, 1270, 1180, 1100-1000 (glycoside), ^{1}H-NMR (DMSO-d_6) δ; 11.6 (1H, brs, C_5-OH), 8.04 (2H, d, J=9, H-2′ and 6′), 6.88 (2H, d, J=9, H-3′ and 5′), 6.42 (1H, d, J=2, H-8), 6.20 (1H, d, J=2, H-6), 5.44 (1H, d, J=7, anomeric).

Isoquercitrin (3)

mp. 236-8℃, Mg/HCl, $FeCl_3$, Molisch test; positive, UV λ_{max}^{MeOH} nm; 257, 359; $\lambda_{max}^{MeOH+AlCl_3}$ 276, 437; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270, 363, 401; $\lambda_{max}^{MeOH+NaOAc}$ 271, 372; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 261, 379; IR ν_{max}^{KBr} cm^{-1}; 3350 (OH),1655 (C=O), 1600 (C=C), 1,100-1,000 (glycoside); ^{1}H-NMR (DMSO-d_6) δ; 11.60 (1H, brs, C_5-OH), 7.56 (1H, d, J=2, H-2′), 7.53 (1H, dd, J=9 and 2, H-6′), 6.92 (1H, d, J=9, H-5′), 6.49 (1H, d, J=2, H-8), 6.27 (1H, d, J=2, H-6), 5.36 (1H, d, J=7, anomeric).

Quercetin 3-O-β-D-sambubioside (4)

Hygroscopic powder, $[\alpha]_D^{25}$ = −55.6° (*c* 0.27, MeOH); UV λ_{max}^{MeOH} nm; 260, 289, 361; $\lambda_{max}^{MeOH+NaOMe}$ 274, 330, 406; $\lambda_{max}^{MeOH+AlCl_3}$ 280, 437; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 277, 366, 405; $\lambda_{max}^{MeOH+NaOAc}$ 273, 400; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 264, 385; ^{1}H-NMR (DMSO-d_6) δ; 12.75 (1H, brs, C_5-OH), 7.75 (1H, dd, J=2.2 and 8.6, H-6′), 7.62 (1H, d, J=2.2, H-2′), 6.93 (1H, d, J=8.6, H-5′), 6.45 (1H, d, J=1.7, H-8), 6.38 (1H, d, J=1.7, H-6), 5.72 (1H, d, J=7, anomeric), 4.93 (1H, d, J=6.8, anomeric).

Acid hydrolysis of 2, 3 and 4

Ten mg of **2**, **3**, and **4** was separately refluxed with 10% H_2SO_4 (15 m*l*) for 4 hr. After cooling, the reaction mixtures were filtered. The aglycones were crystallized from MeOH to give kaempferol from **2**, quercetin from **3** and **4**, respectively. They were confirmed by direct comparisons with authentic samples (TLC, mmp and UV). The filtrates were neutralized with $BaCO_3$, filtered and concentrated *in vacuo*. D-glucose from **2** and **3**, and D-glucose and D-xylose from **4** were detected by TLC.

Mild acid hydrolysis of 4

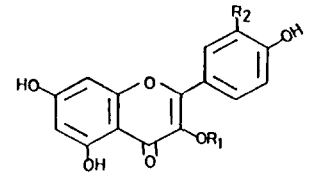
A solution of **4** (40 mg) in 1% H_2SO_4 (5 m*l*) was heated at 95° for 30 min. After cooling, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and chromatographed on silica gel with EtOAc-MeOH-H_2O (600 : 99 : 81) to yield quercetin, isoquercitrin and unreacted compound 4, which were identified with authentic samples.

Chlorogenic acid (5)

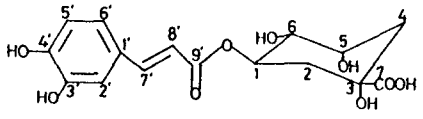
White greyish powder from MeOH, mp. 222-234℃, $FeCl_3$; positive, IR ν_{max}^{KBr}cm^{-1}; 3450 (br. -OH), 1709 (acid), 1642 (α, β-unsaturated ketone), 1600, 1534 (aromatic), 980 (trans); UV λ_{max}^{MeOH} nm; 222, 250, 304, 334; $\lambda_{max}^{MeOH+NaOMe}$ 266, 312 (sh.), 380; Rf value (TLC solvent: EtOAc-MeOH-H_2O (600: 99: 81); 0.20 (cf: 0.30 for neochlorogenic acid); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ; 7.43 (1H, d, J=15.8, H-7′), 7.03 (1H, s, H-2′), 6.90 (1H, d, J=7.9, H-6′), 6.75 (1H, d, J=8.1, H-5′), 6.20 (1H, d, J=15.8, H-8′), 5.19 (1H, br. dd, J=10 and 5, H-1), 4.02 (1H, m, H-5), 3.53 (1H, d, J=9.8, H-6), 2.06-1.84 (4H, m, H-2 and 4); ^{13}C-NMR (DMSO-d_6, 75.5 MHz) δ; 178.3 (C-7), 166.5 (C-9′), 148.6 (C-4′), 145.8 (C-3′), 144.8 (C-7′), 125.6 (C-1′ and C-6′), 121.4 (C-5′), 116.1 (C-2′), 114.8 (C-8′), 77.3 (C-3), 73.0 (C-6), 71.4 (C-1), 71.3 (C-5), 37.6 (C-2 and C-4).

Acetylation of 5

A sample (50 mg) in pyridine and Ac_2O (2 m*l*, each) was allowed to stand at room temperature for 3 days. The reaction mixture was poured into crushed ice and filtered. The precipitate was crystallized from MeOH-H_2O to give white needles (55 mg), mp. 143-5℃, IR ν_{max}^{KBr} cm^{-1}; 1740, 1210 (acetate); MS (m/z, %); M$^+$ (not detected), 480 (18.0), 420 (24.6), 222 (6.0), 180 (39.5), 162 (100); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 7.60 (1H, d, J=16, H-7′), 7.42 (1H, dd, J=2 and 8.5, H-6′), 7.38 (1H, d, J=2, H-2′), 7.31 (1H, d, J=8.5, H-5′), 6.34 (1H, d, J=16, H-8′), 5.57 (1H, br. dd, J=4 and 10, H-5), 5.52 (1H, dd, J=4.1 and 10, H-3), 5.12 (1H, dd, J=3.5 and 10, H-4), 2.72-2.39 (4H, m, 2x-CH_2), 2.31, 2.30 (3H, each, phenolic -OAc), 2.13, 2.07, 2.00 (3H, each, aliphatic -OAc); ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ; 171.7 (C-7), 169.8, 169.6, 169.4, 167.8, 167.7 (each, -O$\underline{C}$OCH$_3$), 165.0 (C-9′), 143.5 (C-3′ and C-7′), 142. 2 (C-4′), 132.7 (C-1′), 126.3 (C-6′), 123.7 (C-5′), 122.6 (C-2′), 118.2 (C-8′), 78.5 (C-1), 71.2 (C-3), 67.6 (C-4), 66.7 (C-5), 36.5 (C-



	R_1	R_2
1	H	OH
2	Glucose	H
3	Glucose	OH
4	Sambubiose	OH



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2), 31.6 (C-6), 20.9, 20.7, 20.5 (each, -OCOC$\underline{H}_3$), 20.3 (-OCOC$\underline{H}_3$×2).

RESULTS AND DISCUSSION

Column chromatography of ethylacetate soluble fraction obtained from the methanolic extract afforded five phenolic compounds **1**, **2**, **3**, **4**, and **5** in the order of increasing polarity.

Compound **1**, mp. 315-6°, showed positive $FeCl_3$, Zn+HCl and Mg+HCl tests and identified as quercetin by direct comparisons with an authentic sample (mp and TLC).

Compound **2**, mp. 224-5°, compound **3**, mp. 236-8° and compound **4**, an hygroscopic powder, showed positive Mg+HCl, Zn+HCl and Molisch tests. Acid hydrolysis yielded kaempferol from **2**, quercetin from **3** and **4** as the aglycone and D-glucose from **2** and **3** and D-glucose and D-xylose from **4** as the sugar.

The ^{1}H-NMR spectra of compounds **2** and **3** showed one anomeric proton signal, indicating the presence of one mole of sugar in **2** and **3**. On the other hands, compound **4** showed two anomeric proton signal in the ^{1}H-NMR spectrum.

The UV spectrum of each compound, exhibiting band I peak at 350-360 nm, was very similar to those reported for a number of 3-hydroxyl substituted flavonols[10]. A bathochromic shift of band I in the presence of $AlCl_3$ or $AlCl_3$+HCl and of band II in the presence of NaOAc indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. And also a bathochromic shift with NaOMe, without decrease in intensity, showed the presence of a free 4'-hydroxyl group. It was, thus, suggested that the sugar might be attached to 3-hydroxyl group. This was further confirmed by the inspection of ^{13}C-NMR spectra (See Table I). The configuration and conformation of sugar moiety was determined by the J value of the anomeric proton signal (see Experimental).

Compounds **2** and **3** were, therefore, identified as kaempferol 3-*O*-β-D-glucoside (astragalin) and quercetin 3-*O*-β-D-glucoside (isoquercitrin), respectively.

Mild acid hydrolysis of **4** gave **3**, mp. 236-8°, which was identified by direct comparison with an authentic sample. It could, therefore, be suggested

Table I. ^{13}C-NMR spectral data of compounds 2, 3, and 4 in DMSO-d_6

Carbon No	2	3	4	Methyl-xyloside[11]
2	156.4*	156.3*	156.3	
3	133.2	133.3	132.8	
4	177.4	177.4	177.1	
5	161.2	161.2	161.2	
6	98.7	98.7	97.9	
7	164.2	164.3	165.3	
8	93.6	93.5	93.5	
9	156.2*	156.2*	155.1	
10	104.0	103.9	104.5	
1′	120.9	121.2	121.8	
2′	130.8	115.2	115.2	
3′	115.1	144.8	145.0	
4′	159.9	148.5	148.8	
5′	115.1	116.2	115.8	
6′	130.8	121.6	121.6	
1″	101.0	100.9	98.9	
2″	74.2	74.1	81.8	
3″	76.4	76.5	76.0	
4″	69.9	70.0	69.5	
5″	77.4	77.6	77.5	
6″	60.9	61.0	60.6	
1‴			103.4	105.1
2‴			73.9	74.0
3‴			76.8	76.9
4‴			69.4	70.4
5‴			65.6	66.3

*Values with the same symbol may be interchanged in the vertical column.

that **4** had a disaccharide chain composed of inner glucose and terminal xylose. The sugar-sugar linkage of disaccharide was deduced from its ^{13}C-NMR. All the carbon signals for xylose were similar to those of methyl xyloside[11], implying a terminal sugar and the signal for C-2 of the glucose as compared with that of **3** was appeared at the low field (81.8 ppm), indicating that the disaccharide should be sambubiose. Therefore, the structure of compound **4** was elucidated as quercetin 3-*O*-β-D-sambubioside.

Compound 5, mp. 222-234℃, showed the presence of a hydroxyl (3450 cm^{-1}), acid (1709 cm^{-1}), α, β-unsaturated ketone (1642 cm^{-1}), aromatic (1600 and 1534 cm^{-1}) in its IR spectrum. It gave a pentaacetate (**5a**), mp. 143-5℃, on acetylation with Ac_2O/pyridine. The MS spectrum of **5a** did not show a molecular ion peak but two prominent ion peaks at m/z 180 ($C_9H_8O_4$, 39.5%) and 162 ($C_9H_8O_4$-H_2O, 100%) suggesting the presence of caffeoyl moiety. Its NMR spectra also showed the signals of caffeoic acid (see Experimental) and two methylenes (36.5 and 31.6 ppm), three oxygen-bearing methine (71.2, 67.6 and 66.7 ppm), one oxygen-bearing tetrahedral carbons (78.5 ppm) and acid (171.7 ppm) ascribable to cyclopolyoxycarboxylic acid, i.e quinic acid. These spectral data were in agreement with those for the structure of caffeoyl quinic acid, which is an ester of caffeic acid with quinic acid. There are a number of isomers of caffeoyl quinic acid known as neochlorogenic acid (5-*O*-caffeoyl quinic acid), band 510 (4-*O*-caffeoyl quinic acid), chlorogenic acid (3-*O*-caffeoyl quinic acid) and isochlorogenic acid (a mixture of 3,4-*O*-dicaffeoyl quinic acid, 3,5-*O*-dicaffeoyl quinic acid). The identity with chlorogenic acid was identified by comparison of ^1H-NMR spectral data with those reported in the literature[12] and finally confirmed by direct comparisons with an authentic sample kindly supplied by Dr. Sakushima. A., Higashi Nippon Gakuen University.

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