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Phenolic Compounds from the Rachis of *Cedrela sinensis*

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Abstract – From the rachis of *Cedrela sinensis* A. Juss., methyl gallate, quercitrin, bis-(p-hydroxyphenyl)ether, adenosine, isoquercitrin, rutin, (+)-catechin and (-)-epicatechin were isolated and characterized by spectral data.

Key words – *Cedrela sinensis* A. Juss.; methyl gallate; quercitrin; isoquercitrin; bis-(p-hydroxyphenyl)ether; adenosine; rutin; (+)-catechin; (-)-epicatechin.

Cedrela sinensis A. Juss. (Meliaceae) is a broadleaf tree which grows in the Jonnam and Kyeongnam in Korea.[1)] The leaves and stems of this plant have been used for the treatments of enteritis, dysentery and itch in oriental medicine.[2)] In this paper, we report the isolation and structure elucidation of the seven phenolic compounds including nucleoside, adenosine.

MATERIALS AND METHODS

Plant material – The rachis of *C. sinensis* was collected in Sunchon on April 12, 1994. A voucher specimen has been deposited in Department of Oriental Medicine Resources, Sunchon National University.

Appratus – The mps were taken on a Thomas Hoober 6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Hitachi 2703-spectrophotometer and the UV spectra were run with CE 599 Universal automatic scanning spectrophotometer. The EI-MS spectra were taken with a Hewlett-Packard 5985B GC/MS spectrometer operating at 70eV. 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). Reversed-phased HPLC was performed on a LiChrospher RP-18 (5 μm) column (4×250 cm) with H_2O-MeOH-AcOH(90:6:4) (flow rate: 1.0ml/min) at 40 ℃. Detection was effected with a Shimadzu SPD-6A spectrophotometric detector at 280 nm.

Extraction, fractionation and isolation – The dried and powdered rachis of *C. sinensis* (600 g) was refluxed with MeOH. The MeOH extract (95 g) was partitioned with $CHCl_3$(12 g), EtOAc(2 g), n-BuOH(20 g) and H_2O(50 g) fractions, respectively. The ethylacetate fraction (2 g) was subjected to chromatograph using SiO_2(30 g) with $CHCl_3$-MeOH-H_2O (25:8:5, lower layer), $CHCl_3$-MeOH-H_2O (7:3:1, lower layer) as solvents to give com-

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pounds 1 (frs. 7-8, 10 mg) , 2 (frs. 53-58, 97 mg) and the mixture of compounds 7 and 8 (frs. 22-30, 40 mg). The n-BuOH soluble fraction (17 g) was also chromatographed on a SiO_2 with $CHCl_3$-MeOH-H_2O (7:3:1, lower layer) and $CHCl_3$-MeOH-H_2O (65:35:10, lower layer) as solvents to give compounds 3 (frs. 27-39, 16 mg), 4 (frs. 54-57, 50 mg), 5 (frs. 88-92, 20 mg) and 6 (frs. 146-169, 66 mg). Then frs. 27-39(16 mg) were purified by repeated column chormatography on Sephadex LH-20 (20 g) with MeOH-H_2O (2:1 and 1:0) to yield compound 3 (12 mg).

Compound 1 - mp 196-198 °; IR, ν_{max}^{KBr} : 3364 (OH), 1694(C=O), 1536, 1443, 1371 cm^{-1}; ^{1}H-NMR (200MHz, DMSO-d_6) δ: 6.93(2H, S, H-2 &6), 3.73(3H, s, $COOCH_3$), ^{13}C-NMR (DMSO-d_6) δ: 166.3(C-7), 145.5(C-3&5), 138.3 (C-4), 119.3(C-1), 108.5(C-2&6), 51.5 ($COOCH_3$)

Compound 2 - mp 174-176 °; IR, ν_{max}^{KBr} : 3430 (OH), 1660(α,β-unsaturated ketone), 1628, 1565, 1504, 1445(aromatic C=C), 1130, 1050, 1018(glycosidic C-O) cm^{-1}; UV, λ_{max} nm: Table I; ^{1}H-NMR(200MHz, DMSO-d_6): Table II; ^{13}C-NMR(50.3MHz, DMSO-d_6): Table III.

Compound 3 - mp 341 °; IR, ν_{max}^{KBr} : 3112 (OH), 1674, 1651(aromatic C=C) cm^{-1}; EI-MS, m/z(rel. int.) 202(M^+, 4.5), 69(100) cm^{-1}; ^{1}H-NMR(200MHz, DMSO-d_6) δ: 11.11(H, br.s, OH), 10.91(1H. br.s, OH), 7.48(2H, d, J=7.6 Hz), 5.54(2H, d, J=7.6Hz); ^{13}C-NMR(50.3MHz, DMSO-d_6) δ: 164.1, 151.3, 142.0(×2), 100.1(×2)

Compound 4 - mp 224-6 °; UV, λ_{max}(MeOH) 208, 261 nm; IR, ν_{max}^{KBr} : 3380(OH, NH_2), 1648 (NH), 1077(C-N) cm^{-1}; EI-MS, m/z(rel. int.) 267(M^+, 1.8), 237(12.6), 178(38.4), 165(11.4), 164(90.4), 136(82.9), 135(100), 134(6.1), 121(7.9), 119(13.9), 108(45.2); ^{1}H-NMR (200MHz, DMSO-d_6) δ: 8.33(1H, s, H-8), 8.12(1H, s, H-2), 5.86(1H, d, J=6.2Hz, H-1'), 4.59(1H, m, H-2'), 4.14(1H, m, H-3'), 3.96(1H, dd, J=6.6, 3.5Hz H-4'), 3.64(1H, m, H-5'a), 3.56(1H, m, H-5'b); ^{13}C-NMR (50.3MHz, DMSO-d6) δ: 156.1(C-6), 152.3(C-2), 149.0(C-4), 139.8(C-8), 119.3(C-5), 87.9(C-1'), 85.8(C-4'), 73.4(C-

Table I. UV spectral data for compounds 2, 5 and 6(λmax, nm)

Sovent	2	5	6
MeOH	259	258	260
	303sh	360	300sh
	352		360
+NaOMe	272	275	271
	328	331	328
	395	395	411
+$AlCl_3$	278	275	275
	306sh	434	305sh
	335		434
	433		
+$AlCl_3$ +HCl	273	269	272
	304sh	360	303
	354	400	366sh
	405		405
+NaOAc	275	270	270
	324sh	381	323
	374		395
+NaOAc+H_3 Bo_3	263	261	260
	303sh	382	399
	369		389

Table II. ^{1}H-NMR spectral data for compounds 2, 5 and 6 in DMSO-d_6

Proton No.	2	5	6
6	6.20(1H,d,J=2.0Hz)	6.32(1H,d,J=1.95Hz)	6.19(1H,d,J=1.9Hz)
8	6.38(1H,d,J=2.0Hz)	6.11(H,d,J=1.95Hz)	6.38(1H,d,J=1.9Hz)
2'	7.29(1H,d,J=2.0Hz)	7.47(1H,d,J=2.0Hz)	7.53(1H,d,J=1.9Hz)
5'	6.85(1H,d,J=8.2Hz)	6.76(1H,d,J=9.3Hz)	6.83(1H,d,J=8.9Hz)
6'	7.25(1H,dd,J=2.0&8.2Hz)	7.47(1H,dd,J=2.0&9.3Hz)	7.53(1H,dd,J=1.9&8.9Hz)
1''	5.24(1H,d,J=1.2Hz)	5.35(1H,d,J=7.7Hz)	5.33(1H,d,J=7.4Hz)
1'''			4.37(1H,s)
$-CH_3$	0.81(3H,d,J=5.5Hz)		0.99(3H,d,J=6.08Hz)

Table III. ^{13}C-NMR spectral data for compounds 2, 5 and 6 in DMSO-d_6

Carbon No.	2	5	6
2	157.2	156.1	156.6
3	134.2	133.3	133.3
4	177.7	177.3	177.3
5	161.3	161.2	161.2
6	98.6	98.6	98.7
7	164.1	164.1	164.2
8	93.6	93.4	93.6
9	156.4	156.2	156.4
10	104.0	103.9	104.1
1′	121.1	121.5	121.6
2′	115.4	115.1	115.3
3′	145.1	144.7	144.7
4′	148.4	148.4	148.4
5′	115.6	116.1	116.3
6′	120.7	121.1	121.2
Glucosyl			
1″	101.8	100.9	101.2
2″	70.0	74.0	74.1
3″	70.3	76.5	76.5
4″	71.1	70.0	70.6
5″	70.5	77.4	75.9
6″	17.4	61.0	67.0
Rhamnosyl			
1‴			100.7
2‴			70.4
3‴			70.0
4‴			71.9
5‴			68.2
6‴			17.7

2′), 70.6(C-3′), 61.6(C-5′)

Compound 5 – mp 234-236 °; IR, ν_{max}^{KBr} : 3340 (OH), 1663(α,β-unsaturated ketone), 1610, 1510, 1494(aromatic C=C), 1052(glycosidic C-O) cm^{-1}; UV, λ_{max} nm; Table I; ^{1}H-NMR (200MHz, DMSO-d_6); Table II; ^{13}C-NMR(50.3 MHz, DMSO-d_6); Table III

Compound 6 – mp 186-188 °; IR, ν_{max}^{KBr} : 3421 (OH), 1653(α,β-unsaturated ketone), 1601, 1506, 1457(aromatic C=C), 1125, 1062(glycosidic C-O) cm^{-1}; UV, λ_{max} nm; Table I; ^{1}H-NMR(200MHz, DMSO-d_6); Table II, ^{13}C-NMR (50.3MHz, DMSO-d_6); Table III

Acid hydrolysis of 2, 5 and 6 – 30 mg of each 2, 5 and 6 were separately refluxed with

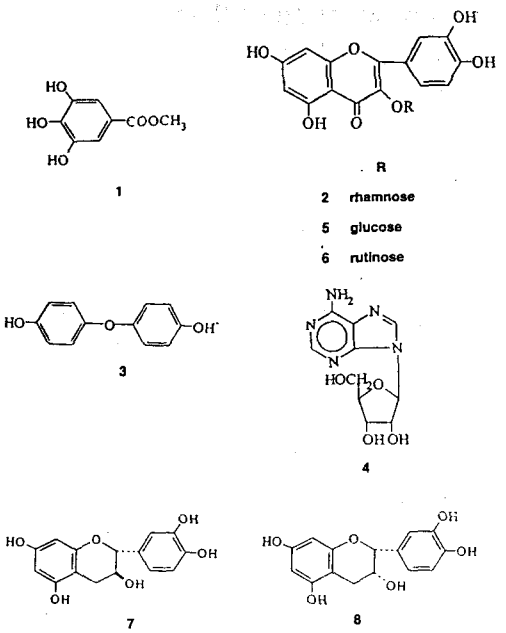

Fig. 1. structures of compounds isolated from the rachis of *Cedrela sinensis*. **1: methyl gallate, 2: quercitrin, 3: bis(p-hydroxyphenyl)ether, 4: adenosine, 5: isoquercitrin, 6: rutin, 7: (+)-catechin, 8: (-)-epicatechin.**

10% H_2SO_4 for 4hr. After cooling, the reaction mixtures were filtered. The aglycones were crystallized from MeOH to give quercetin from 2, 5 and 6, respectively. They were confirmed by direct comparison with authentic sample (TLC, ^{1}H-NMR).

RESULTS AND DISCUSSION

Column chromatography of ethyl acetate and n-butanol soluble fraction obtained from the methanolic extract afforded eight compounds. Compound 1, mp 196-198 °C, displayed hydroxyl (3364 cm^{-1}), carboxyl(1694 cm^{-1}) and double bond(1536, 1443 cm^{-1}) absorptions in its IR spectrum. The ^{1}H-NMR spectrum of compound 1 showed two singlets at δ6.93(2H) and δ3.73(3H) attributable to galloyl and methoxyl protons, respectively. These data were expected that compound 1 is gallic acid methyl ester. A

comparison of the ^{13}C-NMR spectrum of compound 1 with literature data[3] showed it to be methyl gallate.

Compounds 2, 5 and 6 gave a positive reaction in Molisch test and showed glycosidic bond in IR spectra. The UV maxima of compounds 2, 5 and 6, exibiting band I peak at 352-360 nm(Table I) were very similar to those reported for a number of 3-hydroxy substituted flavonol. These compounds showed a bathochromic shift with $AlCl_3$ and $AlCl_3$/HCl in band I, with NaOAc in band II which indicated the presence of free 5- and 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 2, 5 and 6 the hypsochromic shift in band I of the $AlCl_3$ on addition of acid resulted the presence of ortho-dihydroxyl group of B-ring.[4] The ^{1}H-NMR spectra of compounds 2 and 5 showed one anomeric proton signal at δ5.24(J=1.2Hz) and 5.35(J=7.7Hz), respectively. Two anomeric proton signals at δ4.37(s) and 5.33(J=7.4Hz) in the ^{1}H-NMR spectrum of compound 6 indicated that 2 mole of sugars was linked, one of which was assumed to be rhamnose by the diagnostic methyl signal at δ0.99(Table II). The ^{1}H-NMR spectra of compounds 2, 5 and 6 showed two meta-coupled doublets ascribable to H-8 and H-6 of A-ring in the flavonoid skeleton, and an ortho-coupled doublet, a meta-coupled doublet and a ortho, meta-coupled doublet-doublets attributable to H-2′, H-6′ and H-5′ of B-ring, respectively (Table II). These data indicated that compounds 2, 5 and 6 were quercetin glycosides. Acid hydrolysis of 2, 5 and 6 yielded quercetin as their genins. 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, 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 sugar moiety of compounds 2, 5 and 6 were determined to be α-L-rhamnopyranose, β-D-glucopyranose and rutinose, respectively, by the J values of the anomeric proton signals and the ^{13}C-NMR data. From the above results, compounds 2, 5 and 6 were characterized as quercitrin, isoquercitrin and rutin, respectively.

Compound 3, mp 341 °C, showed a molecular ion peak at *m/z* 202 in mass spectrum. The IR spectrum of compound 3 displayed the absorption bands at 3112, 1674 and 1651 cm^{-1}, indicating the presence of hydroxyl group and aromatic ring in the molecule. The ^{1}H-NMR spectrum of compound 3 showed two ortho-coupled doublets with the J values of 7.6 Hz at δ7.48 and 5.54, indicating the presence of a 1,4-disubstituted benzene ring and two singlets at δ11.11 and 10.91 assignable to two phenolic hydroxyl protons, respectively. Two signals(δ 151.3, 164.1) equivalent to one carbon moiety and the other two peaks (δ100.1, 142.0) of two carbon moieties were observed in the ^{13}C-NMR spectrum of compound 3. Comparison with literature data[6] confirmed that compound 3 was bis(p-hydroxyphenyl)ether.

Compound 4, mp 224-5 °C, showed the characteristic bands at 3340(OH, NH_2), 1663(NH) and 1077(C-N) cm^{-1} in the IR spectrum. Its UV spectrum showed the strong absorption peaks at 208 and 261 nm, indicating that compound 4 was a purine nucleoside.[7] The mass spectrum of compound 4 showed a molecular ion at m/z 267 along with a base peak at m/z 135 (M^+- ribosyl

moiety). The ^{1}H-NMR spectrum of compound 4 showed two aromatic singlets at δ8.12 and 8.33 and other signals assignable to the protons of ribosyl moiety. The ^{1}H- and ^{13}C-NMR data of compound 4 were in agreement with those of adenosine[8].

Compounds 7 and 8 were identified to be (+)-catechin (Rt: 9.38) and (-)-epicatechin (Rt: 21.23), respectively, by reversed-phased HPLC on a LiChrospher RP-18 column with H_2O-MeOH-AcOH(90:6:4) using authentic samples.

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