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Phenolic Compounds from the Stems of *Sapium japonicum*

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Abstract – A chemical examination of the stems of *Sapium japonicum* PAX et H_{OFFM} (Euphorbiaceae) has led to the isolation of seven phenolic compounds. On the basis of UV, IR, MS, and NMR spectral data and the chemical reaction, the structures of these compounds were identified as gallic acid (**1**), ellagic acid (**2**), 3,3'-di-*O*-methylellagic acid (**3**), 4-*O*-(β-D-xylopyranosyl)-3,3'-di-*O*-methylellagic acid (**4**), 4-*O*-(α-D-arabinofuranosyl)-3,3'-di-*O*-methylellagic acid (**5**), isoquercitrin (**6**), and geraniin (**7**).
Keywords – *Sapium japonicum*, ellagic acid derivatives, tannin, flavonoid

Introduction

Sapium japonicum P_{AX} et H_{OFFM} (Euphorbiaceae) is a deciduous tree growing widely along the coast of Korea from Seorak Mt. to Baengnyeong island. It is also distributed in the south area of Gyeryong Mt. of Korea (Lee, 1993). The stems of *Sapium sebiferum* belonging to the same genus has been used for a carbuncle, furuncle, and mastitis in China (Pharmacopeia Committee, 1977). It has been reported that the *Sapium* genus contains diterpenes, triterpenes, glycosides, and kauranes (Siems *et al.*, 1993; Ahmad *et al.*, 1991; Pradhan *et al.*, 1984; Kouno *et al.*, 1983; Taylor *et al.*, 1983). *Sapium japonicum* contains piscicidal constituent, phorbol ester (Ohigashi *et al.*, 1972a), and antifungal constituent, methyl 8-hydroxy-5,6-octadienoate (Ohigashi *et al.*, 1972b). We also reported the isolation and structural determination of eleven phenolic compounds from the leaves of *S. japonicum*. (Ahn *et al.*, 1996). Continuing investigation on the ethylacetate fraction of the stems of *S. japonicum* resulted in the isolation of gallic acid, ellagic acid and its three derivatives along with two known phenolic compounds. Ellagic acid and its three derivatives are isolated from the *Sapium* genus for the first time.

Experimental

General experimental procedure – Melting point was determined on a model 510-K melting point apparatus (Buchi company, Swiss) and was not corrected. Optical rotations were taken on Jasco DIP-4 polarimeter. The IR spectra were recorded with Perkin-Elmer spectrophotometer (Model LE 599, U.K.). UV spectra were obtained on Milton Roy spectronic 3000 array. MS spectra were measured on Finnigan Navigator (ESI-MS), Hewlett Packard 5989A (EI-MS) and JEOL JMS-HX/HX110A (FAB-MS) mass spectrometer, respectively. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were run on Varian Unity 300 spectrophotometer and the chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Coupling constant (*J*) were given in Hz. TLC was performed on silica gel 60 F_{254} aluminium sheet (0.2 mm, Merck) and cellulose F aluminium sheet (0.1 mm, Merck). Sephadex LH-20 (25 ~ 100 μ, Pharmacia Fine Chemical Co. Ltd.) and silica gel 60 (70 ~ 230 mesh, AST M 9385, Merck) were used for open column chromatography.

Plant material – The stems of *Sapium japonicum* were collected in Gaya Mt., Chungnam Province, Korea in May of 2000 and identified by Dr. Kyong Soon Lee, Chungbuk National University. A voucher specimen was deposited at Chungbuk National University.

Extraction, fractionation, and isolation – The dried stems (6.75 kg) were cut into small pieces and extracted three times with 80% aqueous acetone at room temperature. The aqueous acetone extract was evaporated to dryness. The dried residue was suspended with water and partitioned with hexane, CH_2Cl_2, and EtOAc. The EtOAc extract (59.9 g) was subjected to silica gel column using a gradient solvent system of CH_2Cl_2 : MeOH (10 : 1 → 1 : 10) to

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give four fractions (E1 ~ E4). The fractions E1 ~ E3 were separately applied over Sephadex LH-20. The elution with a gradient solvent system of H_2O : MeOH (10 : 0 → 0 : 10) gave compound **1** (157 mg), **3** (38 mg), **4** (35 mg), **5** (30 mg), and **7** (220 mg). Fraction (E4) was subjected to Sephadex LH-20 with a gradient solvent system of H_2O : MeOH (10 : 0 → 0 : 10) to afford three subfractions. The second subfraction (E4-2) was re-chromatographed on Sephadex LH-20 with MeOH to yield compound **2** (22 mg) and **6** (111 mg).

Gallic acid (1) : colorless needles (H_2O); mp 215 ~ 220 °C; UV (MeOH) λ_{max} (log ε) nm : 218 (4.99), 273 (4.57); IR (KBr) ν_{max} cm^{-1} : 3400 ~ 2400, 1700, 1610 ~ 1630, 1330 and 1250; EI-MS *m/z* 170 $[M]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 6.95 (2H, s, galloyl-H) 8.84 (1H, s, OH-3), 9.22 (2H, s, OH-2, 4), 12.21 (1H, br s, COOH).

Ellagic acid (2) : white powder (MeOH); mp > 300 °C; UV (MeOH) λ_{max} (log ε) nm : 256 (5.34), 355 (sh 4.56), 368 (4.68); IR (KBr) ν_{max} cm^{-1} : 3300, 1690, 1610 ~ 1580, 1330 and 1200; EI-MS *m/z* 302 $[M]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.45 (2H, s, H-5, 5'), 10.60 (2H, br s, OH-3, 3'), 10.78 (2H, br s, OH-4, 4').

3,3'-Di-*O*-methylellagic acid (3) : yellow powder (MeOH); mp 300 °C; UV (MeOH) λ_{max} (log ε) nm : 248 (5.33), 355 (sh 4.66), 374 (4.74); IR (KBr) ν_{max} cm^{-1} : 3380, 1690, 1600 ~ 1580, 1210; EI-MS *m/z* 330 $[M]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 4.08 (6H, s, OCH_3), 7.55 (2H, s, H-5, 5'), 10.81 (2H, br s, OH-4, 4'); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 111.72 (C-1, 1'), 141.26 (C-2, 2'), 140.28 (C-3, 3'), 152.24 (C-4, 4'), 111.49 (C-5, 5'), 112.17 (C-6, 6'), 158.55 (C-7, 7'), 61.02 (-OMe).

4-*O*-(β-D-Xylopyranosyl)-3,3'-di-*O*-methylellagic acid (4) : white needle crystal (MeOH); mp 226 ~ 230 °C; $[\alpha]_D^{25}$ −12.5° (c = 0.5, DMSO); UV (MeOH) λ_{max} (log ε) nm : 248 (4.64), 355 (sh 3.99), 368 (4.04); IR (KBr) ν_{max} cm^{-1} : 3400, 1750, 1610 ~ 1580; ESI-MS *m/z* : 463 $[M+H]^+$, 485 $[M+Na]^+$; EI-MS *m/z* : 330 $[M-xylose]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 4.09 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 5.18 (1H, d, *J* = 5.1 Hz, H-1"), 7.52 (1H, s, H-5'), 7.76 (1H, s, H-5), 10.90 (1H, br s, OH-4'); ^{13}C-NMR (DMSO-d_6, 75 MHz) spectral data, see Table 1.

4-*O*-(α-L-Arabinofuranosyl)-3,3'-di-*O*-methylellagic acid (5) : white amorphous powder (MeOH); UV (MeOH) λ_{max} (log ε) nm : 248 (4.62), 355 (sh 3.96), 368 (4.01); IR (KBr) ν_{max} cm^{-1} : 3350, 1750, 1580 ~ 1610; ESI-MS *m/z* 461 $[M-H]^-$, EI-MS *m/z* 331 $[M-arabinose]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 4.08 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 5.66 (1H, d, *J* = 1.5 Hz), 7.57 (1H, s, H-5'), 7.79 (1H, s, H-5), 10.91 (1H, s, OH-4'); ^{13}C-NMR (DMSO-d_6, 75 MHz) spectral data, see Table 1.

Table 1. ^{13}C-NMR spectral data of compounds **3-5**

carbon	3	4	5
1	111.72	114.19	114.05
2	141.26	141.61	141.64
3	140.28	141.87	141.99
4	152.24	151.22	150.77
5	111.49	111.87	111.77
6	112.17	111.87	111.88
7	158.55	158.39	158.55
1'	111.72	111.10	111.22
2'	141.26	140.96	140.99
3'	140.28	140.15	140.24
4'	152.24	152.81	152.82
5'	111.49	111.60	111.66
6'	112.17	112.76	112.77
7'	158.55	158.35	158.42
OMe(3')	61.02	61.01	61.07
OMe(3)	61.02	61.64	61.52
xylose			
1"		101.77	
2"		73.03	
3"		76.12	
4"		69.23	
5"		65.79	
arabinose			
1"			107.53
2"			82.14
3"			76.57
4"			86.16
5"			61.70

*Chemical shifts in δ ppm values from TMS.

Geraniin (6) : yellow powder (H_2O); mp 218 ~ 221 °C; $[\alpha]_D^{25}$ −147.0° (c 0.9, MeOH), negative FAB-MS *m/z* 951 $[M-H]^-$; UV (MeOH) λ_{max} (log ε) nm : 222 (5.28), 282 (4.91); IR (KBr) ν_{max} cm^{-1} : 3400 ~ 2400, 1700, 1620; ^{1}H-NMR (acetone-d_6, 300 MHz) δ: 4.28 ~ 4.54 (1H, m, glc-5), 4.68 ~ 5.00 (2H in total, m, glc-3, 6), 5.17 (1H, s, DHHDP-1), 5.40 ~ 5.60 (3H in total, glc-2, 4, 6), 6.53 (1H, s, DHHDP-3'), 6.59 (1H, br s, glc-1), 6.67, 7.11 (each 1H, s, HHDP-H), 7.19 (2H, s, galloyl-H), 7.28 (1H, s, DHHDP-3).

Isoquercitrin (7) : bright yellow powder (H_2O); UV (MeOH) λ_{max} (log ε) nm : 257 (5.12), 359 (4.98); IR (KBr) ν_{max} cm^{-1} : 3400 ~ 2400, 1660, 1600; EI-MS *m/z* 302 $[M-glc]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 5.50 (1H, d, *J* = 7.2 Hz, H-1"), 6.24 (1H, d, *J* = 2.2Hz, H-6), 6.44 (1H, d, *J* = 2.2Hz, H-8), 6.88 (1H, d, *J* = 8.9Hz, H-5'), 7.61 (1H, dd, *J* = 3.3, 8.9Hz, H-6'), 7.62 (1H, d, *J* = 3.3Hz, H-2'); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 156.2 (C-

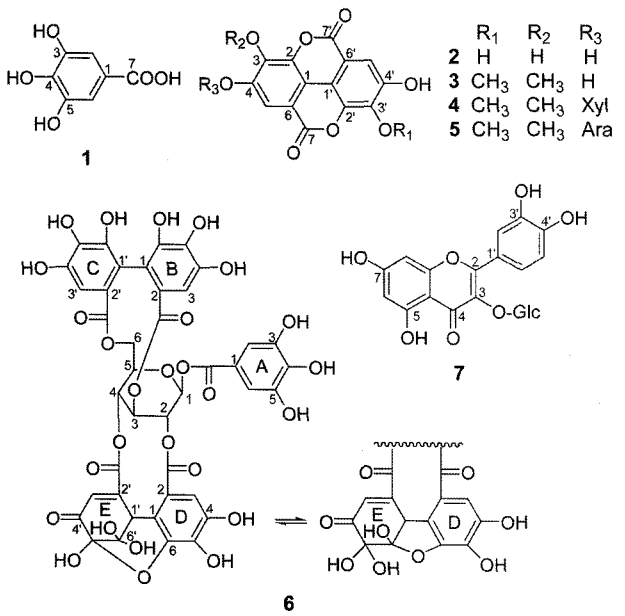

Fig. 1. The chemical structures of compounds **1-7**.

2), 133.3 (C-3), 177.6 (C-4), 161.3 (C-5), 98.8 (C-6), 164.2 (C-7), 93.6 (C-8), 156.5 (C-9), 104.2 (C-10), 121.4 (C-1'), 115.3 (C-2'), 144.8 (C-3'), 148.5 (C-4'), 116.5 (C-5'), 121.6 (C-6'), 101.4 (C-1"), 74.3 (C-2"), 76.8 (C-3"), 70.3 (C-4"), 77.5 (C-5"), 61.3 (C-6").

Results and Discussion

Previous phytochemical investigations on the leaves of *Sapium japonicum* had resulted in the isolation and characterization of eleven known phenolic compounds including gallic acid, geraniin, and isoquercitirn (Ahn *et al.*, 1996). In continuation of our study for chemical constituents of this plant led us to the isolation of seven known phenolic compounds from the stems (Fig. 1).

Compound **2** was identified as $C_{14}H_6O_8$ from EI-MS spectrum (*m/z* 302 $[M]^+$) and NMR data. The IR spectrum displayed characteristic absorptions for hydroxyl groups (3,300 cm^{-1}), α, β-unsaturated lactone functions (1,690 cm^{-1}) and aromatic rings (1,610 cm^{-1}). The ^{1}H-NMR spectrum showed one singlet at δ_H 7.45 (2H) corresponding to proton of aromatic ring, two broad singlet at δ_H 10.60 (2H) and δ_H 10.78 (2H) attributable to hydroxy protons. Based on these results, compound **2** was identified as ellagic acid (Li *et al.*, 1999).

Compound **3** was determined as $C_{14}H_6O_8$ from EI-MS (*m/z* 330 $[M]^+$) and ^{13}C-NMR. The ^{1}H-NMR spectrum showed one singlet at δ_H 4.08 (6H) arising from methoxyl groups, one singlet at δ_H 7.55 (2H) corresponding to proton of aromatic ring, one broad singlet at δ_H 10.81 (2H) attributable to hydroxy proton. The ^{13}C-NMR spectrum of **3** exhibited 16 signals, with two ester carbons at δ_C 158.55 due to α, β-unsaturated lactones, six oxygenated aromatic carbons, four aromatic quaternary carbons, two aromatic methine carbons, and two methoxyl carbons. Accordingly, compound **3** was established as 3,3'-di-*O*-methylellagic acid (Sato, 1987).

Compound **4** was obtained as white needle crystals. The ESI-MS spectrum showed a quasi-molecular ion peak at *m/z* 485 $[M + Na]^+$ and 463 $[M + H]^+$, and EI-MS gave a fragmentation peak at *m/z* 330 $[M - \text{xylose}]^+$. These results together with ^{13}C-NMR spectroscopic analysis proposed the molecular formula $C_{21}H_{18}O_{12}$ for compound **4**. The ^{1}H-NMR spectrum showed two aromatic protons at δ_H 7.52 (1H) and δ_H 7.76 and two methoxyl at δ_H 4.09 (3H) and δ_H 4.11, and a broad singlet at δ_H 10.81 (2H), together with six protons arising from a sugar moiety. The ^{13}C-NMR spectrum exhibited 12 signals, together with two carbonyl carbons at δ_C 158.35 and δ_C 158.39 due to α, β-unsaturated lactones, two methoxyl carbons at δ_C 61.01 and δ_C 61.64, and five sugar carbons whose chemical shift were identical with those of xylose (Table 1). The configuration was concluded to be β-D-xylopyranosyl on the basis of the *J*-value [δ_{H1} 5.18 (d, J = 5.1 Hz)] of the anomeric proton signal and the comparison of their ^{13}C-NMR data (δ_{C1} 101.77, *d*) with those of reported (Harbone *et al.*, 1982). This was further confirmed by acidic hydrolysis of compound **4** to give xylose and 3,3'-di-*O*-methylellagic acid, as the aglycon. The position of attachment of xylose was confirmed by glycosidation shift from ^{13}C-NMR data of compound **4** (Khac *et al.*, 1990). Therefore, compound **4** was identified as 4-*O*-(β-D-xylopyranosyl)-3,3'-di-*O*-methylellagic acid (Khac *et al.*, 1990; Li *et al.*, 1999).

Compound **5** was identified as $C_{21}H_{18}O_{12}$ from the ESI-MS (*m/z* 461 $[M - H]^-$) and EI-MS (*m/z* 331 $[M - \text{arabinose}]^+$) data. The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **5**, almost the same as those of compound **4** except for those of sugar moiety, suggested that a xylosyl group of compound **4** was replaced with other pentose. Acid hydrolysis of compound **5** yielded arabinose, which was further confirmed by co-TLC with authentic sample. The configuration was concluded to be α-L-arabinofuranosyl on the basis of the *J*-value [5.66 (1H, d, J = 1.5 Hz)] of the anomeric proton signal and comparison of their chemical shifts with those of a reference (Harbone *et al.*, 1982). And the position of attachment of arabinose was confirmed by glycosidation shift from ^{13}C-NMR data (Tanaka *et al.*, 2001). Thus, compound **5** was identified to be 4-*O*-(α-L-arabinofuranosyl)-3,3'-di-*O*-methylellagic acid (Tanaka *et al.*, 2001).

Compounds **1**, **6**, and **7** were identified as gallic acid, isoquercitrin, and geraniin, respectively, on the basis of their physical and spectral data comparison with literature values. Ellagic acid (**2**) and its derivatives (**3-5**) were isolated from *Sapium* genus for the first time.

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