Studies on Aldose Reductase Inhibitors from Medicinal Plant of "Sinfito," *Potentilla candicans*, and Further Synthesis of Their Related Compounds[1)]

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For several years we have screened natural products having aldose reductase (AR) inhibitory activity. 3,3′,4-Tri-*O*-methylellagic acid 4′-sulfate potassium salt (2) was isolated from a Mexican herb "Sinfito" (*Potentilla candicans*) as a potent AR inhibitory active constituent. 2 was more potent ($IC_{50}=8.0\times10^{-8}$ M) than ellagic acid, which is one of the natural inhibitors of AR. So we examined the synthesis of ellagic acid derivatives and found that the sulfate group is one of the important function.

Keywords *Potentilla candicans*; Rosaceae; aldose reductase inhibitor; rat lens; ellagic acid; ellagic acid derivative; sulfate salt

Aldose reductase (AR) is the enzyme which, along with the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), catalyzes the reduction of glucose to sorbitol. AR is likely to be responsible for the increased intracellular accumulation of sorbitol found in many tissues, such as lens, nerve, retina and kidney of diabetic animals. It is currently expected that AR inhibitors will play an important role in the management of diabetic complications such as cataract,[1,2)] neuropathy,[3)] retinopathy[4)] and nephropathy.[5)] Carboxylic acids,[6,7)] flavonoids,[8–10)] hydantoin analogues[11,12)] and so forth have hitherto been examined as AR inhibitory drugs. We recently reported the isolation of ellagic acid as a potent AR inhibitor ($IC_{50}=2.0\times10^{-7}$ M) from *Phyllanthus niruri* L. (Euphorbiaceae).[13)]

We screened medicinal plants for lens AR inhibitors and found that "Sinfito" showed a potent inhibitory effect. Sinfito, the root of *Potentilla candicans* (Rosaceae), has traditionally been used for the treatment of diabetic cataract and as an astringent in Mexico. In this paper, we report the isolation and identification of the active compounds inhibiting rat lens AR, and the effect of substituent groups of these derivatives synthesized. The MeOH extract of powdered Sinfito showed a high inhibitory activity towards crude rat lens AR ($IC_{50}=7.0\times10^{-7}$ g/ml). The MeOH solution was concentrated to a small volume to afford a precipitate in good yield. The precipitate, which was more active than the filtrate (Table I), was applied to a column of silica gel to give two compounds, **1** and **2**.

Compound **1**, mp 296—297 °C, gave a positive color reaction to $FeCl_3$. **1** showed a molecular ion peak at *m/z* 344 (M^+: $C_{17}H_{12}O_8$) in the mass spectrum (MS), and its infrared (IR) spectrum suggested the presence of two lactone moieties (1760 and 1730 cm^{-1}). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, the presence of three methoxyl groups (3.96, 4.02 and 4.03 ppm) and two aromatic protons (7.47 and 7.53 ppm) was suggested. On the basis of these data, the structure of compound **1** was supposed to be tri-*O*-methylellagic acid. A nuclear Overhauser effect (NOE) was observed for the aromatic proton signal at δ 7.47 (8.4%) upon irradiation of the methoxyl signal at δ 3.96 in the ^{1}H-NMR spectrum. However, the other aromatic proton signal at δ 7.53 exhibited no NOE with any methoxyl signal. The positions of three methoxyl groups on ellagic acid (**3**) were thus considered to be 3, 3′ and 4 and were further supported by the shifts of ultraviolet (UV) absorption maxima obtained by the addition of shift reagents in the UV spectrum[14)] (Experimental). Consequently, **1** was determined to be 3,3′,4-tri-*O*-methylellagic acid, and this was confirmed by its synthesis.

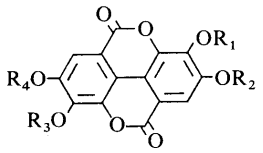
TABLE I. Inhibitory Activity against Rat Lens AR

Sample	Inhibition %		IC_{50} ($\times10^{-6}$ g/ml)
	1×10^{-5}	1×10^{-6} (g/ml)	
MeOH ext.	80.1	64.5	0.7
Filtrate	75.2	48.9	1.0
Precipitate	94.7	87.6	0.07

Compound **2** showed a cluster ion peak $[M+H]^+$ at *m/z* 463 in the positive fast atom bombardment mass spectrum (FAB-MS). The IR spectrum of **2** was very similar to **1**, except for two additional strong absorption bands at 1260 and 1050 cm^{-1}. The ^{1}H-NMR spectrum of **2** showed a similar signal pattern to **1**, but the 5′ aromatic proton signal (8.30 ppm) was shifted more downfield than that of **1** (7.53 ppm). Acid hydrolysis of **2** gave **1**, and equivalent mole ratios of potassium and sulfur were detected in **2** by fluorescence X-ray analysis. These results suggested that **2** has a sulfate group. On the basis of these data, the structure of **2** was determined to be 3,3′,4-tri-*O*-methylellagic acid 4′-sulfate potassium salt and this was confirmed by its synthesis.

Synthesis of 1 and 2 Ellagic acid (**3**) was treated with acetic anhydride and sodium acetate to give 3,3′,4,4′-tetra-*O*-acetylellagic acid (**4**). **4** was hydrolyzed according to the method of Hillis and Yazaki[15)] to give 4,4′-di-*O*-acetylellagic acid (**5**). Methylation of **5** with methyl iodide gave the dimethyl ether (**6**), which was hydrolyzed in the presence of aniline to afford 4′-*O*-acetyl-3,3′-di-*O*-methylellagic acid (**7**). **7** was methylated and then hydrolyzed, affording the 3,3′,4-tri-*O*-methylellagic acid. Its spectral data were consistent with those of the natural compound **1**. **1** was sulfonated with chlorosulfonic acid ($ClSO_3H$) and potassium hydroxide (KOH), and purified with Sephadex LH-20 to afford 3,3′,4-tri-*O*-methylellagic acid 4′-sulfate potassium salt in only 10% yield. But other sulfonylation methods gave less or no reaction product. These spectral data were consistent with those of **2**.

TABLE II. Inhibitory Activity of Ellagic Acid Derivatives against Rat Lens AR



Compd. No.	Substituent				IC_{50} ($\times 10^{-6}$ M)
	R_1	R_2	R_3	R_4	
1	Me	Me	Me	H	>10
2	Me	Me	Me	SO_3K	0.08
3	H	H	H	H	0.2
4	Ac	Ac	Ac	Ac	0.1
5	H	Ac	H	Ac	0.1
6	Me	Ac	Me	Ac	5.0
7	Me	Ac	Me	H	>10
8	Me	H	Me	H	>10
9	H	Me	H	Me	2.0
10	Me	Me	Me	Me	>10
11	Me	Me	Me	Ac	>10
12	Et	Ac	Et	Ac	1.0
13	*n*-Pr	Ac	*n*-Pr	Ac	3.0
14	iso-Pr	Ac	iso-Pr	Ac	2.0
15	Ac	Me	Ac	Me	>10
16	Et	H	Et	H	>10
17	*n*-Pr	H	*n*-Pr	H	5.0
18	iso-Pr	H	iso-Pr	H	>10
19	SO_3K	SO_3K	SO_3K	SO_3K	0.020
20	Me	SO_3K	Me	SO_3K	0.028
21	Et	SO_3K	Et	SO_3K	0.036
22	*n*-Pr	SO_3K	*n*-Pr	SO_3K	0.064
23	iso-Pr	SO_3K	iso-Pr	SO_3K	0.067
24	SO_3K	Me	SO_3K	Me	0.089
Quercitrin[a]					2.0
Sorbinil[b]					0.15

a) Quercitrin was assayed previously, and was tested again as a reference in this study. *b*) Reference 12.

Inhibitory Effect on Crude Rat Lens AR Compounds **1** and **2** were tested for AR inhibitory activity (Table II). **2** exhibited high activity ($IC_{50}=8.0\times10^{-8}$ M), being more potent than both **3** ($IC_{50}=2.0\times10^{-7}$ M) and quercitrin ($IC_{50}=2.0\times10^{-6}$ M), tested as a reference for natural products, and almost as potent as sorbinil,[12] synthetically reported to be a highly potent AR inhibitor. However, **1** which differs from **2** only in the substituent of 4′ position, showed low activity ($IC_{50}>1.0\times10^{-5}$ M). To examine the substituent effect of ellagic acid, we tested some synthetic compounds related to **1** and **2** (Table II). We concluded that partially or completely methylated compounds (**8**—**10**) showed low activity, less than one tenth of ellagic acid, and other alkyl groups (**16**—**18**) showed similarly active patterns. But 4,4′-di-*O*-methylellagic acid (**9**) and 3,3′-di-*O*-*n*-propylellagic acid (**17**) did not decline in activity as other alkylated ones did, while acetyl derivatives of **3** (**4** and **5**) showed rather high activity ($IC_{50}=1.0\times10^{-7}$ M). Analogous derivatives with the same two alkyl groups at the 3,3′-position (**6**, **12**—**14**) showed less activity than **5**. Next, we tried sulfonylation of **3**, and a mixture of products consisting of one to four sulfonyl groups was obtained. It was very difficult to separate into respective components, and only a persulfated one (**19**) could be obtained. **19** exhibited very high activity ($IC_{50}=2.0\times10^{-8}$ M), and the mixture with the average molecular weight had a tendency to be more potent than **3**. Other sulfonyl substituted compounds (**20**—**24**), in which 3,3′ or 4,4′ positions have alkyl groups, showed high activity. To determine the type of inhibition, the kinetics of inhibition of AR by **2** were plotted according to Lineweaver–Burk, and **2** was found to be a non-competitive inhibitor at the concentration of IC_{50} (8.0×10^{-8} M). This was also seen in the case of **3** and other inhibitors, but the inhibition activity of **2** decreased very little when it was tested with the addition of about ten times of bovine serum albumin.

Discussion

Several groups of organic compounds have been known to inhibit AR. As a natural inhibitor we recently reported ellagic acid (**3**)[13] and flavonoids, but this is the first report of ellagic acid derivatives. The potency of **3** is rather high in natural products, but does not seem to be specific. Hydroxy groups of **3** likely combine with other proteins. Thus the inhibitory activity decreases, when the hydroxy groups of **3** are substituted for alkyl groups, *e.g.* the methyl group. While introduction of the acetyl group increases the effect a little, it is decreased with other alkyl substituents as was seen in **5** to **6**, **12**, **13** and **14**. 4,4′-Alkyl substituted ellagic acid (**15**) especially has no inhibitory activity. All sulfonylated compounds (**2**, **19**—**24**) increased the inhibitory activity one or more orders. The sulfonyl group was thus considered to be one of the important substituents increasing the AR inhibitory activity. In a supplementary experiment, **1** was assayed with addition of the equivalent molar concentrations of sulfate ion, but no inhibition activity was found. This fact suggests that sulfate ion is not essential for AR inhibitory activity itself, but strong activity is demonstrated when it is combined with ellagic acid skeleton. Boghosian and MaGuinness[16] reported, in fact, that sulfate ion increases the AR activity. From these facts, we assume that sulfate ion on ellagic acid derivatives links to the active site or some important position of AR, and that sulfate ion containing compound therefore shows strong AR inhibitory activity. The sulfate group is thus considered to be useful and an important substituent for AR inhibitor. An *in vivo* study of inhibition of AR by ellagic acid derivatives is now in progress. We are also examining the effect of some other skeletons and will report the results in the future.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: IR spectra with a Hitachi 260-0611 spectrophotometer and a Jasco A-202 spectrophotometer; UV spectra with a Shimadzu UV-260 spectrophotometer; MS with a JEOL JMS-D 200 spectrometer (70 eV); FAB-MS with a JEOL JMS-DX303 spectrometer (4 keV); and ^{1}H-NMR spectra with a JEOL GX-270 spectrometer (270 MHz) in CD_3OD. Chemical shifts are given in δ (ppm) values referring to internal tetramethylsilane.

Plant Material "Sinfito," in the form of dried chips, was purchased from Mexico and identified as the root of *Potentilla candicans* from specimens of the whole plant.

Bioassay Crude AR was obtained from the supernatant fraction of the homogenate of rat lens according to the method of Kador and Sharpless,[17] and showed a specific activity of 22 unit/mg. One unit was defined as the amount catalyzing the oxidation of 1 μmol of reduced NADPH per minute. Inhibitory activity of the extracts and compounds on AR was assayed by the method previously reported.[18] Samples were dissolved in dimethylsulfoxide (DMSO), which was found to have no effect on the enzyme activity at a concentration below 1%.

Extraction and Isolation Dried chips of the material (5.0 kg) were powdered and extracted with MeOH (50 l × 3, 24 h each) at room temperature. The precipitate obtained after concentration to one fifth of MeOH solution was washed with cold MeOH to yield 23 g of crude mixture. The mixture was chromatographed on a silica gel column eluting first with $CHCl_3$ and then with an increasing amount of MeOH to yield compound **1** (0.9 g). The MeOH eluate was purified with Sephadex LH-20 column eluting with H_2O to give compound **2** (1.6 g).

3,3′,4-Tri-*O*-methylellagic Acid (1) Pale yellow needles, mp 296—297 °C (dec.) (DMSO), yellow-green to $FeCl_3$ reagent. MS *m/z*: 344 (M^+: $C_{17}H_{12}O_8$), 329 ($M^+ - Me$), 301 and 286. UV λ_{max}^{EtOH} nm: 248, 358(sh), 373. $\lambda_{max}^{EtOH+NaOEt}$ nm: 275, 315, 412. $\lambda_{max}^{EtOH+NaOAc}$ nm: 248, 357, 372, 408. IR ν_{max}^{KBr} cm^{-1}: 3430, 1760, 1730, 1600, 1500. ^{1}H-NMR (in CD_3OD) δ: 3.96 (3H, s, OMe), 4.02 (3H, s, OMe), 4.03 (3H, s, OMe), 7.47 (1H, s, H-5), 7.53 (1H, s, H-5′).

3,3′,4-Tri-*O*-methylellagic Acid 4′-Sulfate Potassium Salt (2) Yellow needles, mp 297—298 °C (dec.) (H_2O–EtOH). Positive FAB-MS *m/z*: 463 ($[M+H]^+$: $C_{17}H_{11}O_{11}KS+H$). IR ν_{max}^{KBr} cm^{-1}: 1735, 1730, 1605, 1495, 1260, 1050. ^{1}H-NMR (in CD_3OD) δ: 4.02 (3H, s, OMe), 4.13 (3H, s, OMe), 4.28 (3H, s, OMe), 7.66 (1H, s, H-5), 8.30 (1H, s, H-5′).

3,3′,4,4′-Tetra-*O*-acetylellagic Acid (4) A suspension of **3** (25 g, 82.8 mmol) and sodium acetate (5 g, 61.0 mmol) in acetic anhydride (200 ml) was refluxed at 100—110 °C for 2 h. The reaction mixture was evaporated *in vacuo* and washed with H_2O and acetone. The crude product was recrystallized from dimethylformamide (DMF) to give **4** as colorless needles, mp > 300 °C. Yield 35 g (90%). IR ν_{max}^{KBr} cm^{-1}: 1790, 1780, 1750. MS *m/z*: 470 (M^+: $C_{22}H_{14}O_{12}$).

4,4′-Di-*O*-acetylellagic Acid (5) A suspension of **4** (10 g, 21.3 mmol) in pyridine (200 ml) and H_2O (10 ml) was stirred at room temperature for 20 h. The precipitated reaction mixture was filtered, and washed with H_2O and acetone. The crude product was recrystallized from DMF to give **5** as colorless needles, mp > 300 °C. Yield 7 g (85%). IR ν_{max}^{KBr} cm^{-1}: 3350, 1770, 1745. MS *m/z*: 386 (M^+: $C_{18}H_{10}O_{10}$).

4,4′-Di-*O*-acetyl-3,3′-di-*O*-methylellagic Acid (6) A suspension of **5** (5 g, 13.0 mmol) and K_2CO_3 (1.25 g, 13.4 mmol) in DMF (50 ml) and DMSO (25 ml) was stirred at 40—45 °C for 10 min. CH_3I (1 ml) was added to the mixture and stirred at 40—45 °C for 4 h. The reaction mixture was added dropwise to cold H_2O. The precipitate was filtered off and washed with H_2O. The crude product was recrystallized from MeOH–dioxane to give **6** as colorless needles, mp 297—299 °C. Yield 3 g (56%). IR ν_{max}^{KBr} cm^{-1}: 1780, 1744. MS *m/z*: 414 (M^+: $C_{20}H_{14}O_{10}$).

4′-*O*-Acetyl-3,3′-di-*O*-methylellagic Acid (7) To a suspension of **6** (1 g, 2.4 mmol) in DMF (50 ml) was added aniline (0.4 ml). The mixture was stirred at 105 °C for 3 h. The reaction mixture was evaporated to dryness *in vacuo*. The residue was recrystallized from acetone–MeOH to give **7** as colorless needles, mp 276—278 °C. Yield 0.26 g (29%). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770, 1750. MS *m/z*: 372 (M^+: $C_{18}H_{12}O_9$).

Synthesis of 1 A suspension of **7** (1 g, 2.7 mmol) and K_2CO_3 (1.8 g, 13.0 mmol) in acetone (100 ml) and MeOH (100 ml) was stirred at 40—45 °C for 10 min. To the mixture was added CH_3I (0.4 ml) and stirring continued for 20 h. The reaction mixture was evaporated *in vacuo*. The residue was diluted with H_2O and acidified with 2 N-HCl. The solution was evaporated *in vacuo*, then H_2O was added and the precipitate filtered. The crude product was recrystallized from DMSO to give **1** in 0.6 g yield (65%).

Synthesis of 2 A suspension of **1** (1 g, 2.9 mmol) in pyridine (40 ml) and $ClSO_3H$ (3 ml) was refluxed for 3 h. The reaction mixture was evaporated *in vacuo*. The residue was diluted with MeOH and the precipitate filtered off. The crude product of pyridine salt was diluted with H_2O and an excess of KOH was added. The solution was purified by Sephadex LH-20 column chromatography using H_2O as an eluent and recrystallized from H_2O–EtOH to give **2** in 134 mg yield (10%).

3,3′-Di-*O*-methylellagic Acid (8) A suspension of **6** (1 g, 2.4 mmol) in acetone (25 ml) and MeOH (25 ml) was added to a solution of 10% aqueous NaOH (15 ml). The mixture was stirring continued at 65 °C for 3 min, then H_2O (25 ml) was added and stirred for 10 min. The solution was acidified with 2 N-HCl, and concentrated. The precipitate was filtered, followed by recrystallization from acetone–MeOH to give **8** as colorless needles, mp > 300 °C. Yield 0.86 g (85%). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1720. MS *m/z*: 330 (M^+: $C_{16}H_{10}O_8$).

4,4′-Di-*O*-methylellagic Acid (9) A mixture of methylgallate (20 g, 108.7 mmol) and α,α-dichlorodiphenylmethane (25.6 g, 108 mmol) was heated at 170—180 °C for 5 min. On cooling to room temperature, the mixture was dissolved in hot benzene (200 ml) and hexane (300 ml). After again cooling to room temperature, part of the product crystallized from the solution. The crude product was filtered, and washed with hexane. Recrystallization from H_2O–MeOH gave methyl 3-hydroxy-4,5-diphenylmethylenedioxybenzoate (**25**) in 33.6 g yield. To a suspension of **25** (33.2 g, 95.4 mmol) and K_2CO_3 (38.7 g, 280 mmol) in acetone (200 ml) was added CH_3I (51.6 ml) and the reaction mixture was refluxed for 10 h. The reaction mixture was filtered and evaporated *in vacuo*. The crystalline residue was washed with H_2O, and recrystallized from MeOH–acetone to give methyl 3-methoxy-4,5-diphenylmethylenedioxybenzoate (**26**) in 30.8 g yield. A suspension of **26** (29.0 g, 80.1 mmol) in acetic acid (232 ml) and H_2O (58 ml) was refluxed for 8 h. The reaction mixture was evaporated *in vacuo*, and refluxed in hexane (400 ml) for 1 h. On cooling, the product crystallized from the solution was filtered and washed with hexane. Recrystallization from benzene–hexane gave methyl 3-*O*-methylgallate (**27**) in 14.0 g yield. To a suspension of **27** (13.2 g, 66.7 mmol) in H_2O (94.5 ml) and H_2SO_4 (4.7 ml) was added potassium persulfate (13.0 g) and this was stirred at 100 °C for 1 h. Upon cooling to room temperature, the product crystallized from the solution. The crude product was filtered, and washed with warm H_2O and warm acetone. Recrystallization from acetone–MeOH gave **9** as colorless needles, mp > 300 °C. Yield 1.4 g (12.7%). IR ν_{max}^{KBr} cm^{-1}: 3350, 1720. MS *m/z*: 330 (M^+: $C_{16}H_{10}O_8$).

3,3′,4,4′-Tetra-*O*-methylellagic Acid (10) To a suspension of **3** (0.1 g, 0.3 mmol) in DMF (5 ml) was added sodium hydride (NaH) (400 mg) and this was stirred at 0 °C for 20 min. CH_3I (2 ml) was added to the mixture and stirred at 4 °C for 2 h. The reaction mixture was evaporated *in vacuo*. The residue was diluted with H_2O and extracted with $CHCl_3$. The crude product obtained from the $CHCl_3$ extract was purified by silica gel column chromatography using $CHCl_3$ as an eluent recrystallized from benzene–$CHCl_3$ to give **10** as colorless amorphous powder. Yield 62 mg (52%). IR ν_{max}^{KBr} cm^{-1}: 1735. MS *m/z*: 358 (M^+: $C_{18}H_{14}O_8$).

4′-*O*-Acetyl-3,3′,4-tri-*O*-methylellagic Acid (11) A solution of **1** (0.2 g, 0.6 mol) in pyridine (20 ml) and acetic anhydride (20 ml) was stirred at 60 °C for 3 h. The reaction mixture was evaporated *in vacuo*, and recrystallized from $CHCl_3$ to give **11** as colorless needles, mp 256—257 °C. Yield 0.2 g (91%). IR ν_{max}^{KBr} cm^{-1}: 1780, 1740. MS *m/z*: 386 (M^+: $C_{19}H_{14}O_9$).

4,4′-Di-*O*-acetyl-3,3′-di-*O*-ethylellagic Acid (12) A suspension of **5** (2.5 g, 6.5 mmol) and K_2CO_3 (0.7 g, 5.1 mmol) in DMF (25 ml) and DMSO (13 ml) was stirred at 40—45 °C for 10 min. C_2H_5I (2 ml) was added and the mixture was stirred at 40—45 °C for 4 h. The reaction mixture was added dropwise to cold H_2O. The precipitate was filtered and washed with H_2O. The crude product was recrystallized from dioxane to give **12** as colorless needles, mp > 300 °C. Yield 2.1 g (73%). IR ν_{max}^{KBr} cm^{-1}: 1770, 1750. MS *m/z*: 442 (M^+: $C_{22}H_{18}O_{10}$).

13 and **14** were synthesized as described for **12**.

4,4′-Di-*O*-acetyl-3,3′-di-*O*-*n*-propylellagic Acid (13) Colorless needles (dioxane), mp 282—284 °C. Yield 1.9 g (62%). IR ν_{max}^{KBr} cm^{-1}: 1770, 1745. MS *m/z*: 470 (M^+: $C_{24}H_{22}O_{10}$).

4,4′-Di-*O*-acetyl-3,3′-di-*O*-isopropylellagic Acid (14) Colorless needles (dioxane), mp 282—284 °C. Yield 2.6 g (85%). IR ν_{max}^{KBr} cm^{-1}: 1767, 1746. MS *m/z*: 470 (M^+: $C_{24}H_{22}O_{10}$).

3,3′-Di-*O*-acetyl-4,4′-di-*O*-methylellagic Acid (15) A suspension of **9** (0.1 g, 0.24 mmol) in pyridine (10 ml) and acetic anhydride (10 ml) was stirred at 80 °C for 10 h. The reaction mixture was evaporated *in vacuo*, and the residue was recrystallized from acetone–MeOH to give **15** as colorless needles, mp 298—300 °C. Yield 0.11 g (85%). IR ν_{max}^{KBr} cm^{-1}: 1770, 1747. MS *m/z*: 414 (M^+: $C_{20}H_{14}O_{10}$).

3,3′-Di-*O*-ethylellagic Acid (16) To a suspension of **12** (0.05 g, 0.11 mmol) in MeOH (2 ml) and acetone (2 ml) was added a solution of 10% aqueous NaOH (1 ml). The mixture was stirring continued at 65 °C for 10 min, then H_2O (10 ml) was added and stirred for 30 min. The reaction mixture was acidified with 1 N-HCl, and concentrated. The precipitate was filtered, followed by recrystallization from acetone–MeOH to give **16** as colorless needles, mp > 300 °C. Yield 0.35 g (86%). IR ν_{max}^{KBr} cm^{-1}: 3350, 1720. MS *m/z*: 358 (M^+: $C_{18}H_{14}O_8$).

17 and **18** were synthesized as described for **16**.

3,3′-Di-*O*-*n*-propylellagic Acid (17) Colorless needles (acetone–MeOH), mp 265—267 °C. Yield 0.31 g (76%). IR ν_{max}^{KBr} cm^{-1}: 3450, 1730. MS *m/z*: 386 (M^+: $C_{20}H_{18}O_8$).

3,3′-Di-*O*-isopropylellagic Acid (18) Colorless needles (acetone–MeOH), mp 289—290 °C. Yield 0.09 g (74%). IR ν_{max}^{KBr} cm^{-1}: 3350, 1720. MS *m/z*: 386 (M^+: $C_{20}H_{18}O_8$).

Ellagic Acid Tetra-sulfate Potassium Salt (19) A solution of **3** (1 g, 3.31 mmol) in pyridine (20 ml) and $ClSO_3H$ (10 ml) was refluxed for 5 h. The reaction mixture was evaporated *in vacuo*. The residue was added to MeOH, and the precipitate filtered. The crude product of pyridine salt was dissolved in H_2O containing 0.5% pyridine and an excess of AcOK was added. The solution was purified by Sephadex LH-20 column

chromatography using H_2O as an eluent and recrystallized from H_2O–EtOH to give **19** as pale yellow needles, mp >300 °C. Yield 1.86 g (72%). Positive FAB-MS *m/z*: 775 ($[M+H]^+$: $C_{14}H_2K_4O_{20}S_4+H$). IR ν_{max}^{KBr} cm^{-1}: 1720, 1260, 1020.

20—**23** and **24** were synthesized as described for **19**.

3,3′-Di-*O*-methylellagic Acid 4,4′-Di-sulfate Potassium Salt (20) Pale yellow needles (H_2O–EtOH), mp >300 °C. Yield 63 mg (84%). Positive FAB-MS *m/z*: 567 ($[M+H]^+$: $C_{16}H_8K_2O_{14}S_2+H$). IR ν_{max}^{KBr} cm^{-1}: 1710, 1260, 1040.

3,3′-Di-*O*-ethylellagic Acid 4,4′-Di-sulfate Potassium Salt (21) Pale yellow needles (H_2O–EtOH), mp >300 °C. Yield 18 mg (43%). Positive FAB-MS *m/z*: 595 ($[M+H]^+$: $C_{18}H_{12}K_2O_{14}S_2+H$). IR ν_{max}^{KBr} cm^{-1}: 1740, 1250, 1040.

3,3′-Di-*O*-*n*-propylellagic Acid 4,4′-Di-sulfate Potassium Salt (22) Pale yellow needles (H_2O–EtOH), mp 233—235 °C. Yield 51 mg (35%). Positive FAB-MS *m/z*: 623 ($[M+H]^+$: $C_{20}H_{16}K_2O_{14}S_2+H$). IR ν_{max}^{KBr} cm^{-1}: 1740, 1260, 1040.

3,3′-Di-*O*-isopropylellagic Acid 4,4′-Di-sulfate Potassium Salt (23) Pale yellow needles (H_2O–EtOH), mp >300 °C. Yield 23 mg (43%). Positive FAB-MS *m/z*: 623 ($[M+H]^+$: $C_{20}H_{16}K_2O_{14}S_2+H$). IR ν_{max}^{KBr} cm^{-1}: 1750, 1250, 1040.

4,4′-Di-*O*-methylellagic Acid 3,3′-Di-sulfate Potassium Salt (24) Pale yellow needles (H_2O–EtOH), mp >300 °C. Yield 15 mg (44%). No reaction to $FeCl_3$ reagent. IR ν_{max}^{KBr} cm^{-1}: 1710, 1260, 1035.

Acknowledgement The authors are grateful to Dr. Mujo Kim, Central Research Laboratories of Taiyo Kagaku Co., Ltd., 1-3 Takaramachi, Yokkaichi, Mie 510, Japan, for the FAB-MS measurement.

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