

Studies on Aldose Reductase Inhibitors from Medicinal Plant of "Sinfito," *Potentilla candicans*, and Further Synthesis of Their Related Compounds¹⁾

Satoshi TERASHIMA,^a Mineo SHIMIZU,^{*,b} Hajime NAKAYAMA,^a Masatoshi ISHIKURA,^a Yutaka UEDA,^a Kunihiro IMAI,^a Akio SUZUI^c and Naokata MORITA^b

Toyo Pharmar Co., Ltd.,^a Inami, Higashitonami-gun, Toyama 932-02, Japan, Faculty of Pharmaceutical Sciences, Medical and Pharmaceutical University of Toyama,^b Sugitani, Toyama 930-01, Japan and Daiso Co., Ltd.,^c Otakasu-cho, Amagasaki, Osaka 660, Japan. Received February 15, 1990

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 ($IC_{50}=8.0 \times 10^{-8}$ 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⁾ retinopathy⁴⁾ 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 ($IC_{50}=2.0 \times 10^{-7}$ 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 ($IC_{50}=7.0 \times 10^{-7}$ 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 FeCl₃. **1** showed a molecular ion peak at m/z 344 (M^+ : C₁₇H₁₂O₈) 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 (¹H-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 ¹H-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 spectrum¹⁴⁾ (Experimental). Consequently, **1** was determined to be 3,3',4-tri-*O*-methylellagic acid, and this was confirmed by its synthesis.

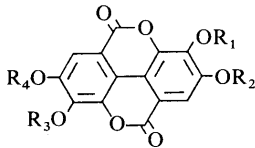
Compound **2** showed a cluster ion peak $[M+H]^+$ at m/z 463 in the positive fast atom bombardment mass spectrum (FAB-MS). The IR spectrum of **2** was very similar to **1**, except for two additional strong absorption bands at 1260 and 1050 cm⁻¹. The ¹H-NMR spectrum of **2** showed a similar signal 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*-acetyllagic acid (**4**). **4** was hydrolyzed according to the method of Hillis and Yazaki¹⁵⁾ to give 4,4'-di-*O*-acetyllagic 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 (ClSO₃H) 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 I. Inhibitory Activity against Rat Lens AR

Sample	Inhibition %		IC ₅₀ ($\times 10^{-6}$ 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

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 ₃ K	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	<i>n</i> -Pr	Ac	<i>n</i> -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	<i>n</i> -Pr	H	<i>n</i> -Pr	H	5.0
18	iso-Pr	H	iso-Pr	H	> 10
19	SO ₃ K	SO ₃ K	SO ₃ K	SO ₃ K	0.020
20	Me	SO ₃ K	Me	SO ₃ K	0.028
21	Et	SO ₃ K	Et	SO ₃ K	0.036
22	<i>n</i> -Pr	SO ₃ K	<i>n</i> -Pr	SO ₃ K	0.064
23	iso-Pr	SO ₃ K	iso-Pr	SO ₃ K	0.067
24	SO ₃ K	Me	SO ₃ K	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 ($IC_{50} = 8.0 \times 10^{-8}$ M), being more potent than both **3** ($IC_{50} = 2.0 \times 10^{-7}$ M) and quercitrin ($IC_{50} = 2.0 \times 10^{-6}$ 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 ($IC_{50} > 1.0 \times 10^{-5}$ 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 ($IC_{50} = 1.0 \times 10^{-7}$ 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 ($IC_{50} = 2.0 \times 10^{-8}$ 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 McGuinness¹⁶⁾ 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 ¹H-NMR spectra with a JEOL GX-270 spectrometer (270 MHz) in CD₃OD. 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 canadensis* 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 μ mol 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 $\times$ 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 CHCl₃ 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 H₂O 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 FeCl₃ reagent. MS m/z : 344 (M^+ : C₁₇H₁₄O₈), 329 (M^+ – Me), 301 and 286. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 248, 358(sh), 373. $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOEt}}$ nm: 275, 315, 412. $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOAc}}$ nm: 248, 357, 372, 408. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3430, 1760, 1730, 1600, 1500. ¹H-NMR (in CD₃OD) 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.) (H₂O–EtOH). Positive FAB-MS m/z : 463 ($[M + H]^+$: C₁₇H₁₁O₁₁KS + H). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1735, 1730, 1605, 1495, 1260, 1050. ¹H-NMR (in CD₃OD) 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*-acetyllagic 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 H₂O and acetone. The crude product was recrystallized from dimethylformamide (DMF) to give **4** as colorless needles, mp > 300 °C. Yield 35 g (90%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1790, 1780, 1750. MS m/z : 470 (M^+ : C₂₂H₁₄O₁₂).

4,4'-Di-*O*-acetyllagic Acid (5) A suspension of **4** (10 g, 21.3 mmol) in pyridine (200 ml) and H₂O (10 ml) was stirred at room temperature for 20 h. The precipitated reaction mixture was filtered, and washed with H₂O and acetone. The crude product was recrystallized from DMF to give **5** as colorless needles, mp > 300 °C. Yield 7 g (85%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350, 1770, 1745. MS m/z : 386 (M^+ : C₁₈H₁₀O₁₀).

4,4'-Di-*O*-acetyl-3,3'-di-*O*-methylellagic Acid (6) A suspension of **5** (5 g, 13.0 mmol) and K₂CO₃ (1.25 g, 13.4 mmol) in DMF (50 ml) and DMSO (25 ml) was stirred at 40–45 °C for 10 min. CH₃I (1 ml) was added to the mixture and stirred at 40–45 °C for 4 h. The reaction mixture was added dropwise to cold H₂O. The precipitate was filtered off and washed with H₂O. The crude product was recrystallized from MeOH–dioxane to give **6** as colorless needles, mp 297–299 °C. Yield 3 g (56%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1780, 1744. MS m/z : 414 (M^+ : C₂₀H₁₄O₁₀).

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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3450, 1770, 1750. MS m/z : 372 (M^+ : C₁₈H₁₂O₉).

Synthesis of 1 A suspension of **7** (1 g, 2.7 mmol) and K₂CO₃ (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 CH₃I (0.4 ml) and stirring continued for 20 h. The reaction mixture was evaporated *in vacuo*. The residue was diluted with H₂O and acidified with 2 N-HCl. The solution was evaporated *in vacuo*, then H₂O 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 ClSO₃H (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 H₂O and an excess of KOH was added. The solution was purified by Sephadex LH-20 column chromatography using H₂O as an eluent and recrystallized from H₂O–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 H₂O (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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400, 3300, 1720. MS m/z : 330 (M^+ : C₁₆H₁₀O₈).

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 H₂O–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 K₂CO₃ (38.7 g, 280 mmol) in acetone (200 ml) was added CH₃I (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 H₂O, 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 H₂O (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 H₂O (94.5 ml) and H₂SO₄ (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 H₂O and warm acetone. Recrystallization from acetone–MeOH gave **9** as colorless needles, mp > 300 °C. Yield 1.4 g (12.7%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350, 1720. MS m/z : 330 (M^+ : C₁₆H₁₀O₈).

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. CH₃I (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 H₂O and extracted with CHCl₃. The crude product obtained from the CHCl₃ extract was purified by silica gel column chromatography using CHCl₃ as an eluent recrystallized from benzene–CHCl₃ to give **10** as colorless amorphous powder. Yield 62 mg (52%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1735. MS m/z : 358 (M^+ : C₁₈H₁₄O₈).

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 CHCl₃ to give **11** as colorless needles, mp 256–257 °C. Yield 0.2 g (91%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1780, 1740. MS m/z : 386 (M^+ : C₁₉H₁₄O₉).

4,4'-Di-*O*-acetyl-3,3'-di-*O*-ethylellagic Acid (12) A suspension of **5** (2.5 g, 6.5 mmol) and K₂CO₃ (0.7 g, 5.1 mmol) in DMF (25 ml) and DMSO (13 ml) was stirred at 40–45 °C for 10 min. C₂H₅I (2 ml) was added and the mixture was stirred at 40–45 °C for 4 h. The reaction mixture was added dropwise to cold H₂O. The precipitate was filtered and washed with H₂O. The crude product was recrystallized from dioxane to give **12** as colorless needles, mp > 300 °C. Yield 2.1 g (73%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1750. MS m/z : 442 (M^+ : C₂₂H₁₈O₁₀).

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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1745. MS m/z : 470 (M^+ : C₂₄H₂₂O₁₀).

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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1767, 1746. MS m/z : 470 (M^+ : C₂₄H₂₂O₁₀).

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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1747. MS m/z : 414 (M^+ : C₂₀H₁₄O₁₀).

3,3'-Di-*O*-methylellagic 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 H₂O (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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350, 1720. MS m/z : 358 (M^+ : C₁₈H₁₄O₈).

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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3450, 1730. MS m/z : 386 (M^+ : C₂₀H₁₈O₈).

3,3'-Di-*O*-isopropylellagic Acid (18) Colorless needles (acetone–MeOH), mp 289–290 °C. Yield 0.09 g (74%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350, 1720. MS m/z : 386 (M^+ : C₂₀H₁₈O₈).

Ellagic Acid Tetra-sulfate Potassium Salt (19) A solution of **3** (1 g, 3.31 mmol) in pyridine (20 ml) and ClSO₃H (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 H₂O containing 0.5% pyridine and an excess of AcOK was added. The solution was purified by Sephadex LH-20 column

chromatography using H₂O as an eluent and recrystallized from H₂O–EtOH to give **19** as pale yellow needles, mp > 300 °C. Yield 1.86 g (72%). Positive FAB-MS *m/z*: 775 ([M + H]⁺: C₁₄H₂K₄O₂₀S₄ + H). IR $\nu_{\max}^{\text{KBr}}$ 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 (H₂O–EtOH), mp > 300 °C. Yield 63 mg (84%). Positive FAB-MS *m/z*: 567 ([M + H]⁺: C₁₆H₈K₂O₁₄S₂ + H). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1710, 1260, 1040.

3,3'-Di-O-ethylellagic Acid 4,4'-Di-sulfate Potassium Salt (21) Pale yellow needles (H₂O–EtOH), mp > 300 °C. Yield 18 mg (43%). Positive FAB-MS *m/z*: 595 ([M + H]⁺: C₁₈H₁₂K₂O₁₄S₂ + H). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1740, 1250, 1040.

3,3'-Di-O-*n*-propylellagic Acid 4,4'-Di-sulfate Potassium Salt (22) Pale yellow needles (H₂O–EtOH), mp 233–235 °C. Yield 51 mg (35%). Positive FAB-MS *m/z*: 623 ([M + H]⁺: C₂₀H₁₆K₂O₁₄S₂ + H). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1740, 1250, 1040.

3,3'-Di-O-isopropylellagic Acid 4,4'-Di-sulfate Potassium Salt (23) Pale yellow needles (H₂O–EtOH), mp > 300 °C. Yield 23 mg (43%). Positive FAB-MS *m/z*: 623 ([M + H]⁺: C₂₀H₁₆K₂O₁₄S₂ + H). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1750, 1250, 1040.

4,4'-Di-O-methylellagic Acid 3,3'-Di-sulfate Potassium Salt (24) Pale yellow needles (H₂O–EtOH), mp > 300 °C. Yield 15 mg (44%). No reaction to FeCl₃ reagent. IR $\nu_{\max}^{\text{KBr}}$ 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.

References

- 1) Part III in the series "Studies on Aldose Reductase Inhibitors from Natural Products." For Part II ref. 13).
- 2) J. H. Kinoshita, P. F. Kador and M. Catiles, *J. Am. Med. Assoc.*, **246**, 257 (1981).
- 3) a) K. H. Gabbay and J. J. Snider, *Diabetes*, **21**, 295 (1972); b) R. J. Toung, D. J. Ewing and B. F. Clarke, *ibid.*, **32**, 938 (1983).
- 4) W. G. Robinson Jr., P. F. Kador and J. H. Kinoshita, *Science*, **221**, 1177 (1983).
- 5) A. Beyer-Mears, E. Cruz, T. Edelist and E. Varagiannis, *Pharmacology*, **32**, 52 (1986).
- 6) P. F. Kador, J. H. Kinoshita and N. E. Sharpless, *J. Med. Chem.*, **28**, 841 (1985).
- 7) J. DeRuiter, B. E. Swearingen, V. Wanderkar and C. A. Mayfield, *J. Med. Chem.*, **32**, 1033 (1989).
- 8) S. D. Varma and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
- 9) J. Okuda, I. Miwa, K. Inagaki, T. Horie and M. Nakayama, *Biochem. Pharmacol.*, **31**, 3807 (1982).
- 10) M. Shimizu, T. Ito, S. Terashima, T. Hayashi, M. Arisawa and N. Morita, *Phytochemistry*, **23**, 1885 (1984).
- 11) I. Miwa, M. Hirano, K. Inagaki, C. Belbeoc'h and J. Okuda, *Biochem. Pharmacol.*, **36**, 2789 (1987).
- 12) R. Sarges, R. C. Schnur, J. L. Belletire and M. J. Peterson, *J. Med. Chem.*, **31**, 230 (1988).
- 13) M. Shimizu, S. Horie, S. Terashima, H. Ueno, T. Hayashi, M. Arisawa, S. Suzuki, M. Yoshizaki and N. Morita, *Chem. Pharm. Bull.*, **37**, 2531 (1989).
- 14) L. Jurd, *J. Am. Chem. Soc.*, **81**, 4610 (1971).
- 15) W. E. Hillis and Y. Yazaki, *Phytochemistry*, **12**, 2969 (1973).
- 16) R. A. Boghosian and E. T. McGuinness, *Biochim. Biophys. Acta*, **567**, 278 (1979).
- 17) P. E. Kador and N. E. Sharpless, *Biophys. Chem.*, **8**, 81 (1978).
- 18) M. Shimizu, S. Horie, M. Arisawa, T. Hayashi, S. Suzuki, M. Yoshizaki, M. Kawasaki, S. Terashima, H. Tsuji, S. Wada, H. Ueno, N. Morita, L. H. Berganza, E. Ferro and I. Basualdo, *Chem. Pharm. Bull.*, **35**, 1234 (1987).