Five New *C*-Methyl Flavonoids, the Potent Aldose Reductase Inhibitors from *Matteuccia orientalis* TREV.

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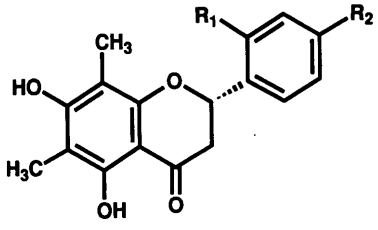
Five new compounds, matteuorien, matteuorienin, and matteuorienate A (7), B (8), and C (9) were isolated together with five known compounds from the MeOH extract of the rhizome of *Matteuccia orientalis* TREV. The structures of these compounds were determined by the use of spectroscopic methods including two dimensional (2D)-NMR experiments and chemical methods, except for the configuration at the C-3‴ of matteuorienate A (7), B (8), and C (9). Among them, matteuorienate A, B, and C showed very strong aldose reductase inhibitory activity. A structure–activity relationship study showed that a carboxyl group played an important part in aldose reductase inhibitory activity in these three compounds.

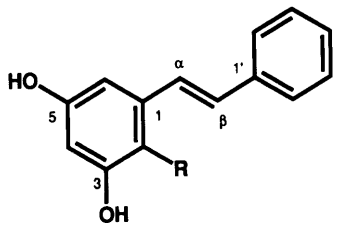
Key words matteuorienate A; matteuorienate B; matteuorienate C; aldose reductase inhibitors; *C*-methyl flavonoid; *Matteuccia orientalis*

In animal tissues, the reduction of aldoses occurs by the action of an enzyme called aldose reductase. It can convert a number of D-aldoses into respective alcohols and uses NADPH as a coenzyme. There is another enzyme called polyol dehydrogenase which oxidizes L-sorbitol to D-fructose using NAD^+ as a coenzyme. The combination of aldose reductase activity on glucose to produce sorbitol, and polyol dehydrogenase acting on sorbitol to produce fructose, constitutes what is called a polyol pathway. The aldose reductase normally has a low affinity for glucose and therefore requires a relatively high concentration of that sugar for significant activity. In the diabetic condition, sufficient glucose may enter the tissues, and the pathway operates to produce both sorbitol and fructose. Sorbitol remains within the tissue and fructose exits from the cells with some difficulty. In diabetics, the relative sorbitol concentration is very high while the concentration of myo-inositol is found to be decreased reciprocally. As the myo-inositol concentration decreases, the activity of the Na^+/K^+ ATPase is found to be decreased, which has been a suspected cause of the diabetic complications of neuropathy for a long time.[1] Some aldose reductase inhibitors have increased the concentration of myo-inositol in the nerve tissue, so that one of the approaches for the treatment of diabetic complications has been based on aldose reductase inhibitors.

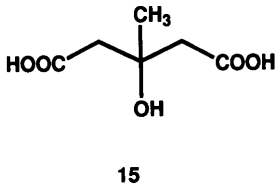
The rhizome of *Matteuccia orientalis* is a traditional Chinese medicine called Guan Zhong (Kuan-chung in



1 : $R_1 = R_2 = H$
2 : $R_1 = H$, $R_2 = OCH_3$
3 : $R_1 = OH$, $R_2 = OCH_3$
6 : $R_1 = R_2 = H$, Δ^2



4 : R = H
5 : R = COOH



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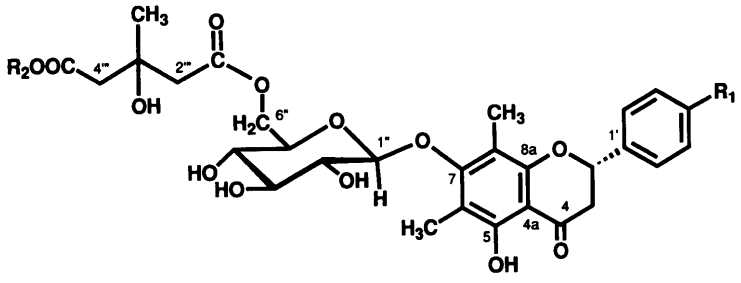
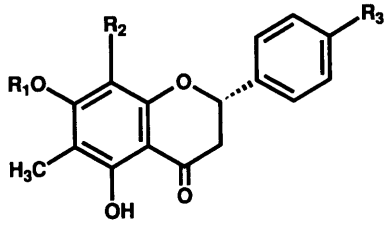


7 : $R_1 = OCH_3$, $R_2 = H$
8 : $R_1 = R_2 = H$
9 : $R_1 = R_2 = H$, Δ^2
11 : $R_1 = OCH_3$, $R_2 = CH_3$
12 : $R_1 = H$, $R_2 = CH_3$



10 : $R_1 = H$, $R_2 = Glc$, $R_3 = OH$
13 : $R_1 = Glc$, $R_2 = CH_3$, $R_3 = OCH_3$
14 : $R_1 = Glc$, $R_2 = CH_3$, $R_3 = H$

Chart 1

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Japan).[2] The $CHCl_3$ extract from *M. orientalis* showed a very strong hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats and an active principle 2′-hydroxymatteucinol was isolated from this extract.[3] It was considered worthwhile to study whether or not 2′-hydroxymatteucinol or some other constituents present in *M. orientalis* inhibit the polyol pathway. For this, $CHCl_3$, MeOH and water extracts of *M. orientalis* were examined for their inhibitory activity against aldose reductase isolated from rat lens. In the preceding communication,[4] we reported the isolation and structure elucidation of two *C*-methyl flavanones, matteuorienate A and B, from the MeOH extract of this plant, both of which showed potent aldose reductase inhibitory activity. In this paper, we wish to present the isolation and structure elucidation of these new *C*-methyl flavonoids in detail, as well as the aldose reductase inhibitory activity of matteuorienate A, B, and C, together with structure–activity relationship.

Materials and Methods

All melting points were determined with a Kofler-type apparatus and were uncorrected. IR spectra were taken on a Hitachi 260-10 IR spectrophotometer in a KBr disc, and the absorbence is expressed in cm^{-1}. UV spectra were taken on a Shimadzu UV 2200 UV-visible spectrophotometer in MeOH, and the λ_{max} is expressed in nanometers (nm). Optical rotation was measured on a JASCO DIP-4 automatic polarimeter at 25 °C. ^1H- and ^{13}C-NMR spectra were taken on a JEOL GX-400 and Fourier-transform NMR spectrometer with tetramethylsilane (TMS) as an internal standard for ^1H-NMR, and chemical shifts are expressed in δ value. ^1H–^1H correlation spectroscopy (COSY), ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY spectra were obtained using the usual pulse sequences, and data processing was performed with standard JEOL software. Mass spectra and high-resolution MS were taken on a JEOL JMS-SX 102A (ionization voltage, 70 eV; accelerating voltage, 5.0 kV) mass spectrometer using a direct inlet system. CD spectra were measured between 220 and 400 nm in MeOH or $CHCl_3$. NADPH, NADP, mercaptoethanol, glyceraldehyde and Wako gel C-200 were from Wako Pure Chemical Industries, Ltd., Osaka, Japan, and TLC and preparative TLC were carried out on precoated Merck Kieselgel F_{254} plates (0.25 or 0.5 mm). Other chemicals were of analytical grade.

Plant Materials The rhizomes of *Matteuccia orientalis* were collected from Yatsuo, Toyama prefecture, Japan in 1991. The plant was identified by an expert and the voucher sample was preserved in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Japan, for reference.

Extraction and Isolation The shade dried rhizomes (6.5 kg) were exhaustively extracted by percolation with $CHCl_3$ at room temperature to give a $CHCl_3$ extract (390 g). The residue was successively extracted with MeOH and water in a manner similar to that above, and yielded MeOH (406 g) and water (450 g) extracts, respectively. The methanol extract of the rhizome of *M. orientalis* showed a strong inhibitory activity against aldose reductase. Then the MeOH extract (155 g) was applied into the Iatrobeads column chromatography and eluted with the 10%, 20%, 30%, 50% MeOH in $CHCl_3$ and MeOH to give fraction 1 (10.42 g), fraction 2 (48.09 g), fraction 3 (62.48 g), fraction 4 (20.45 g), and fraction 5 (24.92 g), respectively. Further purification of fraction 1 by silica gel column chromatography was followed by preparative TLC, and five known compounds, demethoxymatteucinol (**1**) (705 mg), matteucinol (**2**) (1.3 g), 2′-hydroxymatteucinol (**3**) (980 mg), pinosylvin (**4**) (18 mg), and pinosylvic acid (**5**) (12 mg) were isolated together with a new compound, matteuorien (**6**) (7 mg). Fraction 2 (20 g), obtained by eluting 20% MeOH in $CHCl_3$ was again subjected to silica gel column chromatography and fractionated into fractions A (1.33 g), B (7.35 g), C (9.23 g), D (1.17 g) and E (645 mg). A portion of fraction B (60 mg) was again purified by recycled HPLC (column: YMC ODS SH343-5, carrier phase: MeOH : H_2O : TFA (60 : 40 : 0.1), flow rate: 6.0 ml/min) to obtain two new compounds, matteuorienate A (**7**) (31.6 mg), and matteuorienate B (**8**) (22.6 mg). Fraction D (200 mg) was subjected to Sephadex LH-20 column chromatography and finally purified by HPLC under similar conditions to those described above to give matteuorienate C (**9**) (4.6 mg). A new compound, matteuorienin (**10**) (19 mg) was isolated from fraction 3 (10.5 g) by repeated Sephadex LH-20 column chromatography.

Demethoxymatteucinol (**1**)[3]: CD ($CHCl_3$, $c=0.047$): $[\theta]_{347}$ +2131 (max), $[\theta]_{316}$ +2815 (max), $[\theta]_{290}$ −21538 (max), $[\theta]_{244}$ +1531 (max).

Matteucinol (**2**)[3]: CD ($CHCl_3$, $c=0.035$): $[\theta]_{340}$ +3536 (max), $[\theta]_{312}$ +2115 (max), $[\theta]_{290}$ −25261 (max), $[\theta]_{250}$ +2731 (max).

2′-Hydroxymatteucinol (**3**)[3]: CD ($CHCl_3$, $c=0.051$): $[\theta]_{355}$ +3291 (max), $[\theta]_{313}$ +2933 (max), $[\theta]_{291}$ −23571 (max), $[\theta]_{250}$ +3015 (max).

Pinosylvin (**4**): Light pink plates; mp 157 °C; ^1H-NMR (DMSO-d_6) δ: 6.17 (1H, t, $J=2.1$ Hz, C_4-H), 6.45 (2H, d, $J=2.1$ Hz, C_2-H, C_6-H), 7.04 (2H, s, C-H_α, C-H_β), 7.25 (1H, t, $J=7.7$ Hz, $C_{4'}$-H), 7.35 (2H, t, $J=7.7$ Hz, $C_{3'}$-H, $C_{5'}$-H), 7.57 (2H, d, $J=7.7$ Hz, $C_{2'}$-H, $C_{6'}$-H), 9.28 (2H, br s, C_3-OH, C_5-OH); ^{13}C-NMR (DMSO-d_6) δ: 102.4 (d, C_4), 104.7 (d, C_2, C_6), 126.4 (d, $C_{2'}$, $C_{6'}$), 127.4 (d, C_β), 127.8 (d, C_α), 128.6 (d, $C_{3'}$, $C_{5'}$), 128.9 (d, $C_{4'}$), 137.0 (s, $C_{1'}$), 138.7 (s, C_1), 158.5 (s, C_3, C_5).

Pinosylvic acid (**5**): Dark brown plates; mp 168—170 °C; ^1H-NMR (DMSO-d_6) δ: 6.15 (1H, d, $J=2.5$ Hz, C_4-H), 6.47 (1H, d, $J=2.5$ Hz, C_6-H), 6.81 (1H, d, $J=16.5$ Hz, C-H_α), 7.25 (1H, t, $J=7.5$ Hz, $C_{4'}$-H), 7.36 (2H, t, $J=7.5$ Hz, $C_{3'}$-H, $C_{5'}$-H), 7.51 (2H, d, $J=7.5$ Hz, $C_{2'}$-H, $C_{6'}$-H), 8.20 (1H, d, $J=16.5$ Hz, C-H_β); ^{13}C-NMR (DMSO-d_6) δ: 102.1 (d, C_4), 104.8 (d, C_6), 107.1 (s, C_2), 126.4 (d, $C_{3'}$, $C_{5'}$), 127.3 (d, $C_{4'}$), 128.1 (d, C_α), 128.6 (d, $C_{2'}$, $C_{6'}$), 130.6 (d, C_β), 137.7 (s, $C_{1'}$), 141.3 (s, C_1), 160.3 (s, C_5), 164.5 (s, C_3), 172.5 (s, C=O); EI-MS *m/z*: 256 (M^+), 238 ($M^+ - H_2O$), 212 ($M^+ - CO_2$), 165, 141, 82.

Matteuorien (**6**): Yellow powder; UV λ_{max} nm (ε): 197 (11200), 216 (14300), 321 (7620); IR ν_{max} (KBr) cm^{-1}: 3375, 2935, 1750, 1660, 1640, 1605, 1135, 1110; ^1H- and ^{13}C-NMR (DMSO-d_6) in Tables 1 and 2; EI-MS *m/z*: 282 (M^+), 266 ($M^+ - H_2O$), 152, 130.

Matteuorienate A (**7**): Light yellow powder slightly changed to light green; $[\alpha]_D$ +8.6° (MeOH, $c=0.2$); UV λ_{max} nm (ε): 207 (6200), 227 (5700), 284 (220), 362 (980); IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1730, 1710; ^1H- and ^{13}C-NMR (acetone-d_6) in Tables 1 and 2; Positive ion FAB-MS *m/z*: 621 $(M+H)^+$; Molecular formula $C_{30}H_{37}O_{14}$ from High-resolution FAB-MS (Found 621.2175, Calcd 621.2183). CD (MeOH, $c=0.045$): $[\theta]_{344}$ +5423 (max), $[\theta]_{290}$ −33007 (max), $[\theta]_{354}$ +9002 (max).

Matteuorienate B (**8**): Light yellow powder slightly changed to light green; $[\alpha]_D$ +16.3° (MeOH, $c=0.1$); UV λ_{max} nm (ε): 211 (11900), 232 (5800), 287 (480), 360 (210); IR ν_{max} (KBr) cm^{-1}: 3380, 2950, 1725, 1720; ^1H- and ^{13}C-NMR (acetone-d_6) in Tables 1 and 2; Positive ion FAB-MS *m/z*: 591 $(M+H)^+$; Molecular formula $C_{29}H_{35}O_{13}$ from High-resolution FAB-MS (Found 591.2070, Calcd 591.2077). CD (MeOH, $c=0.052$): $[\theta]_{341}$ +5323 (max), $[\theta]_{291}$ −34357 (max), $[\theta]_{245}$ +8922 (max).

Matteuorienate C (**9**): Light yellow powder slightly changed to light green; $[\alpha]_D$ −54.0° (MeOH, $c=0.09$); UV λ_{max} nm (ε): 197 (11200), 216 (14300), 321 (7620); IR ν_{max} (KBr) cm^{-1}: 3375, 2945, 1750, 1665, 1635, 1600, 1135 1110; ^1H- and ^{13}C-NMR (acetone-d_6) in Tables 1 and 2; Positive ion FAB-MS *m/z*: 589 $(M+H)^+$; Molecular formula $C_{29}H_{33}O_{13}$ from High-resolution FAB-MS (Found 589.2048, Calcd 589.2027).

Matteuorienin (**10**): Light yellow powder slightly changed to light green. $[\alpha]_D$ −24.0° (MeOH, $c=0.08$); UV λ_{max} nm (ε): 217 (5200), 242 (6300), 280 (530), 355 (1050); IR ν_{max} (KBr) cm^{-1}: 3375, 2960, 1735, 1515; ^1H- and ^{13}C-NMR (DMSO-d_6) in Tables 1 and 2; Positive ion FAB-MS *m/z*: 449 $(M+H)^+$; Molecular formula $C_{22}H_{25}O_{10}$ from High-resolution FAB-MS (Found 449.1469, Calcd 449.1448); CD (MeOH, $c=0.057$): $[\theta]_{355}$ +6114 (max), $[\theta]_{288}$ −29893 (max), $[\theta]_{247}$ +4284 (max).

Methylation of Matteuorienate A (7) and B (8) with Diazomethane Matteuorienate A (**7**) (20 mg) gave methyl matteuorienate A (**11**) (2 mg) with diazomethane in MeOH solution. The reaction mixture was purified by preparative TLC. By the same method, **8** (10 mg) gave methyl matteuorienate B (**12**) (1.1 mg). However, it is interesting to note that the main product was matteucinol 7-*O*-β-glucopyranoside (**13**) (14 mg) and demethoxymatteucinol 7-*O*-β-glucopyranoside (**14**) (6 mg) during the methylation of **7** and **8**, respectively.

Acid Hydrolysis of Matteuorienate A (7), B (8), and C (9) Matteuorienate A (**7**) (22 mg) was refluxed with 5% hydrochloric acid (3 ml) for 2 h. The reaction mixture was extracted with ethyl acetate and the aglycone was purified by preparative TLC to give matteucinol (5 mg), and it was identified by comparing it with the ^1H-NMR of the authentic sample. The aqueous layer was lyophilized and subjected to preparative

TLC with the solvent system of EtOAc:MeOH:H_2O:HOAc (65:15:15:30) to obtain two compounds. They were identified as glucose by comparing them with the standard sample of D-glucose with co-TLC and **15** by the NMR spectra in comparison with the authentic sample. By a similar method, **8** (12 mg) gave **1**, **15**, and glucose. In the hydrolysis of **9** (4.3 mg), **6**, **15**, and glucose were obtained.

Alkali Hydrolysis of a Mixture of Matteuorienate A (7) and B (8) A mixture of matteuorienate A and B (almost 1:1) (500 mg) was refluxed with 5% KOH for 3 h in a water bath. The reaction mixture was extracted with EtOAc, and a portion of organic layer was again purified by HPLC to give **13** and **14**. The aqueous layer was lyophilized and again dissolved in MeOH. The methanol soluble portion gave almost pure **15**.

Isolation of Crude Enzyme The eye lens was isolated from five-week-old male Wistar rats, weighing 140—150 g. The lenses were collected in a chilled phosphate buffer (pH 6.2) containing NADP and mercaptoethanol.[5] The lens was homogenized and centrifuged at $10000 \times g$ for 15 min. The supernatant was considered to be a crude aldose reductase and was preserved at −80 °C until it was used in the experiment.

Measurement of Aldose Reductase Inhibitory Activity The frozen enzyme was melted in ice-cold water 2 h before starting the experiment. The assay was performed by the method of Kador *et al.*, with slight modification.[6] The substrate was used as a glyceraldehyde. The reaction mixture (1 ml) consisted of a phosphate buffer (970 μl) containing glyceraldehyde (10 mM) and NADPH (0.1 mM), DMSO (10 μl) or the sample in DMSO, and 20 μl of the crude enzyme. The reaction was carried out at 25 °C and a time course of 200 s was observed at 340 nm. The experiment was repeated until two concurrent readings were observed.

Results and Discussion

Matteuorienate A (**7**) was obtained as a pale yellow amorphous solid, $[\alpha]_D$ +8.6° (MeOH, $c=0.2$), and showed a quasi molecular ion peak at m/z 621 $(M+H)^+$ in positive ion FAB-MS. The molecular formula was determined to be $C_{30}H_{36}O_{14}$ by high-resolution FAB-MS. It showed IR absorptions at 3400, 2900, 1730, 1710 cm^{-1}, which was a similar pattern to that of **2**, but a sharp peak at 1730 cm^{-1} and a shoulder peak at 1710 cm^{-1} were different than that of **2** due to the presence of an additional carbonyl group. On comparing the ^{1}H-NMR spectrum with **2**, except for the signals of sugar and two methylene and methyl groups, other signals were almost similar to those of matteucinol. ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectrum showed 28 carbon signals. Sixteen carbon signals followed a similar pattern to that of matteucinol with a slight shift: six carbon signals were of sugar (δ_C 64.54, 71.62, 75.29, 75.75, 78.08, 105.38), two signals for carbonyl carbons (δ_C 171.98, 173.86), two methylene carbons (δ_C 45.60, 46.51), one methyl carbon (δ_C 28.05) and one tertiary aliphatic carbon (δ_C 70.52) bonded with oxygen were observed. All the proton signals of sugar in **7** were assigned from the ^{1}H–^{1}H COSY spectrum and the carbon signals of the sugar were assigned by ^{1}H–^{13}C COSY spectrum. Detailed NMR spectral analysis suggested that **7** is composed of **2**, 3-hydroxy-3-methylglutaric acid (dicrotalic acid) (**15**) and a sugar. The ^{1}H-signals of the sugar at positions C_1 and C_6 were shifted downfield, suggesting that the sugar was in between **2** and **15**. The connectivity between matteucinol (**2**), sugar, and 3-hydroxy-3-methylglutaric acid (**15**) was confirmed by the ^{1}H–^{13}C long-range COSY and nuclear Overhauser effect (NOE) experiments. The proton at δ_H 4.35 assigned for the $C_{6''}$-H_a showed a cross peak with the carbon at δ_C 171.98. Some other significant ^{1}H–^{13}C long-range correlations are shown by arrows (Chart 2). On irradiating the methyl signals at δ_H 2.11 and 2.13, respectively, an NOE was observed at the anomeric proton of the sugar at δ_H 4.80 (Chart 2).

The acid hydrolysis of matteuorienate A (**7**) gave **2**, **15**, and glucose. The alkali hydrolysis of **7** yielded **13** and **15**. Matteucinol 7-*O*-β-glucopyranoside (**13**) showed the $[\alpha]_D$ −24° (MeOH), and a positive Cotton effect was observed in the CD spectrum of **7**, suggesting that the absolute configuration at the C-2 position is *S*.[7] This result suggested that the absolute configuration of matteuorienate at the C-2 position is *S*. Matteuorienate A (**7**) was methylated with diazomethane to give **11** as the minor product, which was isolated by preparative TLC followed by recycled HPLC, and was identified by NMR. The complete assignment of each ^{1}H- and ^{13}C-NMR signal is shown in Tables 1 and 2. All the spectral and chemical information suggested that matteuorienate A is matteucinol 7-*O*-[6″-*O*-(3‴-hydroxy-3‴-methylglutaryl)]-β-glucopyranoside (**7**) and is a new compound from the natural source (Chart 1).

Matteuorienate B (**8**) was obtained as a pale yellow amorphous solid, with positive ion FAB-MS showing a quasi-molecular ion peak at m/z 591 $(M+H)^+$. The molecular formula was calculated to be $C_{29}H_{34}O_{13}$ by high-resolution FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **8** were almost similar to those of **7**. However, both the ^{1}H- and ^{13}C-NMR spectra of **7** showed the absence of a

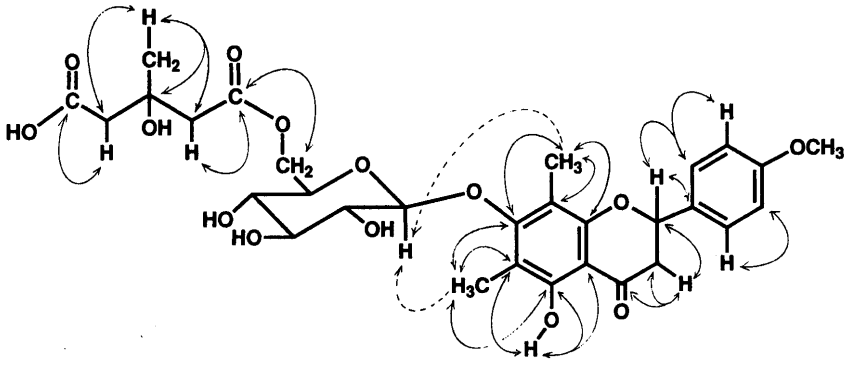

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Chart 2.

(⌒) Some significant ^{1}H–^{13}C long-range correlations observed in ^{1}H–^{13}C long-range COSY spectrum. (⋰⋱) NOE observed.

Table 1. ^{1}H-NMR Data of Matteuorien (**6**), Matteuorienate A (**7**), Matteuorienate B (**8**), Matteuorienate C (**9**), and Matteuorienin (**10**)

Proton	6[a]	7[b,c]	8[b,c]	9[b,c]	10[a,c]
2	—	5.51, dd (12.5, 2.5)	5.61, dd (12.0, 2.5)	—	5.44, dd (13.0, 3.5)
3a	6.96, s	2.85, dd (17.0, 2.5)	2.91, dd (17.0, 2.5)	7.09, s	2.78, dd (17.0, 3.5)
3b	—	3.22, dd (17.0, 12.5)	3.21, dd (17.0, 12.5)	—	3.02, dd (17.0, 13.0)
6-CH_3	2.06, s	2.11, s	2.13, br s	2.17, s	2.48, s
8-CH_3	2.31, s	2.13, s	2.15, br s	2.41, s	—
5-OH	13.00, s	12.20, s	12.14, s	12.91, s	12.55, s
2′	8.08, dd (7.5, 2.0)	7.51, d (8.5)	7.61, d (7.0)	8.11, br d (7.0)	7.35, d (8.5)
3′	7.60, m	7.00, d (8.5)	7.46, t (7.0)	7.63, t (7.0)	6.78, d (8.5)
4′	7.60, m	—	7.39, t (7.0)	7.62, t (7.0)	—
5′	7.60, m	7.00, d (8.5)	7.46, t (7.0)	7.63, t (7.0)	6.78, d (8.5)
6′	8.08, dd (7.5, 2.0)	7.51, d (8.5)	7.61, d (7.0)	8.11, br d (7.0)	7.35, d (8.5)
1″	—	4.80, d (7.0)	4.81, d (7.0)	4.70, d, (7.5)	4.63, d (10.0)
2″	—	3.58, m	3.59, m	3.34, m	3.50, dd (10.0, 8.5)
3″	—	3.49, m	3.49, m	3.22, m	3.20, dd (8.5, 5.0)
4″	—	3.54, m	3.55, m	3.31, m	3.25, m
5″	—	3.48, m	3.48, m	3.17, m	3.22, m
6a″	—	4.35, d (12.0)	4.36, d (12.0)	4.06, dd (12.0, 7.0)	3.56, dd (12.0, 5.0)
6b″	—	4.24, br d (12.0)	4.24, br d (12.0)	4.21, br d (12.0)	3.63, br d (12.0)
2‴	—	2.64, m	2.06, m	2.41, d (13.0)	—
4‴	—	2.64, m	2.06, m	2.41, d (13.0)	—
3‴-CH_3	—	1.30, s	1.30, s	1.10, s	—

Chemical shifts in δ ppm, measured in *a*) DMSO-d_6 or *b*) acetone-d_6 *c*) ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectra were measured.

Table 2. ^{13}C-NMR Data of Matteuorien (**6**), Matteuorienate A (**7**), Matteuorienate B (**8**), Matteuorienate C (**9**), and Matteuorienin (**10**)

Carbon	6[a]	7[b,c]	8[b,c]	9[b,c]	10[a,c]
2	162.82 (s)	79.84 (d)	80.00 (d)	163.63 (s)	77.91 (s)
3	104.72 (d)	44.17 (t)	44.33 (t)	105.16 (d)	42.85 (t)
4	182.20 (s)	199.61 (s)	199.36 (s)	182.88 (s)	197.03 (s)
4a	102.05 (s)	106.50 (s)	106.50 (s)	107.11 (s)	104.23 (s)
5	156.00 (s)	160.02 (s)	160.05 (s)	155.98 (s)	160.03 (s)
6	107.18 (s)	113.00 (s)	113.15 (s)	113.91 (s)	103.85 (s)
7	160.00 (s)	162.78 (s)	162.84 (s)	158.84 (s)	162.74 (s)
8	103.79 (s)	111.93 (s)	111.99 (s)	114.58 (s)	101.74 (s)
8a	156.00 (s)	159.05 (s)	158.90 (s)	152.16 (s)	158.25 (s)
1′	131.14 (s)	132.52 (s)	140.68 (s)	130.88 (s)	129.48 (s)
2′	129.21 (d)	129.15 (d)	129.97 (d)	129.24 (d)	127.42 (d)
3′	126.33 (d)	115.25 (d)	127.54 (d)	126.45 (d)	115.12 (d)
4′	131.87 (d)	161.27 (s)	129.72 (d)	132.15 (d)	157.27 (s)
5′	126.33 (d)	115.25 (d)	127.54 (d)	126.45 (d)	115.12 (d)
6′	129.21 (d)	129.15 (d)	129.97 (d)	129.24 (d)	127.42 (d)
1″	—	105.38 (d)	105.38 (d)	104.35 (d)	74.49 (d)
2″	—	75.75 (d)	75.75 (d)	73.87 (d)	71.78 (d)
3″	—	78.08 (d)	78.08 (d)	75.99 (d)	77.91 (d)
4″	—	71.62 (d)	71.62 (d)	73.62 (d)	69.29 (d)
5″	—	75.29 (d)	75.32 (d)	69.80 (d)	80.99 (d)
6″	—	64.54 (t)	64.57 (t)	63.06 (t)	60.19 (t)
1‴	—	171.98 (s)	171.98 (s)	170.22 (s)	—
2‴	—	46.51 (t)	46.51 (t)	45.33 (t)	—
3‴	—	70.52 (s)	70.52 (s)	68.07 (s)	—
4‴	—	45.60 (t)	45.63 (t)	45.06 (t)	—
5‴	—	173.86 (s)	173.89 (s)	172.23 (s)	—
6-$\underline{C}H_3$	8.00 (q)	9.51 (q)	9.47 (q)	8.93 (q)	7.10 (q)
8-$\underline{C}H_3$	8.34 (q)	10.73 (q)	10.14 (q)	9.17 (q)	—
3‴-$\underline{C}H_3$	—	28.05 (q)	28.05 (q)	27.11 (q)	—
4′-O$\underline{C}H_3$	—	56.01 (q)	—	—	—

Chemical shifts in δ ppm, measured in *a*) DMSO-d_6 or *b*) acetone-d_6. The multiplicities of carbon signals were determined by means of the DEPT method, and indicated as s, d, t, and q for singlet, doublet, triplet and quartet, respectively. *c*) ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectra were measured.

methoxy group in ring B. Therefore, in the ^{1}H- and ^{13}C-NMR spectra, the signals at the 2′ and 6′ as well as at 3′ and 5′ were not equivalent like those found in matteuorienate A (**7**). The ^{1}H-NMR spectrum showed five aromatic protons of ring B at δ_H 7.39 (1H, t, J=7.0 Hz, $C_{4'}$-H), 7.46 (2H, t, J=7.0 Hz, $C_{3'}$-, $C_{5'}$-H), 7.61 (2H, d, J=7.0 Hz, $C_{2'}$-, $C_{6'}$-H). The acid hydrolysis of **8** yielded **1**, **15**, and glucose. The aglycone was identified as demethoxymatteucinol (**1**) by comparing the ^{1}H-NMR with that of the authentic sample.[3] Methylation with the diazomethane, **8**, gave **12**. It also showed a positive Cotton effect, hence the configuration at the C-2 position was determined to be *S* by the same method as that of **7**.[5] The complete assignment of each ^{1}H- and ^{13}C-NMR signal is shown in Tables 1 and 2. The structure of matteuorienate B should be assigned as demethoxymatteucinol 7-*O*-[6″-*O*-(3‴-hydroxy-3‴-methylglutaryl)]-β-glucopyranoside (**8**).

Matteuorienate C (**9**) was obtained as a dark yellow amorphous solid, and showed a quasi-molecular ion peak at m/z 589 $(M+H)^+$ in positive ion FAB-MS. High resolution FAB-MS confirmed the molecular formula to be $C_{29}H_{32}O_{13}$. It showed stronger UV absorptions than those of **7** or **8**. The ^{1}H- and ^{13}C-NMR spectra of **9** were almost similar to those of **8**. However, a comparative study of the ^{1}H- and ^{13}C-NMR spectra with **7** and **8** suggested that there was one additional double bond (δ_H 7.09, 1H, s; δ_C 163.63, 105.16) at C_2 and C_3 which was also supported due to the absence of the ABX type protons in C_2 and C_3 found for **7** and **8**. The ^{13}C-NMR spectrum of **9** showed that the carbonyl carbon at C_4 (δ_C 182.88) was shifted upfield compared to that of **7** or **8**. The ^{1}H-NMR spectrum showed that five aromatic protons in the ring B at δ_H 7.62 (1H, t, J=7.0 Hz), 7.63 (2H, t, J=7.0 Hz) and 8.11 (2H, br d, J=7.0 Hz) were slightly shifted downfield compared with that of **8**. The chemical shifts for two methyls at δ_H 2.17 and 2.41 assigned for the C_6 and C_8-methyls, respectively, were quite separate, in contrast to the close overlapping found in **7** or **8**. Acid hydrolysis of **9** gave **6**, **15**, and glucose. The complete assignment of

each ^{1}H- and ^{13}C-NMR signal was due to ^{1}H–^{1}H, ^{1}H–^{13}C, and ^{1}H–^{13}C long-range COSY, which is shown in Tables 1 and 2. Based on the above results, the structure of matteuorienate C should be represented by the formula **9**.

Matteuorienin (**10**), a dark yellow amorphous solid. The positive ion FAB-MS showed a quasi-molecular ion peak at m/z 449 $(M+H)^+$ and the molecular formula was confirmed as $C_{22}H_{24}O_{10}$ by high-resolution FAB-MS. A part of the ^{1}H- and ^{13}C-NMR spectra of **10** was almost similar to that of **7**. In ^{1}H- and ^{13}C-NMR spectra, the signals assigned for 2′ and 6′ as well as 3′ and 5′ were found to be equivalent like those found in **7**. Four protons of ring B at δ_H 7.35 (2H, d, $J=8.5$ Hz) and 6.78 (2H, d, $J=8.5$ Hz) were found to be slightly shifted upfield compared with **7**. It showed only one *C*-methyl (δ_H 2.48, δ_C 7.10) in the ring A in contrast to the other compounds. Six carbon signals in the ^{13}C-NMR spectrum of **10** were observed due to the presence of a sugar. A hydroxyl proton at C_5-OH (δ_H 12.55) showed a cross-peak with carbon at δ_C 103.85, which was assigned to C_6. The anomeric proton (δ_H 4.63) showed a cross-peak with the carbon at δ_C 74.49 in ^{1}H–^{13}C COSY spectrum, suggesting that the anomeric carbon is connected directly with a carbon atom, and it was observed in ^{1}H–^{13}C long-range COSY that an anomeric proton showed a cross-peak with δ_C 101.74 (C_8). The *J*-value analysis suggested the sugar is a glucose and the sugar carbon signal pattern of **10** was also similar with that of vitexin.[8] All other ^{1}H- and ^{13}C-NMR signals were similar to that of the **2** except the signals due to the glucose and methoxy group. The absolute configuration at C-2 was determined to be *S*, since it showed a positive Cotton effect.[5] The complete assignment of each ^{1}H- and ^{13}C-NMR signal was due to ^{1}H–^{1}H, ^{1}H–^{13}C, and ^{1}H–^{13}C long-range COSY which is shown in Tables 1 and 2. All these spectral data suggested that compound **10** was a *C*-glucosyl derivative of matteucinol formed due to the replacement of a C_8-methyl group by glucose and also to the absence of methoxy group in $C_{4'}$, and it was named a matteuorienin.

Matteuorien (**6**) was obtained as a dark yellow amorphous solid. EI-MS showed a molecular ion peak at m/z 282 (M^+) corresponding to the molecular formula $C_{17}H_{14}O_4$. It showed very strong UV absorption like **9**. A part of the ^{1}H- and ^{13}C-NMR spectra of **6** was almost similar to that of **9**, but signals due to the sugar and 3-hydroxy-3-methylglutaryl group were not observed in the ^{1}H- and ^{13}C-NMR spectra. The complete assignment of each ^{1}H- and ^{13}C-NMR signal is shown in Tables 1 and 2. Acid hydrolysis of **9** gave **6**, so that **6** was the aglycone of **9**.

Pinosylvin (**4**), a pink colored crystalline solid, showed a melting point at 157 °C. EI-MS showed a molecular ion peak at m/z 212 (M^+) corresponding to the molecular formula $C_{14}H_{12}O_2$. IR spectrum showed the absence of a carbonyl function. From the ^{1}H- and ^{13}C-NMR spectral analysis and comparison with data in the literature, **4** was identified as a dihydroxystilbene, pinosylvin.[9]

Pinosylvic acid (**5**), a dark brown crystalline solid, mp 168—170 °C. EI-MS showed a molecular ion peak at m/z 256 (M^+) corresponding to the molecular formula $C_{15}H_{12}O_4$. IR spectrum showed the presence of a carbonyl function. The ^{1}H- and ^{13}C-NMR spectral data and comparison with data in the literature suggested that **5** was pinosylvic acid which has been reported to be an intermediate during the biosynthesis of pinosylvin (**4**).[10]

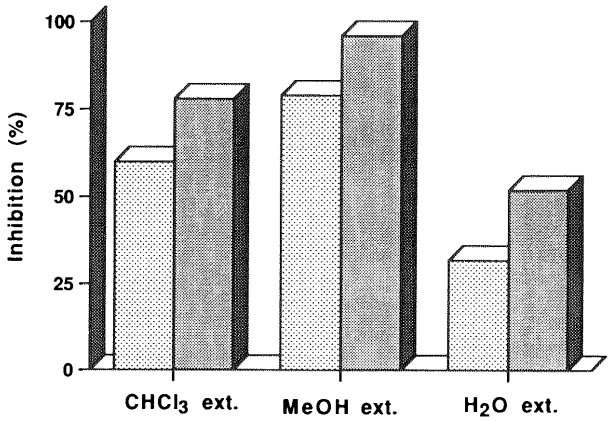

Fig. 1. The Aldose Reductase Inhibitory Activity of Chloroform, Methanol and Water Extracts of *Matteuccia orientalis*

▨, 100 μg/ml; □, 500 μg/ml.

Aldose Reductase Inhibitory Activity of Matteuorienate A (7), B (8), and C (9) The aldose reductase inhibitory activity of $CHCl_3$, MeOH and water extracts was examined at the concentration of 100 and 500 μg/ml. Of the three extracts, the MeOH extract showed very strong inhibitory activity (Fig. 1). Three new compounds, **7**, **8**, and **9**, were isolated from the active fraction of MeOH extract. The activity of these compounds were compared with the activity of epalrestat and quercetin. Epalrestat is a drug recently introduced in Japan which has strong aldose reductase inhibitory activity,[11] and quercetin is a natural flavonoid usually referred to as an aldose reductase inhibitor[12] as a positive control. The IC_{50} of **7**, **8**, and **9**, epalrestat, and quercetin were 1.0, 1.0, 2.3, 0.2, and 6.6 μM, respectively, under similar experimental conditions (Fig. 2).

The results of this experiment showed that **7**, **8**, and **9** were less active than epalrestat, but were found to be the most potent inhibitor of aldose reductase known yet from natural sources. Plasma comprises about 4% albumin, and in most cases, any drug administered in the animal body is bound to albumin or other proteins present in the blood, if they are not selective, and hence their activity is remarkably decreased. To find whether or not these compounds selectively react with the aldose reductase, the inhibitory activity of **7**, **8**, **9**, epalrestat, and quercetin were examined in a reaction mixture containing 1% bovine serum albumin (BSA) which showed an IC_{50} value of 3.6, 3.7, 6.4, 3.2, and 34.7 μM, respectively (Fig. 2). These results showed that these compounds were as potent as epalrestat in inhibiting the activity of aldose reductase in the presence of 1% BSA.

Structure–Activity Relationship The literature survey showed that most of the aldose reductase inhibitors are carboxyl group-containing compounds.[11,13] In the present investigation also, these three new compounds, **7**, **8**, and **9**, were found to be carboxyl group-bearing compounds, and all these compounds showed a very strong inhibitory activity against aldose reductase, which was

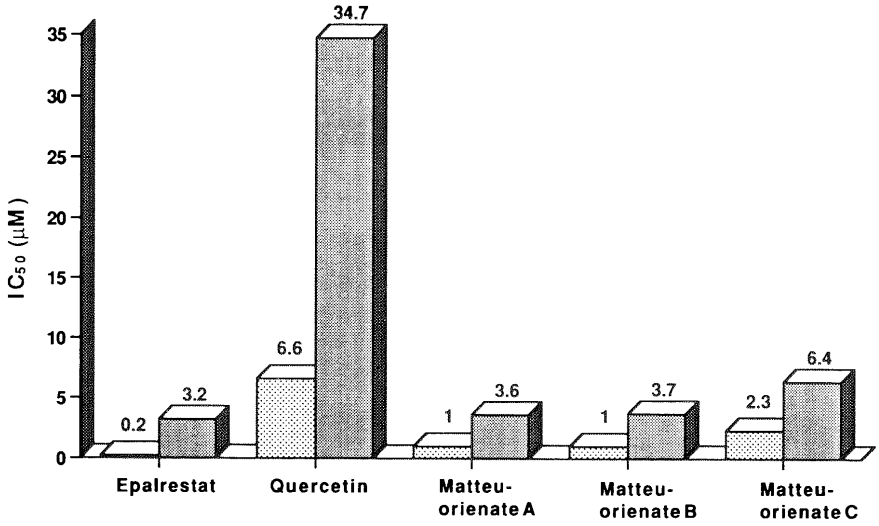

Fig. 2. Aldose Reductase Inhibitory Activity of Epalrestat, Quercetin, Matteuorienate A (**7**), Matteuorienate B (**8**), Matteuorienate C (**9**) in Absence and Presence of Bovine Serum Albumin (BSA)

▨, in absence of BSA; □, in presence of 1% BSA. Aldose reductase was obtained from rat lens. Glyceraldehyde was taken as a substrate.

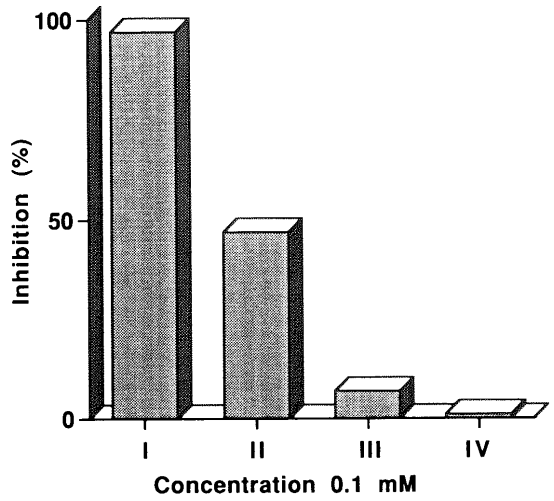

Fig. 3. Aldose Reductase Inhibitory Activities of Matteuorienate A (**7**) (I), Methyl Matteuorienate A (**11**) (II), Matteucinol 7-*O*-β-Glucopyranoside (**13**) (III), and 3-Hydroxy-3-methyl Glutaric Acid (**15**) (IV)

isolated from the eye lens of rats.

In order to determine the structure–activity relationship, the aldose reductase inhibitory activity of **7** and its derivatives, such as **11**, **13** and **15**, was examined. Methyl matteuorienate A (**11**) and the other derivatives, **13** and **15**, showed less than 50% inhibitory activity in a concentration of 0.1 mM (Fig. 3). When the carboxyl group in **7** was blocked by a methyl ester group, almost 50% inhibitory activity was decreased. The hydrolyzed products, **13** and **15**, did not show inhibitory activity. The same result was obtained for **8**. Other compounds, **1**, **2**, **3**, **7** and **10**, showed less than 50% inhibition in a concentration of 0.1 mM. All these results suggested that the carboxyl group in the matteuorienate frame is very important to showing aldose reductase inhibitory activity. The present study suggests that matteuorienate A, B, and C (**7**, **8**, **9**) are the strongest inhibitors of aldose reductase known from a natural source.

Conclusion

This drug comprises one of the constituents of traditional Chinese medicine, "Kuan Chung," but has never been used for the treatment of diabetes. However, a new compound, 2′-hydroxymatteucinol (**3**) from $CHCl_3$ extract showed strong hypoglycemic activity in the STZ-induced diabetic rats and normal mice.

On the other hand, the MeOH extract showed strong aldose reductase inhibitory activity. Aldose reductase inhibitory activity-guided chemical analysis led to the isolation of the five new compounds: matteuorien (**6**), matteuorienate A (**7**), matteuorienate B (**8**), matteuorienate C (**9**), and matteuorienin (**10**). However, the configuration at C-3‴ of **7**, **8**, and **9** remains to be explained. The aldose reductase inhibitory activity of matteuorienate A, B, and C, when compared with the epalrestat, showed that these compounds were as active as epalrestat in presence of 1% albumin. The experiment on several derivatives of these compounds showed that the carboxyl group is an important part in the matteuorienate frame in terms of showing aldose reductase inhibitory activity. The rhizome of *M. orientalis* will probably be applicable for the treatment of diabetics and diabetic complications.

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References

1) Rifkin H., Porte D., "Diabetes Mellitus Theory and Practice," 4th ed., 1990, pp. 28—30.
2) Hutoh M., *Shoyakugaku Zasshi*, **15**, 167—196 (1961).
3) Basnet P., Kadota S., Shimizu M., Xu H. X., Namba T., *Chem. Pharm. Bull.*, **41**, 1790—1795 (1993).
4) Kadota S., Basnet P., Hase K., Namba T., *Chem. Pharm. Bull.*, **42**, 1712—1714 (1994).
5) Terashima S., Shimizu M., Nakayama H., Ishikura M., Ueda Y., Imai K., Suzui A., Morita N., *Chem. Pharm. Bull.*, **38**, 2733—2736 (1990).
6) Kador P. F., Sharpless N. E., *Biophys. Chem.*, **8**, 81—85 (1978).
7) Gaffield W., *Tetrahedron*, **26**, 4093—4108 (1970).

8) Harborne J. B., Mabry T. J., "The Flavonoides, Advances in Research," Chapman and Hall, 1982, p. 62.
9) Cardona L., Fernandez I., Gracia B., Pedro J. R., *Tetrahedron*, **42**, 2725—2730 (1986).
10) Asakawa Y., *Bull. Chem. Soc. Jpn.* **44**, 2761—2766 (1971).
11) Sarges R., Oates P. J., *Prog. Drug Res.*, **40**, 99—161 (1993).
12) Shimizu M., Ito T., Terashima S., Hayashi T., Arisawa M., Morita N., Kurokawa S., Ito K., Hashimoto Y., *Phytochemistry*, **23**, 1885—1888 (1984); Varma S. D., Mikuni I., Konishita J. H., *Science*, **188**, 1215—1216 (1975).
13) Lee Y. S., Pearlstein R., Kodar P. F., *J. Med. Chem.*, **37**, 787—792 (1994).