MATTEUORIENATE A AND B, TWO NEW AND POTENT ALDOSE REDUCTASE INHIBITORS FROM *MATTEUCCIA ORIENTALIS* (HOOK.) TREV.

Shigetoshi KADOTA,* Purusotam BASNET, Koji HASE, and Tsuneo NAMBA

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-01, Japan

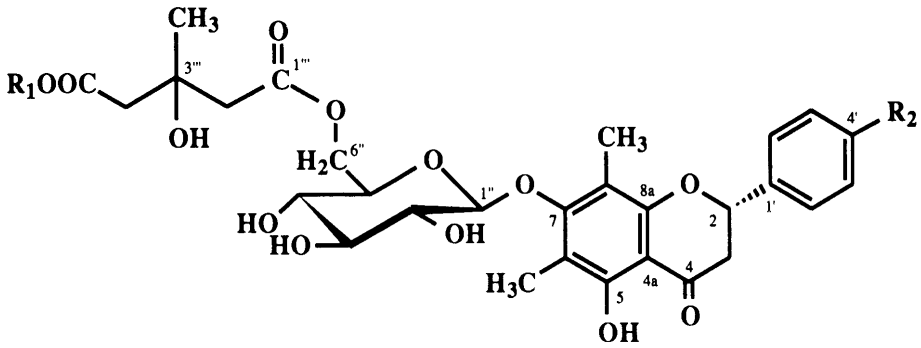
Matteuorienate A and B, two new *C*-methyl flavanone derivatives, were isolated from the *Matteuccia orientalis* (Hook.) Trev. (Aspidiaceae), and their structures were determined by the use of spectroscopic methods including 2D-NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and ^{1}H-^{13}C long-range COSY) experiments and chemical methods. Both the compounds were found to be very strong inhibitors of aldose reductase among the natural products.

KEYWORDS matteuorienate A; matteuorienate B; aldose reductase; *Matteuccia orientalis*

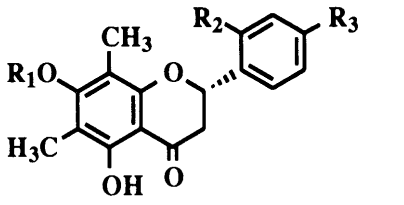
In the previous paper,[1] we reported the isolation and the structure determination of 2'-hydroxymatteucinol (**1**) as an active principle of a significant hypoglycemic activity in STZ-induced diabetic rats, from the $CHCl_3$ extract of *Matteuccia orientalis*. On continuing our study, we further isolated two new *C*-methyl flavonoids due to screening the aldose reductase inhibitory activity from the MeOH extract, and both compounds showed very strong inhibition to aldose reductase activity. This paper deals with the structure elucidation of two new compounds, matteuorienate A (**2**) and matteuorienate B (**3**), and their aldose reductase inhibitory activity.

The rhizome (6.5 kg) of *M. orientalis* was extracted with $CHCl_3$ followed by MeOH and water to obtain the $CHCl_3$ (310 g), MeOH (660 g) and water (760 g) extracts, respectively. Among the three extracts, the MeOH extract showed a strong inhibitory activity against aldose reductase,[2,3] and the MeOH extract was fractionated into four fractions by an Iatrobead column chromatography. The second fraction (48 g) obtained by eluting with 5% MeOH in $CHCl_3$ was again subjected to a silica gel column chromatography to give seven fractions. Finally, a portion (60 mg) of an active fraction (fr. 4) (1.6 g) was purified by HPLC {column: YMC ODS SH343-5, eluent: MeOH : H_2O : TFA (60 : 40 : 0.1), flow rate: 6.0 ml/min} recycled twice to obtain matteuorienate A (**2**) (31.6 mg) and matteuorienate B (**3**) (22.6 mg).

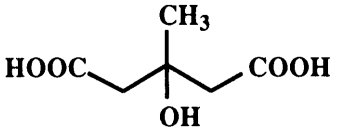
Matteuorienate A (**2**), a pale yellow amorphous solid, showed a quasi molecular ion peak at *m/z*: 621 $(M+1)^+$ in the positive ion FAB-MS corresponding to the molecular formula of $C_{30}H_{36}O_{14}$. It was found to be optically active, $[\alpha]_D$ +8.6° (MeOH, c = 0.2), and its IR spectrum showed a similar pattern to that of matteucinol (**4**)[1]; but a sharp peak at λ_{max} 1730 cm^{-1} and a shoulder peak at λ_{max} 1710 cm^{-1} were characteristically different, suggesting that **2** has two additional carbonyl groups in comparing with **4**. Its ^{1}H-NMR spectrum[4] was also partially similar to that of **4** except for the signals due to a sugar and two methylene and a methyl groups. The ^{13}C-NMR and DEPT spectra[4] showed 28 carbon signals: 16 carbon signals showed similar patterns to those of matteucinol (**4**) with slight shifts, and six carbon signals were due to sugar (δ_C 64.54, 71.62, 75.29, 75.75, 77.08, and 105.38); two carbonyl carbons (δ_C 171.98 and 173.86), two methylene carbons (δ_C 45.60 and 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 the sugar in **2** were assigned by the ^{1}H-^{1}H COSY spectrum, and the carbon signals of the sugar moiety were assigned by the ^{1}H-^{13}C COSY spectrum. Detailed NMR spectral analyses suggested that **2** is composed of **4**, **5**, and a sugar. The ^{1}H- signals of the sugar at C6"-H (δ_H 4.24 and 4.35) were found down field when compared with **6** (δ_H 3.3), suggesting that **5** is substituted at the C6" position. The connectivity among matteucinol (**4**), sugar and dicrotalic acid (**5**) was clarified by ^{1}H-^{13}C long-range COSY and



2 : R_1 = H, R_2 = OCH_3
3 : R_1 = R_2 = H
9 : R_1 = CH_3, R_2 = OCH_3
10 : R_1 = CH_3, R_2 = H



1 : R_1 = H, R_2 = OH, R_3 = OCH_3
4 : R_1 = R_2 = H, R_3 = OCH_3
6 : R_1 = β-glucopyranosyl, R_2 = H, R_3 = OCH_3
7 : R_1 = R_2 = R_3 = H
8 : R_1 = β-glucopyranosyl, R_2 = R_3 = H



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NOE experiments. The sugar proton at δ_H 4.24 assigned to C6"-Ha[4] showed a cross peak with the carbon at δ_C 171.98. Although we did not find the cross peak between C1"-H and C7 in the ^1H-^{13}C long-range COSY, on irradiating the methyl signals at δ_H 2.11 and 2.13, the NOE was observed at the anomeric proton of the sugar at δ_H 4.80.

The structure of this compound was further confirmed by the acid hydrolysis as the usual method to obtain **4**, **5**, and glucose. These compounds were purified by preparative TLC of silica gel and were identified by comparing the ^1H-NMR spectrum and/or co-TLC with authentic samples. All these spectral data suggested that this compound is 6"-*O*-dicrotalyl-7-*O*-β-glucopyranosyl matteucinol and named matteuorienate A (**2**).

Matteuorienate B (**3**), a pale yellow amorphous solid, showed a quasi molecular ion peak at *m/z*: 591 $(M+1)^+$ in the positive ion FAB-MS corresponding to the molecular formula of $C_{29}H_{34}O_{13}$. The ^1H- and ^{13}C- NMR spectra[5] of **3** were similar to those of **2**, but both the ^1H- and ^{13}C- NMR spectra of **3** showed the absence of methoxy group in ring B. Five protons in ring B were found at δ_H 7.39 (1H, t, *J* = 7.0 Hz, C4'-H), 7.46 (2H, t, *J* = 7.0 Hz, C3'-H and C5'-H), 7.61 (2H, d, *J* = 7.0 Hz, C2'-H and C6'-H) in the ^1H- NMR spectrum, showing the different signal patterns at 2' and 6' as well as 3' and 5' as in **2**. This was further confirmed by the acid hydrolysis. The acid hydrolysis of **3** yielded **5**, demethoxymatteucinol (**7**)[1] and glucose, which were identified by comparing the ^1H-NMR and/or co-TLC with the authentic samples. On the basis of these findings, the structure of matteuorienate B was determined to be represented by formula **3**.

In order to determine the structure-activity relationship, alkali hydrolysis and methylation were carried out on a mixture of **2** and **3** (approximately 1:1 mixture).[6] The alkali hydrolysis yielded dicrotalic acid (**5**), 7-*O*-β-glucopyranosyl matteucinol (**6**) and 7-*O*-β-glucopyranosyl demethoxymatteucinol (**8**). These compounds were isolated by preparative TLC followed by the recycled HPLC, and identified by NMR spectra. Also, the methylation with diazomethane yielded a mixture of methylated esters **9** and **10** as the minor products,[7] and they were isolated by preparative TLC followed by the HPLC and identified by NMR spectra.

Both these new compounds, matteuorienate A (**2**) and matteuorienate B (**3**), showed very strong aldose reductase inhibitory activity, isolated from the eye lens of rats.[2,3] The activities of **2** and **3** were compared with that of quercetin, which is usually referred as the positive control of the aldose reductase.[8] The IC_{50} values of **2**, **3**, and quercetin were 1.0×10^{-6} M, 1.0×10^{-6} M, and 6.6×10^{-6} M, respectively. The results of the experiment showed that compounds **2** and **3** were very strong inhibitors of aldose reductase from natural sources. In addition, we examined the activity in presence of bovine serum albumin (BSA) in order to clarify the interaction of **2** and **3** with other proteins besides aldose reductase. The matteuorienate A (**2**) and

matteuorienate B (**3**) and quercetin showed the IC_{50} as 3.6 x 10^{-6} M, 3.7 x 10^{-6} M, and 3.47 x 10^{-5} M, respectively, in the presence of 1% BSA. The matteucinol (**4**), 7-*O*-*β*-glucopyranosyl matteucinol (**6**), demethoxymatteucinol (**7**) and 7-*O*-*β*-glucopyranosyl demethoxymatteucinol (**8**) showed inhibition of 25.2%, 16.6%, 19.5% and 7.3 %, respectively, by the concentration of 1.0 x 10^{-4} M, and dicrotalic acid (**5**) and methyl esters (**9** and **10**) did not show aldose reductase inhibitory activity. The result of this experiment clearly suggests that the activity of **2** and **3** is not only strong but highly selective to aldose reductase rather than albumin. Further studies of *in vivo* and of structure-activity relationships are in progress in our laboratory, but the results of the experiments obtained in the present study suggest that **2** and **3** might be a possible drug for the treatment of a complication of diabetes mellitus.

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4) Matteuorienate A (**2**): a pale yellow amorphous, ^{1}H-NMR (acetone-d_6) δ: 1.30 (3H, s, $C_{3'''}$-C$\underline{H}_3$), 2.11 (3H, s, C_6-C$\underline{H}_3$), 2.13 (3H, s, C_8-C$\underline{H}_3$), 2.64 (H, m, $C_{2'''}$ and $C_{4'''}$ -CH_2), 2.85 (1H, dd, J = 17.0, 2.5 Hz, C_3-H_{eq}), 3.22 (1H, dd, J = 17.0, 12.5 Hz, C_3-H_{ax}), 3.48 (1H, m, $C_{5''}$-H), 3.49 (1H, m, $C_{4''}$-H), 3.54 (1H, m, $C_{2''}$-H), 3.58 (1H, m, $C_{3''}$-H), 3.83 (3H, s, $C_{4'}$-OCH_3), 4.24 (1H, br d, J = 12.0 Hz, $C_{6''}$-Ha), 4.35 (1H, br d, J = 12.0 Hz, $C_{6''}$-Hb), 4.80 (1H, d, J = 7.0 Hz, $C_{1''}$-H), 5.51 (1H, dd, J = 12.5, 2.5 Hz, C_2-H), 7.00 (2H, d, J = 8.5 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.51 (2H, d, J = 8.5 Hz, $C_{2'}$-H and $C_{6'}$-H), 12.20 (1H, s, C_5-O$\underline{H}$); ^{13}C NMR (acetone-d_6) δ: 9.51 (q, 6-C$\underline{H}_3$), 10.73 (q, 8-C$\underline{H}_3$), 28.05 (q, 3'''-C$\underline{H}_3$), 44.17 (t, C_3), 45.60 (t, $C_{4'''}$), 46.51 (t, $C_{2'''}$), 56.01 (q, 4'-O$\underline{C}H_3$), 64.54 (d, $C_{6''}$), 70.52 (s, $C_{3'''}$), 71.62 (d, $C_{5''}$), 75.29 (d, $C_{4''}$), 75.75 (d, $C_{3''}$), 77.08 (d, $C_{2''}$), 79.84 (d, C_2), 105.38 (d, $C_{1''}$), 106.50 (s, C_{4a}), 111.93 (s, C_8), 113.0 (s, C_6), 115.25 (d, $C_{3'}$ and $C_{5'}$), 129.15 (d, $C_{2'}$ and $C_{6'}$), 132.52 (s, $C_{1'}$), 159.05 (s, C_{8a}), 160.02 (s, C_5), 161.27 (s, $C_{4'}$), 162.78 (s, C_7), 171.98 (s, $C_{1'''}$), 173.86 (s, $C_{5'''}$), 199.61 (s, C_4). Assignments of ^{1}H- and ^{13}C-NMR signals were accomplished by means of the ^{1}H- ^{1}H COSY and the ^{1}H- ^{13}C and ^{1}H- ^{13}C long-range COSY methods.

5) Matteuorienate B (**3**): a pale yellow amorphous, ^{1}H-NMR (acetone-d_6) δ: 1.30 (3H, s, $C_{3'''}$-C$\underline{H}_3$), 2.13 (3H, br s, C_8-C$\underline{H}_3$), 2.15 (3H, br s, C_6-C$\underline{H}_3$), 2.91 (1H, dd, J = 17.0, 2.5 Hz C_3-H_{eq}), 3.21 (1H, dd, J = 17.0, 12.5 Hz, C_3-H_{ax}), 3.48 (1H, m, $C_{5''}$-H), 3.49 (1H, m, $C_{4''}$-H), 3.55 (1H, m, $C_{2''}$-H), 3.59 (1H, m, $C_{3''}$-H), 4.24 (1H, br d, J = 12.0 Hz, $C_{6''}$-Ha), 4.36 (1H, d, J = 12.0 Hz, $C_{6''}$-Hb), 4.81 (1H, d, J = 7.0 Hz, $C_{1''}$-H), 5.61 (1H, dd, J = 12.0, 2.5 Hz, C_2-H), 7.39 (1H, t, J = 7.0 Hz, $C_{4'}$-H), 7.46 (2H, t, J = 7.0 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.61 (2H, d, J = 7.0 Hz, $C_{2'}$-H and $C_{6'}$-H), 12.14 (1H, s, C_5-O$\underline{H}$); ^{13}C-NMR (acetone-d_6) δ: 9.47 (q, 6-C$\underline{H}_3$), 10.14 (q, 8-C$\underline{H}_3$), 28.05 (q, 3'''-C$\underline{H}_3$), 44.33 (t, C_3), 45.63 (t, $C_{4'''}$), 46.51 (t, $C_{2'''}$), 64.57 (t, $C_{6''}$), 70.52 (s, $C_{3'''}$), 71.62 (d, $C_{4''}$), 75.32 (d, $C_{5''}$), 75.75 (d, $C_{2''}$), 78.08 (d, $C_{3''}$), 80.00 (d, C_2), 105.38 (d, $C_{1''}$), 106.50 (s, C_{4a}), 111.99 (s, C_8), 113.15 (s, C_6), 127.54 (d, $C_{3'}$ and $C_{5'}$), 129.72 (d, $C_{4'}$), 129.97 (d, $C_{2'}$ and $C_{6'}$), 140.68 (s, $C_{1'}$), 158.90 (s, C_{8a}), 160.05 (s, C_5), 162.84 (s, C_7), 171.98 (s, $C_{1'''}$), 173.89 (s, $C_{5'''}$), 199.36 (s, C_4). Assignments of ^{1}H- and ^{13}C-NMR signals were accomplished by the same methods as **2**.

6) It was very difficult to separate the mixture of matteuorienate A (**2**) and matteuorienate B (**3**) even by HPLC.

7) The major products were a mixture of **6** and **8.** They were purified by preparative TLC followed by HPLC, and identified by NMR spectra.

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