2'-Hydroxymatteucinol, a New C-Methyl Flavanone Derivative from *Matteuccia orientalis*; Potent Hypoglycemic Activity in Streptozotocin (STZ)-Induced Diabetic Rat

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The CHCl₃ extract of *Matteuccia orientalis* showed very strong hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats. A new C-methyl flavanone derivative, 2'-hydroxymatteucinol (3) was isolated from the hypoglycemic activity bearing fraction, along with two known compounds, demethoxymatteucinol (1) and matteucinol (2). The structures of these isolated compounds were elucidated by spectroscopic methods. One of the compounds isolated from CHCl₃ extract, 2'-hydroxymatteucinol (3), showed dose-dependent hypoglycemic activity, and a blood sugar lowering effect was observed even at the dose of 10 mg/kg (p.o.) in STZ-induced diabetic rats.

**Keywords** *Matteuccia orientalis*; Aspidiaceae; hypoglycemic activity; C-methyl flavanone; 2'-hydroxymatteucinol

![Diagram](chart1.png)

1. R₁ = R₂ = R₃ = R₄ = H
2. R₁ = R₂ = R₃ = H, R₄ = OCH₃
3. R₁ = R₂ = H, R₃ = OCH₃, R₄ = OCH₃
3a: R₁ = H, R₂ = Ac, R₃ = OAc, R₄ = OCH₃
3b: R₁ = R₂ = Ac, R₃ = OAc, R₄ = OCH₃

**Chart 1**

**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 absorbance frequency 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 28°C. ¹H- and ¹³C-NMR spectra were taken on a JEOL GX-400 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-¹³C COSY and ¹H-¹³C long-range COSY spectra were obtained using the usual pulse sequences, and data processing was performed with standard JEOL software. Mass spectra (MS) and high-resolution MS were taken on a JEOL JMX DX-300 mass spectrometer using a direct inlet system. The blood glucose analysis of rats was carried out on a Reflotron kit using a standard Reflotron glucose strip (Boehringer Mannheim Co.) based on the glucose oxidation method. Column chromatography was done with Wakogel C-200 (Wako Pure Chemical Co., Osaka, Japan), and TLC and preparative TLC were carried out on precoated Merck Kieselgel F₂₅₄ plates (0.25 or 0.5 mm). Other chemicals, STZ (Sigma), heparin (Wako, Japan), tobutamide (Chugai, Japan), and buformine (Kodama, Japan) were of analytical grade.

**Plant Materials**

The rhizomes of *Matteuccia orientalis* were collected in Yatsuo, Toyama Prefecture, Japan in October 1990. The plant was properly identified by an expert, and a voucher sample was preserved for reference in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Japan.

**Extraction and Isolation**

The fresh rhizomes were chopped into small pieces and dried in the shade. The shade dried rhizomes (6.5 kg) were exhaustively extracted by percolation with CHCl₃ (20 l × 3) at room temperature. Evaporation of the extract (60 l) in vacuo at 40°C yielded a CHCl₃ extract (390 g). The insoluble mass was successively extracted with MeOH (20 l × 3) and water (20 l × 3) in a manner similar to that above, and yielded MeOH (406 g) and water (450 g) extracts respectively. The CHCl₃ extract showed only three spots on the silica gel TLC using the solvent system, EtOAc–hexane (2:8). The CHCl₃ extract (160 g) was subjected to column chromatography using Silica gel G (5 kg) and eluted with hexane by increasing the polarity with an increase in the concentration of CHCl₃. Repeated silica gel column chromatography followed by crystallization gave three pure compounds, demethoxymatteucinol (1) (0.74% of crude drug) matteucinol (2) (1.23%) and 2'-hydroxymatteucinol (3) (0.25%).

**Demethoxymatteucinol (1)**

1H-NMR (DMSO-d₆): δ: 12.40 (1H, s, C₅-H), 9.70 (1H, s, C₆-H), 7.63 (2H, d, J = 7.5 Hz, C₂-H, C₃-H), 7.48 (2H, d, J = 7.5 Hz, C₂-H, C₃-H), 7.19 (1H, br t, J = 7.5 Hz, C₄-H), 7.59 (1H, d, J = 13.0 Hz, C₅-H), 6.93 (1H, d, J = 13.0 Hz, C₅-H), 5.34 (1H, d, J = 12.7 Hz, C₆-H), 3.84 (3H, s, C₁-OCH₃), 3.05 (1H, dd, J = 17.0, 12.7 Hz, C₆-H), 2.80 (1H, d, J = 17.0, 3.3 Hz, C₅-H), 2.05 (6H, br s, C₆-C₇, C₆-C₈, C₆-C₉), 13C-NMR (DMSO-d₆): Table 1.

**Matteucinol (2)**

1H-NMR (DMSO-d₆): δ: 12.28 (1H, s, C₅-H), 9.62 (1H, s, C₆-H), 7.38 (2H, d, J = 8.8 Hz, C₂-H, C₃-H), 6.94 (2H, d, J = 8.8 Hz, C₂-H, C₃-H), 5.34 (1H, d, J = 12.7 Hz, C₆-H), 3.84 (3H, s, C₁-OCH₃), 3.05 (1H, dd, J = 17.0, 12.7 Hz, C₆-H), 2.80 (1H, d, J = 17.0, 3.3 Hz, C₅-H), 2.05 (6H, br s, C₆-C₇, C₆-C₈, C₆-C₉), 13C-NMR (DMSO-d₆): Table 1.

**2'-Hydroxymatteucinol (3)**

Light yellow crystals, mp 244–248°C (MeOH), [α]D₂₀ = -163.1° (c = 0.2, MeOH). UV λ_max 290 nm (log ε): 340 (3.52),

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peritoneally. Blood samples were collected 6 h after the last dose (i.p. or p.o.) was administered in the cases where time was not mentioned in the Table. A blood sample was taken by syringe from a tail vein and immediately transferred into a tube which had been rinsed with heparin. The glucose level in the blood sample was analysed within an hour using a commercial Reftoltron kit.

Statistical Analysis All values expressed as the mean ± S.E. were obtained from a number of experiments (n). The Student's t-test for unequal variation between the control the experimental samples was carried out for statistical evaluation of the differences; p-values of 0.05 or less were considered significant.

Results and Discussion

Five doses of the CHCl₃ extract of M. orientalis when administered intraperitoneally in doses of 200 mg/kg each twice a day, lowered the blood glucose level 39.21% (p < 0.001) in STZ-induced diabetic rats, while the MeOH and water extracts of this plant did not show any significant hypoglycemic activity under similar experimental conditions (Table II). The hypoglycemic activity of the CHCl₃ extract of M. orientalis was also compared with a mixture of tolbutamide and buformine, used as the positive control.

The results showed that five doses of a mixture of tolbutamide (200 mg/kg) and buformine (2 mg/kg) lowered the blood glucose level by 28.79% (p < 0.02) under similar experimental conditions. In this set of the experiments, blood samples were collected 6 h after the last dose of drug administration.

The results obtained in the above experiment clearly showed that the CHCl₃ extract of M. orientalis contains an active principle or principles responsible for lowering the blood sugar level. Therefore, chemical analysis of this fraction was carried out. TLC examination of the CHCl₃ extract showed three spots in the chromatogram, indicating that only three compounds comprise the main constituents in the CHCl₃ extract. These three compounds were isolated by repeated column chromatography followed by crystallization. Spectral analysis showed that three compounds, 1, 2 and 3, isolated from the active fraction, were flavanone derivatives. Two of them, 1 and 2, have already been isolated.
from this plant, while compound 3 was a new C-methyl flavanone derivative. A literature survey revealed that there was no complete or well-illustrated NMR spectral data for compounds 1 and 2 to date and further, some of the positions were assigned incorrectly so that it would be worthwhile to discuss the complete NMR spectral assignment of these compounds.

**Demethoxymateucinol (I)** Pale yellow needles, mp 211 °C (CHCl₃) [lit. 200—202 °C (MeOH)]. A molecular ion peak at m/z 284 and its high-resolution MS suggested the molecular formula to be C₁₇H₁₆O₄. The UV absorptions at λ_max 210 (2.17), 295 (4.42) and 360 (3.97) and IR absorptions at ν_max 3250 (OH), 1630 (CO) and 1600 (br, aromatic) cm⁻¹ were due to the flavanone. Its ¹H-NMR spectrum showed a set of five protons coupling with one another at δ_H 7.63 (2H, br_d, J = 7.5 Hz), 7.48 (2H, br_t, J = 7.5 Hz), 7.45 (1H, br_t, J = 7.5 Hz) in one aromatic ring. When the spectrum was measured in the CDCl₃, the signal for all five protons were observed as multiplets at δ_H 7.50—7.35. The signal at δ_H 5.39 (1H, dd, J = 13.0, 3.3 Hz) showed a correlation with the geminal protons at δ_H 3.03 (1H, dd, J = 17.5, 13.0 Hz) and 2.82 (1H, dd, J = 17.5, 3.3 Hz). In addition, broad singlets for two methyl groups at δ_H 2.05 and two singlets, one for each proton in the low field at δ_H 12.40 and 9.70 due to the hydroxyl protons, were also observed. The ¹³C-NMR spectrum of 1 showed 15 signals: three signals due to the oxygen substituted aromatic carbons at δ_C 162.58 (s), 158.57 (s), 157.22 (s), seven signals due to hydrogen or carbon substituted aromatic carbons at δ_C 139.23 (s), 128.62 (d), 128.34 (d), 126.23 (d), 103.53 (s), 102.72 (s), 101.80 (s), and a signal each for the oxygen substituted sp³ carbon at δ_C 77.96 (d), sp³ methylene at δ_C 42.26 (t), carbonyl carbon at δ_C 196.33 (s), and two methyl carbons at δ_C 8.30 (q), and 7.65 (q). The carbon signal intensity at δ_C 128.62 and 126.23 was twice as big as other relative carbon signals due to a set of two equivalent carbons for each signal. When the ¹³C-NMR spectrum of 1 was compared with the spectrum of pinocembrin, there was a very close similarity. Regarding all this information and comparison with data from previous literature, compound 1 was found to be the demethoxymateucinol reported by Murakami et al. from *Wagneriopteris japonica*. However, some carbon assignments in our experiment compared to those of Murakami et al. for the C₆₂, C₅, C₆, C₇, C₈, C₉, 6-CH₃ and 8-CH₃ were found to be different for this compound. The ¹H-¹³C long-range COSY spectrum of 1 gave very good information for establishing the complete assignment of carbons (Fig. 1). There was correlation between the hydroxyl protons at δ_H 3.48 and the hydrogen at δ_H 3.37, and the carbon at δ_C 162.58 (s).
12.40 (C₂-OH) with three carbons at δC 158.57, 103.53 and 101.80, which were assigned to C₅, C₆ and C₄α, respectively. These assignments were also in support, due to the correlation between the hydroxyl proton at δH 9.70 (C₇-OH) with two carbons at δC 103.53 and 102.72, which can be assigned to carbons at the C₅ and C₆ positions, respectively (Fig. 1). The 1H-NMR signals of the two methyl groups at the C₅ and C₆ positions were very close, but the carbon signals were very different, and by comparing the correlation between the C₅ and C₆ carbons (assigned with the help of hydroxyl protons) and the two methyl groups, the methyl group at the slightly lower field was assigned as C₅-CH₃ and another was C₆-CH₃. With the help of 1H-13C COSY and 1H-13C long-range COSY, the methyl carbon signals in the high field were assigned as δC 8.30 (q) and 7.65 (q) for C₅-CH₃ and C₆-CH₃, respectively. The other main 1H-13C long-range correlations are shown by the arrows (Fig. 1). Compound 1 was found to be levorotatory, ([α]D = -40.0°) so that the configuration at the C₅ position was assigned as (S) by comparison with the literature. The result of this spectral information obviously suggests that the assignment by Murakami et al. for the carbons discussed above must be revised.

**Matteucinol (2)** Light yellow needles, mp 182°C (CHCl₃), showed M⁺ at m/z 314 and was found to be optically active [α]D = -20.5°. The molecular formula was calculated as C₁₉H₁₉O₄ according to high-resolution MS. Compound 2 gave a pattern of UV and IR absorption signals similar to that of 1. The 1H- and 13C-NMR signals were also almost similar to that of 1, but some differences were noticed due to one additional signal at δH 3.84 of the methoxyl protons, and to the signals of the ring B protons in the 1H-NMR spectrum. Four aromatic protons were observed in the 1H-NMR spectrum at δH 7.38 (2H, d, J = 8.8 Hz) and 6.94 (2H, d, J = 8.8 Hz) instead of five, as in 1, so that 2 was suggested to be a methoxy derivative of 1. Both the 1H-NMR and 13C-NMR spectra suggested that there were two sets of equivalent carbons and protons in ring B, and it is possible only when the methoxy group is in the C₄α-position. The complete assignment of the carbon signals were determined using distortionless enhancement by polarization transfer (DEPT), 1H-13C COSY and 1H-13C long-range COSY as shown in Table I. This compound has already been reported from this plant, but this is the first complete NMR spectral information that we know of so far.

**2'-Hydroxyamatteucinol (3)** Pale yellow crystals, mp 244—248°C (MeOH), showed M⁺ at m/z 330 and was found to be optically active [α]D = -163.1°C. The molecular formula was calculated as C₁₉H₁₉O₆ according to high-resolution MS. Compound 3 also gave a pattern of UV and IR absorption signals almost the same as that of 1 and 2. Its 1H-NMR spectrum showed signals due to one proton at δH 7.05 (1H, d, J = 2.5 Hz) and two protons at δH 6.75—6.85 (2H, m). The 1H-1H COSY experiment showed that these three protons were coupled with one another, suggesting that they are in one aromatic ring. However, due to the overlapping of two proton signals at δH 6.75—6.85 (2H, m), it was difficult to assign the position of these protons in ring B, while the other signals were found to be very close to those of 2. In the 13C-NMR spectrum, one more signal was also observed at a low field, due to the oxygen substitution, when compared to 2. By examination of both the 1H- and 13C-NMR spectra, one more hydroxyl group was observed in ring B as compared with 2. Hence, 3 was suggested to be a hydroxyl derivative of 2, due to the replacement of one hydrogen by a hydroxyl group. This was further supported by preparing a triacetate.
flavanone derivatives (1, 2, 3) and the structures of 1, 2 and 3 were found to be different only regarding the substituents in ring B. Among these three compounds isolated from the CHCl₃ extract of M. orientalis, 2'-hydroxymatuecinol (3) was found to be the most effective in lowering the blood glucose level (Fig. 4).

The hypoglycemic activity of 2'-hydroxymatuecinol (3) was compared with tolbutamide under similar experimental conditions. In this experiment, five intraperitoneal doses of 100 mg/kg each of tolbutamide and two different doses, 100 and 50 mg/kg each of 2'-hydroxymatuecinol (3), twice a day, were received by the STZ-induced diabetic rats. Blood samples were collected in each case 6h after the last dose of drug administration. It was observed that the blood glucose lowering effect of 2'-hydroxymatuecinol (3), even at a dose of 50 mg/kg, was more significant (28.73%, p < 0.001) than the 100 mg/kg dose of tolbutamide (30.73%, p < 0.01) in the STZ-induced diabetic rats (Table III).

It was observed that the intraperitoneal drug administra-

![Fig. 4. Effect of CHCl₃ Extract, Demethylmatuecinol (1), Matuecinol (2), and 2'-Hydroxymatuecinol (3) from Mattucea orientalis on Blood Glucose Level of Normal Mice](image)

![Table III. Effect of Various Doses of 2'-Hydroxymatuecinol (3) (5 Doses Each, Twice a Day, i.p.) on Blood Glucose Level in STZ-Induced Diabetic Rats](table)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose level in mg/dl</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before i.p.</td>
<td>after i.p.</td>
<td></td>
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<td>Control</td>
<td>380.5 ± 24.4</td>
<td>376.0 ± 21.6</td>
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<td>100</td>
<td>381.8 ± 28.0</td>
<td>260.5 ± 56.2 (1)</td>
<td>30.73</td>
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<tr>
<td>100</td>
<td>363.6 ± 33.8</td>
<td>222.4 ± 45.4 (2)</td>
<td>38.17</td>
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<td>100</td>
<td>365.3 ± 33.1</td>
<td>251.3 ± 20.6 (3)</td>
<td>28.73</td>
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<td>100</td>
<td>365.3 ± 33.1</td>
<td>251.3 ± 20.6 (10)</td>
<td>28.73</td>
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</tbody>
</table>

a) Glucose level before administering the drugs or saline. b) Decrease in blood glucose level relative to the level before i.p. administration expressed as a % in comparison with the control. Results are due to mean ± S.E. Significantly different from control value, c) p < 0.01, d) p < 0.001.
Table IV. Effects of Various Doses of 2-Hydroxymateculin (3) (Five Doses Each, p.o., Twice a Day) on Blood Glucose Level in STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Glucose level (mg/dl)</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before p.o.a</td>
<td>after p.o.</td>
</tr>
<tr>
<td>Control 1</td>
<td>100</td>
<td>40.4 ± 14.9</td>
<td>45.1 ± 10.2</td>
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<td></td>
<td>(100.0 ± 3.3)</td>
<td>(99.4 ± 2.2)</td>
<td>(98.4 ± 2.2)</td>
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<tr>
<td>Control 2</td>
<td>25</td>
<td>419.2 ± 23.5</td>
<td>299.3 ± 25.5</td>
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<td></td>
<td>(100.0 ± 5.6)</td>
<td>(71.3 ± 6.1)</td>
<td>(71.3 ± 6.1)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>422.8 ± 32.9</td>
<td>379.0 ± 20.5</td>
</tr>
</tbody>
</table>

Results are due to mean ± S.E. of six experiments, n = 6. a) Glucose level before administering the drugs or saline. b) Decrease in blood glucose level relative to the level before p.o. administration expressed in % in comparison with the control. Significantly different from control value, c) p < 0.001, d) p < 0.01, e) p < 0.05.

tion was very effective in lowering the blood glucose level of the STZ-induced diabetic rats. Thus, in the next step, the oral hypoglycemic activity of 2'-hydroxymateculin was observed. When various doses ranging from 100 to 5 mg/kg of 2'-hydroxymateculin (3) were administered orally, a dose-dependent response was observed in STZ-induced diabetic rats. In this experiment, five groups of STZ-induced diabetic rats received doses of 100, 50, 25, 10, and 5 mg/kg of 2'-hydroxymateculin (3), respectively, each with five doses, twice a day, administered orally. Blood samples were collected 6 h after the administration of the last dose. The results, shown in the Table IV, suggest that 2'-hydroxymateculin (3) is significantly effective in lowering blood glucose levels with doses of 100 mg/kg (p < 0.001), 50 mg/kg (p < 0.01) and 25 mg/kg (p < 0.05). Five doses of the oral administration of 10 mg/kg also lowered the blood glucose level by 16.85%, but the Student's t-test calculation did not show any significant difference between the control and experimental groups (p < 0.5).

From the results obtained in this study, it is clear that the most active principle of CHC-l extract of M. orientalis for lowering the blood glucose level is a new C-methyl flavanone derivative, 2'-hydroxymateculin (3). The blood glucose lowering effect of 2'-hydroxymateculin (3) is much more effective, and the effect lasting as compared with tolbutamide as the positive control. It has also become clear from the present investigation that 2'-hydroxymateculin (3) definitely lowers the blood glucose level significantly in normal mice as well as in STZ-induced diabetic rats.

A survey of the literature showed that a compound having a very close structure to 2'-hydroxymateculin (3) exhibits strong hypolipidemic and choreatic activity, but there has been no report on its antidiabetic activity. It has also been found that this type of compound is a very strong inhibitor of cAMP, and it is well known that a high level of insulin sharply reduced the cAMP level. Based on these information sequences, 2'-hydroxymateculin (3) probably reduces the blood glucose level by stimulating insulin secretion. Additional pharmacological studies, such as a glucose tolerance test and including the insulin and glucagon-releasing effects of 2'-hydroxymateculin (3), are now in progress in our laboratory.

The results of the present investigation showed that 2'-hydroxymateculin (3), isolated from the CHC-l extract of M. orientalis, lowered the blood glucose level in animal experiments. Also, chemical analysis showed that plant is a potential source of this compound because of its high yield. Hence, 2'-hydroxymateculin (3) could be a possible hypoglycemic agent in the treatment of clinical diabetes.

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References and Notes
1) Although this drug is included in the "Kuan-chung," it is not in common use as Kuan-chung in the Japanese market. See M. Hutoh, Shoyakugaku Zasshi, 15, 167 (1961).
3) K. Mohri, T. Takemoto, Y. Kondo, Yakugaku Zasshi, 102, 310 (1982).