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

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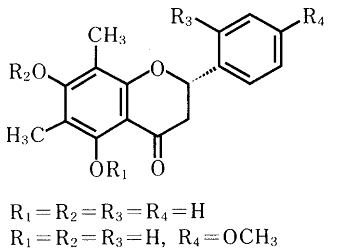
The $CHCl_3$ extract of *Matteccia orientalis* showed very strong hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats. A new *C*-methyl flavanone derivative, 2′-hydroxymatteucinol (3) was isolated from the hypoglycemic activity bearing fraction, along with two known compounds, demethoxymatteucinol (1) and matteucinol (2). The structures of these isolated compounds were elucidated by spectroscopic methods. One of the compounds isolated from $CHCl_3$ 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 *Matteccia orientalis*; Aspidiaceae; hypoglycemic activity; *C*-methyl flavanone; 2′-hydroxymatteucinol

Matteccia orientalis (HOOK.) TREV. belonging to the Aspidiaceae, vernacularly known as inugansoku, has been distributed in the hilly region of Japan and grows especially in shady and wet places. The rhizome of this plant is a traditional Chinese medicine called Guan Zhong (Kuan-chung in Japan).[1] No report or traditional use of this plant in connection with the treatment of diabetes has been found, but in our preliminary screening of some Chinese crude drugs for antidiabetic properties, this plant showed a very strong blood sugar level lowering effect in streptozotocin (STZ)-induced diabetic rats. Therefore, it was thought worthwhile to find and study the active principle/principles responsible for lowering the blood glucose level. In this paper, we wish to present the chemical analysis of the $CHCl_3$ extract of *M. orientalis* from which one new and two known *C*-methyl flavanone derivatives were isolated. The structures of these isolates were elucidated by spectroscopic evidence, and one of the compounds, 2′-hydroxymatteucinol (3) showed a very strong hypoglycemic effect in both normal mice and STZ-induced diabetic rats.



1 : $R_1=R_2=R_3=R_4=H$
2 : $R_1=R_2=R_3=H$, $R_4=OCH_3$
3 : $R_1=R_2=H$, $R_3=OH$, $R_4=OCH_3$
3a : $R_1=H$, $R_2=Ac$, $R_3=OAc$, $R_4=OCH_3$
3b : $R_1=R_2=Ac$, $R_3=OAc$, $R_4=OCH_3$

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 ^{13}C-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–^{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 (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 Wako gel C-200 (Wako Pure Chemical Co., 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, STZ (Sigma), heparin (Wako, Japan), tolbutamide (Chugai, Japan), and buformine (Kodama, Japan) were of analytical grade.

Plant Materials The rhizomes of *M. 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 $CHCl_3$ (20 l × 3) at room temperature. Evaporation of the extract (60 l) *in vacuo* at 40 °C yielded a $CHCl_3$ 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 $CHCl_3$ extract showed only three spots on the silica gel TLC using the solvent system, EtOAc–hexane (2 : 8). The $CHCl_3$ 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 $CHCl_3$. 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-d_6) δ: 12.40 (1H, s, C_5-OH), 9.70 (1H, s, C_7-OH), 7.63 (2H, br d, $J=7.5$ Hz, $C_{2'}$-H, $C_{6'}$-H), 7.48 (2H, br t, $J=7.5$ Hz, $C_{3'}$-H, $C_{5'}$-H), 7.45 (1H, br t, $J=7.5$ Hz, $C_{4'}$-H), 5.39 (1H, dd, $J=13.0$, 3.3 Hz, C_2-H), 3.03 (1H, dd, $J=17.5$, 13.0 Hz, C_3-H_α), 2.82 (1H, dd, $J=17.5$, 3.3 Hz, C_3-H_β), 2.05 (6H, br s, C_6-CH_3, C_8-CH_3). ^{13}C-NMR (DMSO-d_6): Table I.

Matteucinol (2) ^1H-NMR (DMSO-d_6) δ: 12.28 (1H, s, C_5-OH), 9.62 (1H, s, C_7-OH), 7.38 (2H, d, $J=8.8$ Hz, $C_{2'}$-H, $C_{6'}$-H), 6.94 (2H, d, $J=8.8$ Hz, $C_{3'}$-H, $C_{5'}$-H), 5.34 (1H, dd, $J=12.7$, 3.0 Hz, C_2-H), 3.84 (3H, s, $C_{4'}$-OCH_3), 3.05 (1H, dd, $J=17.0$, 12.7 Hz, C_3-H_α), 2.80 (1H, dd, $J=17.0$, 3.0 Hz, C_3-H_β), 2.07 (3H, s, C_8-CH_3), 2.05 (3H, s, C_6-CH_3). ^{13}C-NMR (DMSO-d_6): Table I.

2′-Hydroxymatteucinol (3) Light yellow crystals, mp 244—248 °C (MeOH); $[\alpha]_D$ $-163.1°$ ($c=0.2$, MeOH). UV $\lambda_{max}^{Me_2CO}$ nm (log ε): 340 (3.52), 291 (4.21), 222 (4.32). IR ν_{max}^{KBr} cm^{-1}: 3300 (phenolic), 2920, 1640 (CO), 1600 (br, aromatic). EIMS (m/z): 330 (M^+), 312, 284, 180, 152. High-resolution MS m/z: Found 330.3065, Calcd for $C_{18}H_{18}O_6$ (M^+) 330.3062. ^{1}H-NMR (DMSO-d_6) δ: 12.35 (1H, s, C_5-OH), 9.65 (1H, br s, C_7-OH), 9.30 (1H, s, $C_{2'}$-OH), 7.05 (1H, d, $J=2.5$ Hz, $C_{3'}$-H), 6.75—6.85 (2H, m, $C_{5'}$-H, $C_{6'}$-H), 5.60 (1H, dd, $J=13.0$, 3.0 Hz, C_2-H), 3.80 (3H, s, $C_{4'}$-OCH_3), 3.10 (1H, dd, $J=17.0$, 13.0 Hz, C_3-H_α), 2.75 (1H, dd, $J=17.0$, 3.0 Hz, C_3-H_β), 2.05 (3H, s, C_8-CH_3), 2.03 (3H, s, C_6-CH_3). ^{13}C-NMR (DMSO-d_6): Table I.

Acetylation of 2′-Hydroxymatteucinol (3) For acetylation, after treating **3** (208 mg) with a mixture of pyridine (4 ml) and acetic anhydride (4 ml) for 6 h at room temperature, the major product recovered was a diacetate derivative **3a** (109 mg), with triacetate derivative **3b** (15 mg) as a minor product. In this experiment, the reaction mixture was extracted with $CHCl_3$ after being treated with water and purified by preparative TLC.

7,2′-*O*-Diacetyl-2′-hydroxymatteucinol (3a) ^{1}H-NMR ($CDCl_3$) δ: 12.33 (1H, s, C_5-OH), 7.17 (1H, d, $J=2.5$ Hz, $C_{3'}$-H), 7.05 (1H, d, $J=8.0$ Hz, $C_{6'}$-H), 6.90 (1H, dd, $J=8.0$, 2.5 Hz, $C_{5'}$-H), 5.45 (1H, dd, $J=13.0$, 3.0 Hz, C_2-H), 3.81 (3H, s, $C_{4'}$-OCH_3), 3.02 (1H, dd, $J=17.0$, 13.0 Hz, C_3-H_α), 2.80 (1H, dd, $J=17.0$, 3.0 Hz, C_3-H_β), 2.35, 2.25 (each 3H, s, C_7-, $C_{2'}$-$OCOCH_3$), 1.98 (3H, s, C_8-CH_3), 1.90 (3H, s, C_6-CH_3). ^{13}C-NMR ($CDCl_3$): Table I.

5,7,2′-*O*-Triacetyl-2′-hydroxymatteucinol (3b) ^{1}H-NMR ($CDCl_3$) δ: 7.15 (1H, d, $J=2.5$ Hz, $C_{3'}$-H), 7.05 (1H, d, $J=8.0$ Hz, $C_{6'}$-H), 6.90 (1H, dd, $J=8.0$, 2.5 Hz, $C_{5'}$-H), 5.50 (1H, dd, $J=13.0$, 3.0 Hz, C_2-H), 3.80 (3H, s, $C_{4'}$-OCH_3), 2.93 (1H, dd, $J=17.0$, 13.0 Hz, C_3-H_α), 2.68 (1H, br d, $J=17.0$ Hz, C_3-H_β), 2.42 (3H, s, C_5-$OCOCH_3$), 2.32, 2.25 (each 3H, s, C_7, $C_{2'}$-$OCOCH_3$), 2.05 (3H, s, C_8-CH_3), 1.94 (3H, s, C_6-CH_3).

Animals and Treatment Male Sprague-Dawley rats, 5 weeks old, weighing 120—140 g, and male ddY mice, 5 weeks old, weighing 25—30 g were purchased from the Shizuoka Laboratory Animal Center (Japan) and maintained under a 12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were fed with CE 2 (Clea, Japan) and given water *at libitum*. Diabetes was introduced into the 16 h fasted rat by a single intravenous injection of 50 mg/kg STZ in a citrate buffer (pH 4.5) according to the protocol of Like and Rossini (1976).[2] The blood glucose level was checked on the third and fourth days after injection with STZ. Those animals with a high blood glucose level (more than 300 mg/dl and less than 550 mg/dl) were divided into groups. Drugs or physiological saline were administered to each group orally or intraperitoneally. 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 Reflotron kit.

Table I. ^{13}C-NMR (100 MHz) Spectral Data for Demethoxymatteucinol (**1**), Matteucinol (**2**), 2′-Hydroxymatteucinol (**3**) and 7,2′-*O*-Diacetyl-2′-hydroxymatteucinol (**3a**)

Carbon	**1**[a]	**2**[a]	**3**[a]	**3a**[b]
2	77.96 (d)	77.70 (d)	73.74 (d)	74.42 (d)
3	42.26 (t)	42.05 (t)	41.30 (t)	42.79 (t)
4	196.33 (s)	196.50 (s)	196.77 (s)	196.46 (s)
4a	101.80 (s)	101.75 (s)	101.58 (s)	105.99 (s)
5	158.57 (s)	158.52 (s)	158.41 (s)	157.66 (s)
6	103.53 (s)	103.37 (s)	103.32 (s)	111.43 (s)
7	162.58 (s)	162.48 (s)	162.36 (s)	158.72 (s)
8	102.72 (s)	102.56 (s)	102.56 (s)	109.42 (s)
8a	157.22 (s)	157.28 (s)	157.49 (s)	157.15 (s)
1′	139.23 (s)	131.05 (s)	126.02 (s)	131.43 (s)
2′	126.23 (d)	127.75 (d)	147.69 (s)	140.87 (s)
3′	128.62 (d)	113.83 (d)	112.09 (d)	112.42 (d)
4′	128.34 (d)	159.28 (s)	152.24 (s)	155.75 (s)
5′	128.62 (d)	113.83 (d)	113.99 (d)	114.52 (d)
6′	126.23 (d)	127.75 (d)	116.05 (d)	123.81 (d)
6-CH_3	8.30 (q)	8.19 (q)	8.19 (q)	8.60 (q)
8-CH_3	7.65 (q)	7.54 (q)	7.54 (q)	8.09 (q)
4′-OCH_3	—	55.05 (q)	55.32 (q)	55.63 (q)
$CO\underline{C}H_3 \times 2$	—	—	—	20.35 (q)
	—	—	—	20.35 (q)
$\underline{C}OCH_3 \times 2$	—	—	—	169.47 (s)
	—	—	—	167.89 (s)

Chemical shifts in δ ppm, measured in *a*) DMSO-d_6 *b*) $CDCl_3$ where the multiplicities of carbon signals were determined by means of the DEPT method, and indicated as s, d, t, and q. For all these compounds ^{1}H–^{13}C COSY, ^{1}H–^{13}C long range COSY spectra were measured.

Statistical Analysis All values expressed as the mean ± S.E. were obtained from a number of experiments (n). The Student's t-test for unpaired observation 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 $CHCl_3$ 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 $CHCl_3$ 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 $CHCl_3$ 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 $CHCl_3$ extract showed three spots in the chromatogram, indicating that only three compounds comprise the main constituents in the $CHCl_3$ 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 drivatives. Two of them, **1** and **2**, have already been isolated

Table II. Effect of Extracts of *M. orientalis* (5 Doses Each, Twice a Day, i.p.) on Blood Glucose Level in STZ-Induced Diabetic Rats

Group	Dose (mg/kg)	(n)	Blood glucose level in mg/dl		Decrease (%)[b] after i.p.
			before i.p.[a]	after i.p.	
Control 1	—	6	355.5 ± 20.1 (100.0 ± 5.7)	342.0 ± 18.4 (96.2 ± 5.2)	—
Positive control	—	6	429.2 ± 42.4 (100.0 ± 9.9)	294.0 ± 22.4[c] (68.5 ± 5.2)	28.79
Control 2	—	6	460.8 ± 30.7 (100.0 ± 6.7)	472.3 ± 13.5 (102.5 ± 2.9)	—
$CHCl_3$ ext.	200	6	455.2 ± 26.8 (100.0 ± 5.9)	283.7 ± 21.9[d] (62.3 ± 4.8)	39.21
Control 3	—	5	393.4 ± 41.3 (100.0 ± 6.7)	390.8 ± 29.9 (102.5 ± 2.9)	—
MeOH ext.	200	5	392.8 ± 32.3 (100.0 ± 5.9)	396.4 ± 20.2 (62.3 ± 4.8)	−1.61
Water ext.	200	5	393.8 ± 31.4 (100.0 ± 8.0)	377.0 ± 40.4 (95.7 ± 10.3)	3.63

Positive control group (5 doses administration of mixture of 200 mg/kg of tolbutamide and 2 mg/kg of buformine, twice a day, i.p.). *a*) Glucose level before administering the drugs or saline. *b*) Decrease in blood glucose level relative to the level before i.p. administration expressed in % in comparison with the control. Results are due to mean ± S.E. Significantly different from control value, *c*) $p<0.02$, *d*) $p<0.001$.

from this plant,[3)] 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,[4—6)] 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.

Demethoxymatteucinol (1) Pale yellow needles, mp 211 °C ($CHCl_3$) [lit. 200—202 °C (MeOH)][7)] showed a molecular ion peak at m/z 284 and its high-resolution MS suggested the molecular formula to be $C_{17}H_{16}O_4$. 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.[8)] 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 $CDCl_3$, 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 ^{13}C-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 sp^3 carbon at δ_C 77.96 (d), sp^3 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 ^{13}C-NMR spectrum of **1** was compared with the spectrum of pinocembrin,[9)] there was a very close similarity. Regarding all this information and comparison with data from previous literature,[10,11)] compound **1** was found to be the demethoxymatteucinol reported by Murakami *et al.*[12)] from *Wagneriopteris japonica*. However, some carbon assignments in our experiment compared to those of Murakami *et al.*[11)] for the C_{4a}, C_5, C_6, C_7, C_8, C_{8a}, 6-CH_3 and 8-CH_3 were found to be different for this compound. The ^1H–^{13}C 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

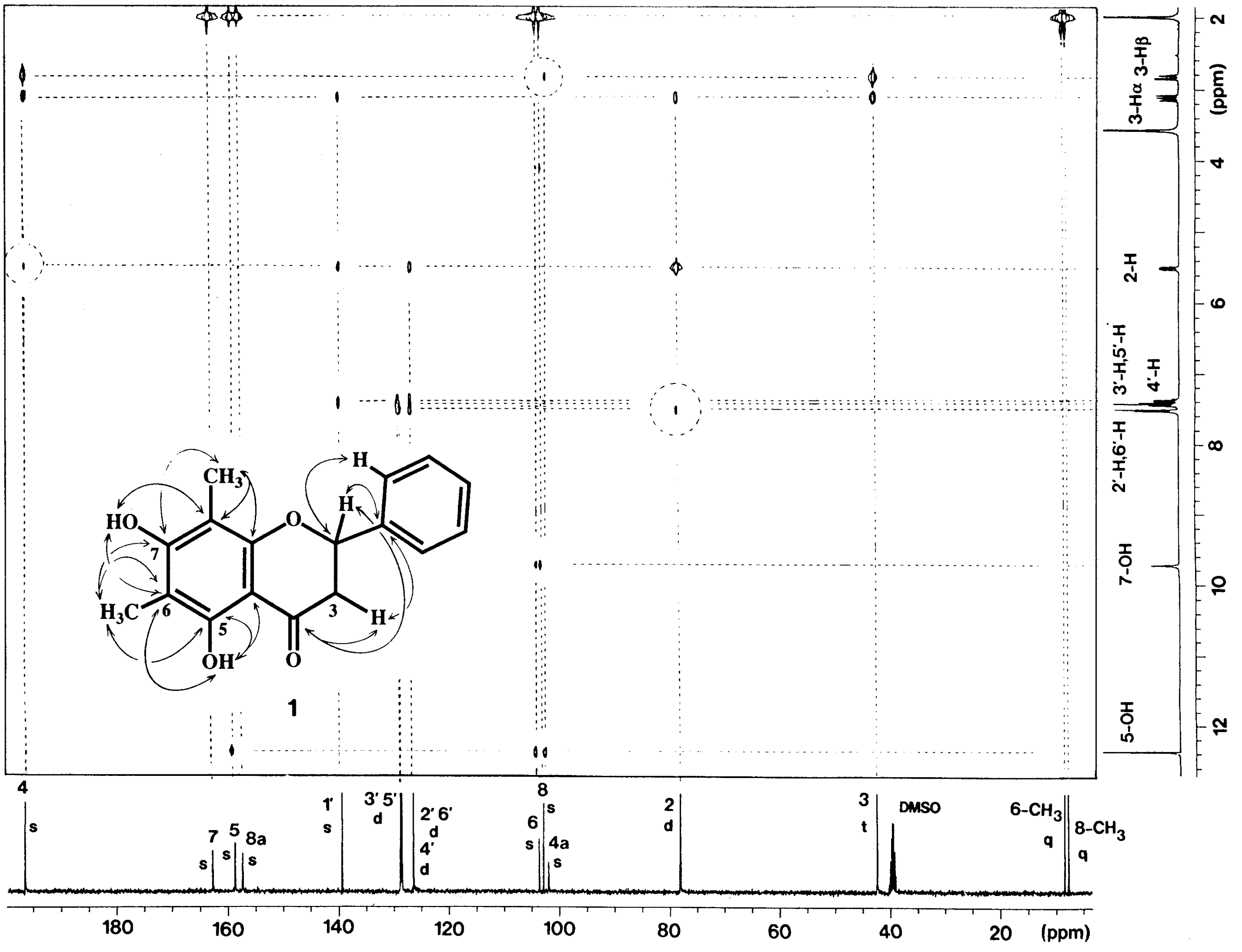
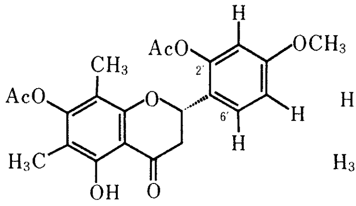


Fig. 1. ^1H–^{13}C Long-Range Shift Correlation Spectrum of Demethoxymatteucinol (1) in DMSO-d_6 ($J_{CH}=10.0$ Hz)

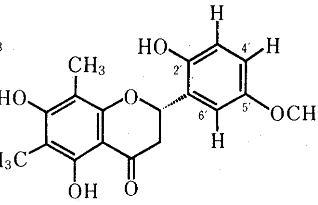
12.40 (C_5-OH) with three carbons at δ_C 158.57, 103.53 and 101.80, which were assigned to C_5, C_6 and C_{4a}, respectively. These assignments were also in support, due to the correlation between the hydroxyl proton at δ_H 9.70 (C_7-OH) with two carbons at δ_C 103.53 and 102.72, which can be assined to carbons at the C_6 and C_8 positions, respectively (Fig. 1). The ^{1}H-NMR signals of the two methyl groups at the C_6 and C_8 positions were very close, but the carbon signals were very different, and by comparing the correlation between the C_6 and C_8 carbons (assigned with the help of hydroxyl protons) and the two methyl groups, the methyl group at the slighly lower field was assigned as C_8-CH_3 and another was C_6-CH_3. With the help of ^{1}H-^{13}C COSY and ^{1}H–^{13}C long-range COSY, the methyl carbon signals in the high field were assigned as δ_C 8.30 (q) and 7.65 (q) for C_8-CH_3 and C_6-CH_3, respectively. The other main ^{1}H–^{13}C long-range correlations are shown by the arrows (Fig. 1). Compound **1** was found to be levorotatory, ($[\alpha]_D$ $-46.0°$) so that the configuration at the C_2 position was assigned as (*S*) by comparison with the literature.[8] The result of this spectral information obviously suggests that the assignment by Murakami *et al.*[12] for the carbons discussed above must be revised.

Matteucinol (2) Light yellow needles, mp 182 °C ($CHCl_3$), showed M^+ at *m*/*z* 314 and was found to be optically active $[\alpha]_D$ $-20.5°$. The molecular formula was calculated as $C_{18}H_{18}O_5$ according to high-resolution MS. Compound **2** gave a pattern of UV and IR absorption signals similar to that of **1**. The ^{1}H- and ^{13}C-NMR signals were also almost similar to that of **1**, but some differences were noticed due to one additional signal at δ_H 3.84 of the methoxyl protons, and to the signals of the ring B protons in the ^{1}H-NMR spectrum. Four aromatic protons were observed in the ^{1}H-NMR spectrum at δ_H 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 ^{1}H-NMR and ^{13}C-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 $C_{4'}$-position. The complete assignment of the carbon signals were determined using distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{13}C COSY and ^{1}H–^{13}C 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′-Hydroxymatteucinol (3) Pale yellow crystals, mp 244—248 °C (MeOH), showed M^+ at *m*/*z* 330 and was found to be optically active $[\alpha]_D$ -163.1 °C. The molecular formula was calculated as $C_{18}H_{18}O_6$ 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 ^{1}H-NMR spectrum showed signals due to one proton at δ_H 7.05 (1H, d, $J=2.5$ Hz) and two protons at δ_H 6.75—6.85 (2H, m). The ^{1}H–^{1}H 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 δ_H 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 ^{13}C-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 ^{1}H- and ^{13}C-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



3a



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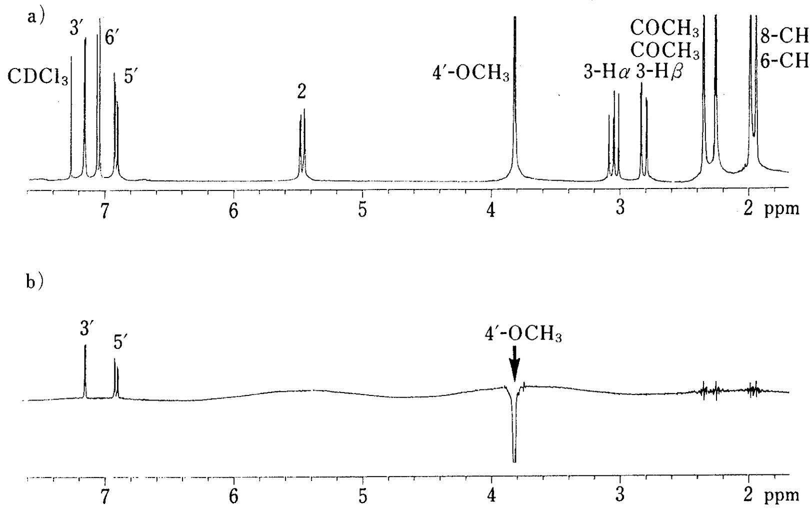
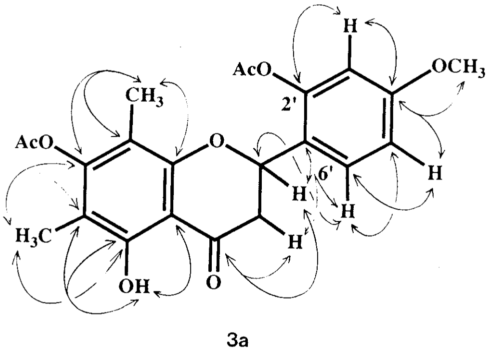


Fig. 2. ^{1}H-NMR (Normal and NOE) Spectra of 7,2′-*O*-Diacetyl-2′-hydroxy-matteucinol (**3a**)

a) Normal spectrum, b) NOE different spectrum on irradiation at δ_H 3.81.

derivative (**3b**), but a diacetate derivative (**3a**) was found to be the major product on acetylation. Both of the acetate derivatives (**3a**, **3b**) of **3** gave ^{1}H-NMR signals of one *meta*-coupling proton at δ_H 7.17 (1H, d, $J=2.5$ Hz), [this signal was δ_H 7.15 (1H, d, $J=2.5$ Hz) for **3b**] one *ortho*-coupling proton at δ_H 7.05 (1H, d, $J=8$ Hz) and one *ortho*- and *meta*-coupling proton at δ_H 6.90 (1H, dd, $J=8.0$, 2.5 Hz). All this information suggests that ring B of **3** was occupied by a set of three protons at the 1, 3 and 4 positions, and two substituents were hydroxyl and methoxyl groups. The correct position of the hydroxyl and methoxyl groups was assigned according to the nuclear Overhauser effect (NOE) and ^{1}H–^{13}C long-range COSY experiments of **3a**. On irradiation of the signal of the methoxyl group of **3a** at δ_H 3.81, the NOE effect was observed on the *meta*-coupling proton at δ_H 7.17 and on the *ortho*- and *meta*-coupling protons at δ_H 6.90 (Fig. 2) which clearly suggested that a methoxy group lies in between these protons. It was not possible to assign the correct positions of these substituents using only the NOE experiment. Next, we measured the ^{1}H–^{13}C long-range COSY spectrum of **3a** where the *meta*-coupling proton showed a correlation with two carbons at δ_C 155.75 and 140.87 which were assigned for $C_{4'}$ and $C_{2'}$, respectively. In addition, the *ortho*-coupling proton gave a correlation with the carbons at δ_C 131.43 which were assigned for $C_{1'}$, and several other important ^{1}H–^{13}C long-range correlations are shown by arrows in Fig. 3. All these observations clearly suggest that compound **3** is 2′-hydroxymatteucinol. The complete assignment of all the carbons is shown in Table I. The compound methoxymatteucin (**4**) isolated from this plant by Mohri *et al.*[2] showed a close similarity to **3**. Based on all this information, 2′-hydroxymatteucinol (**3**) was found to be a new compound.

The hypoglycemic activity of the $CHCl_3$ extract and its three main consitituents was tested in normal mice, each with a single intraperitonial dose of 100 mg/kg. In this experiment, blood samples were collected 6 and 24 h after the drug administration. All these compounds, including the $CHCl_3$ extract, showed significant hypoglycemic activity 6 h after the drug administration and a significant effect remained up to 24 h after the drug administration. This result suggests that compounds **1**, **2** and **3** were significantly effective in lowering the blood glucose level. Chemical analysis showed that more than 90% of the constituents in the $CHCl_3$ extract were the *C*-methyl 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 $CHCl_3$ extract of *M. orientalis*, 2′-hydroxymatteucinol (**3**) was found to be the most effective in lowering the blood glucose level (Fig. 4).



3a

Fig. 3. Significant Long-Range Correlations Observed in the ^{1}H–^{13}C Long-Range COSY Spectrum of 7,2′-*O*-Diacetyl-2′-hydroxymatteucinol (**3a**) in $CDCl_3$

The hypoglycemic activity of 2′-hydroxymatteucinol (**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′-hydroxymatteucinol (**3**), twice a day, were received by the STZ-induced diabetic rats. Blood samples were collected in each case 6 h after the last dose of drug administration. It was observed that the blood glucose lowering effect of 2′-hydroxymatteucinol (**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-

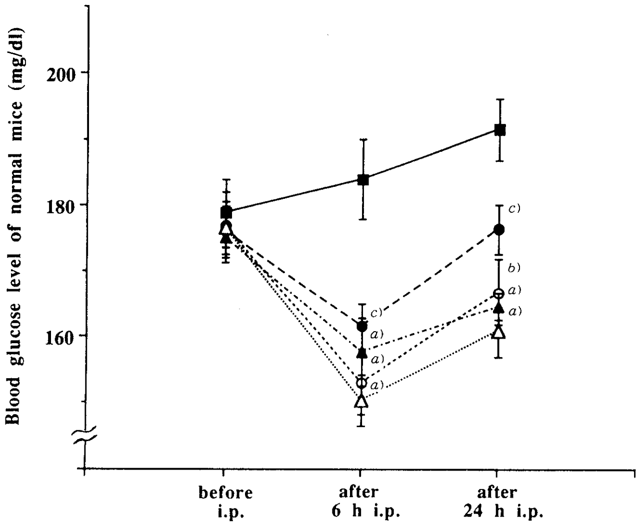


Fig. 4. Effect of $CHCl_3$ Extract, Demethoxymatteucinol (**1**), Matteucinol (**2**), and 2′-Hydroxymatteucinol (**3**) from *Matteccia orientalis* on Blood Glucose Level of Normal Mice

—■—, control; ---○---, $CHCl_3$ extract; –·–▲–·–, demethoxymatteucinol (**1**); ––●––, matteucinol (**2**); ---△---, 2′-hydroxymatteucinol (**3**). Results are due to mean ± S.E., $n=6$, each group was treated with 100 mg/kg of drugs i.p. Significantly different from the control group with *a*) $p<0.001$, *b*) $p<0.01$, *c*) $p<0.05$.

Table III. Effect of Various Doses of 2′-Hydroxymatteucinol (**3**) (5 Doses Each, Twice a Day, i.p.) on Blood Glucose Level in STZ-Induced Diabetic Rats

Group	Dose (mg/kg)	(n)	Blood glucose level in mg/dl		Decrease (%)[b] after i.p.
			before i.p.[a]	after i.p.	
Control	—	6	380.5 ± 24.4 (100.0 ± 6.4)	376.0 ± 21.6 (98.8 ± 5.7)	—
Tolbutamide	100	6	381.8 ± 28.0 (100.0 ± 7.3)	260.5 ± 56.2[c] (68.2 ± 19.9)	30.73
3	100	6	363.6 ± 33.8 (100.0 ± 9.3)	222.4 ± 45.4[d] (61.2 ± 12.5)	38.17
3	50	6	356.3 ± 33.1 (100.0 ± 9.3)	251.3 ± 20.2[d] (70.5 ± 5.7)	28.73

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′-Hydroxymatteucinol (**3**) (Five Doses Each, *p.o.*, Twice a Day) on Blood Glucose Level in STZ-Induced Diabetic Rats

Group	Dose (mg/kg)	Glucose level (mg/dl)		Decrease (%)$^{b)}$ after *p.o.*
		before *p.o.*$^{a)}$	after *p.o.*	
Control 1	—	454.4±14.9	451.9±10.2	—
		(100.0±3.3)	(99.4±2.2)	
3	100	448.4±21.2	323.3±20.5$^{c)}$	27.46
		(100.0±4.7)	(72.1±4.6)	
3	50	447.4±18.6	378.6± 8.9$^{d)}$	14.80
		(100.0±4.2)	(84.6±2.0)	
Control 2	—	419.7±22.0	383.8±22.4	—
		(100.0±5.2)	(91.4±5.3)	
3	25	419.2±23.5	299.3±25.6$^{e)}$	21.99
		(100.0±5.6)	(71.3±6.1)	
3	10	415.5±25.7	316.0±30.4	16.85
		(100.0±6.2)	(76.0±7.3)	
3	5	422.8±32.9	379.0±20.5	1.97
		(100.0±7.8)	(89.6±4.8)	

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′-hydroxymatteucinol was observed. When various doses ranging from 100 to 5 mg/kg of 2′-hydroxymatteucinol (**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′-hydroxymatteucinol (**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′-hydroxymatteucinol (**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 $CHCl_3$ extract of *M. orientalis* for lowering the blood glucose level is a new C-methyl flavanone derivative, 2′-hydroxymatteucinol (**3**). The blood glucose level lowering effect of 2′-hydroxymatteucinol (**3**) is much more effective, and the effect long-lasting as compared with tolbutamide as the positive control. It has also become clear from the present investigation that 2′-hydroxymatteucinol (**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′-hydroxymatteucinol (**3**) exhibits strong hypolipemic and choleretic activity,[13] 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,[14] and it is well known that a high level of insulin sharply reduced the cAMP level.[15] Based on these information sequences, 2′-hydroxymatteucinol (**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′-hydroxymatteucinol (**3**), are now in progress in our laboratory.

The results of the present investigation showed that 2′-hydroxymatteucinol (**3**), isolated from the $CHCl_3$ 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′-hydroxymatteucinol (**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 commom use as Kuan-chung in the Japanese market. See M. Hutoh, *Shoyakugaku Zasshi*, **15**, 167 (1961).
2) A. A. Like, A. A. Rossini, *Science*, **193**, 415 (1976).
3) K. Mohri, T. Takemoto, Y. Kondo, *Yakugaku Zasshi*, **102**, 310 (1982).
4) H. R. Arthur, *J. Chem. Soc.*, **1955**, 3740.
5) D. H. Miles, J. Melderos, L. Chen, V. Chittawang, C. Swithenbank, Z. Lidert, A. M. Payne, P. A. Hedin, *Chem. Abstr.*, **114**, 201745*t* (1991).
6) H. R. Arthur, S. W. Tam, *J. Chem. Soc.*, **1960**, 3197.
7) P. Richomme, S. Sinbandhit, B. David, A. Hamid, A. Hadi, J. Bruneton, *J. Nat. Prod.*, **53**, 294 (1990).
8) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
9) J. B. Harborne, T. J. Mabry, "The Flavonoides, Advances in Research," Chapman and Hall, 1982, p. 99.
10) M. Iinuma, S. Matsuura, K. Kusuda, *Chem. Pharm. Bull.*, **28**, 708 (1980).
11) H. Wagner, V. M. Chari, J. Sonnenbichler, *Tetrahedron Lett.*, **1976**, 1799.
12) H. Wada, N. Tanaka, T. Murakami, T. Uchida, K. Kozawa, Y. Saiki, C. M. Chen, *Yakugaku Zasshi*, **108**, 740 (1988).
13) E. Bonbardelli, B. Gabetta, M. J. Magistretti, *Chem. Abstr.*, **102**, 131808*s* (1985).
14) A. Beretz, R. Anton, J. C. Stoclet, *Experientia*, **34**, 1054 (1978).
15) R. K. Murray, D. K. Granner, P. A. Mayes, V. W. Rodwell, "Harper's Biochemistry," 22nd ed. Prentice Hall International, London, 1990, p. 536.