# Hyaluronidase Inhibitors from Takuran, *Lycopus lucidus*

Toshihiro Murata,*[,a] Mai Watahiki,[a] Yu Tanaka,[b] Toshio Miyase,[b] and Fumihiko Yoshizaki[a]

[a] *Department of Pharmacognosy, Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan: and* [b] *School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.* Received November 22, 2009; accepted December 25, 2009; published online January 5, 2010

**Takuran is a traditional herbal medicine that is produced from the herbal plant *Lycopus lucidas* Turcz. (Lamiaceae). Takuran is used as a treatment for diseases in women. From Takuran, four new phenylpropanoids along with 18 known compounds were isolated, and their structures were elucidated by spectroscopic analyses. Five phenylpropanoids isolated from the plant showed hyaluronidase inhibitory activity comparable to that of rosmarinic acid.**

**Key words** phenylpropanoid; hyaluronidase inhibitor; anti-allergic; *Lycopus lucidas*; Lamiaceae

Takuran is used in traditional Japanese, Korean, and Chinese medicine. It is produced from *Lycopus lucidus* Turcz. (Lamiaceae), an herbaceous perennial that grows on lakesides and riversides of East Asia and North America. Previous studies on the constituents of *L. lucidus* have resulted in the isolation of caffeic acid derivatives,[1)] flavonoids,[2,3)] and terpenoids.[4)] Takuran is used for the treatment of menstrual disorder, menstrual cramps, and cardiovascular diseases.[5)] Recently, several biological activities of *L. lucidus* were reported such as anti-allergic effects,[6)] cholesterol acyltransferase inhibitory activity,[4)] and inhibitory effects on high glucose-induced vascular inflammation.[7)] Hyaluronidase is an enzyme that degrades hyaluronic acid. Hyaluronidase inhibition is known to be related to various conditions and is expected to be useful for treating certain diseases,[8)] including allergies. In previous studies, it was found that there are correlations between allergic reactions and hyaluronidase inhibitory activity.[9,10)] Rosmarinic acid, a phenylpropanoid, was reported to have various biological activities[11)] including as a hyaluronidase inhibitor.[12)] It is also known that many derivatives of rosmarinic acid; dimmers, trimers, and tetramers of caffeic acid; as well as oligomers of phenylpropanoids are found in Lamiaceae and other plants.

In this paper, 4 new phenylpropanoids and 18 known compounds were isolated from Takuran, and six phenylpropanoids were tested for their hyaluronidase inhibitory activities. Flakes of Takuran, produced from dried aerial parts of *L. lucidus*, were extracted with acetone–water (8 : 2). After the concentrated extract had been partitioned between water and diethyl ether, the water layer was fractionated by column chromatography (using a porous polymer gel column and reverse-phase columns) to afford four new phenylpropanoids (**3**, **5**, **7**, **9**), six known phenylpropanoids (**1**, **2**, **4**, **6**, **8**, **10**), and 12 known compounds. Rosmarinic acid (**1**), clinopodic acid C (**2**), and clinopodic acid E (**4**) were isolated from *Clinopodium chinense* var. *parviflorum* in our previous study.[13)] The structures of the other known compounds were identified by comparing their spectroscopic data with those in the literature as schizotenuin A (**6**),[14)] 3-*O*-(caffeoyl)-rosmarinic acid (**8**),[15)] rosmarinic acid ethyl ester (**10**),[16)] apigenin (**11**),[17)] acacetin (**12**),[17)] acacetin-7-*O*-$\beta$-D-glucuronopyranoside (**13**),[18)] luteolin (**14**),[17)] scutellarin (**15**),[19)] hispidulin (**16**),[20)] pectolinarigenin-7-*O*-$\beta$-D-glucopyranoside (**17**),[21)] pectolinarigenin-7-*O*-$\beta$-D-glucuronopyranoside (**18**),[22)] pectolinarigenin-7-*O*-$\beta$-D-glucuronopyranoside methyl ester (**19**),[22)] verbascoside (**20**),[23)] 3-*O*-[$\beta$-D-xylopyranosyl-(1→6)-$\beta$-D-glucopyranosyl]-(3*R*)-1-octen-3-ol (**21**),[24)] and (−)-syringaresinol (**22**).[25)]

The $^1$H- and $^{13}$C-NMR spectra of lycopic acid A (**3**) and B (**5**) were similar to those of **2** and **4**, respectively, which each had an additional 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid moiety. For **3**, the molecular formula $C_{36}H_{30}O_{16}$ was confirmed on the basis of HR-FAB-MS (*m*/*z* 719.1606, Calcd for $C_{36}H_{31}O_{16}$, 719.1611). In its nuclear Overhauser effect (NOE) spectra (Fig. 2), methylene protons at $\delta$ 2.75 (1H, d, $J$=6.0, H-7‴) and 2.77 (1H, d, $J$=6.0 Hz, H-7‴) were found to be correlated with the aromatic protons at $\delta$ 6.67 (1H, d, $J$=2 Hz, H-2‴) and 6.43 (1H, dd, $J$=8.5, 2.0 Hz, H-6‴). These protons and their corresponding carbons showed the presence of a 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid moiety. The H-8‴ proton was correlated with the carbonyl carbon at $\delta$ 166.8 (C-9″) in the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 2). The absolute configurations of C-8′ and C-8‴ were determined to be *R* from the retention time of the amide derivative of 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid, which was obtained by acidic hydrolysis of **3**, in (*S*)-2-phenylglycine methyl ester.[13)] The absolute stereochemistry of C-7″ was found to be *S* from the circular dichroism (CD) spectrum, which showed a positive Cotton effect at 220—260 nm.[26)] The coupling constant between H-7″ and H-8″ ($J$=3.0 Hz) indicated a *cis*-orientation.[27,28)] These data suggested that the structure of **3** was as shown in Fig. 1.

For **5**, the molecular formula $C_{36}H_{30}O_{16}$ was confirmed on the basis of HR-FAB-MS (*m*/*z* 719.1595, Calcd for $C_{36}H_{31}O_{16}$, 719.1611). In its NOE spectra, methylene protons at $\delta$ 2.89 (2H, d, $J$=6.0 Hz, H-7‴) and an oxygenated methine proton at $\delta$ 5.11 (1H, t, $J$=6.0 Hz, H-8‴) were found to be correlated with the aromatic protons at $\delta$ 6.75 (1H, d, $J$=2.0 Hz, H-2‴) and 6.55 (1H, dd, $J$=8.5, 2.0 Hz, H-6‴). These protons and their corresponding carbons showed the presence of a 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid moiety. The H-8‴ proton was determined to be correlated with the carbonyl carbon at $\delta$ 167.5 (C-9″) in the HMBC spectrum. The absolute stereochemistry of C-7″ was indicated to be *R* from the CD spectrum, which showed a negative Cotton effect at 230—260 nm.[26)] The absolute stereochemistry of C-7″ and C-8″ were both *R* as indicated by the

* To whom correspondence should be addressed. e-mail: murata-t@tohoku-pharm.ac.jp

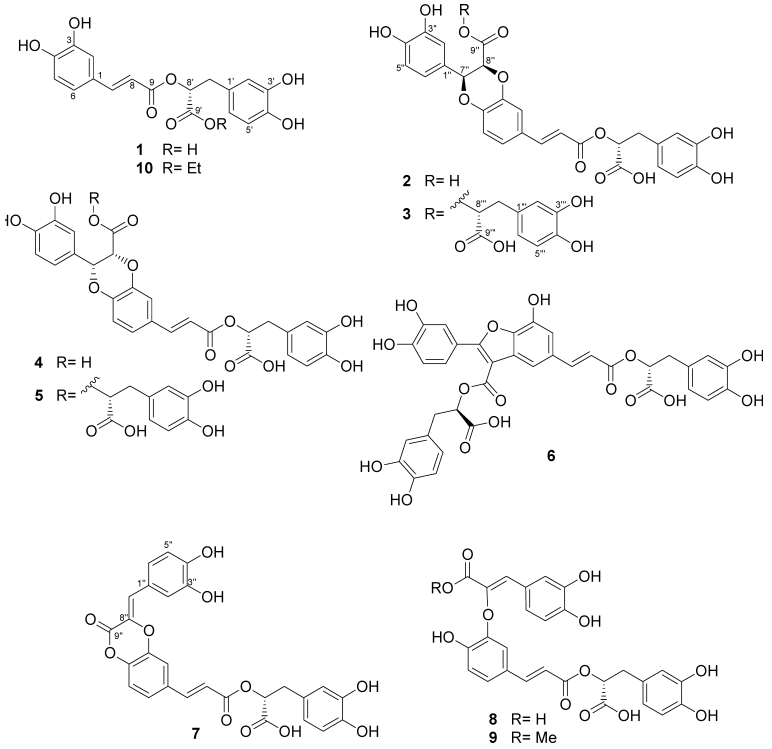


Fig. 1. Structures of Phenylpropanoids (Compounds **1**—**10**)

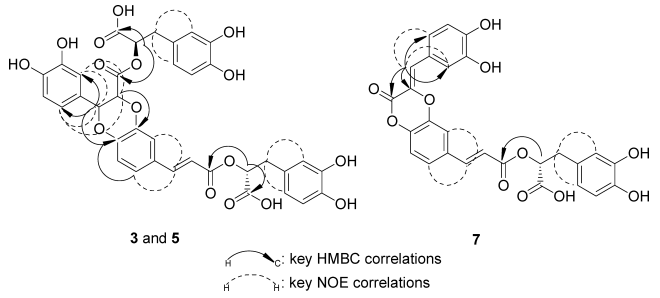


Fig. 2. Key HMBC and NOE Correlations for Compounds **3**, **5**, and **7**

finding that the coupling constant between H-7″ and H-8″ in the proton NMR spectrum ($J$=5.0 Hz) was identical to that of clinopodic acid E.[13] Two 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid moieties were determined to be *R* from the retention time of the amide derivative as in the case of **3**.[13]

Lycopic acid C (**7**) showed a pseudomolecular ion peak at *m*/*z* 519.0927 (Calcd for $C_{27}H_{19}O_{11}$, 519.0927) on the HR-FAB-MS spectrum. The $^1$H- and $^{13}$C-NMR spectra suggested the presence of three phenylpropanoid moieties. A similar compound, melitric acid B, was isolated from *Melissa officinalis* L. and had three phenylpropanoid moieties and the same molecular weight.[29] However, the $^1$H- and $^{13}$C-NMR spectra of **7** did not match those of melitric acid B. For **7**, there were three sets of ABX protons. The two ABX proton sets at $\delta$ 7.58 (1H, d, $J$=2.0 Hz, H-2), 7.15 (1H, d, $J$=8.0 Hz, H-5), 7.35 (1H, dd, $J$=8.0, 2.0 Hz, H-6), 7.76 (1H, d, $J$=2.0 Hz, H-2′), 6.70 (1H, d, $J$=8.0 Hz, H-5′), and 6.63 (1H, dd, $J$=8.0, 2.0 Hz, H-6′); two *trans*-olefinic protons at $\delta$ 7.66 (1H, d, $J$=16.0 Hz, H-7) and 6.55 (1H, d, $J$=16.0 Hz, H-8); and 14 aromatic, two carbonyl, and two aliphatic carbons

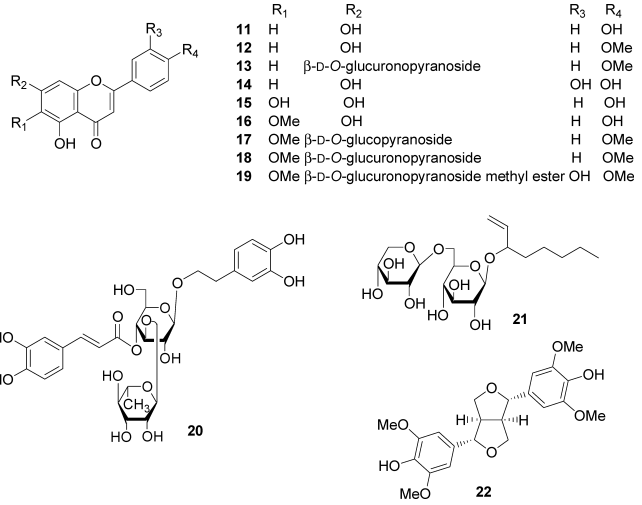


Fig. 3. Structures of Known Compounds (**11**—**22**)

indicated the presence of rosmarinic acid moiety. The C-8′ configuration was established as *R* using the same method as used for **3** and **5**. A singlet olefinic proton at $\delta$ 6.98 (1H, H-7″) was found to be correlated with aromatic protons at $\delta$ 7.61 (1H, d, $J$=2.0 Hz, H-2″) and 7.19 (1H, dd, $J$=8.5, 2.0 Hz, H-6″) in the NOE spectra. In the HMBC spectrum, the H-7″ proton was indicated to be long range coupled with the carbons at $\delta$ 125.8 (C-8″) and 157.7 (C-9″). Treatment of **7** with water for three days gave 3-*O*-(caffeoyl)rosmarinic acid (**8**),[15] and treatment with MeOH for 3 d gave the compound **9**. These hydrolysis and methyl esterification products suggested that **7** has a 8-substituted caffeic acid moiety bound to the 3,4-substituted rosmarinic acid moiety as shown in Fig. 1.

Table 1. Hyaluronidase Inhibitory Activities of Phenylpropanoids (Compounds **1**—**6**) Isolated from Takuran

| Compound | $IC_{50}$ ($\mu$M) |
|---|---|
| Rosmarinic acid (**1**) | 309 |
| Clinopodic acid C (**2**) | 80.1 |
| Lycopic acid A (**3**) | 134 |
| Clinopodic acid E (**4**) | 82.8 |
| Lycopic acid B (**5**) | 141 |
| Scizotenuin A (**6**) | 241 |

Values represent the mean of 3 observations.

Compound **9** showed an $[M-H]^-$ at *m/z* 551.1183 in the HR-FAB-MS, which indicated the molecular formula $C_{28}H_{24}O_{12}$. The $^1$H- and $^{13}$C-NMR spectra of **9** were similar to those of **8**[15] except for the presence of a methyl signal at $\delta$ 3.70 (3H, s). In its HMBC spectrum, the methyl protons were found to be long range coupled with the C-9″ carbon at $\delta$ 164.3, and the aromatic proton at $\delta$ 7.31 (1H, s, H-7″) was determined to be long range coupled with the C-8″ carbon at $\delta$ 138.5 and C-9″. These data showed that **9** was 3-*O*-(caffeoyl)rosmarinic acid-9″-methylester.

Although compounds **8** and **9** were isolated from the acetone extract of Takuran, **7** changed to **8** and **9** in water and MeOH under moderate conditions. So, they may have been artifacts.

Hyaluronidase inhibitory activity was measured for compounds **1**—**6** as shown in Table 1. Rosmarinic acid was reported as a hyaluronidase inhibitor in *Melissa officinalis* L.,[12] so it was regarded to be a positive control. Compounds **2**—**5** showed higher activity ($IC_{50}$ 80.1, 134, 82.8, and 141 $\mu$M, respectively) than that of rosmarinic acid (**1**, $IC_{50}$ 309 $\mu$M). Compound **6** was showed activity ($IC_{50}$ 241 $\mu$M) comparable to that of rosmarinic acid. Aqueous extracts of *L. lucidus* inhibited mast cell-mediated immediate-type allergic reactions.[6] However, there is little available knowledge regarding the constituents of these extracts. Potent hyaluronidase inhibitors are assumed to suppress the degranulation of mast cells and possess anti-allergic activity.[9,10,12] Thus, the inhibitors (**2**—**6**) observed in this paper are expected to be anti-allergic components of *L. lucidus* and could be useful components for the care and prophylaxis of allergies and other diseases.

## Experimental

**General Procedure** Optical rotations were recorded on a Jasco P-2300 polarimeter, and CD spectra were recorded on a Jasco J-700 spectropolarimeter. $^1$H-NMR (400 MHz), $^{13}$C-NMR (100 MHz), $^1$H–$^1$H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC) (optimized for $^1J_{C-H}$=145 Hz), and HMBC (optimized for $^nJ_{C-H}$=8 Hz) spectra were recorded on a Jeol JNM-AL400 FT-NMR spectrometer, and chemical shifts were given as $\delta$ values with TMS as an internal standard. HR-FAB-MS data were obtained on a Jeol JMS700 mass spectrometer, using a *m*-nitrobenzyl alcohol or a glycerol matrix. A porous polymer gel (Mitsubishi Chemical, Diaion HP-20, 60×300 mm) and an octadecyl silica (ODS) column (Cosmosil 140 $C_{18}$-OPN, Nacalai Tesque, 150 g) were used for column chromatography. Preparative Yamazen Cartridge Column Chromatography (YCCC) was performed on a Jasco 2089 instrument (column, Ultra Pack ODS-SM-50C-M, Yamazen, 37×100 mm; detector, UV at 210 nm). Preparative HPLC was performed on a Jasco 2089 instrument and detected by UV at 210 nm (columns, Inertsil ODS-3, GL Sciences, 30×500 mm; ODS-100V, Tosoh, 20×250 mm; 5$C_{18}$-AR-II, Cosmosil, 20×250 mm; Capcell-Pak Ph, Shiseido, 20×250 mm; YMC-Pack ODS-AM, YMC, 10×300 mm; Mightysil RP-18 GP, Kanto Chemical, 10×250 mm).

**Plant Material** Takuran, produced from dried *Lycopus lucidus* Turcz., was purchased from Uchida Wakanyaku Co., Ltd. (Tokyo, Japan; the product number: 76Q3308). A voucher specimen is deposited at the herbarium of Tohoku Pharmaceutical University, No. 20080531.

**Extraction and Isolation** Takuran, aerial plant parts (480 g) of *L. lucidus*, were extracted with acetone–water (8 : 2), (14 l) twice at 60 °C for 6 h. The extract was then concentrated at reduced pressure, suspended in water (1.5 l), and extracted with ether (1.0 l) three times. The water layer (61.5 g) was dissolved in water and passed through a porous polymer gel column and eluted with water, 50% MeOH, and MeOH. The MeOH eluate (1.66 g) was separated using HPLC columns [Capcel pak Ph, mobile phase, acetonitrile–0.2% trifluoroacetic acid (TFA) in water (35 : 65); 5$C_{18}$-AR-II, mobile phase, acetonitrile–0.2% TFA in water (35 : 65), (40 : 60)] to give compounds **2** (3.8 mg), **4** (7.2 mg), **7** (2.6 mg), **9** (1.6 mg), **11** (2.2 mg), **12** (2.6 mg), **14** (8.5 mg), **15** (1.9 mg), **17** (1.9 mg), **18** (2.8 mg), and **22** (5.9 mg). The 50% MeOH eluate (16.4 g) was chromatographed on a reversed-phase column using ODS and then eluted with 30%, 40%, 50%, 80% MeOH, and MeOH (fractions 1A—1E). Fraction 1B (1.85 g) was subjected to YCCC [mobile phase, methanol–0.2% TFA (45 : 55) and (50 : 50)] to give 14 fractions (Frs. 2A—2N) and compound **1** (75.8 mg). Fractions 2C and 2D (59.2 mg) were subjected to HPLC [Capcel pak Ph, mobile phase, methanol–0.2% TFA in water (35 : 65)] to yield compound **20** (2.1 mg). Fractions 2E, 2F and 2G (141.6 mg) were subjected to HPLC [5$C_{18}$-AR-II, mobile phase, acetonitrile–0.2% TFA (25 : 75)] to yield compound **8** (1.6 mg). Fractions 2I, 2J, and 2K (392.4 mg) were subjected to HPLC [Intersil ODS-3, mobile phase, acetonitrile–0.2% TFA in water (27.5 : 72.5), and 5$C_{18}$-AR-II, mobile phase, methanol–0.2% TFA (50 : 50)] to yield compounds **4** (10.4 mg) and **21** (5.1 mg). Fraction 1C was subjected to HPLC [ODS-100V, mobile phase, methanol–0.2% TFA in water (60 : 40); Capcel pak Ph, mobile phase, acetonitrile–0.2% TFA (35 : 65); 5$C_{18}$-AR-II, mobile phase, acetonitrile–0.2% TFA (35 : 65); ODS-AM, mobile phase, acetonitrile–0.2% TFA (35 : 65)] to yield compounds **3** (31.8 mg), **5** (54.0 mg), **6** (119.7 mg), **10** (2.5 mg), **13** (19.9 mg), **16** (19.3 mg), and **19** (1.4 mg).

Lycopic Acid A (**3**): Colorless amorphous solid, $[\alpha]_D^{21}$ −6.6° (*c*=0.16, MeOH), UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 287 (4.72), 320 (4.68). CD (*c*=0.033, MeOH) nm ([$\theta$]): 227 (27900), 231 (24100), 246 (45900), 293 (−8800), 295 (−6200), 328 (−14400). HR-FAB-MS (positive): *m/z* 719.1606 $[M+H]^+$ (Calcd for $C_{36}H_{31}O_{16}$: 719.1611). $^1$H-NMR: (acetone-$d_6$, 400 MHz) $\delta$: 7.34 (1H, d, *J*=2.0 Hz, H-2), 6.99 (1H, d, *J*=8.5 Hz, H-5), 7.26 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 7.63 (1H, d, *J*=16.0 Hz, H-7), 6.47 (1H, d, *J*=16.0 Hz, H-8), 6.87 (1H, d, *J*=2.0 Hz, H-2′), 6.75 (1H, d, *J*=8.5 Hz, H-5′), 6.69 (1H, dd, *J*=8.5, 2.0 Hz, H-6′), 3.05 (1H, dd, *J*=15.0, 8.5 Hz, H-7′), 3.14 (1H, dd, *J*=15.0, 4.0 Hz, H-7′), 5.24 (1H, m, H-8′), 6.89 (1H, d, *J*=2.0 Hz, H-2″), 6.81 (1H, d, *J*=8.5 Hz, H-5″), 6.43 (1H, dd, *J*=8.5, 2.0 Hz, H-6″), 5.43 (1H, d, *J*=3.0 Hz, H-7″), 5.17 (1H, d, *J*=3.0 Hz, H-8″), 6.67 (1H, d, *J*=2.0 Hz, H-2‴), 6.73 (1H, d, *J*=8.5 Hz, H-5‴), 6.43 (1H, dd, *J*=8.5, 2.0 Hz, H-6‴), 2.75 (1H, d, *J*=6.0 Hz, H-7‴) , 2.77 (1H, d, *J*=6.0 Hz, H-7‴), 4.92 (1H, t, *J*=6.0 Hz, H-8‴). $^{13}$C-NMR: (acetone-$d_6$, 100 MHz) $\delta$: 129.3 (C-1), 117.5 (C-2), 143.4 (C-3), 145.9 (C-4), 118.3 (C-5), 123.5 (C-6), 144.8 (C-7), 116.7 (C-8), 166.6 (C-9), 129.1 (C-1′), 117.3 (C-2′), 145.7 (C-3′), 144.8 (C-4′), 116.0 (C-5′), 121.7 (C-6′), 37.5 (C-7′), 73.8 (C-8′), 171.0 (C-9′), 127.5 (C-1″), 114.4 (C-2″), 145.7 (C-3″), 146.5 (C-4″), 116.0 (C-5″), 119.6 (C-6″), 75.9 (C-7″), 75.8 (C-8″), 166.8 (C-9″), 128.1 (C-1‴), 117.5 (C-2‴), 145.7 (C-3‴), 144.9 (C-4‴), 116.0 (C-5‴), 122.0 (C-6‴), 37.0 (C-7‴), 74.5 (C-8‴), 169.9 (C-9‴).

Lycopic Acid B (**5**): Colorless amorphous solid, $[\alpha]_D^{21}$ 73.6° (*c*=0.19, MeOH), UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 287 (4.84), 320 (4.78). CD (*c*=0.019, MeOH) nm ([$\theta$]): 222 (44700), 245 (−14300), 263 (1500), 274 (−6200), 295 (38100) nm. HR-FAB-MS (positive): *m/z* 719.1595 $[M+H]^+$ (Calcd for $C_{36}H_{31}O_{16}$: 719.1611). $^1$H-NMR: (acetone-$d_6$, 400 MHz) $\delta$: 7.31 (1H, d, *J*=2.0 Hz, H-2), 6.95 (1H, d, *J*=8.5 Hz, H-5), 7.23 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 7.61 (1H, d, *J*=16.0 Hz, H-7), 6.45 (1H, d, *J*=16.0 Hz, H-8), 6.87 (1H, d, *J*=2.0 Hz, H-2′), 6.76 (1H, d, *J*=8.0 Hz, H-5′), 6.69 (1H, dd, *J*=8.0, 2.0 Hz, H-6′), 3.05 (1H, dd, *J*=14.5, 8.5 Hz, H-7′), 3.14 (1H, dd, *J*=14.5, 4.0 Hz, H-7′), 5.24 (1H, dd, *J*=8.5, 4.0 Hz, H-8′), 6.98 (1H, d, *J*=1.5 Hz, H-2″), 6.84 (1H, d, *J*=8.5 Hz, H-5″), 6.84 (1H, dd, *J*=8.5, 1.5 Hz, H-6″), 5.21 (1H, d, *J*=5.0 Hz, H-7″), 5.06 (1H, d, *J*=5.0 Hz, H-8″), 6.75 (1H, d, *J*=2.0 Hz, H-2‴), 6.74 (1H, d, *J*=8.0 Hz, H-5‴), 6.55 (1H, dd, *J*=8.0, 2.0 Hz, H-6‴), 2.89 (2H, d, *J*=6.0 Hz, H-7‴), 5.11 (1H, t, *J*=6.0 Hz, H-8‴). $^{13}$C-NMR: (acetone-$d_6$, 100 MHz) $\delta$: 129.1 (C-1), 117.5 (C-2), 143.4 (C-3), 145.7 (C-4), 118.6 (C-5), 123.5 (C-6), 145.7 (C-7), 116.8 (C-8), 166.6 (C-9), 129.3 (C-1′), 117.4 (C-2′), 145.7 (C-3′), 144.9 (C-4′), 116.0 (C-5′), 121.7 (C-6′), 37.5 (C-7′), 74.7 (C-8′), 171.1 (C-9′), 128.2 (C-1″), 115.2 (C-2″), 146.1 (C-3″), 146.8 (C-4″), 116.2 (C-5″), 120.0 (C-6″), 76.5 (C-7″), 76.5 (C-8″), 167.5 (C-9″), 128.3 (C-1‴), 117.4 (C-2‴), 146.0 (C-3‴), 144.9 (C-4‴),

116.0 (C-5‴), 121.8 (C-6‴), 37.1 (C-7‴), 73.9 (C-8‴), 170.1 (C-9‴).

Lycopic Acid C (**7**): Pale brown amorphous solid, $[\alpha]_D^{21}$ 98.2° ($c$=0.55, MeOH), UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 247 (4.64), 284 (4.67), 323 (4.61). CD ($c$=0.005, MeOH) CD ($c$=0.033, MeOH) nm ([$\theta$]): 207 (−20100), 221 (18500), 230 (12400), 245 (20600), 271 (13000), 292 (32400) nm. HR-FAB-MS (negative): $m/z$ 519.0927 $[M-H]^-$ (Calcd for $C_{27}H_{19}O_{11}$: 519.0927). $^1$H-NMR: (acetone-$d_6$, 400 MHz) $\delta$: 7.58 (1H, d, $J$=2.0 Hz, H-2), 7.15 (1H, d, $J$=8.0 Hz, H-5), 7.35 (1H, dd, $J$=8.0, 2.0 Hz, H-6), 7.66 (1H, d, $J$=16.0 Hz, H-7), 6.55 (1H, d, $J$=16.0 Hz, H-8), 6.76 (1H, d, $J$=2.0 Hz, H-2′), 6.70 (1H, d, $J$=8.0 Hz, H-5′), 6.63 (1H, dd, $J$=8.0, 2.0 Hz, H-6′), 3.03 (1H, dd, $J$=14.5, 8.5 Hz, H-7′), 3.13 (1H, dd, $J$=14.5, 4.5 Hz, H-7′), 5.23 (1H, dd, $J$=8.5, 4.5 Hz, H-8′), 7.61 (1H, d, $J$=2.0 Hz, H-2″), 6.85 (1H, d, $J$=8.5 Hz, H-5″), 7.19 (1H, dd, $J$=8.5, 2.0 Hz, H-6″), 6.98 (1H, s, H-7″). $^{13}$C-NMR: (acetone-$d_6$, 100 MHz) $\delta$: 133.4 (C-1), 116.4 (C-2), 141.5 (C-3), 142.4 (C-4), 118.7 (C-5), 125.4 (C-6), 145.3 (C-7), 119.1 (C-8), 167.6 (C-9), 129.3 (C-1′), 117.6 (C-2′), 146.2 (C-3′), 145.4 (C-4′), 116.4 (C-5′), 121.8 (C-6′), 37.9 (C-7′), 75.0 (C-8′), 173.4 (C-9′), 126.0 (C-1″), 118.4 (C-2″), 146.5 (C-3″), 148.9 (C-4″), 116.6 (C-5″), 125.8 (C-6″), 121.2 (C-7″), 125.8 (C-8″), 157.7 (C-9″).

3-*O*-(Caffeoyl)rosmarinic Acid Methyl Ester (**9**): Colorless amorphous solid, $[\alpha]_D^{21}$ 65.5° ($c$=0.55, MeOH), UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 275 (sh), 324 (4.77). CD ($c$=0.005, MeOH) CD ($c$=0.033, MeOH) nm ([$\theta$]): 206 (−13000), 251 (21700), 280 (8900), 297 (20900), 328 (22000) nm. HR-FAB-MS (negative): $m/z$ 551.1183 $[M-H]^-$ (Calcd for $C_{28}H_{23}O_{12}$: 553.1189). $^1$H-NMR: (acetone-$d_6$, 400 MHz) $\delta$: 7.40 (1H, d, $J$=2.0 Hz, H-2), 6.84 (1H, d, $J$=8.5 Hz, H-5), 7.15 (1H, dd, $J$=8.5, 2.0 Hz, H-6), 7.53 (1H, d, $J$=16.0 Hz, H-7), 6.25 (1H, d, $J$=16.0 Hz, H-8), 6.80 (1H, d, $J$=2.0 Hz, H-2′), 6.72 (1H, d, $J$=8.0 Hz, H-5′), 6.62 (1H, dd, $J$=8.0, 2.0 Hz, H-6′), 3.00 (1H, dd, $J$=14.5, 8.5 Hz, H-7′), 3.08 (1H, dd, $J$=14.5, 4.5 Hz, H-7′), 5.19 (1H, dd, $J$=8.5, 4.5 Hz, H-8′), 7.10 (1H, d, $J$=2.0 Hz, H-2″), 7.01 (1H, d, $J$=8.5 Hz, H-5″), 7.29 (1H, dd, $J$=8.5, 2.0 Hz, H-6″), 7.31 (1H, s, H-7″). 3.70 (3H, s, H-OMe). $^{13}$C-NMR: (acetone-$d_6$, 100 MHz) $\delta$: 127.3 (C-1), 117.8 (C-2), 145.9 (C-3), 148.3 (C-4), 116.3 (C-5), 125.0 (C-6), 145.9 (C-7), 115.7 (C-8), 166.6 (C-9), 129.1 (C-1′), 117.3 (C-2′), 145.6 (C-3′), 144.7 (C-4′), 116.0 (C-5′), 121.6 (C-6′), 37.4 (C-7′), 73.7 (C-8′), 171.0 (C-9′), 127.3 (C-1″), 115.0 (C-2″), 145.8 (C-3″), 150.5 (C-4″), 117.7 (C-5″), 125.5 (C-6″), 128.8 (C-7″), 138.5 (C-8″), 164.3 (C-9″), 52.5 (C-OMe).

**(*S*)-PGME and (*R*)-PGME Esters of 3-(3,4-Dihydroxyphenyl)-2-hydroxypropanoic Acid** To 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid (5 mg, each) obtained from rosmarinic acid[13] in *N*,*N*-dimethylformamide (DMF) (1.0 ml) was added (*S*)-phenylglycine methyl ester (PGME) or (*R*)-PGME (10 mg), and then benzotriazol-1-yl-oxy-tris-pyrrolidinophonium hexafluorophosphate (PyBOP) (15 mg), 1-hydroxybenzotriazole (HOBT) (5 mg), and *N*-methylmorpholine (20 $\mu$l) were added and the mixture was stirred for 10 h at room temperature. The reactions gave (*S*)-amide and (*R*)-amide.[13] The retention time of (*S*)-amide was 19.4 min and that of (*R*)-amide was 20.1 min. The analytical HPLC was performed on a Shiseido Capcell Pak C18 column (4.6×250 mm) using acetonitrile–0.2% TFA in water (22.5 : 77.5) as the solvent (flow rate, 1 ml/min; detector, UV 210 nm).

**Acidic Hydrolysis of Compounds 3, 5, 7, and 9 and Condensation with (*S*)-PGME** Each compound (compounds **3** and **5**: 2 mg; **7** and **9**: 0.5 mg) was dissolved in 7% HCl (1 ml) and stirred for 2 h at 90 °C. After concentration, the residues were dissolved in DMF and (*S*)-PGME (5 mg), PyBOP (7 mg), HOBT (3 mg), and *N*-methylmorpholine (15 $\mu$l) were added. The mixtures were then stirred for 10 h at room temperature to give (*S*)-amide; $t_R$=19.4 min in the HPLC analysis [column, Shiseido Capcell Pak C18 (4.6×250 mm); solvent, acetonitrile–0.2% TFA in water (22.5 : 77.5); flow rate, 1 ml/min; detector, UV 210 nm].

**Hyaluronidase Inhibitory Assay** The inhibitory activity of hyaluronidase was determined by the Morgan-Elson method, which was modified by Davidson and Aronson.[10,30—32] Samples dissolved in 0.1 M acetate buffer (0.2 ml) and hyaluronidase (Type IV-S: From Bovine Testes, 2140 units/mg solid, Sigma Chemical Co., St. Louis, U.S.A.) in buffer (final concentration: 4000 unit/ml, 0.1 ml) were mixed and the mixture was incubated at 37 °C for 20 min. Then, compound 48/80 (Sigma Chemical Co., St. Louis, U.S.A.) in buffer (final concentration: 0.3 mg/ml, 0.2 ml) was added and incubated at 37 °C for 20 min. After hyaluronic acid potassium salt (Sigma Chemical Co., St. Louis, U.S.A.) in buffer (final concentration: 0.4 mg/ml, 0.5 ml) had been added, the mixture was incubated at 37 °C for 40 min. Then, the reaction was stopped by adding 0.4 M NaOH and bolate solution and then boiling the mixture in a water bath for 3 min. *p*-Dimethylaminobenzaldehyde (Wako Pure Chemical Industries Ltd., Osaka, Japan) acetate solution (6 ml) was then added and incubated at 37 °C for 20 min. Acetate buffer was added in place of the sample as a control, and the buffer was added in place of hyaluronidase in buffer as a blank. The enzyme inhibitory activity (%) was calculated as follows: inhibitory activity (%)=[(Control $Abs_{600\,nm}$−$Control_{blank}Abs_{600\,nm}$)−(Sample $Abs_{600\,nm}$−$Sample_{blank}Abs_{600\,nm}$)]/(Control $Abs_{600\,nm}$−$Control_{blank}Abs_{600\,nm}$)×100.

**Acknowledgments** We thank Mr. S. Sato and Mr. T. Matsuki of Tohoku Pharmaceutical University for assisting with the MS measurements.

**References**

1) Takahashi Y., Nagumo S., Noguchi M., Nagai M., *Nat. Med.*, **53**, 273 (1999).
2) Malik A., Yuldashev M. P., *Chem. Nat. Compd.*, **38**, 104—105 (2002).
3) Woo E. R., Piao M. S., *Arch. Pharm. Res.*, **27**, 173—176 (2004).
4) Lee W. S., Im K. R., Park Y. D., Sung N. D., Jeong T. S., *Biol. Pharm. Bull.*, **29**, 382—384 (2006).
5) Mitsuhashi H., Okada M., Nunome S., Terabayashi S., Miki E., Fujita T., Yamauchi T., Itokawa H., "Illustrated Medicinal Plants of the World in Colour," Hokuryukan Co., Ltd., Tokyo, 1988, p. 450.
6) Shin T. Y., Kim S. H., Suk K., Ha J. H., Kim I., Lee M. G., Jun C. D., Kim S. Y., Lim J. P., Eun J. S., Shin H. Y., Kim H. M., *Toxycol. Appl. Pharmacol.*, **209**, 255—262 (2005).
7) Lee Y. J., Kang D. G., Kim J. S., Lee H. S., *Vascul. Pharmacol.*, **48**, 38—46 (2008).
8) Girish K. S., Kemparaju K., Nagaraju S., Vishwanath B. S., *Curr. Med. Chem.*, **16**, 2261—2288 (2009).
9) Kakegawa H., Matsumoto H., Satoh T., *Chem. Pharm. Bull.*, **33**, 642—646 (1985).
10) Kakegawa H., Matsumoto H., Satoh T., *Chem. Pharm. Bull.*, **40**, 1439—1442 (1992).
11) Petersen M., Simmonds M. S. J., *Phytochemistry*, **62**, 121—125 (2003).
12) Ippoushi K., Yamaguchi Y., Itou H., Azuma K., Higashio H., *Food Sci. Technol. Res.*, **6**, 74—77 (2000).
13) Murata T., Sasaki K., Sato K., Yoshizaki F., Yamada H., Mutoh H., Umehara K., Miyase T., Warashina T., Aoshima H., Tabata H., Matsubara K., *J. Nat. Prod.*, **72**, 1379—1384 (2009).
14) Matsuta M., Kanita R., Saito Y., Yamashita A., *Natural Medicines*, **50**, 204—211 (1996).
15) Dapkevicius A., van Beek T. A., Lelyveld G. P., van Veldhuizen A., de Groot A., Linssen J. P. H., Venskutonis R., *J. Nat. Prod.*, **65**, 892—896 (2002).
16) Woo E., Piao M. S., *Arch. Pharm. Res.*, **27**, 173—176 (2004).
17) Agrawal P. K., Rastogi R. P., *Heterocycles*, **16**, 2181—2236 (1981).
18) Bucar F., Kartnig T., *Planta Med.*, **61**, 378—380 (1995).
19) Wang Y., Xue X., Xiao Y., Zang F., Xu Q., Liang X., *J. Sep. Sci.*, **31**, 1669—1676 (2008).
20) Nagano T., Abe F., Kinjo J., Okabe H., *Biol. Pharm. Bull.*, **25**, 875—879 (2002).
21) Yim S., Kim H. J., Lee I., *Arch. Pharm. Res.*, **26**, 128—131 (2003).
22) Arisawa M., Fukuta M., Shimizu M., Morita N., *Chem. Pharm. Bull.*, **27**, 1252—1254 (1979).
23) Miyase T., Koizumi A., Ueno A., Noro T., Kuroyanagi M., Fukushima S., Akiyama Y., Takemoto T., *Chem. Pharm. Bull.*, **30**, 2732—2737 (1982).
24) Yamamura S., Ozawa K., Ohtani K., Kasai R., Yamasaki K., *Phytochemistry*, **48**, 131—136 (1998).
25) Min Y. D., Choi S. U., Lee, K. R., *Arch. Pharm. Res.*, **29**, 627—632 (2006).
26) Kim N. C., Graf T. N., Sparacino C. M., Wani M. C., Wall M. E., *Org. Biomol. Chem.*, **1**, 1684—1689 (2003).
27) Silva M. S., Barbosa-Filho J. M., Yohida M., Gottlieb O. R., *Phytochemistry*, **28**, 3477—3482 (1989).
28) Fang J. M., Lee C. K., Cheng Y. S., *Phytochemistry*, **31**, 3659—3661 (1992).
29) Agata I., Kusakabe H., Hatano T., Nishibe S., Okuda T., *Chem. Pharm. Bull.*, **41**, 1608—1611 (1993).
30) Reissig J. L., Strominger J. L., Leloir L. F., *J. Biol. Chem.*, **217**, 959—966 (1955).
31) Davidson E. A., Aronson N. N., *J. Biol. Chem.*, **242**, 437—440 (1967).
32) Maeda Y., Yamamoto M., Masui T., Sugiyama K., Yokota M., Nakagomi K., Tanaka H., Takahashi I., Kobayashi T., Kobayashi E., *Syokueishi*, **31**, 233—237 (1990).