Structures of New β-Carboline-Type Alkaloids with Antiallergic Effects from *Stellaria dichotoma*[1,2]

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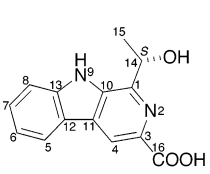
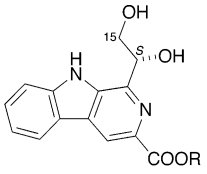
The aqueous ethanolic extract from the roots of *Stellaria dichotoma* showed an antiallergic effect on ear passive cutaneous anaphylaxis (PCA) reaction in mice (in vivo) and inhibitory activity on the release of β-hexosaminidase in RBL-2H3 cells (in vitro). From the aqueous ethanolic extract, new β-carboline-type alkaloids, dichotomines A (**1**), B (**2**), C (**3**), and D (**4**) and dichotomides I (**5**) and II (**6**), were isolated. The structures of the new constituents (**1**−**6**) were determined on the basis of chemical and physicochemical evidence, including the application of the modified Mosher's method. The effects of the isolated constituents on the release of β-hexosaminidase in RBL-2H3 cells were examined, and **3** was found to show inhibitory activity (IC_{50} = 62 μM). Moreover, **3** also inhibited the releases of antigen-IgE-mediated TNF-α and IL-4 (19 and 15 μM, respectively) in RBL-2H3 cells, both of which participate in the late phase of type I allergic reactions.

The Caryophyllaceae plant *Stellaria dichotoma* L. var. *lanceolata* Bge. (Chinese name "Yin Chai Hu") is distributed in Ningxie and neighboring provinces of China, and the roots of this plant are used as a folk medicine for the treatment of fever.[3] Previously, flavonoids,[3,4] sterols,[3] and cyclic peptides[5−8] have been isolated from the roots of this plant.

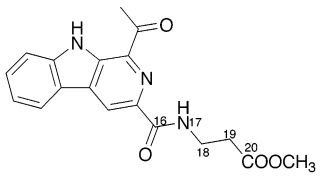
In the course of our characterization studies on bioactive constituents of Chinese natural medicines,[2] the aqueous ethanolic extract from the roots of *S. dichotoma* was found to show an antiallergic effect on ear passive cutaneous anaphylaxis (PCA) reaction in mice and inhibitory activity on the release of β-hexosaminidase in RBL-2H3 cells. From this herbal extract, six new β-carboline-type alkaloids, dichotomines A (**1**), B (**2**), C (**3**), and D (**4**) and dichotomides I (**5**) and II (**6**), were isolated together with 18 known compounds. This paper deals with the isolation and structure elucidation of the new alkaloids (**1**−**6**) as well as the antiallergic activity of the aqueous ethanolic extract on ear PCA reactions in mice. In addition, we examined inhibitory effects of these isolates on the release of β-hexosaminidase and antigen-IgE-mediated TNF-α and IL-4 from RBL-2H3 cells.



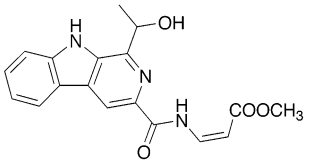
dichotomine A (**1**)



dichotomine B (**2**): R=H
dichotomine C (**3**): R=CH_3
dichotomine D (**4**): R=$(CH_2)_3CH_3$



dichotomide I (**5**)



dichotomide II (**6**)

Results and Discussion

The 95% aqueous EtOH extract from the roots of *S. dichotoma* L. var. *lanceolata* purchased in Shenyang, Liaoning Province, the People's Republic of China, was partitioned into an EtOAc−H_2O mixture to furnish an EtOAc-soluble fraction and aqueous layer. The aqueous layer was extracted with *n*-BuOH to give *n*-BuOH- and H_2O-soluble fractions. The aqueous EtOH extract inhibited the PCA reactions in mice [inhibition (%) at 1000 mg/kg (po) = 46.2], as shown in Table S1, and also the release of β-hexosaminidase from RBL-2H3 cells (IC_{50} = 960 μg/mL). The *n*-BuOH-soluble fraction was subjected to normal-phase and reversed-phase silica gel CC and repeated HPLC to give dichotomines A−D (**1**−**4**). The EtOAc-soluble portion was also subjected to normal-phase and reversed-phase silica gel CC and repeated HPLC to give dichotomides I (**5**) and II (**6**) and 18 known compounds (**7**−**24**, Chart S1).

Dichotomine A (**1**) was presumed to possess a nitrogen function on the basis of TLC examination using Dragendorff's reagent. The EIMS of **1** showed a molecular ion peak at *m*/*z* 256 $[M]^+$, and the molecular formula $C_{14}H_{12}N_2O_3$ was determined by HREIMS. Absorption maxima in the UV spectrum of **1** were observed at 236 and 269 nm, suggestive of the β-carboline skeleton.[9] The IR spectrum of **1** showed absorption bands at 3400 and 1710 cm^{-1} ascribable to hydroxyl and carboxyl functions. The ^1H and ^{13}C NMR spectra[10] of **1** showed signals assignable to a

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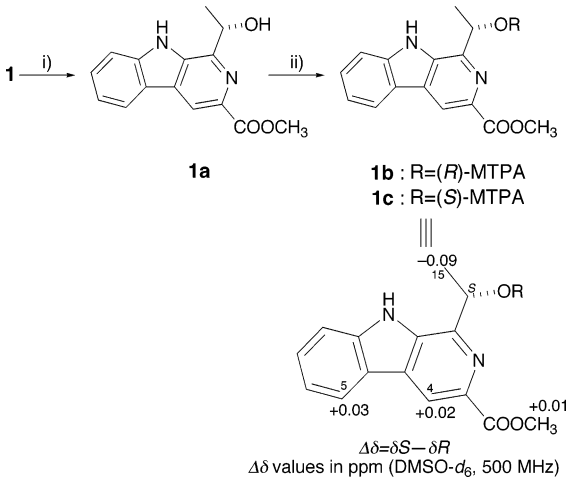
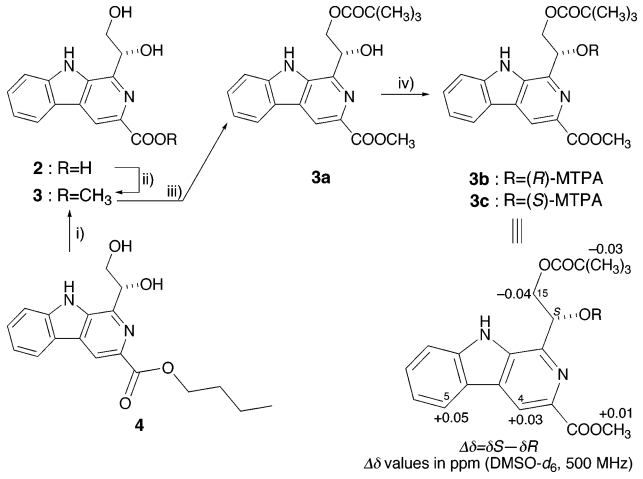
Scheme 1[a]



[a] Reagents and conditions: (i) $TMSCHN_2$−MeOH, rt, 2 h; (ii) (*R*)- or (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2, rt, 10 h.

Scheme 2[a]



[a] Reagents and conditions: (i) 0.1% NaOMe-MeOH, rt, 7 h; (ii) $TMSCHN_2$−MeOH, rt, 2 h; (iii) pivaloyl chloride−pyridine, 0 °C, 2 h; (iv) (*R*)- or (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2, rt, 10 h.

secondary methyl [δ 1.57 (H_3-15)], a methine bearing an oxygen function [δ 5.26 (H-14)], five aromatic protons [δ 7.29, 7.58, 7.73, 8.36, 8.81 (H-6, 7, 8, 5, 4)], and an amino proton [δ 11.70 (NH-9)]. The $^1H-^1H$ COSY experiment on **1** indicated the presence of two partial structures (C-5−C-8, C-14−C-15), as shown in Figure S1. In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs of **1** (H-4 and C-10, 16; H-5 and C-13; H-6 and C-12; H-7 and C-13; H-8 and C-12; H3-15 and C-1). The absolute stereochemistry of **1** was determined by application of modified Mosher's method.[11] Namely, methylation of **1** with trimethylsilyldiazomethane ($TMSCHN_2$) gave the methyl ester derivative (**1a**), which was treated with (*R*)- or (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-(dimethylamino)pyridine (4-DMAP) to yield the (*R*)- and (*S*)-MTPA esters (**1b** and **1c**), respectively. As shown in Scheme 1, the signals due to protons attached to C-4 and C-5 and the 16-methoxyl protons in the (*S*)-MTPA ester (**1c**) were observed at lower fields compared with those of the (*R*)-MTPA ester (**1b**) [$\Delta\delta$: positive], while the signal due to protons of C-15 in **1c** was observed at higher fields compared with those of **1b** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 14-position of **1** was determined to be *S*.

The ^1H and ^{13}C NMR spectra[10] of dichotomine B (**2**) showed signals assignable to a methylene and a methine bearing an oxygen function [δ 3.85, 5.16 (H_2-15 and H-14)], five aromatic protons [δ 7.30, 7.59, 7.74, 8.37, 8.84 (H-6, 7, 8, 5, 4)], and an amino proton [δ 11.80 (NH-9)]. The proton and carbon signals in the ^1H and ^{13}C NMR spectra of **2** were found to be superimposable on those of **1**, except for the 14- and 15-positions. The $^1H-^1H$ COSY experiment of **2** indicated the presence of partial structures as shown in Figure S1, so that **2** was deduced to be the 15-hydroxyl derivative of dichotomine A (**1**).

The ^1H and ^{13}C NMR spectra[10] of dichotomine C (**3**) resembled those of **2**, except for the signals due to an ester methyl group [δ 3.97 (−OCH_3)]. The structure of **3** was clarified by $^1H-^1H$ COSY and HMBC experiments (Figure S1). Finally, $TMSCHN_2$ methylation of **2** gave the methyl ester (**3**) (Scheme 2).

Absolute configurations of the 14-positions in **2** and **3** were determined by application of the modified Mosher's method.[11] Thus, the 15-pivaloyl ester (**3a**), which was obtained by selective esterification of the 15-primary hydroxyl group in **3** with pivaloyl chloride, was derived to the (*R*)- and (*S*)-MTPA esters (**3b**, **3c**). As shown in Scheme 2, the signals due to protons attached to C-4 and C-5 and the 16-methoxyl protons in the (*S*)-MTPA ester (**3c**) were observed at lower fields compared with those of the (*R*)-MTPA ester (**3b**) [$\Delta\delta$: positive], while signals due to protons of C-15 and the pivaloyl group in **3c** were observed at higher fields compared with those of **3b** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 14-position of **2** and **3** was determined to be *S*.

Dichotomine D (**4**)[12] had an absorption maximum at 283 nm in the UV spectrum, and the IR spectrum of **4** showed absorption bands at 3320 and 1716 cm^{-1}. The ^1H and ^{13}C NMR spectra[10] of **4** were similar to those of **3**, except for signals due to the 16-carboxyl ester moiety [δ 0.86 (3H), 1.43, 1.73, 4.51 (2H each), −OC_4H_9]. Treatment of **4** with 0.1% sodium methoxide (NaOMe)−MeOH gave the methyl ester (**3**).

The EIMS of dichotomide I (**5**) showed a molecular ion peak at *m/z* 339 $[M]^+$, and the molecular formula $C_{18}H_{17}N_3O_4$ was determined by HREIMS. The UV spectrum of **5** showed absorption maxima at 220 and 286 nm, and its IR spectrum showed absorption bands at 1732, 1669, and 1653 cm^{-1} assignable to carboxyl, carbonyl, and amide functions. The ^1H and ^{13}C NMR spectra[10] of **5** showed signals assignable to a methyl [δ 2.94 (H_3-15)], two methylenes [δ 2.75, 3.86 (H_2-19, 18)], a methoxyl group [δ 3.76 (−OCH_3)], five aromatic protons [δ 7.40, 7.62, 7.65, 8.22, 9.10 (H-6, 8, 7, 5, 4)], and two amino protons [δ 8.63, 10.40 (NH-17, 9)]. The signals due to the β-carboline moiety in the ^1H and ^{13}C NMR spectra of **5** were similar to those of **1**−**4**, except for the signals due to the amide-ester and acetyl moieties. As shown in Figure S1, the $^1H-^1H$ COSY experiment on **5** indicated the presence of two partial structures (C-5−C-8, N-17−C-18−C-19). In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs of **5** (H-4 and C-10, 16; H-5 and C-13; H-6 and C12; H-7 and C-13; H-8 and C-12; H_3-15 and C-1, 14; H_2-18 and C-16, 20; H_2-19 and C-20; −OCH_3 and C-20). Thus, the structure of **5** was determined to be as shown.

The molecular formula of dichotomide II (**6**)[13] was determined to be $C_{18}H_{17}N_3O_4$ by HREIMS analysis. The UV spectrum of **6** showed absorption maxima at 226, 284, and 324 nm. The ^1H and ^{13}C NMR spectra[10] of **6** showed signals assignable to a secondary methyl [δ 1.72 (H_3-15)], a methoxyl group [δ 3.75 (−OCH_3)], a methine bearing an

oxygen function [δ 5.31 (H-14)], *cis*-substituted olefins [δ 5.31, 7.78 (H-19, 18)], five aromatic protons [δ 7.30, 7.59, 7.78, 8.40, 8.92 (H-6, 7, 8, 5, 4)], and two amino protons [δ 11.80, 12.40 (NH-9, 17)]. The proton and carbon signals of **6** were superimposable on those of **1**, except for the signal due to the amide-ester moiety. The ^{1}H−^{1}H COSY and the HMBC experiments on **6** unambiguously characterized the structure of **6**.

Inhibitory effects of the principal constituents (**1**−**4**, **7**−**12**, **14**) on the release of β-hexosaminidase in RBL-2H3 cells were examined. Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in in vitro experiments on immediate allergic reactions. β-Hexosaminidase is also stored in the secretory granules of mast cells and is released concomitantly with histamine when mast cells are immunologically activated.[14,15] Therefore it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. As a part of our characterization studies on the bioactive components of natural medicines, we previously reported several inhibitors of the release of β-hexosaminidase such as diarylheptanoids,[16,17] sesquiterpenes,[18] diterpenes,[19] flavonoids,[20] anthraquinones,[21] stilbenes,[22] phenanthrenes,[22] and phenylpropanoids.[23] Among the principal constituents from *S. dichotoma*, **3** showed inhibitory activity (IC_{50} = 62 μM), and the activity was stronger than that of two antiallergic compounds, tranilast (IC_{50} = 0.49 mM) and ketotifen fumarate (IC_{50} = 0.22 mM).[21,23] However, none of the other isolates (**1**, **2**, **4**, **7**−**12**, **14**) were active in this assay [inhibition (%) at 100 μM: 1.2−11.2%, Table S2].

After challenge with an antigen, sensitized animals and atopic individuals exhibit early phase responses, such as the appearance of wheals and flares on the skin and bronchoconstriction of the airways, and late phase responses such as edema and erythema usually persist over a 6−24 h period at the site of challenge in the skin and airways.[24−27] The early phase responses are mainly due to small molecule chemical mediators (e.g., histamine, serotonin) from mast cells. Mast cells also produce cytokines including TNF-α, IL-4, and IL-5, and these cytokines play an important role in the late phase reactions.[24−27] From natural resources, several flavones[20] and phenylpropanoids[23] were reported to inhibit releases of TNF-α and IL-4. However, there have been no reports of the inhibitory effects of β-carboline-type alkaloids on the releases of TNF-α and IL-4 from mast cells. In the present study, the effects of **3**, which exhibited inhibitory effects against the release of β-hexosaminidase, on releases of TNF-α and IL-4 from RBL-2H3 cells 4 h after challenge were examined. As a result, **3** inhibited releases of TNF-α and IL-4 with IC_{50} values of 19 and 15 μM. These findings suggest that **3** is more effective against the late phase reactions in type I allergy than that of the early phase.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JEOL JNM LA-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM LA-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; HPLC, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); HPLC column, YMC-Pack ODS-A (250 × 20 mm i.d.); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with Dragendorff's reagent or 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The roots of *Stellaria dichotoma* L. var. *lanceolata* were purchased in Shenyang, Liaoning Province, the People's Republic of China, in March 2003, and identified by Prof. Yingjie Chen (Shenyang Pharmaceutical University, People's Republic of China). A voucher of the plant is on file in our laboratory (2003.03 Shenyang-02).

Extraction and Isolation. The dried roots of *S. dichotoma* L. var. *lanceolata* (7.0 kg) were cut and extracted three times with 95% aqueous EtOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the aqueous EtOH extract (1500 g, 21.4%). The extract (1300 g) was partitioned in an EtOAc−H_2O (1:1, v/v) mixture. The aqueous layer was extracted with *n*-BuOH, and removal of the solvent in vacuo from the EtOAc-, *n*-BuOH-, and H_2O-soluble portions yielded 126 g (2.1%), 405 g (6.7%), and 769 g (12.6%) of the residue, respectively.

Normal-phase silica gel CC [3.0 kg, $CHCl_3$−MeOH−H_2O (50:3:1−10:3:1 lower layer−6:4:1, v/v/v)−MeOH] of the *n*-BuOH-soluble fraction (200 g) gave seven fractions [1 (30.3 g), 2 (28.7 g), 3 (30.8 g), 4 (29.5 g), 5 (24.8 g), 6 (23.1 g), and 7 (32.8 g)]. Fraction 2 (28.7 g) was separated by reverse-phase silica gel CC [100 g, MeOH−H_2O (70:30, v/v)−MeOH] to furnish five fractions [2-1 (5.0 g), 2-2 (6.5 g), 2-3 (6.6 g), 2-4 (6.0 g), 2-5 (4.6 g)]. Fraction 2-2 (6.3 g) was separated by HPLC [MeOH−H_2O (50:50, v/v)] to give **3** (56 mg, 0.0016%). Fraction 2-4 (6.0 g) was further purified by HPLC [MeOH−H_2O (70:30, v/v)] to give **2** (30 mg, 0.0009%) and **4** (15 mg, 0.0004%). Fraction 7 (32.8 g) was separated by reverse-phase silica gel CC [1.0 kg, MeOH−H_2O (20:80−40:60−70:30, v/v)−MeOH] to furnish eight fractions [7-1 (1.2 g), 7-2 (3.4 g), 7-3 (3.2 g), 7-4 (4.2 g), 7-5 (4.4 g), 7-6 (4.5 g), 7-7 (4.7 g), and 7-8 (7.2 g)]. Fraction 7-3 (3.2 g) was separated by [MeOH−H_2O (25:75, v/v)] to give **1** (52 mg, 0.0014%).

Normal-phase silica gel CC [3.0 kg, *n*-hexane−EtOAc (100:1−50:1−20:1−10:1−1:1−1:5−1:10, v/v)−MeOH] of the EtOAc-soluble portion (100 g) gave 12 fractions [1 (0.8 g), 2 (2.0 g), 3 (14.1 g), 4 (4.2 g), 5 (1.4 g), 6 (2.1 g), 7 (9.6 g), 8 (8.5 g), 9 (8.9 g), 10 (9.1 g), 11 (14.1 g), and 12 (25.2 g)]. Fraction 2 (2.0 g) was purified by recrystallization from MeOH to give **15**[3] (10.0 mg, 0.0002%). Fraction 4 (4.2 g) was separated by reversed-phase silica gel CC [120 g, MeOH−H_2O (40:60−60:40−90:10, v/v)−MeOH] to furnish seven fractions [4-1 (0.1 g), 4-2 (0.3 g), 4-3 (0.3 g), 4-4 (0.8 g), 4-5 (0.2 g), 4-6 (0.3 g), and 4-7 (2.2 g)]. Fraction 4-5 (0.2 g) was further separated by HPLC [MeOH−H_2O (30:70, v/v)] to give **19**[28] (6 mg, 0.0001%), **20**[28] (15 mg, 0.0003%), **22**[28] (36 mg, 0.0006%), **23**[28] (60 mg, 0.0011%), and **24**[28] (11 mg, 0.0002%). Fraction 6 (2.1 g) was separated by reversed-phase silica gel CC [60 g, MeOH−H_2O (30:70−50:50−90:10, v/v)−MeOH] to furnish six fractions [6-1 (0.3 g), 6-2 (0.4 g), 6-3 (0.2 g), 6-4 (0.3 g), 6-5 (0.2 g), and 6-6 (0.7 g)]. Fraction 6-2 (0.4 g) was further separated by HPLC [YMC-Pack ODS-A, MeOH−H_2O (20:80, v/v)] to give **9**[28] (52 mg, 0.0009%) and **11**[28] (9 mg, 0.0002%). Fraction 6-3 (0.2 g) was separated by HPLC [MeOH−H_2O (45:55, v/v)] to give **16**[29] (37 mg, 0.0007%). Fraction 6-5 (0.2 g) was separated by HPLC [MeOH−H_2O (65:35, v/v)] to give **12**[30] (37 mg, 0.0007%). Fraction 8 (8.5 g) was separated by reversed-phase silica gel CC [250 g, MeOH−H_2O (5:95−20:80−50:50, v/v)−MeOH] to give six fractions [8-1 (0.9 g), 8-2 (1.4 g), 8-3 (0.7 g), 8-4 (0.8 g), 8-5 (3.4 g), and 8-6 (1.3 g)]. Fraction 8-2 (1.4 g) was separated by HPLC [MeOH−H_2O (20:80, v/v)] to give **14**[31] (88 mg, 0.0016%). Fraction 8-6 (1.3 g) was separated by HPLC [MeOH−H_2O (70:30, v/v)] to give **6** (5 mg, 0.00001%). Fraction 9 (8.9 g) was separated by reversed-phase silica gel CC [300 g, MeOH−H_2O (5:95−20:80−90:10, v/v)−MeOH] to furnish

nine fractions [9-1 (0.2 g), 9-2 (0.7 g), 9-3 (0.2 g), 9-4 (0.3 g), 9-5 (1.5 g), 9-6 (1.1 g), 9-7 (2.2 g), 9-8 (1.5 g), and 9-9 (1.2 g)]. Fraction 9-2 (0.7 g) was further separated by HPLC [MeOH−H_2O (20:80, v/v)] to give **7**[32] (72 mg, 0.0013%) and **8**[33] (7 mg, 0.00001%). Fraction 9-4 (0.3 g) was separated by HPLC [MeOH−H_2O (35:65, v/v)] to give **13**[28] (40 mg, 0.0007%). Fraction 9-6 (1.1 g) was separated by HPLC [MeOH−H_2O (45:55, v/v)] to give **21**[28] (270 mg, 0.0049%). Fraction 9-8 (1.5 g) was purified by HPLC [MeOH−H_2O (60:40, v/v)] to give **5** (6 mg, 0.00001%). Fraction 10 (9.1 g) was separated by reversed-phase silica gel CC [300 g, MeOH−H_2O (20:80−50:50−90:10, v/v)−MeOH] to furnish 10 fractions [10-1 (0.1 g), 10-2 (0.2 g), 10-3 (0.4 g), 10-4 (0.7 g), 10-5 (0.3 g), 10-6 (1.2 g), 10-7 (1.3 g), 10-8 (0.5 g), 10-9 (3.5 g), and 10-10 (0.9 g)]. Fraction 10-5 (0.3 g) was further separated by HPLC [MeOH−H_2O (30:70, v/v)] to give **18**[28] (54 mg, 0.0010%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values[3,29−33] or commercial samples.[28]

Dichotomine A (1): yellow powder; $[\alpha]_D^{27}$ −9.7° (*c* 0.85, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (4.14), 269 (4.24) nm; IR (KBr) ν_{max} 3400, 3320, 1710, 1460, 1377, 1256, 1163 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.57 (3H, d, J = 7.4 Hz, H_3-15), 5.26 (1H, m, H-14), 7.29, 7.58 (1H each, both m, H-6, 7), 7.73, 8.36 (1H each, both d, J = 7.0 Hz, H-8, 5), 8.81 (1H, s, H-4), 11.70 (1H, br s, NH-9); ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 23.2 (CH_3, C-15), 69.5 (CH, C-14), 112.4 (CH, C-8), 115.7 (CH, C-4), 119.7 (CH, C-6), 120.5 (C, C-12), 121.5 (CH, C-5), 128.1 (CH, C-7), 128.2 (C, C-11), 133.3 (C, C-10), 135.1 (C, C-3), 140.8 (C, C-13), 147.3 (C, C-1), 166.2 (C, C-16); EIMS *m*/*z* 256 [M]$^+$ (13), 238 (13), 223 (10), 55 (100); HREIMS *m*/*z* 256.0842 (calcd for $C_{14}H_{12}N_2O_3$, 256.0848).

Dichotomine B (2): yellow powder; $[\alpha]_D^{27}$ −19.0° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (3.91), 269 (4.04) nm; IR (KBr) ν_{max} 3420, 3300, 1719, 1458, 1375, 1250, 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.85 (2H, m, H_2-15), 5.16 (1H, m, H-14), 7.30, 7.59 (1H each, both m, H-6, 7), 7.74, 8.37 (1H each, both d, J = 7.6 Hz, H-8, 5), 8.84 (1H, s, H-4), 11.80 (1H, br s, NH-9); ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 65.3 (CH_2, C-15), 73.6 (CH, C-14), 112.6 (CH, C-8), 115.9 (CH, C-4), 119.9 (CH, C-6), 120.7 (C, C-12), 121.8 (CH, C-5), 128.4 (CH, C-7), 128.4 (C, C-11), 134.8 (C, C-10), 135.4 (C, C-3), 140.9 (C, C-13), 145.2 (C, C-1), 166.5 (C, C-16); EIMS *m*/*z* 272 [M]$^+$ (8), 254 (64), 194 (6), 73 (100); HREIMS *m*/*z* 272.0802 (calcd for $C_{14}H_{12}N_2O_4$, 272.0797).

Dichotomine C (3): yellow powder; $[\alpha]_D^{27}$ −16.6° (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.56) nm; IR (KBr) ν_{max} 3300, 1723, 1456, 1352, 1258 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.97 (3H, s, −OCH3), 4.61 (2H, m, H_2-15), 5.94 (1H, m, H-14), 7.39, 7.60 (1H each, both m, H-6, 7), 7.75, 8.34 (1H each, both d, J = 7.3 Hz, H-8, 5), 9.09 (1H, s, H-4), 12.60 (1H, br s, NH-9); ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 52.1 (CH_3, −OCH_3), 67.2 (CH_2, C-15), 75.9 (CH, C-14), 112.8 (CH, C-8), 117.2 (CH, C-4), 120.5 (CH, C-6), 121.8 (C, C-12), 121.8 (CH, C-5), 128.8 (CH, C-7), 129.3 (C, C-11), 136.3 (C, C-10), 136.4 (C, C-3), 141.8 (C, C-13), 146.8 (C, C-1), 166.7 (C, C-16); EIMS *m*/*z* 286 [M]$^+$ (11), 256 (73), 223 (81), 195 (100); HREIMS *m*/*z* 286.0959 (calcd for $C_{15}H_{14}N_2O_4$, 286.0593).

Dichotomine D (4): yellow powder; $[\alpha]_D^{27}$ −1.8° (*c* 0.75, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 283 (3.66) nm; IR (KBr) ν_{max} 3320, 1716, 1456, 1348, 1250 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ [0.86 (3H, t, J = 7.3 Hz), 1.43, 1.73 (2H each, both m), 4.51 (2H, t, J = 6.8 Hz), −OC_4H_9], 4.66 (2H, m, H_2-15), 5.94 (1H, t, J = 5.4 Hz, H-14), 7.39, 7.63 (1H each, both m, H-6, 7), 7.73, 8.34 (1H each, both d, J = 7.8 Hz, H-8, 5), 9.20 (1H, s, H-4), 12.60 (1H, br s, NH-9); ^{13}C NMR (CD_3OD, 125 MHz) δ_C [14.3 (CH_3), 19.9 (CH_2), 31.5 (CH_2), 65.3 (CH_2), −OC_4H_9], 67.6 (CH_2, C-15), 76.2 (CH, C-14), 113.1 (CH, C-8), 117.4 (CH, C-4), 120.7 (CH, C-6), 122.2 (C, C-12), 122.3 (CH, C-5), 129.1 (CH, C-7), 129.7 (C, C-11), 136.7 (C, C-10), 137.1 (C, C-3), 142.2 (C, C-13), 147.2 (C, C-1), 166.6 (C, C-16); EIMS *m*/*z* 328 [M]$^+$ (13), 298 (84), 223 (100), 195 (93); HREIMS *m*/*z* 328.1428 (calcd for $C_{18}H_{20}N_2O_4$, 328.1423).

Dichotomide I (5): yellow powder; UV (MeOH) λ_{max} (log ϵ) 220 (3.43), 286 (3.49) nm; IR (KBr) ν_{max} 3300, 1732, 1669, 1653, 1559, 1464, 1362, 1250, 1183 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.75 (2H, t, J = 6.2 Hz, H_2−19), 2.94 (3H, s, H_3-15), 3.76 (3H, s, −OCH_3), 3.86 (2H, dt, J = 1.0, 6.2 Hz, H_2-18), 7.40, 7.65 (1H each, both m, H-6, 7), 7.62, 8.22 (1H each, both d, J = 7.8 Hz, H-8, 5), 8.63 (1H, br s, NH-17), 9.10 (1H, s, H-4), 10.40 (1H, br s, NH-9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 25.6 (CH_3, C-15), 34.1 (CH_2, C-19), 34.9 (CH_2, C-18), 51.8 (CH_3, −OCH_3), 112.0 (CH, C-8), 118.2 (CH, C-4), 120.8 (C, C-12), 121.3 (CH, C-6), 122.1 (CH, C-5), 129.5 (CH, C-7), 132.4 (C, C-11), 133.4 (C, C-1), 136.1 (C, C-10), 138.9 (C, C-3), 141.3 (C, C-13), 164.4 (C, C-16), 172.7 (C, C-20), 202.1 (C, C-14); EIMS *m*/*z* 339 [M]$^+$ (31), 237 (30), 210 (100), 182 (40); HREIMS *m*/*z* 339.1223 (calcd for $C_{18}H_{17}N_3O_4$, 339.1219).

Dichotomide II (6): yellow powder; $[\alpha]_D^{19}$ +7.2° (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 226 (4.02), 284 (4.07), 324 (3.85) nm; IR (KBr) ν_{max} 3200, 1701, 1664, 1624, 1597, 1491, 1460, 1429, 1389, 1298, 1211, 1181 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.72 (3H, d, J = 6.5 Hz, H_3−15), 3.75 (3H, s, −OCH_3), 5.31 (1H, m, H-14), 5.31 (1H, d, J = 8.9 Hz, H-19), 7.30, 7.59 (1H each, both m, H-6, 7), 7.78 (1H, d, J = 8.9 Hz, H-18), 7.78, 8.40 (1H each, both d, J = 7.8 Hz, H-8, 5), 8.92 (1H, s, H-4), 11.80 (1H, br s, NH-9), 12.40 (1H, br d, J = ca. 1 Hz, NH-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 22.1 (CH_3, C-15), 50.9 (CH_3, −OCH_3), 69.1 (CH, C-14), 95.9 (CH, C-19), 112.6 (CH, C-8), 114.5 (CH, C-4), 119.9 (CH, C-6), 121.4 (C, C-12), 121.7 (CH, C-5), 128.3 (CH, C-7), 128.7 (C, C-11), 134.4 (C, C-10), 137.4 (C, C-3), 137.4 (CH, C-18), 140.7 (C, C-13), 147.3 (C, C-1), 162.6 (C, C-16), 167.9 (C, C-20); EIMS *m*/*z* 339 [M]$^+$ (71), 280 (45), 211 (100), 169 (33); HREIMS *m*/*z* 339.1223 (calcd for $C_{18}H_{17}N_3O_4$, 339.1219).

Methylation of 1 and 2. A solution of **1** (3.0 mg) in MeOH (0.4 mL) was treated with trimethylsilyldiazomethane ($TMSCHN_2$) (10% in hexane, ca. 0.4 mL), and the whole mixture was stirred at room temperature for 2 h. Removal of the solvent under reduced pressure gave **1a** (2.9 mg, 92%). Through a similar procedure, **3** (1.9 mg, 90%) was obtained from **2** (2.0 mg) using $TMSCHN_2$ (ca. 0.2 mL) in MeOH (0.2 mL).

Compound 1a: yellow powder; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.00 (3H, d, J = 7.4 Hz, H_3-15), 4.05 (3H, s, −$COOCH_3$), 4.27 (1H, m, H-14), 7.57, 7.69 (1H each, both m, H-6, 7), 7.82, 8.40 (1H each, both d, J = 7.0 Hz, H-8, 5), 9.05 (1H, s, H-4), 9.24 (1H, br s, NH-9).

Pivaloylation of 3. A solution of **3** (5.1 mg) in pyridine (2.0 mL) was treated with pivaloyl chloride (10 μL), and the mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into ice−water, and the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a resudue, which was purified by HPLC [MeOH−H_2O (80:20, v/v)] to give **3a** (3.3 mg, 50%).

Compound 3a: yellow powder; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.27 [9H, s, −$OCOC(CH_3)_3$], 4.05 (3H, s, −OCH_3), 4.85 (2H, m, H_2-15), 5.50 (1H, m, H-14), 7.26, 7.37 (1H each, both m, H-6, 7), 7.64, 8.21 (1H each, both d, J = 7.3 Hz, H-8, 5), 8.85 (1H, s, H-4), 10.16 (1H, br s, NH-9).

Preparation of the (*R*)-MTPA Esters (1b, 3b) and (*S*)-MTPA Esters (1c, 3c) from 1a and 3a. A solution of **1a** or **3a** (1.2 mg each) in CH_2Cl_2 (1.0 mL) was treated with (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid [(*R*)-MTPA, 3.5 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl, 3.0 mg) and 4-(dimethylamino)pyridine (4-DMAP, 2.5 mg), and the mixture was stirred at room temperature for 10 h. After cooling, the reaction mixture was poured into ice−water, and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was separated by HPLC [MeOH−H_2O (80:20, v/v)] to give **1b** (1.2 mg, 54%) or **3b** (0.5 mg, 26%), respectively. Using a similar procedure, (*S*)-MTPA esters [**1c** (1.1 mg, 41%) or **3c** (0.6 mg, 31%)] were obtained

from **1a** or **3a** (1.2 mg each), respectively, using (*S*)-MTPA (3.5 mg), EDC·HCl (3.0 mg), and 4-DMAP (2.5 mg).

Compound 1b: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.90 (3H, d, J = 7.4 Hz, H_3-15), 3.68 (3H s, $-OCH_3$), 4.05 (3H, s, $-COOCH_3$), 4.30 (1H, m, H-14), [7.36 (1H, m), 7.41 (2H, dd-like), 7.51 (2H, dd-like), Ph-H], 7.33, 7.49 (1H each, both m, H-6, 7), 7.64, 8.16 (1H each, both d, J = 7.0 Hz, H-8, 5), 8.81 (1H, s, H-4), 8.93 (1H, br s, NH-9).

Compound 1c: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.81 (3H, d, J = 7.4 Hz, H_3-15), 3.53 (3H, s, $-OCH_3$), 4.06 (3H, s, $-COOCH_3$), 4.30 (1H, m, H-14), 7.32, 7.47 (1H each, both m, H-6, 7), [7.36 (1H, m), 7.41 (2H, dd-like), 7.51 (2H, dd-like), Ph-H], 7.63, 8.19 (1H each, both d, J = 7.0 Hz, H-8, 5), 8.83 (1H, s, H-4), 9.02 (1H, br s, NH-9).

Compound 3b: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.67 (3H, s, $-OCH_3$), 4.04 (3H, s, $-COOCH_3$), 4.30 (2H, m, H_2-15), 5.94 (1H, m, H-14), [7.36 (1H, m), 7.41 (2H, dd-like), 7.51 (2H, dd-like), Ph-H], 7.40, 7.51 (1H each, both m, H-6, 7), 7.68, 8.16 (1H each, both d, J = 7.3 Hz, H-8, 5), 8.83 (1H, s, H-4), 9.35 (1H, br s, NH-9).

Compound 3c: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.58 (3H, s, $-OCH_3$), 4.05 (3H, s, $-COOCH_3$), 4.26 (2H, m, H_2-15), 5.94 (1H, m, H-14), [7.37 (1H, m), 7.41 (2H, dd-like), 7.52 (2H, dd-like), Ph-H], 7.40, 7.44 (1H each, both m, H-6, 7), 7.64, 8.21 (1H each, both d, J = 7.3 Hz, H-8, 5), 8.86 (1H, s, H-4), 9.46 (1H, br s, NH-9).

Treatment of 4 with 0.1% NaOMe−MeOH. A solution of **4** (7.0 mg) in 0.1% NaOMe−MeOH (2.0 mL) was stirred at room temperature for 7 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form), and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by HPLC [MeOH−H_2O (60:40, v/v)] to give **3** (5.1 mg, 88%).

Bioassay. Animals. Male ddY mice were purchased from Kiwa Laboratory Animal (Wakayama, Japan). The animals were maintained at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chow (MF, Oriental Yeast, Japan) for 1 week. Test samples were suspended with 5% acacia solution, and the solution was administered orally at 10 mL/kg in each experiment, while vehicle was given orally at 10 mg/kg in the corresponding control group.

Effects on Ear Passive Cutaneous Anaphylaxis (PCA) Reaction in Mice. The ear PCA reactions were performed according to the method reported previously with slight modification.[22,23] Briefly, 10 μL of anti-DNP IgE diluted in PBS (20 μg/mL) or PBS alone (normal group) was injected intradermally into both ears of male ddY mice (5−6 weeks old). Forty-seven hours later, test compounds suspended in 5% acacia solution were administrated orally. After 1 h, 0.25 mL of PBS which contained 2% Evans blue and 0.25 mg of DNP-BSA was injected into the vein. Thirty minutes later, mice were killed by cervical dislocation, and both ears were removed and incubated with 1 M KOH solution overnight at 37 °C to dissolve them. The solution was then mixed with 4.5 mL of a mixture of acetone−0.2 M H_3PO_4 (13:5, v/v). After centrifugation at 4000 rpm for 10 min, absorbance was measured at 620 nm using a spectrophotmeter (Beckmann DU 530). Tranilast was used as a reference compound.

Inhibitory Effect on the Release of β-Hexosaminidase in RBL-2H3 Cells. The inhibitory effects of the test samples on the release of β-hexosaminidase from RBL-2H3 cells [Cell No. JCRB0023, obtained from Health Science Research Resources Bank (Osaka, Japan)] were evaluated by a method reported previously.[16−23] Briefly, RBL-2H3 cells [2×10^5 cells/well in Eagle's minimum essential medium (MEM) in a 24-well microplate] were sensitized with anti-DNP IgE. Then the medium was replaced with Siraganian buffer with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA (incubation buffer) and incubated for 10 min at 37 °C. Test sample solution was added to each well and incubated for 10 min, followed by the addition of antigen (DNP-BSA, final concentration 10 μg/mL). Ten minutes after stimulation by the antigen, the supernatant (50 μL) was transferred into a 96-well microplate and incubated with 50 μL of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μL of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured using a microplate reader at 405 nm. The test sample was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the incubation buffer (final DMSO concentration 0.1%). The percent inhibition of the release of β-hexosaminidase by the test material was calculated, and IC_{50} values were determined graphically.

Inhibitory Effect on Antigen-Induced TNF-α and IL-4 Release in RBL-2H3 Cells. Inhibitory effects of test samples on the release of TNF-α and IL-4 in RBL-2H3 cells were evaluated by the method reported previously.[20,23] Briefly, RBL-2H3 cells (2×10^5 cells/well in MEM) were sensitized with anti-DNP IgE. Four hours after stimulation by the antigen in the presence of test samples, TNF-α and IL-4 concentrations in the medium were determined using commercial ELISA kits (TNF-α, rat, ELISA system, code 3012, Biosource International Co., Ltd.; IL-4, rat, ELISA system, code 2737, Amersham Pharmacia Biotech Co., Ltd.). The inhibition % of the release of TNF-α or IL-4 by the test sample was calculated, and IC_{50} values were determined graphically.

Statistics. Values are expressed as mean ± SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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Supporting Information Available: ^{1}H−^{1}H COSY and HMBC correlations of **1**−**6**, structures of known constituents from the dried roots of *S. dichotoma* L. var. *lanceolata*, and inhibitory effects of aqueous EtOH extract on ear PCA reactions in mice (in vivo) and of constituents on release of β-hexosaminidase from RBL-2H3 cells (in vitro). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dedicated to the memory of the late Dr. Yingjie Chen, Professor, Shenyang Pharmaceutical University.
(2) This paper is number 13 in our series Bioactive Constituents of Chinese Natural Medicines. For paper number 12, see ref 22.
(3) Yasukawa, K.; Yamanouchi, S.; Takido, M. *Yakugaku Zasshi* **1981**, *101*, 64−66.
(4) Yasukawa, K.; Yamanouchi, S.; Takido, M. *Yakugaku Zasshi* **1982**, *102*, 292−294.
(5) Morita, H.; Kayashita, T.; Shishido, A.; Takeya, K.; Itokawa, H.; Shiro, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2353−2356.
(6) Morita, H.; Kayashita, T.; Shishido, A.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1996**, *52*, 1165−1176.
(7) Morita, H.; Takeya, K.; Itokawa, H. *Phytochemistry* **1997**, *45*, 841−845.
(8) Morita, H.; Shishido, A.; Kayashita, T.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1997**, *60*, 404−407.
(9) Ohmoto, T.; Koike, K. *Chem. Pharm. Bull.* **1982**, *30*, 1204−1209.
(10) The ^{1}H and ^{13}C NMR spectra of **1**−**6** were assigned with the aid of homo- and heterocorrelation spectroscopy (^{1}H−^{1}H, ^{13}C−^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
(11) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(12) Compound **4** could not be produced by treatment of **2** with *n*-BuOH under reflux for more than 24 h. This suggested that **4** was not artificially produced during the isolation procedure.
(13) We were not able to determine the absolute configuration of the 15-position in **6** due to the small amount isolated.
(14) Schwartz, L. B.; Lewis, R. A.; Seldin, D.; Austen, K. F. *J. Immunol.* **1981**, *126*, 1290−1294.
(15) Marquardt, D. L.; Wasserman, S. I. *J. Immunol.* **1983**, *131*, 934−939.
(16) Matsuda, H.; Morikawa, T.; Tao, J.; Ueda, K.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 208−215.
(17) Morikawa, T.; Tao, J.; Ueda, K.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2003**, *51*, 62−67.
(18) Morikawa, T.; Matsuda, H.; Toguchida, I.; Ueda, K.; Yoshikawa, M. *J. Nat. Prod.* **2002**, *65*, 1468−1474.
(19) Morikawa, T.; Matsuda, H.; Sakamoto, Y.; Ueda, K.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 1045−1049.
(20) Matsuda, H.; Morikawa, T.; Ueda, K.; Managi, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2002**, *10*, 3123−3128.
(21) Tao, J.; Morikawa, T.; Ando, S.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2003**, *51*, 654−662.
(22) Matsuda, H.; Morikawa, T.; Xie, H.; Yoshikawa, M. *Planta Med.*, in press.

(23) Matsuda, H.; Morikawa, T.; Managi, H.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3197−3202.
(24) Kimata, M.; Inagaki, N.; Nagai, H. *Planta Med.* **2000**, *66*, 25−29.
(25) Pelletier, C.; Guerin-Marchand, C.; Iannascoli, B.; Marchand, F.; David, B.; Weyer, A.; Blank, U. *Inflamm. Res.* **1998**, *47*, 493−500.
(26) Sewell, W. A.; Scurr, L. L.; Orphanides, H.; Kinder, S.; Ludowyke, R. I. *Clin. Diagn. Lab. Immunol.* **1998**, *5*, 18−23.
(27) Saito, H.; Yamada, T.; Tachinoto, H. *Saishin Igaku* **1998**, *51*, 2795−2801.
(28) The known compounds (**9**−**11**, **13**, **17**−**24**) were identified by comparison of their physical data (^{1}H and ^{13}C NMR, MS) with commercial samples.
(29) Tanaka, H.; Ichino, K.; Ito, K. *Chem. Pharm. Bull.* **1985**, *33*, 2602−2604.
(30) Schneiders, G. E.; Stevenson, R. *J. Org. Chem.* **1981**, *46*, 2969−2971. (b) Miski, M.; Jakupovic, J. *Phytochemistry* **1990**, *29*, 1995−1998.
(31) Umezawa, B.; Hoshino, O.; Sawaki, S.; Mori, K. *Chem. Pharm. Bull.* **1980**, *28*, 1003−1005. (b) **14**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.59, 2.87 (2H each, both t, J = 7.6 Hz, H_2-8, 7), 3.66, 3.83 (3H each, both s, $-OCH_3$), 6.65 (1H, d, J = 1.3 Hz, H-2), 6.69 (1H, d, J = 7.8 Hz, H-5), 6.83 (1H, dd, J = 1.3, 7.8 Hz, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 30.6 (C-7), 36.0 (C-8), 51.6, 55.8 ($C-OCH_3$), 110.9 (C-6), 114.3 (C-5), 120.6 (C-2), 133.2 (C-1), 143.8 (C-3), 146.3 (C-4), 173.3 (C-9); EIMS m/z 210 $[M]^+$ (30), 137 (100).
(32) Hearn, M. T. W. *Aust. J. Chem.* **1976**, *29*, 107−113.
(33) Hiermann, A.; Kedwani, S.; Schramm, H. W.; Seger, C. *Fitoterapia* **2002**, *73*, 22−27.

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