# Medicinal Flowers. I. Aldose Reductase Inhibitors and Three New Eudesmane-Type Sesquiterpenes, Kikkanols A, B, and C, from the Flowers of *Chrysanthemum indicum* L.

Masayuki YOSHIKAWA,* Toshio MORIKAWA, Toshiyuki MURAKAMI, Iwao TOGUCHIDA, Shoichi HARIMA, and Hisashi MATSUDA

*Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.*
Received September 24, 1998; accepted December 9, 1998

**The methanolic extract from the flowers of *Chrysanthemum indicum* L., Chrysanthemi Indici Flos, was found to show inhibitory activity against rat lens aldose reductase. By bioassay-guided separation, the active components, such as flavone and flavone glycosides, were isolated from the extract together with three new eudesmane-type sesquiterpenes, kikkanols A, B, and C. The structures of kikkanols A, B, and C were elucidated on the basis of chemical and physicochemical evidence, which included application of the modified Mosher's method.**

**Key words** kikkanol; *Chrysanthemum indicum*; aldose reductase inhibitor; medicinal flower; eudesmane-type sesquiterpene; flavonoid

The flowers of *Chrysanthemum indicum* L. and related Chrysanthemum plants (Compositae) have been used as a Chinese natural medicine, Chrysanthemi Indici Flos [Yagikka (野菊花) in Japanese], which is prescribed for antiinflammatory, analgesic, and antipyretic purposes and the treatment of eye disease in Chinese traditional preparations. As chemical constituents of this plant, several bisabolane- and guaiane-type sesquiterpenes have been isolated from the flowers and aerial parts of European *C. indicum*,[1] and the composition of the essential oil and isolation of a flavonoid have been also reported.[2] In addition, several flavonoids and sesquiterpenes have been isolated from the Chinese natural medicine.[3] However, the pharmacological activity and bioactive constituents of this natural medicine are left uncharacterized.

In the course of our studies on bioactive principles of natural medicines[4] and medicinal foodstuffs,[5] we have found that the methanolic extract from the flowers of *C. indicum* exhibited potent inhibitory activity against rat lens aldose reductase. From the methanolic extract, active components such as flavone and flavone glycosides were isolated by bioassay-guided separation using aldose reductase inhibitory activity together with three new eudesmane-type sesquiterpenes called kikkanols A (**1**), B (**2**), and C (**3**). In this paper, we describe the characterization of the inhibitors from the flowers of *C. indicum* against rat lens aldose reductase and the structure elucidation of kikkanols (**1**—**3**).

The flowers of Chinese *C. indicum* were extracted with methanol under reflux. Since the methanolic extract was found to exhibit inhibitory activity against rat lens aldose reductase, the methanolic extract was partitioned in an ethyl acetate/water mixture to give an ethyl acetate-soluble portion and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble portion and a water-soluble portion. As is apparent from Table 1, the ethyl acetate-soluble and 1-butanol-soluble portions showed inhibitory activity ($IC_{50}$ 1.3 $\mu$g/ml and 3.5 $\mu$g/ml, respectively). The ethyl acetate-soluble portion was subjected to silica gel and ODS column chromatography and finally HPLC to furnish kikkanols A (**1**, 0.0008% from the natural medicine), B (**2**, 0.0003%), and C (**3**, 0.0007%), *cis*-spiroketalenolether polyyne[6] (**4**, 0.079%), *trans*-spiroketalenolether polyyne[6] (**5**, 0.015%), clovanediol[7] (**6**, 0.0017%), caryolane 1,9 $\beta$-diol[8] (**7**, 0.0007%), oplopanone[9] (**8**, 0.012%), eupatilin[7] (**9**, 0.0008%), and luteolin[10] (**10**, 0.0016%). From the 1-butanol-soluble portion, luteolin 7-*O*-$\beta$-D-glucopyranoside[10] (**11**, 0.13%), luteolin 7-*O*-$\beta$-D-glucopyranosiduronic acid[11] (**12**, 0.048%), acacetin 7-*O*-(6″-$\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucopyranoside[12] (**13**, 0.014%), and chlorogenic acid[13] (**14**, 0.058%) were isolated by silica gel and ODS column chromatography.

**Structures of Kikkanols A (1), B (2), and C (3)** Kikkanol A (**1**) was isolated as a colorless needles of mp 165—167 °C with positive optical rotation ($[\alpha]_D^{27}$ +57.2°). The electron impact (EI)-MS of **1** showed a molecular ion ($M^+$) peak at *m*/*z* 254 in addition to fragment ion peaks at *m*/*z* 236 ($M^+$–$H_2O$) and *m*/*z* 123 (base peak). The molecular formula $C_{15}H_{26}O_3$ of **1** was determined from the molecular ion peak observed in the EI-MS and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl and olefin functions at 3532, 3456, 1647, and 1073 $cm^{-1}$. The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR (Table 2) spectra of **1** showed signals assignable to a tertiary methyl [$\delta$ 1.66 (s, 14-$H_3$)], two secondary methyls [$\delta$ 1.02, 1.03 (both d, $J$=6.4 Hz, 12, 13-$H_3$)], two methines bearing a hydroxyl group [$\delta$ 4.54 (br s, 6-H), 4.78 (dd, $J$=6.4, 10.1 Hz, 1-H)], and an *exo*-methylene [$\delta$ 5.17, 5.84 (both dd, $J$=2.1, 2.1 Hz, 15-$H_2$)] together with four methylenes (2, 3, 8, 9-$H_2$), two methines (7, 11-H), and three quaternary carbons (4, 5, 10-C).

The plane structure of **1** was constructed on the basis of

Table 1. Inhibitory Activity of MeOH Extract, AcOEt-, 1-BuOH-, and $H_2O$-Soluble Fractions from *C. indicum* on Rat Lens Aldose Reductase

| | $IC_{50}$ ($\mu$g/ml) |
|---|---|
| MeOH extract | 3.5 |
| AcOEt-soluble fraction | 1.3 |
| 1-BuOH-soluble fraction | 3.5 |
| $H_2O$-soluble fraction | 113 |

* To whom correspondence should be addressed.

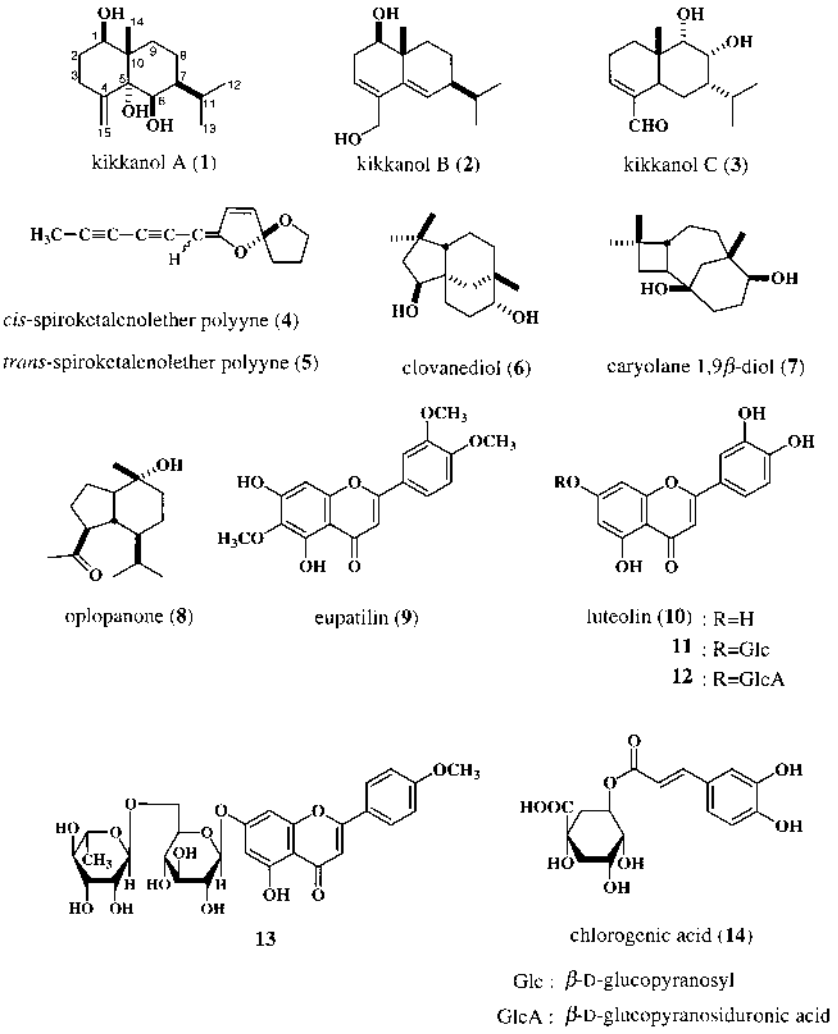


Chart 1

$^1$H–$^1$H correlation spectroscopy (H–H COSY) and heteronuclear multiple bond correlation (HMBC) experiments. Thus, the H–H COSY experiment on **1** indicated the presence of two partial structures as shown in Fig. 1: from C-1—C-3, from C-6—C-9 and C-7—C-13. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **1** (4-C and 3, 15-$H_2$; 5-C and 6-H, 14-$H_3$; 10-C and 1-H, 14-$H_3$), so that the connectivities of the quaternary carbons in **1** were clarified. The above-mentioned evidence led us to confirm the skeleton of kikkanol A (**1**) to be 4(15)-eudesmene 1,5,6-triol.

The $^1$H-NMR nuclear overhauser effect spectroscopy (NOESY) experiment on **1** showed NOE correlations between the signals of following proton pairs (1-H and 3$\alpha$, 9$\alpha$-H; 7-H and 6, 8$\alpha$, 9$\alpha$-H; 14-$H_3$ and 8$\beta$, 9$\beta$-H), as depicted in Fig. 1. The coupling patterns of the 1-proton (dd, $J$=6.4, 10.1 Hz) and 6-proton (br s) indicated the configurations of the 1- and 6-hydroxyl groups to be equatorial and axial, respectively. Furthermore, examination of the pyridine-$d_5$ induced solvent shift in $^1$H-NMR spectrum,[14] showed that signals due to the 1-[$\delta$ 3.96 (dd, $J$=5.3, 11.6 Hz)], 3$\alpha$-[$\delta$ 2.71 (m)], 6-[$\delta$ 3.99 (br s)], and 7-[$\delta$ 1.67 (m)] protons in the $CD_3OD$ solution were markedly deshielded in the pyridine-$d_5$ solution at $\delta$ 4.78 (dd, $J$=6.4, 10.1 Hz, 1-H), 3.21 (br ddd, 3$\alpha$-H), 4.54 (br s, 6-H), and 2.25 (br ddd, 7-H). Thus, the

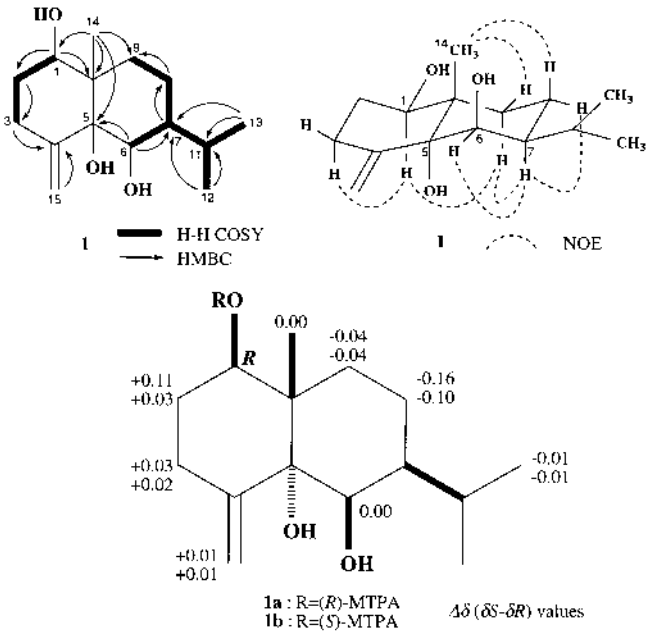


Fig. 1

Table 2. $^{13}$C-NMR Data on Kikkanols A (**1**), B (**2**), B 15-Pivalate (**2a**), and C (**3**)

| Position | **1**[a)] | **2**[b)] | **2a**[b)] | **3**[b)] |
|---|---|---|---|---|
| C-1 | 73.0 | 75.6 | 75.8 | 25.8 |
| C-2 | 31.9 | 31.5 | 31.6 | 24.4 |
| C-3 | 31.8 | 122.7 | 124.9 | 150.4 |
| C-4 | 151.5 | 138.8 | 138.7 | 144.8 |
| C-5 | 77.0 | 135.5 | 131.5 | 35.3 |
| C-6 | 73.3 | 126.5 | 126.7 | 27.8 |
| C-7 | 44.6 | 42.4 | 42.5 | 42.2 |
| C-8 | 21.3 | 20.2 | 20.5 | 72.5 |
| C-9 | 32.0 | 33.7 | 33.7 | 78.1 |
| C-10 | 42.6 | 37.9 | 37.9 | 35.0 |
| C-11 | 28.9 | 32.2 | 32.3 | 28.3 |
| C-12 | *21.5 | *19.6 | *19.7 | *21.1 |
| C-13 | *21.1 | *18.9 | *19.1 | *20.5 |
| C-14 | 14.0 | 16.3 | 16.3 | 20.8 |
| C-15 | 108.9 | 64.2 | 65.0 | 194.2 |
| ($\underline{C}H_3$)CO- | | | 27.0 | |
| ($CH_3$)$\underline{C}$O- | | | 178.2 | |

Measured in *a*) pyridine-$d_5$, *b*) $CDCl_3$ at 125 MHz. * Assignments may be interchangeable within the same column.

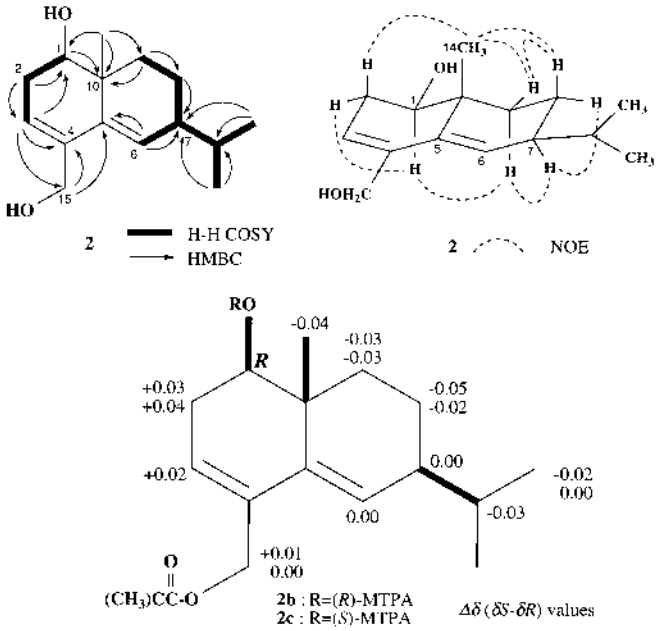


Fig. 2

configuration of the 5-hydroxyl group was shown to be $\alpha$-axial. On the basis of these findings, the relative stereostructure of **1** was elucidated.

The absolute configuration of **1** was determined by application of the modified Mosher's method[15] for the 1-mono-(*R*)- and (*S*)-2-methoxy-2-trifluorophenylacetate (MTPA esters, **1a** and **1b**), which were prepared by selective esterification of the 1-equatorial hydroxyl group in **1** with (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid in the presence of 1-ethyl 3-(3-dimethylaminopropyl)-carbodiimide·HCl (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP). Here again, the relative stereostructures of the 1-, 6-, and 7-positions in **1** were confirmed by examination of the $^1$H-NMR data of **1a** and **1b**. Thus, the $^1$H-NMR ($CDCl_3$) spectra of **1a** and **1b** showed signals assignable to the 1-axial, 6-equatorial, and 7-axial protons [**1a**: $\delta$ 5.46 (dd, $J$=4.6, 11.7 Hz, 1-H), 3.99 (d, $J$=2.3 Hz, 6-H), 1.36 (dddd, $J$=1.6, 2.3, 4.4, 12.7 Hz, 7-H); **1b**: $\delta$ 5.46 (dd, $J$=4.9, 11.6 Hz, 1-H), 3.99 (d, $J$=3.1 Hz, 6-H), 1.36 (dddd, $J$=1.9, 3.1, 4.9, 12.1 Hz, 7-H)]. As shown in Fig. 1, the signals due to protons attached to the 2, 3, and 15-carbons in the (*S*)-MTPA ester (**1b**) were observed at a lower field compared with those of the (*R*)-MTPA ester (**1a**) [$\Delta\delta$: positive], while the signals due to protons on the 8, 9, and 12-carbons in **1b** were observed at higher fields compared with those of **1a** [$\Delta\delta$: negative]. Thus, the absolute configuration at the 1-carbon in **1** has been shown to be *R* and the absolute stereostructure of kikkanol A (**1**) has been determined as shown.

Kikkanol B (**2**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{22}$ −28.0°). The molecular formula $C_{15}H_{24}O_2$ of **2** has been determined for the molecular ion peak at *m/z* 236 ($M^+$) in the EI-MS of **2** and by high-resolution MS measurement. The IR spectrum of **2** showed absorption bands ascribable to hydroxyl and olefin functions at 3460, 1653, and 1060 $cm^{-1}$, while its UV spectrum indicated the presence of a hetero-annular diene chromophore from a characteristic triplet with absorption maxima at 238 (log $\varepsilon$ 3.93), 271 (3.05), and 336 (3.05) nm. The $^1$H-NMR ($CDCl_3$) and $^{13}$C-NMR (Table 2) spectra of **2** showed signals assignable to a tertiary methyl [$\delta$ 0.92 (s, 14-$H_3$)], two secondary methyls [$\delta$ 0.89, 0.93 (both d, $J$=6.7 Hz, 12, 13-$H_3$)], a hydroxymethyl [$\delta$ 4.20, 4.29 (ABq, $J$=13.2 Hz, 15-$H_2$)], a methine bearing a hydroxyl group [$\delta$ 3.54 (dd, $J$=5.8, 10.1 Hz, 1-H)], and two olefin [$\delta$ 5.66 (d, $J$=2.1 Hz, 6-H), 5.69 (br d, 3-H)] together with three methylenes, two methines, and three quaternary carbons. The connectivities of the $^1$H–$^1$H and the quaternary carbons in **2** was clarified by H–H COSY and HMBC experiments as shown in Fig. 2. Thus, long-range correlations were observed between the following protons and carbons of **2** (4-C and 3-H, 15-$H_2$; 5-C and 6-H, 15-$H_2$; 10-C and 1-H, 9-$H_2$, 14-$H_3$) in the HMBC experiment. On the basis of this evidence, the plane structure of **2** was characterized to be 3,5(6)-eudesmadien-1,15-diol.

Next, the relative stereostructure of **2** was elucidated by a NOESY experiment, which showed NOE correlations between the following proton pairs (1-H and 2$\alpha$, 9$\alpha$-H; 7-H and 8$\alpha$, 9$\alpha$-H; 14-$H_3$ and 2$\beta$, 8$\beta$-H), as depicted in Fig. 2. In order to characterize the absolute stereostructure of **2**, the 15-pivaloyl ester (**2a**), which was obtained by selective esterification of the 15-primary hydroxyl group in **2** with pivaloyl chloride, was subjected to the modified Mosher's method. As shown in Fig. 2, the signals due to protons attached to the 2, 3, and 15-carbons in the 1-mono-(*S*)-MTPA ester (**2c**) were observed at higher fields compared with those of the 1-mono-(*R*)-MTPA (**2b**) [$\Delta\delta$: positive], while the signals due to protons of 8, 9, 11, and 12-carbons in **2c** were observed at lower fields compared with those of **2b** [$\Delta\delta$ : negative]. Consequently, the absolute configuration at the 1-position of **2** has been elucidated as *R* and the absolute stereostructure of kikkanol B (**2**) was determined to be as shown.

Kikkanol C (**3**) was also isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{22}$ +7.7°). Here again, the molecular formula $C_{15}H_{24}O_3$ of **3** was determined from the EI-MS [*m/z* 252 ($M^+$)] and by high-resolution MS measurement. In the UV spectrum of **3**, an absorption maximum was observed at 234 nm (log $\varepsilon$ 3.97), suggestive of an $\alpha,\beta$-unsaturated carbonyl function. The IR spectrum of **3** showed absorption

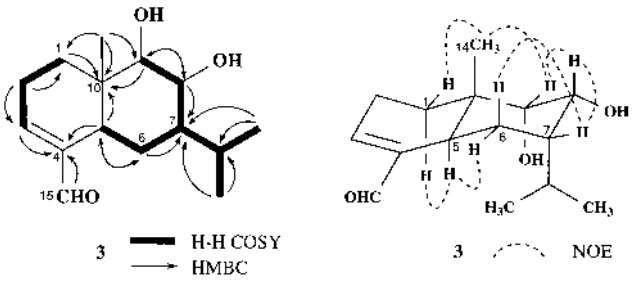


Fig. 3

Table 3. Inhibitory Activity of Constituents from *C. indicum* on Rat Lens Aldose Reductase

| Compounds | $IC_{50}$ ($\mu$M) |
|---|---|
| Kikkanol A (**1**) | >100 (3.3) |
| Kikkanol C (**3**) | >100 (9.4) |
| *cis*-Spiroketalnenolether polyyne (**4**) | >100 (24.2) |
| *trans*-Spiroketalnenolether polyyne (**5**) | >100 (21.1) |
| Clovanediol (**6**) | 96 |
| Caryolane 1,9$\beta$-diol (**7**) | 45 |
| Oplopanone (**8**) | >100 (24.2) |
| Eupatilin (**9**) | 25 |
| Luteolin (**10**) | 0.45 |
| Luteolin 7-*O*-$\beta$-D-glucopyranoside (**11**) | 0.99 |
| Luteolin 7-*O*-$\beta$-D-glucopyranosiduronic acid (**12**) | 3.1 |
| Acacetin 7-*O*-(6″-$\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucopyranoside (**13**) | 4.7 |
| Chlorogenic acid (**14**) | 1.8 |
| Epalrestat | 0.072 |

( ): values in parenthesis represent the (%) inhibition at 100 $\mu$M.

bands assignable to hydroxyl and $\alpha,\beta$-unsaturated carbonyl functions at 3456, 1669, 1634, and 1024 $cm^{-1}$. The $^1$H-NMR ($CDCl_3$) and $^{13}$C-NMR (Table 2) spectra of **3** showed signals due to a tertiary methyl [$\delta$ 0.89 (s, 14-$H_3$)], two secondary methyls [$\delta$ 0.95, 0.96 (both d, $J$=6.1 Hz, 12, 13-$H_3$)], two methine bearing a hydroxyl group [$\delta$ 3.49 (d, $J$=2.7 Hz, 9-H), 4.09 (br d, 8-H)], an olefin [$\delta$ 6.70 (dd, $J$=3.4, 3.7 Hz, 3-H)], and an aldehyde [$\delta$ 9.42 (s, 15-H)] together with three methylenes, two methines, and two quaternary carbons. The 8,9-dihydroxy-3-eudesmen-15-al structure of **3** was elucidated on the basis of H–H COSY and HMBC experiments as shown in Fig. 3. Furthermore, the relative stereostructure of **3** was clarified by a NOESY experiment, which showed NOE correlations between the following proton pairs (1$\alpha$-H and 5-H; 1$\beta$-H and 14-$H_3$; 5-H and 6$\alpha$-H; 6$\beta$-H and 7-H; 7-H and 8-H; 8-H and 9-H; 9-H and 14-$H_3$). This evidence allowed us to confirm the stereostructure of kikkanol C (**3**).[16]

**Inhibitory Effect of Constituents from Flowers of *C. indicum* on Rat Lens Aldose Reductase** Aldose reductase as a key enzyme in the polyol pathway is reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract. Since the flowers of *C. indicum* has been proved useful for the treatment of eye disease in Chinese traditional medicine, we examined the inhibitory activity of extracts (*vide ante*) and the components isolated from the active fractions on rat lens aldose reductase. As shown in Table 3, a flavone, luteolin (**10**), three flavone glycosides (**11**—**13**), and chlorogenic acid (**14**) were potent inhibitories of rat lens aldose reductase, but their activity was weaker than that of a commercial synthetic aldose reductase inhibitor, epalrestat.[17] Another flavone, eupatilin (**9**), and two sesquiterpenes (**6**, **7**) exhibited less activity than luteolin (**10**) and flavone glycosides (**11**—**13**). Other sesquiterpenes (**1**, **3**, **8**) and two polyacetylenes (**4**, **5**) exhibited little activity. Taking this data into account, these results indicate that flavonoids and chlorogenic acid are active principles of this natural medicine.

**Experimental**

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter ($l$=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GC-MATE mass spectrometer; $^1$H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; $^{13}$C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60$F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 60$F_{254}$ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 60$WF_{254S}$ (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous $H_2SO_4$ and heating.

**Extraction and Isolation** Dried flowers of *C. indicum* (5.8 kg, cultivated in China and purchased from Koshiro Co. Ltd., Osaka) were finely minced and extracted with methanol under reflux. Evaporation of the solvent under reduced pressure gave the MeOH extract (1650 g). The MeOH extract (1600 g) was partitioned in a AcOEt–$H_2O$ (1 : 1) mixture, and the aqueous layer was further extracted with 1-BuOH. Removal of the solvent under reduced pressure from the AcOEt-, 1-BuOH-, and water-soluble portions yielded 438 g, 226 g, and 900 g of residues, respectively.

The AcOEt-soluble portion (150 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, *n*-hexane–AcOEt→$CHCl_3$–MeOH→$CHCl_3$–MeOH–$H_2O$] to afford twelve fractions [fr. 1 (14.2 g), fr. 2 (10.7 g), fr. 3 (5.6 g), fr. 4 (5.6 g), fr. 5 (10.8 g), fr. 6 (7.3 g), fr. 7 (8.1 g), fr. 8 (3.7 g), fr. 9 (3.4 g), fr. 10 (6.3 g), fr. 11 (56.4 g), fr. 12 (17.9 g)]. Fraction 3 (5.6 g) was further subjected to reversed-phase silica gel column chromatography to furnish *cis*-spiroketalenolether polyyne (**4**, 1.5 g). Fraction **5** (10.8 g) was separated by reversed-phase silica gel column chromatography and, finally, HPLC [YMC-pack ODS-A, MeOH–$H_2O$] to give *trans*-spiroketalenolether polyyne (**5**, 285 mg). Fraction 8 (3.7 g) was purified by reversed-phase silica gel column chromatography and finally HPLC [YMC-pack ODS-A, MeOH–$H_2O$] to furnish kikkanols A (**1**, 15 mg), B (**2**, 6 mg), and C (**3**, 13 mg), oplopanone (**8**, 228 mg) and eupatilin (**9**, 15 mg). Fraction 10 (6.3 g) was subjected to reversed-phase silica gel column chromatography and finally HPLC to furnish clovanediol (**6**, 32 mg), caryolane 1,9$\beta$-diol (**7**, 14 mg), and luteolin (**10**, 32 mg). The 1-BuOH-soluble portion (182 g) was also subjected to ordinary-phase silica gel column chromatography [$CHCl_3$–MeOH–$H_2O$] to afford seven fractions [fr. 1 (7.5 g), fr. 2 (38.6 g), fr. 3 (21.9 g), fr. 4 (28.2 g), fr. 5 (14.2 g), fr. 6 (22.2 g), fr. 7 (32.3 g)]. Fraction 3 (21.9 g) was further subjected to reversed-phase silica gel column chromatography to furnish luteolin 7-*O*-$\beta$-D-glucopyranoside (**11**, 5.9 g), chlorogenic acid (**14**, 610 mg), and acacetin 7-*O*-(6″-$\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucopyranoside (**13**, 800 mg). Fraction 5 (14.2 g) was purified by reversed-phase silica gel column chromatography to furnish luteolin 7-*O*-$\beta$-D-glucopyranosiduronic acid (**12**, 2.2 g). These constituents were identified by comparison of their physical data with those of authentic samples (**6**, **9**, **13**) or with reported values.[6,8—12]

Kikkanol A (**1**): Colorless fine crystals from *n*-hexane–AcOEt, mp 165—167 °C, $[\alpha]_D^{27}$ +57.2 ° ($c$=2.1, MeOH). High-resolution EI-MS: Calcd for $C_{15}H_{26}O_3$ ($M^+$): 254.1882. Found: 254.1899. IR (film): 3532, 3456, 2958, 1647, 1073 $cm^{-1}$. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.02, 1.03 (3H each, both d, $J$=6.4 Hz, 12 and 13-$H_3$), 1.66 (3H, s, 14-$H_3$), 1.84 (1H, m, 8$\alpha$-H), 1.96 (1H, m, 8$\beta$-H), 1.98 (1H, dq, $J$=3.7, 6.4 Hz, 11-H), 2.11 (2H, m, 2-$H_2$), 2.25 (1H, br ddd, 7-H), 2.26 (1H, m, 9$\beta$-H), 2.33 (1H, ddd-like, 3$\beta$-H), 2.34 (1H, m, 9$\alpha$-H), 3.21 (1H, br ddd, 3$\alpha$-H), 4.54 (1H, br s, 6-H), 4.78 (1H, dd, $J$=6.4,

10.1 Hz, 1-H), 5.17, 5.84 (1H each, both dd, $J$=2.1, 2.1 Hz, 15-$H_2$); ($CD_3OD$) $\delta$: 0.95 (3H, s, 14-$H_3$), 0.95, 0.97 (3H each, both d, $J$=6.2 Hz, 12 and 13-$H_3$), 1.56, 1.58 (1H each, both m, 8-$H_2$), 1.61, 1.63 (1H each, both m, 9-$H_2$), 1.65 (1H, m, 11-H), 1.67 (1H, m, 7-H), 1.68 (2H, m, 2-$H_2$), 2.11 (1H, m, 3$\beta$-H), 2.71 (1H, m, 3$\alpha$-H), 3.96 (1H, dd, $J$=5.3, 11.6 Hz, 1-H), 3.99 (1H, br s, 6-H), 4.91, 5.26 (1H each, both dd-like, 15-$H_2$). $^{13}$C-NMR (pyridine-$d_5$) $\delta_c$: given in Table 2. EI-MS $m/z$ (%): 254 ($M^+$, 2), 236 ($M^+-H_2O$, 6), 123 (100).

Kikkanol B (**2**): Colorless oil, $[\alpha]_D^{22}$ −28.0° ($c$=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{24}O_2$ ($M^+$): 236.1776. Found: 236.1774. UV (MeOH, nm, log $\varepsilon$) 238 (3.93), 271 (3.05), 336 (3.05). IR (film) 3460, 2962, 1653, 1060 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.89, 0.93 (3H each, both d, $J$=6.7 Hz, 12 and 13-$H_3$), 0.92 (3H, s, 14-$H_3$), 1.33 (1H, m, 9$\alpha$-H), 1.43 (1H, dddd, $J$=3.4, 11.0, 13.7, 16.4 Hz, 8$\beta$-H), 1.68 (1H, m, 8$\alpha$-H), 1.71 (1H, m, 11-H), 1.90 (1H, ddd, $J$=3.4, 3.4, 12.5 Hz, 9$\beta$-H), 2.11 (1H, m, 7-H), 2.18 (1H, m, 2$\beta$-H), 2.43 (1H, ddd, $J$=5.5, 5.8, 13.6 Hz, 2$\alpha$-H), 3.54 (1H, dd, $J$=5.8, 10.1 Hz, 1-H), 4.20, 4.29 (2H, ABq, $J$=13.2 Hz, 15-$H_2$), 5.66 (1H, d, $J$=2.1 Hz, 6-H), 5.69 (1H, br d, 3-H). $^{13}$C-NMR ($CDCl_3$) $\delta_c$: given in Table 2. EI-MS $m/z$ (%): 236 ($M^+$, 34), 218 ($M^+-H_2O$, 79), 193 (100).

Kikkanol C (**3**): Colorless oil, $[a]_D^{22}$ +7.7° ($c$=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{24}O_3$ ($M^+$): 252.1725. Found: 252.1727. UV (MeOH, nm, log $\varepsilon$): 234 (3.97). IR (film): 3456, 2939, 1669, 1636, 1024 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, s, 14-$H_3$), 0.95, 0.96 (3H each, both d, $J$=6.1 Hz, 12 and 13-$H_3$), 1.04 (1H, ddd, $J$=5.8, 12.5, 12.8 Hz, 6$\beta$-H), 1.15 (1H, ddd, $J$=3.3, 5.8, 13.7 Hz, 1$\alpha$-H), 1.50 (1H, m, 7-H), 1.53 (1H, m, 11-H), 1.79 (1H, ddd, $J$=3.3, 3.7, 12.8 Hz, 6$\alpha$-H), 2.40 (2H, m, 2-$H_2$), 2.50 (1H, m, 1$\beta$-H), 2.52 (1H, m, 5-H), 3.49 (1H, d, $J$=2.7 Hz, 9-H), 4.09 (1H, br d, 8-H), 6.70 (1H, dd, $J$=3.4, 3.7 Hz, 3-H), 9.42 (1H, s, 15-H). $^{13}$C-NMR ($CDCl_3$) $\delta_c$: given in Table 2. EI-MS: $m/z$ (%): 252 ($M^+$, 6), 234 ($M^+-H_2O$, 19), 166 (100).

**Preparation of the (*R*)-MTPA Ester (1a) and the (*S*)-MTPA Ester (1b) from Kikkanol A (1)** A solution of **1** (2.0 mg, 7.9 $\mu$mol) in $CH_2Cl_2$ (1.0 ml) was treated with (*R*)-MTPA (9.2 mg, 39.4 $\mu$mol) in the presence of EDC·HCl (7.6 mg, 39.4 $\mu$mol) and 4-DMAP (2.9 mg, 23.6 $\mu$mol) and the mixture was heated under reflux for 6 h. After cooling, it was poured into ice-water and the whole was extracted with AcOEt. The AcOEt 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 purified by silica gel column chromatography (0.2 g, *n*-hexane–AcOEt=5 : 1) to give **1a** (1.7 mg) and **1** (0.9 mg). Through a similar procedure, **1b** (1.2 mg) was prepared from **1** (2.0 mg) using (*S*)-MTPA (9.2 mg), EDC·HCl (7.6 mg) and 4-DMAP (2.9 mg).

**1a**: Colorless oil, $^1$H-NMR ($CDCl_3$) $\delta$: 0.97, 0.98 (3H each, both d, $J$=6.6 Hz, 12 and 13-$H_3$), 1.01 (3H, s, 14-$H_3$), 1.36 (1H, dddd, $J$=1.6, 2.3, 4.4, 12.7 Hz, 7-H), 1.43 (1H, m, 8-H), 1.56 (1H, m, 11-H), 1.65 (1H, m, 8-H), 1.66 (2H, m, 9-$H_2$), 1.71 (1H, m, 2-H), 1.99 (1H, m, 2-H), 2.25 (1H, ddd, $J$=4.5, 5.1, 13.4 Hz, 3-H), 2.75 (1H, ddd-like, 3-H), 3.52 (3H, s, OMe), 3.99 (1H, d, $J$=2.3 Hz, 6-H), 5.04, 5.30 (1H each, both s, 15-$H_2$), 5.46 (1H, dd, $J$=4.6, 11.7 Hz, 1-H), 7.39—7.54 (5H, m, Ph).

**1b**: Colorless oil, $^1$H-NMR ($CDCl_3$) $\delta$: 0.96, 0.97 (3H each, both d, $J$=6.4 Hz, 12 and 13-$H_3$), 1.01 (3H, s, 14-$H_3$), 1.27 (1H, dddd, $J$=3.6, 4.9, 7.3, 14.1 Hz, 8-H), 1.36 (1H, dddd, $J$=1.9, 3.1, 4.9, 12.1 Hz, 7-H), 1.55 (1H, m, 8-H), 1.56 (1H, m, 11-H), 1.62 (2H, m, 9-H2), 1.82 (1H, dddd, $J$=5.8, 8.5, 11.6, 13.7 Hz, 2-H), 2.02 (1H, dddd, $J$=4.9, 6.4, 7.3, 13.7 Hz, 2-H), 2.28 (1H, ddd, $J$=5.8, 7.3, 14.4 Hz, 3-H), 2.77 (1H, ddd, $J$=6.4, 8.5, 14.4 Hz, 3-H), 3.52 (3H, s, OMe), 3.99 (1H, d, $J$=3.1 Hz, 6-H), 5.05, 5.31 (1H each, both s, 15-$H_2$), 5.46 (1H, dd, $J$=4.9, 11.6 Hz, 1-H), 7.38—7.53 (5H, m, Ph).

**Treatment of 2 with Pivaloyl Chloride and Pyridine** A solution of **2** (2.1 mg, 8.9 $\mu$mol) in pyridine (0.5 ml) was treated with pivaloyl chloride (30 $\mu$l) and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was treated in the usual manner to give a residue, which was purified by silica gel column chromatography (0.5 g, *n*-hexane–AcOEt=5 : 1) to furnish **2a** (2.2 mg).

**2a**: Colorless oil, $^1$H-NMR ($CDCl_3$) $\delta$: 0.89, 0.93 (3H each, both d, $J$=6.7 Hz, 12 and 13-$H_3$), 0.91 (3H, s, 14-$H_3$), 1.19 (9H, s, pivaloyl-$CH_3$), 1.30 (1H, m, 9-H), 1.42 (1H, m, 8-H), 1.65 (2H, m, 8 and 11-H), 1.89 (1H, ddd, $J$=3.1, 3.3, 12.2 Hz, 9-H), 2.06 (1H, m, 7-H), 2.18 (1H, ddd, $J$=1.9, 10.1, 18.6 Hz, 2-H), 2.43 (1H, ddd, $J$=5.8, 6.1, 18.6 Hz, 2-H), 3.53 (1H, dd, $J$=6.1, 10.1 Hz, 1-H), 4.63, 4.71 (2H, ABq, $J$=12.5 Hz, 15-$H_2$), 5.58 (1H, d, $J$=1.8 Hz, 6-H), 5.69 (1H, dd, $J$=1.9, 5.8 Hz, 3-H). $^{13}$C-NMR ($CDCl_3$) $\delta_c$: given in Table 2. EI-MS $m/z$ (%): 320 ($M^+$, 3), 302 ($M^+-H_2O$, 1), 218 (100).

**Preparation of the (*R*)-MTPA Ester (2b) and the (*S*)-MTPA Ester (2c) from Kikkanol B (2)** A solution of **2a** (1.0 mg, 3.1 $\mu$mol) in $CH_2Cl_2$ (0.5 ml) was treated with (*R*)-MTPA (7.5 mg, 32.0 $\mu$mol) in the presence of EDC·HCl (6.1 mg, 32.0 $\mu$mol) and 4-DMAP (2.3 mg, 19.2 $\mu$mol) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above gave a product which was purified by silica gel column chromatography (0.5 g, *n*-hexane–AcOEt=5 : 1) to yield **2b** (1.1 mg). **2c** (1.0 mg) was obtained from **2a** (1.0 mg) using (*S*)-MTPA (7.5 mg), EDC·HCl (6.1 mg) and 4-DMAP (2.7 mg) by the same procedure.

**2b**: Colorless oil, $^1$H-NMR ($CDCl_3$) $\delta$: 0.89, 0.91 (3H each, both d, $J$=7.3 Hz, 12 and 13-$H_3$), 0.92 (3H, s, 14-$H_3$), 1.19 (9H, s, pivaloyl-$CH_3$), 1.35 (1H, m, 9-H), 1.40 (1H, m, 8-H), 1.67 (1H, m, 11-H), 1.74 (1H, m, 8-H), 2.02 (1H, m, 9-H), 2.05(1H, m, 7-H), 2.34 (1H, m, 2-H), 2.63 (1H, m, 2-H), 3.53 (3H, s, OMe), 4.64, 4.71 (2H, ABq, $J$=12.8 Hz, 15-$H_2$), 4.94 (1H, dd, $J$=5.8, 10.7 Hz, 1-H), 5.65 (1H, d, $J$=2.7 Hz, 6-H), 5.68 (1H, dd-like, 3-H), 7.41—7.55 (5H, m, Ph).

**2c**: Colorless oil, $^1$H-NMR ($CDCl_3$) $\delta$: 0.87, 0.91 (3H each, both d, $J$=7.4 Hz, 12 and 13-$H_3$), 0.88 (3H, s, 14-$H_3$), 1.19 (9H, s, pivaloyl- $CH_3$), 1.32 (1H, m, 9-H), 1.35 (1H, m, 8-H), 1.64 (1H, m, 11-H), 1.72 (1H, m, 8-H), 1.99 (1H, m, 9-H), 2.05(1H, m, 7-H), 2.37 (1H, m, 2-H), 2.67 (1H, m, 2-H), 3.54 (3H, s, OMe), 4.65, 4.71 (2H, ABq, $J$=13.2 Hz, 15-$H_2$), 4.95 (1H, dd, $J$=5.8, 10.3 Hz, 1-H), 5.65 (1H, d, $J$=2.1 Hz, 6-H), 5.70 (1H, dd-like, 3-H), 7.40—7.55 (5H, m, Ph).

**Bioassay** Aldose reductase activity was assayed by the method described in a previous paper.[4a] The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM $Li_2SO_4$, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 $\mu$l of enzyme fraction, with or without 25 $\mu$l of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 $\mu$l 0.5 N HCl. Then, 0.5 ml 6 N NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a spectrofluorometer (Type 650-10, Hitachi, Japan) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

## References and Notes


1) *a*) Hausen B. M., Schulz K. H., Jarchow O., Klaska K. H., Schmalle H., *Naturwissenschaften*, **62**, 585—586 (1975) [*Chem. Abstr*., **84**, 57278*k* (1976)]; *b*) Hausen B. M., Schulz K. H., *Arch. Dermatol. Res*., **255**, 111—121 (1976) [*Chem. Abstr*., **85**, 18792*z* (1976)]; *c*) Stoyanova-Ivanova B., Hinkovska V. K., Mladenova K., Iochkova I., Lashev L., Dimitrov G., *Int. Conf. Chem. Biotechnol. Biol. Act. Nat. Prod*., **3**, 209—214 (1981) [*Chem. Abstr*., **97**, 36144*s* (1982)]; *d*) Mladenova K., Tsankova E., Stoianova-Ivanova B., *Planta Med*., **51**, 284—285 (1985); *e*) Mladenova K., Tsankova E., Kostova I., Stoianova-Ivanova B., *ibid*., **53**, 118—119 (1987); *f*) Mladenova K., Tsankova E., Van Hung D., *ibid*., **53**, 553—555 (1987); *g*) Mladenova K., Tsankova E., Van Hung D., Budesinsky M., *Dokl. Bolg. Akad. Namk*., **41**, 81—83 (1988) [*Chem. Abstr*., **109**, 226742*z* (1988)]; *h*) Mladenova K., Mikhailova R., Tsutsulova A., Beremliiski K., Stoianova-Ivanova B., *ibid*., **42**, 39—41 (1989) [*Chem. Abstr*., **112**, 83832*c* (1989)].

2) *a*) Uchio Y., Tomosue K., Nakayama M., Yamamura A., Waki T., *Phytochemistry*, **20**, 2691—2693 (1981); *b*) Stoianova-Ivanova B., Budzikiewicz H., Koumanova B., Tsoutsoulova A., Mladenova K., Brauner A., *Planta Med*., **49**, 236—239 (1983); *c*) Chatterjee A., Sarkar S., Saha S. K., *Phytochemistry*, **20**, 1760—1761 (1981).

3) *a*) He Y., Li R., Shen L., *Beijing Yixueyuan Xuebao*, **14**, 259—261 (1982) [*Chem. Abstr*., **98**, 68830*z* (1983)]; *b*) Chen Z., Xu P., *Yaoxue Xuebaao*, **22**, 67—69 (1987) [*Chem. Abstr*., **106**, 135326*d* (1987)]; *c*) Yu D. Q., Xie F. Z., *ibid*., **22**, 837—840 (1987) [*Chem. Abstr*., **108**, 164702*e* (1988)]; *d*) Yu D. Q. Xie F. Z. He W. Y., Liang X. T., *ibid*., **27**, 191—196 (1992) [*Chem. Abstr*., **117**, 108087*g* (1992)]; *e*) Chen Y., Li X., *Linchan Huaxue Yu Gongye*, **12**, 159—163 (1992) [*Chem. Abstr*., **119**, 34075*u* (1993)]; *f*) Ma R., Guan J., Wang X., Fang H., *Sepu*, **12**, 47—49 (1994) [*Chem. Abstr*., **120**, 227081*x* (1994)]; *g*) Gong F., Wang G., Yuan X., Wu X., *Tianran Chanwu Yanjiu Yu Kaifa,* **6**, 32—34 (1994) [*Chem. Abstr*., **122**, 183220*b* (1995)].

4) *a*) Yoshikawa M., Shimada H., Nishida N., Li Y., Toguchida I., Yamahara J., Matsuda H., *Chem. Pharm. Bull*., **46**, 113—119 (1998); *b*) Yoshikawa M., Murakami T., Yashiro K., Yamahara J., Matsuda H., Saijoh R., Tanaka O., *ibid*., **46**, 647—654 (1998); *c*) Yoshikawa M.,

Murakami T., Yashiro K., Matsuda H., *ibid*., **46**, 1339—1340 (1998); *d*) Matsuda H., Li Y., Murakami T., Matsumura N., Yamahara J., Yoshikawa M., *ibid*., **46**, 1399—1403 (1998); *e*) Yoshikawa M., Murakami T., Yamahara J., Matsuda H., *ibid*., **46**, 1764—1769 (1998).

5) *a*) Yoshikawa M., Murakami T., Komatsu H., Yamahara J., Matsuda H., *Heterocycles*, **47**, 397—405 (1998); *b*) Yoshizumi S., Murakami T., Kadoya M., Matsuda H., Yamahara J., Yoshikawa M., *Yakugaku Zasshi*, **118**, 188—192 (1998); *c*) Yoshikawa M., Murakami T., Komatsu H., Matsuda H., *Chem. Pharm. Bull*., **46**, 812—816 (1998); *d*) Komatsu H., Murakami T., Matsuda H., Yoshikawa M., *Heterocycles*, **48**, 703—710 (1998); *e*) Yoshikawa M., Murakami T., Shimada H., Fukada N., Matsuda H., Sashida Y., Yamahara J., *Heterocycles*, **48**, 869—873 (1998); *f*) Yoshikawa M., Murakami T., Shimada H., Yoshizumi S., Saka M., Yamahara J., Matsuda H., *Chem. Pharm. Bull*., **46**, 1008—1014 (1998); *g*) Yoshikawa M., Murakami T., Kadoya M., Yamahara J., Matsuda H., *ibid*., **46**, 1758—1763 (1998).
6) *a*) Zeisberg R., Bohlmann F., *Chem. Ber*., **107**, 3800—3805 (1974); *b*) Bohlmann F., Zdero C., *Phytochemistry*, **21**, 2543—2549 (1982).
7) Yoshikawa M., Shimada H., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull*., **44**, 1656—1662 (1996).
8) Heymann H., Tezuka Y., Kikuchi T., Supriyatna S., *Chem. Pharm. Bull*., **42**, 138—146 (1994).
9) Takeda K., Minato H., Ishikawa M., *Tetrahedron Suppl*., **7**, 219—225 (1966).
10) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahedron*, **34**, 1389—1397 (1978).
11) Morita N., Shimizu M., Arisawa M., Kitanaka S., *Yakugaku Zasshi*, **94**, 875—877 (1974).
12) Hu C., Chen K., Shi Q., Kilkuskie R. E., Cheng Y., Lee K., *J. Nat. Prod*., **57**, 42—51 (1994).
13) Yoshikawa M., Shimada H., Saka M., Yoshizumi S., Yamahara J., Matsuda H., *Chem. Pharm. Bull*., **45**, 464—469 (1997).
14) Kitagawa I., Yoshikawa M., Yoshioka I., *Tetrahedron Lett*., **5**, 469—472 (1974).
15) Ohtani I., Kusumi T., Kashman H., Kakisawa H., *J. Am. Chem. Soc*., **113**, 4092—4096 (1991).
16) Since the esterification of **3** with MTPA in the presence of EDC·HCl and 4-DMAP or benzoyl chloride in pyridine are unsuccessful so far, we could not characterize the absolute stereostructure of **3**.
17) Terashima H., Hama K., Yamamoto R., Tsuboshima M., Kikkawa R., Hatanaka I., Shigeta Y., *J. Pharmacol Exp. Ther*., **229**, 226—230 (1984).