Medicinal Flowers. IV.[1] Marigold. (2): Structures of New Ionone and Sesquiterpene Glycosides from Egyptian *Calendula officinalis*

Toshiyuki MARUKAMI, Akinobu KISHI, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607–8412, Japan.
Received February 20, 2001; accepted April 16, 2001

Following the characterization of hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and the structure elucidation of calendasaponins A, B, C, and D, two new ionone glucosides (officinosides A and B), and two sesquiterpene oligoglycosides (officinosides C and D), were isolated from the flowers of Egyptian *Calendula officinalis*. The structures of the officinosides were elucidated on the basis of chemical and physicochemical evidence.

Key words officinoside; *Calendula officinalis*; marigold; ionone glucoside; sesquiterpene oligoglycoside; Compositae

In the course of our studies on the bioactive constituents of medicinal flowers,[1,2] we have found that the methanolic extract and its 1-butanol-soluble fraction from the flowers of Egyptian *Calendula* (*C.*) *officinalis* exhibited hypoglycemic, gastric emptying inhibitory, and gastroprotective effects. Thus far, we have isolated four oleanene-type triterpene oligoglycosides (calendasaponins A—D), two new ionone glucosides [officinosides A (**1**) and B (**2**)], and two new sesquiterpene oligoglycosides [officinosides C (**3**) and D (**4**)], from the 1-butanol-soluble fraction together with eight known saponins, one sesquiterpene glucoside, and seven flavonol glycosides. In the preceding paper,[1] we reported the isolation and structure elucidation of calendasaponins A—D. Furthermore, we described the inhibitory activities of the principle saponins from the flowers of *C. officinalis* on the increase of serum glucose levels in oral glucose-loaded rats, on gastric emptying in carboxymethyl cellulose sodium salt test meal-loaded mice, and on ethanol- or indomethacin-induced gastric mucosal lesions in rats and also discussed the structure requirements for these activities. This paper offers the structure elucidation of officinosides A (**1**), B (**2**), C (**3**), and D (**4**).

Structures of Ionone Glucosides, Officinosides A (1) and B (2) Officinoside A (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{22}$ +13.0°). In the negative- and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 387 $(M-H)^-$ and *m/z* 411 $(M+Na)^+$, respectively. High-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **1** to be $C_{19}H_{32}O_8$. The IR spectrum of **1** showed absorption bands at 1671 cm^{-1} ascribable to olefin and strong absorption bands at 3432, 1076, and 1038 cm^{-1} suggestive of a glycosidic structure. Acid hydrolysis of **1** with 5% aqueous sulfuric acid–1,4-dioxane liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolidine derivative.[3] Enzymatic hydrolysis of **1** with β-glucosidase liberated (3*S*,5*R*,8*S*,9ξ)-5,8-epoxy-6-megastigmene-3,9-diol (**5**). Since the stereostructure of **5** was tentatively presented,[4] we investigated the total structure of **1** including the aglycone moiety. The ^{1}H-NMR (**1**: pyridine-d_5, **5**: $CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1** and **5**, which were assigned by various NMR experiments,[5] indicated the presence of three singlet methyls [**1**: δ 1.13, 1.42, 1.92 (all s, 12, 11, 13-H_3), **5**: δ 1.19, 1.32, 1.61 (all s, 12, 11, 13-H_3)], a doublet methyl [**1**: δ 1.43 (d, J=6.6 Hz, 10-H_3), **5**: δ 1.17 (d, J=6.4 Hz, 10-H_3)], three methines bearing an oxygen function [**1**: δ 3.94 (m, 9-H), 4.49 (m, 3-H), 4.76 (br d, J=*ca.* 7 Hz, 8-H), **5**: δ 3.81 (dd, J=3.1, 6.4 Hz, 9-H), 4.24 (dd-like, 3-H), 4.71 (br d, J=*ca.* 5 Hz, 8-H)], and an olefinic methine [**1**: δ 5.76 (br s, 7-H), **5**: δ 5.37 (br s, 7-H)] together with a β-D-glucopyranosyl moiety [δ 4.87 (d, J=8.4 Hz, 1′-H)] in **1**. As shown in Fig. 1, the planar structure of the aglycone and the position

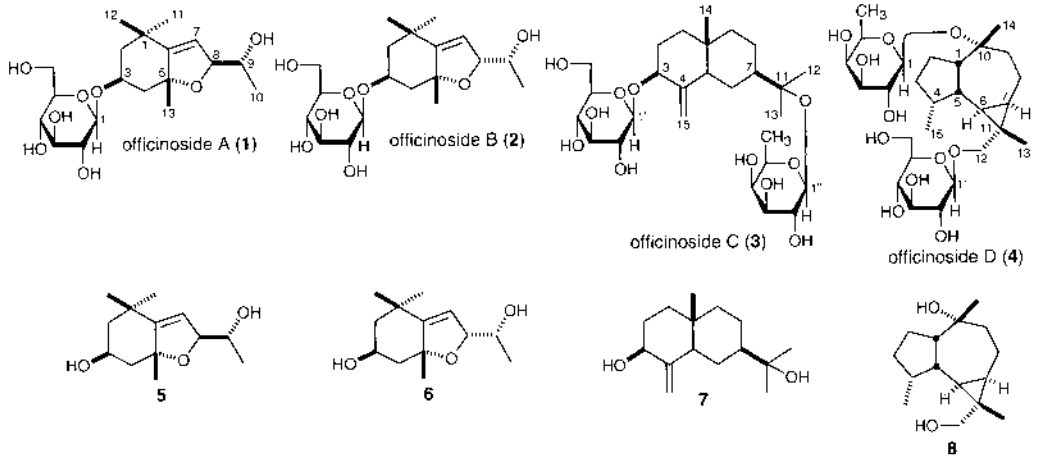

Chart 1

* To whom correspondence should be addressed. e-mail: shoyaku@mb.kyoto-phu.ac.jp

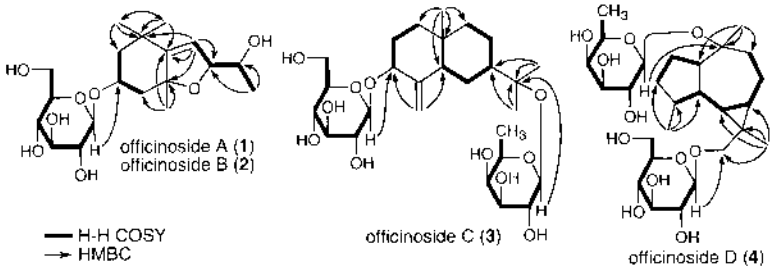

Fig. 1. H–H COSY and HMBC Correlations of **1**—**4**

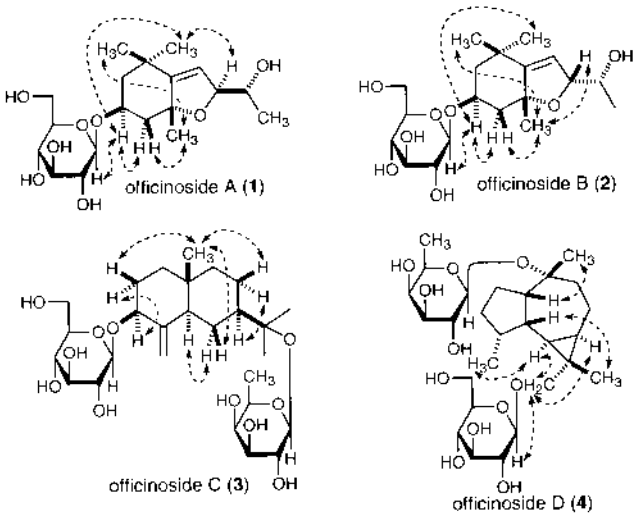

Fig. 2. NOE Correlations of **1**—**4**

Table 1. ^{13}C-NMR Data of Officinosides A (**1**), B (**2**), C (**3**), and D (**4**), **5**, and **6**

	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[b]		**1**[a]	**2**[a]	**3**[a]	**4**[a]
C-1	34.0	34.1	40.1	55.0	33.8	33.8	C-1′	102.6	102.6	103.1	103.8
2	45.8	45.8	31.9	25.8	46.6	46.7	2′	75.4	75.4	75.7	75.9
3	73.8	73.8	78.9	29.3	67.6	67.6	3′	78.9	78.9	78.7	78.7
4	44.2	44.3	150.2	38.7	47.5	48.0	4′	71.9	71.9	71.9	72.0
5	87.3	87.3	48.7	38.7	86.7	87.2	5′	78.3	78.3	78.5	78.5
6	154.6	154.6	22.7	18.7	155.4	155.4	6′	63.0	63.0	63.0	63.0
7	119.0	118.0	48.9	26.8	116.1	117.4	C-1″			98.8	98.1
8	87.6	87.4	25.4	19.2	86.1	86.8	2″			72.4	72.5
9	70.7	70.1	41.3	37.1	68.4	70.9	3″			75.6	75.2
10	20.0	20.0	36.0	81.2	17.8	18.8	4″			72.8	72.8
11	28.8	28.7	79.2	23.4	28.9	28.5	5″			70.9	71.0
12	31.4	31.5	23.8	79.4	31.3	31.4	6″			17.5	17.4
13	29.2	29.3	25.0	12.6	28.5	28.7					
14			16.7	27.6							
15			104.7	16.6							

a) 125 MHz, pyridine-d_5 or *b*) $CDCl_3$.

of a glycoside linkage in **1** were confirmed by ^{1}H–^{1}H COSY and HMBC experiments, which showed long-range correlations between the following protons and carbons: 11, 12-H_3 and 1, 2, 6-C; 13-H_3 and 4, 5, 6-C; 10-H_3 and 8, 9-C; 7-H and 1, 5, 6-C; 8-H and 7, 9-C; 1′-H and 3-C. On the basis of this evidence and the ^{1}H–^{1}H COSY data (Fig. 1), the planar structure of **2** and **6** were characterized. NOE correlations were observed in the pNOESY experiment between the following protons: 3-H and 4α, 1′-H; 11-H_3 and 2α, 3-H; 13-H_3 and 12-H_3, 8-H. This evidence led us to elucidate the relative stereostructure of **2** to be as shown.

In order to clarify the absolute stereostructures of **1** and **2**, the aglycones, **5** and **6**, were subjected to a modified Mosher's method.[6] Namely, **5** and **6** were treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetate (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardoiimide (EDC·HCl) and 4-dimethylaminopyridine (DMAP) to give the 3,9-di-(*R*)-MTPA ester (**5a**, **6a**) and the 3,9-di-(*S*)-MTPA ester (**5b**, **6b**), respectively. As shown in Fig. 3, the signals due to protons attached to the 2, 7, 8, 11, and 12-positions in **5a** and **6a** were observed at lower fields ($\Delta\delta$: negative) as compared to those of **5b** and **6b**, while the signal due to the 4 and 10-positions in **5a** and **6a** was observed at higher fields ($\Delta\delta$: positive) as compared to those of **5b** and **6b**. On the basis of the above evidence, the absolute stereostructures of officinosides A and B were determined to be (3*S*,5*R*,8*S*,9*R*)-5,8-epoxy-6-megastigmene-3,9-diol 3-*O*-β-D-glucopyranoside (**1**) and (3*S*,5*R*,8*R*,9*R*)-5,8-epoxy-6-megastigmene-3,9-diol

Officinoside B (**2**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{22}$ +1.2°) and its IR spectrum was similar to that of **1**. The molecular formula $C_{19}H_{32}O_8$ of **2**, which was the same as that of **1**, was characterized from the negative- and positive-ion FAB-MS [*m/z* 387 $(M-H)^-$ and *m/z* 411 $(M+Na)^+$] and by high-resolution MS measurement. Acid hydrolysis of **2** with 5% aqueous sulfuric acid–1,4-dioxane furnished D-glucose,[3] while a new aglycone (**6**) was obtained by enzymatic hydrolysis. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[5] of **6** showed signals assignable to three singlet methyls [δ 1.18, 1.33, 1.60 (all s, 12, 11, 13-H_3)], a doublet methyl [δ 1.18 (d, *J*=6.4 Hz, 10-H_3)], two methylenes [δ 1.47 (m), 1.82 (dd, *J*=3.7, 14.0 Hz) (2-H_2), 1.75 (ddd, *J*=1.8, 4.0, 14.0 Hz), 2.15 (ddd-like) (4-H_2)], three methines bearing an oxygen function [δ 3.57 (dd-like, 9-H), 4.22 (dd-like, 3-H), 4.52 (br d, *J*=*ca*. 6 Hz, 8-H)], and an olefin methine [δ 5.30 (br s, 7-H)], while those of **2** indicated the presence of an aglycone part and a β-D-glucopyranosyl moiety [δ 4.90 (d, *J*=8.4 Hz, 1′-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** and **6** were shown to be superimposable on those of **1** and **5**, except for the signals due to the 7, 8, and 9-positions. The HMBC experiment of **2** showed long-range correlations between the following protons and carbons: 11, 12-H_3 and 1, 2, 6-C; 13-H_3 and 4, 5, 6-C; 10-H_3 and 8, 9-C; 7-H and 1, 5, 6-C; 8-H and 7, 9-C; 1′-H and 3-C. On the basis of this evidence and the ^{1}H–^{1}H COSY data (Fig. 1), the planar structure of **2** and **6** were characterized. NOE correlations were observed in the pNOESY experiment between the following protons: 3-H and 4α, 1′-H; 11-H_3 and 2α, 3-H; 13-H_3 and 12-H_3, 8-H. This evidence led us to elucidate the relative stereostructure of **2** to be as shown.

In order to clarify the absolute stereostructures of **1** and **2**, the aglycones, **5** and **6**, were subjected to a modified Mosher's method.[6] Namely, **5** and **6** were treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetate (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardoiimide (EDC·HCl) and 4-dimethylaminopyridine (DMAP) to give the 3,9-di-(*R*)-MTPA ester (**5a**, **6a**) and the 3,9-di-(*S*)-MTPA ester (**5b**, **6b**), respectively. As shown in Fig. 3, the signals due to protons attached to the 2, 7, 8, 11, and 12-positions in **5a** and **6a** were observed at lower fields ($\Delta\delta$: negative) as compared to those of **5b** and **6b**, while the signal due to the 4 and 10-positions in **5a** and **6a** was observed at higher fields ($\Delta\delta$: positive) as compared to those of **5b** and **6b**. On the basis of the above evidence, the absolute stereostructures of officinosides A and B were determined to be (3*S*,5*R*,8*S*,9*R*)-5,8-epoxy-6-megastigmene-3,9-diol 3-*O*-β-D-glucopyranoside (**1**) and (3*S*,5*R*,8*R*,9*R*)-5,8-epoxy-6-megastigmene-3,9-diol

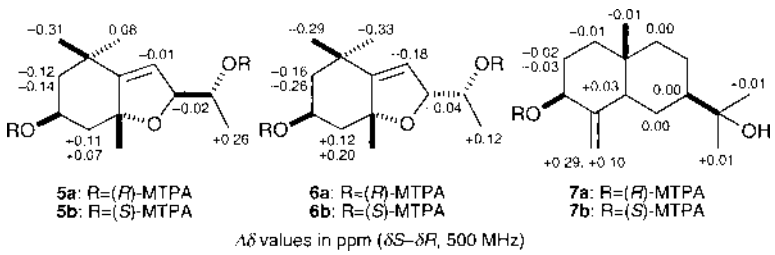

Fig. 3

3-*O*-*β*-D-glucopyranoside (**2**).

Officinoside C (**3**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −7.0°), and its IR spectrum showed absorption bands due to hydroxyl and *exo*-methylene functions at 3432 and 1655 cm^{-1}. In the negative- and positive-ion FAB-MS of **3**, quasimolecular ion peaks were observed at *m/z* 545 $(M-H)^-$ and *m/z* 569 $(M+Na)^+$ and the molecular formula, $C_{27}H_{46}O_{11}$, of **3** was determined by high-resolution MS measurement. Acid hydrolysis of **3** with 5% aqueous H_2SO_4–1,4-dioxane liberated D-glucose and D-fucose,[3] while enzymatic hydrolysis of **3** with naringinase furnished selin-4(15)-en-3*β*,11-diol (**7**).[7] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[5] of **3** showed signals due to a selin-4(15)-en-3*β*,11-diol moiety [*δ* 0.71, 1.39, 1.42 (s, 14, 12, 13-H_3), 4.46 (m, 3-H), 4.79, 6.81 (br s, 15-H_2)], a *β*-D-glucopyranosyl moiety [*δ* 5.03 (d, *J*=7.6 Hz, 1′-H)], and a *β*-D-fucopyranosyl moiety [*δ* 1.51 (d, *J*=6.1 Hz, 6″-H_3), 4.81 (d, *J*=7.4 Hz, 1″-H)]. The planar structure and the glycoside linkages of **3** were constructed on the basis of ^{1}H–^{1}H COSY and HMBC. Thus, the ^{1}H–^{1}H COSY experiment for **3** indicated the presence of the partial structures from the 1-position to the 3-position and from the 5-position to the 9-position. In the HMBC experiment, long-range correlations were observed between the 15-protons and the 3, 4, 5-carbons, between the 14-protons and the 1, 5, 9, 10-carbons, between the 12, 13-protons and the 11, 7-carbons, between the 1′-proton and the 3-carbon, and between the 1″-proton and the 11-carbon. The relative stereostructure of **3** was characterized by pNOESY experiment, which showed NOE correlations between the following protons: 14-H_3 and 2*β*, 6*β*, 8*β*, 9*β*-H; 2*α*-H and 3-H; 8*α*-H and 7, 9*α*-H. Since the absolute stereostructure of **7** was not yet determined, **7** was subjected to a modified Mosher's method.[6] Consequently, the signals due to protons attached to the 1 and 2-carbons in the ^{1}H-NMR spectrum of the 3-(*S*)-MTPA ester (**7b**) were observed at higher field as compared to those of the 3-(*R*)-MTPA ester (**7a**) (*Δδ*: negative), while the signal due to protons on the 15-carbon of the 3-(*S*)-MTPA (**7b**) was observed at lower field than those of the 3-(*R*)-MTPA (**7a**) (*Δδ*: positive). On the basis of the above evidence, the absolute stereostructure of officinoside C was elucidated to be 12-*O*-*β*-D-fucopyranosyl selin-4(15)-en-3*β*,11-diol 3-*O*-*β*-D-glucopyranoside (**3**).

Officinoside D (**4**), isolated as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −14.5°), gave the quasimolecular ion peaks at *m/z* 545 $(M-H)^-$ and *m/z* 569 $(M+Na)^+$ in the negative- and positive-ion FAB-MS and the molecular formula was defined as $C_{27}H_{46}O_{11}$ from the high-resolution MS analysis. Acid hydrolysis of **4** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) liberated D-fucose and D-glucose,[3] while enzymatic hydrolysis of **4** with naringinase liberated flourensadiol (**8**).[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[5] of **4** showed a flourensadiol moiety [*δ* 0.31 (ddd-like, 6-H), 0.89 (d, *J*=6.7 Hz, 15-H_3), 1.07 (ddd-like, 7-H), 1.24, 1.36 (s, 13, 14-H_3), 3.29, 4.16 (d, *J*=10.1 Hz, 12-H_2)], a *β*-D-fucopyranosyl moiety [*δ* 1.47 (d, *J*=6.4 Hz, 6′-H_3), 4.80 (d, *J*=7.7 Hz, 1′-H)], and a *β*-D-glucopyranosyl moiety [*δ* 4.87 (d, *J*=7.9 Hz, 1″-H)]. In the HMBC experiment of **4**, long-range correlations were observed between the 1′-proton of the *β*-D-fucopyranosyl moiety and the 10-carbon and between the 1″-proton of the *β*-D-glucopyranosyl moiety and 12-carbon. Furthermore, in the pNOESY experiment, NOE correlations were observed between the following protons: 12-H_2 and 6, 7, 1″-H; 13-H_3 and 8*β*, 5-H; 14-H_3 and 1, 8*β*, 9*β*-H; 15-H_3 and 6-H. On the basis of the above evidence, officinoside D was determined to be 12-*O*-*β*-D-fucopyranosyl flourensadiol 10-*O*-*β*-D-glucopyranoside (**4**).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Officinosides A (1), B (2), C (3), and D (4) from the Dried Flowers of *C. officinalis* L. Officinosides A (**1**), B (**2**), C (**3**), and D (**4**) were isolated from the dried flowers of *C. officinalis* cultivated in Egypt, as described earlier.[1]

Officinoside A (**1**): A white powder, $[\alpha]_D^{22}$ +13.0° (*c*=0.6, MeOH). IR (KBr): 3432, 1671, 1076, 1038 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{32}O_8Na$ $(M+Na)^+$: 411.1995. Found: 411.2008. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 1.13, 1.42, 1.92 (3H each, all s, 12, 11, 13-H_3), 1.43 (3H, d, *J*=6.6 Hz, 10-H_3), 1.52 (1H, dd, *J*=3.3, 13.8 Hz, 2*α*-H), 1.98 (1H, dd, *J*=4.0, 13.8 Hz, 4*β*-H), 2.05 (1H, br d, *J*=*ca.* 14 Hz, 2*β*-H), 2.62 (1H, br d, *J*=*ca.* 14 Hz, 4*α*-H), 3.94 (1H, m, 9-H), 4.49 (1H, m, 3-H), 4.76 (1H, br d, *J*=*ca.* 7 Hz, 8-H), 4.87 (1H, d, *J*=8.4 Hz, 1′-H), 5.76 (1H, br s, 7-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 387 $(M-H)^-$, 225 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 411 $(M+Na)^+$.

Officinoside B (**2**): A white powder, $[\alpha]_D^{26}$ +1.2° (*c*=0.5, MeOH). IR (KBr): 3432, 1670, 1078, 1036 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{32}O_8Na$ $(M+Na)^+$: 411.1995. Found: 411.1981. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 1.14, 1.42, 1.90 (3H each, all s, 12, 11, 13-H_3), 1.43 (3H, d, *J*=6.4 Hz, 10-H_3), 1.52 (1H, dd, *J*=3.6, 13.8 Hz, 2*α*-H), 1.97 (1H, dd, *J*=4.3, 13.8 Hz, 4*β*-H), 2.06 (1H br d, *J*=*ca.* 14 Hz, 2*β*-H), 2.59 (1H, br d, *J*=*ca.* 14 Hz, 4*α*-H), 4.01 (1H, dd, *J*=5.2, 6.4 Hz, 9-H), 4.48 (1H, m, 3-H), 4.90 (1H, d, *J*=8.4 Hz, 1′-H), 4.94 (1H, br d, *J*=*ca.* 5 Hz, 8-H), 5.56 (1H, br s, 7-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 387 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 411 $(M+Na)^+$.

Officinoside C (**3**): A white powder, $[\alpha]_D^{27}$ −7.0° (*c*=0.7, MeOH). IR (KBr): 3432, 1655, 1074, 1038 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{27}H_{46}O_{11}Na$ $(M+Na)^+$: 569.2938. Found: 569.2953. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 0.71, 1.39, 1.42 (3H each, all s, 14, 12, 13-H_3), 1.06 (1H, m, 9*α*-H), 1.09 (1H, m, 1*α*-H), 1.31 (1H, m, 1*β*-H), 1.34 (1H, m, 8*β*-H), 1.35 (1H, m, 6*α*-H), 1.42 (1H, m, 9*β*-H), 1.51 (3H, d, *J*=6.1 Hz, 6″-H_3), 1.57 (1H, m, 5-H), 1.59 (1H, m, 7-H), 1.77 (1H, m, 2*α*-H), 1.81 (1H, m, 6*β*-H), 1.96 (1H, m, 8*α*-H), 2.11 (1H, m, 2*β*-H), 4.46 (1H, m, 3-H), 4.79, 6.81 (1H each, both br s, 15-H_2), 4.81 (1H, d, *J*=7.4 Hz, 1″-H), 5.03 (1H, d, *J*=7.6 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1.

Negative-ion FAB-MS: *m/z* 545 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 569 $(M+Na)^+$.

Officinoside D (**4**): A white powder, $[\alpha]_D^{26}$ −14.5° (*c*=0.3, MeOH). IR (KBr): 3432, 2940, 1167, 1075 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{27}H_{46}O_{11}Na$ $(M+Na)^+$: 569.2938. Found: 569.2924. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.31 (1H, dd-like, 6-H), 0.89 (3H, d, *J*=6.7 Hz, 15-H_3), 1.07 (1H, ddd-like, 7-H), 1.24, 1.36 (3H each, both s, 13, 14-H_3), 1.25 (1H, m, 3α-H), 1.47 (3H, d, *J*=6.4 Hz, 6′-H_3), 1.56 (2H, m, 2-H_2), 1.62 (1H, m, 8α-H), 1.65 (1H, m, 9β-H), 1.72 (1H, m, 3β-H), 1.92 (1H, m, 9α-H), 1.95 (1H, m, 4-H), 2.07 (1H, dd-like, 8β-H), 2.22 (1H, m, 5-H), 2.24 (1H, m, 1-H), 3.29, 4.16 (1H each, both d, *J*=10.1 Hz, 12-H_2), 4.80 (1H, d, *J*=7.7 Hz, 1′-H), 4.87 (1H, d, *J*=7.9 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 545 $(M-H)^-$, 383 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 569 $(M+Na)^+$.

Acid Hydrolysis of and Officinosides (1—4) A solution of **1**—**4** (5 mg each) in 5% aq. H_2SO_4–1,4-dioxane (2 ml, 1 : 1, v/v) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH- form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i) from **1**—**4**; D-fucose (ii) from **3** and **4**; GLC conditions: column: Supeluco STB™-1, 30 m×0.25 mm (i.d.) capillary column, column temperature: 230 °C, He flow rate: 15 ml/min, t_R: i: 24.2 min, ii: 17.2 min.

Enzymatic Hydrolysis of Officinoside A (1) A solution of **1** (12 mg) in 0.2 M acetate buffer (pH 4.4, 2.0 ml) was treated with β-glucosidase (Oriental Yeast Co., 20 mg) and the whole mixture was stirred at 38 °C for 48 h. The reaction mixture was poured into EtOH and removal of the solvent under reduced pressure gave a product. The product was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (30 : 3 : 1, lower layer, v/v)] to give (3*S*,5*R*,8*S*,9ξ)-5,8-epoxy-6-megastigmene-3,9-diol (**5**, 4.3 mg, 61.5%), which was identified by comparison of the ^{1}H-NMR data and $[\alpha]_D$ with reported values.[4)]

5: An amorphous powder, $[\alpha]_D^{23}$ +10.6 °C (*c*=0.1, $CHCl_3$). IR (film): 3453, 1632, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.17 (3H, d, *J*=6.4 Hz, 10-H_3), 1.19, 1.32, 1.61 (3H, all s, 12, 11, 13-H_3), 1.50 (1H, m), 1.84 (1H, dd, *J*=4.0, 13.6 Hz) (2-H_2), 1.75 (1H, ddd, *J*=2.0, 4.0, 13.6 Hz), 2.15 (1H, ddd-like) (4-H_2), 3.81 (1H, dd, *J*=3.1, 6.4 Hz, 9-H), 4.24 (1H, dd-like, 3-H), 4.71 (1H, br d, *J*=*ca*. 5 Hz, 8-H), 5.37 (1H, br s, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1.

Preparation of the (*R*)-MTPA Ester (5a) and the (*S*)-MTPA Ester (5b) from (3*S*,5*R*,8*S*,9ξ)-5,8-Epoxy-6-megastigmene-3,9-diol (5) A solution of **5** (0.8 mg) in CH_2Cl_2 (0.5 ml) was treated with (*R*)-MTPA (50 mg) in the presence of EDC·HCl (50 mg) and 4-DMAP (20 mg), and the mixture was stirred at 50 °C under an N_2 atmosphere for 2 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$ and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified on a normal-phase silica gel column [0.6 g, *n*-hexane–AcOEt (5 : 1, v/v) to give **5a** (1.1 mg, 47%). Through a similar procedure, **5b** (0.9 mg, 44%) was prepared from **5** (0.7 mg) by the use of (*S*)-MTPA (50 mg), EDC·HCl (50 mg), and 4-DMAP (20 mg).

5a: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.17, 1.23, 1.28 (3H each, all s, 12, 13, 11-H_3), 1.26 (3H, d, *J*=5.8 Hz, 10-H_3), 1.68 (1H, dd, *J*=3.8, 15.2 Hz), 2.05 (1H, dd-like) (2-H_2), 1.82 (1H, dd, *J*=3.8, 15.2 Hz), 2.38 (1H, dd-like) (4-H_2), 3.54, 3.58 (3H each, both s, MTPA-OMex$_2$), 5.54 (1H, dd-like, 8-H), 5.70 (1H, br s, 7-H), 7.40—7.55 (10H).

5b: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.86, 1.20, 1.25 (3H each, all s, 12, 11, 13-H_3), 1.52 (3H, d, *J*=5.8 Hz, 10-H_3), 1.54 (1H, m), 1.93 (1H, dd-like) (2-H_2), 1.89 (1H, dd, *J*=4.1, 15.1 Hz), 2.49 (1H, dd-like) (4-H_2), 3.58 (6H, s, MTPA-OMex$_2$), 5.52 (1H, dd-like, 8-H), 5.69 (1H, br s, 7-H), 7.41—7.55 (10H).

Enzymatic Hydrolysis of Officinoside B (2) A solution of **2** (13 mg) in 0.2 M acetate buffer (pH 4.4, 2.0 ml) was treated with β-glucosidase (Oriental Yeast Co., 20 mg) and the whole mixture was stirred at 38 °C for 48 h. The reaction mixture was poured into EtOH and removal of the solvent under reduced pressure gave a product. The product was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (30 : 3 : 1, lower layer, v/v)] to give (3*S*,5*R*,8*R*,9ξ)-5,8-epoxy-6-megastigmene-3,9-diol (**6**, 3.5 mg, 46.2%).

6: An amorphous powder, $[\alpha]_D^{25}$ −61.1° (*c*=0.1, $CHCl_3$). IR (film): 3453, 1632, 1028 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.18 (3H, d, *J*=6.4 Hz, 10-H_3), 1.18, 1.33, 1.60 (3H, all s, 12, 11, 13-H_3), 1.47 (1H, m), 1.82 (1H, dd, *J*=3.7, 14.0 Hz) (2-H_2), 1.75 (1H, ddd, *J*=1.8, 4.0, 14.0 Hz), 2.15 (1H, ddd-like) (4-H_2), 3.57 (1H, dd-like, 9-H), 4.22 (1H, dd-like, 3-H), 4.52 (1H, br d, *J*=*ca*. 6 Hz, 8-H), 5.30 (1H, br s, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1.

Preparation of the (*R*)-MTPA Ester (6a) and the (*S*)-MTPA Ester (6b) from (3*S*,5*R*,8*R*,9ξ)-5,8-Epoxy-6-megastigmene-3,9-diol (6) A solution of **6** (1.0 mg) in CH_2Cl_2 (0.5 ml) was treated with (*R*)-MTPA (50 mg) in the presence of EDC·HCl (50 mg) and 4-DMAP (20 mg), and the mixture was stirred at 50 °C under an N_2 atmosphere for 2 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$ and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified on a normal-phase silica gel column [0.6 g, *n*-hexane–AcOEt (5 : 1, v/v) to give **6a** (0.5 mg, 17%). Through a similar procedure, **6b** (1.4 mg, 48%) was prepared from **6** (1.0 mg) by the use of (*S*)-MTPA (50 mg), EDC·HCl (50 mg), and 4-DMAP (20 mg).

6a: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.01, 1.11, 1.14 (3H each, all s, 11,12, 13-H_3), 1.25 (3H, d, *J*=5.8 Hz, 10-H_3), 1.76 (1H, dd, *J*=3.8, 15.2 Hz), 1.80 (1H, dd-like) (2-H_2), 1.76 (1H, dd, *J*=3.8, 15.2 Hz), 1.99 (1H, dd-like) (4-H_2), 3.53, 3.57 (3H each, both s, MTPA-OMex$_2$), 5.38 (1H, dd-like, 8-H), 5.24 (1H, br s, 7-H), 7.38—7.55 (10H).

6b: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.68, 0.82, 1.35 (3H each, all s, 11, 12, 13-H_3), 1.37 (3H, d, *J*=5.8 Hz, 10-H_3), 1.50 (1H, m), 1.64 (1H, dd-like) (2-H_2), 1.96 (1H, dd, *J*=4.1, 15.1 Hz), 2.11 (1H, dd-like) (4-H_2), 3.56 (6H, s, MTPA-OMex$_2$), 5.06 (1H, br s, 7-H), 5.42 (1H, dd-like, 8-H), 7.36—7.52 (10H).

Enzymatic Hydrolysis of Officinoside C (3) A solution of **3** (10 mg) in 0.2 M acetate buffer (pH 4.0, 2.0 ml) was treated with naringinase (Sigma Co., Ltd., 20 mg) and the whole mixture was stirred at 40 °C for 24 h. The reaction mixture was poured into EtOH and removal of the solvent under reduced pressure gave a product. The product was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (30 : 3 : 1, lower layer, v/v)] to give selin-4(15)-en-3β,11-diol (**7**, 4.0 mg, 91.8%), which were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[7)]

Preparation of the (*R*)-MTPA Ester (7a) and the (*S*)-MTPA Ester (7b) from Selin-4(15)-en-3β,11-diol (7) A solution of **7** (0.6 mg) in CH_2Cl_2 (0.5 ml) was treated with (*R*)-MTPA (50 mg) in the presence of EDC·HCl (50 mg) and 4-DMAP (20 mg), and the mixture was stirred at 50 °C under an N_2 atmosphere for 3 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$ and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified on a normal-phase silica gel column [0.6 g, *n*-hexane–AcOEt (5 : 1, v/v) to give **7a** (0.4 mg, 35%). Through a similar procedure, **7b** (0.4 mg, 19%) was prepared from **7** (1.1 mg) by the use of (*S*)-MTPA (50 mg), EDC·HCl (50 mg), and 4-DMAP (20 mg).

7a: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.72 (3H, s, 14-H_3), 0.85 (2H, m, 9-H), 0.87 (2H, m, 1-H_2), 1.20 (6H, s, 12, 13-H_3), 1.64 (1H, dd-like, 5-H), 1.66 (2H, m, 7-H), 1.81 (2H, m, 6-H_2), 1.69 (1H, dd-like), 2.04 (1H, m) (2-H_2), 3.61 (3H, s, MTPA-OMe), 4.51, 4.66 (1H each, both br s, 15-H_2), 5.40 (1H, m, 3-H), 7.38—7.58 (5H).

7b: A white powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.71 (3H, s, 14-H_3), 0.85 (2H, m, 9-H), 0.86 (2H, m, 1-H_2), 1.21 (6H, s, 12, 13-H_3), 1.61 (1H, dd-like, 5-H), 1.66 (2H, m, 7-H), 1.81 (2H, m, 6-H_2), 1.66 (1H, dd-like), 2.02 (1H, m) (2-H_2), 3.56 (3H, s, MTPA-OMe), 4.61, 4.95 (1H each, both br s, 15-H_2), 5.39 (1H, m, 3-H), 7.38—5.58 (5H).

Enzymatic Hydrolysis of Officinoside D (4) A solution of **4** (20 mg) in 0.2 M acetate buffer (pH 4.0, 2.0 ml) was treated with naringinase (40 mg) and the whole mixture was stirred at 40 °C for 24 h. The reaction mixture was poured into EtOH and removal of the solvent under reduced pressure gave a product. The product was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (30 : 3 : 1, lower layer, v/v)] to give flourensadiol (**8**, 7.6 mg, 87%), which was identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[8)]

References and Notes

1) Part III: Yoshikawa M., Murakami T., Kishi A., Kageura T., Matsuda H., *Chem. Pharm. Bull.*, **49** (7), 863—870 (2001).

2) *a*) Yoshikawa M., Morikawa T., Murakami T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull*., **47**, 340—345 (1999); *b*) Yoshikawa M., Morikawa T., Toguchida I., Harima S., Matsuda H., *ibid*., **48**, 651—656 (2000).
3) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull*., **34**, 1843—1845 (1986).
4) Behr D., Wahlberg I., Nishida T., Enzell C. R., *Acta Chem. Scand. B*, **33**, 701—704 (1979).
5) The ^{1}H- and ^{13}C-NMR spectra of **1**—**8** were assigned on the basis of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), homo- and hetero-nuclear Hartmann–Hahn spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA), heteronucler multiple bond correlation (HMBC), phase-sensitive nuclear Overhauser effect spectroscopy (pNOESY) experiments.
6) Ohtani I., Kusumi T., Kashman H., Kakisawa H., *J. Am. Chem. Soc*., **113**, 4092—4096 (1991).
7) Pascual-T. De J., Bellido I. S., Gonzales M. S., *Tetrahedron*, **36**, 371—376 (1980).
8) Kingston D. G. I., *Phytochemistry*, **14**, 2033—2037 (1975).