Immunosuppressive Components from an Ascomycete, *Diplogelasinospora grovesii*

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Two known fungal metabolites, macrophin and colletodiol, and a new stereoisomer of colletodiol named 10-*epi*-colletodiol, were isolated as immunosuppressive principles from an Ascomycete, *Diplogelasinospora grovesii*. The IC_{50} values of the major active component among them, macrophin, were calculated to be 0.4 and 0.3 μg/ml against concanavalin A- and lipopolysaccharide-induced proliferations of mouse spleen lymphocytes, respectively. A new natural product, 4,8-dimethyl-1,5-dioxacyclooctane-2,6-dione, and a known fungal metabolite, isosclerone, which showed no immunosuppressive activity, were also isolated from this fungus.

Key words fungal metabolite; Ascomycete; *Diplogelasinospora grovesii*; immunosuppressant; macrophin; 10-*epi*-colletodiol

In our screening project on immunomodulatory components of fungi, several immunosuppressive compounds have so far been isolated from Basidiomycetes,[1a,b] and Ascomycetes.[1c,d] Recently, it was found that the AcOEt layer of the acetone extract of an Ascomycete, *Diplogelasinospora grovesii* CAILLEUX, appreciably suppressed proliferation (blastogenesis) of mouse spleen lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partition followed by repeated chromatographic fractionation afforded five components tentatively named DG-1 (**1**), DG-2 (**2**), DG-3 (**3**), DG-4 (**4**) and DG-5 (**5**), among which **2**, **3**, and **4** showed immunosuppressive activity. From the chemical and spectral data, DG-2 and -4 were deduced to be identical with macrophin (**2**)[2] and colletodiol (**4**),[3] respectively, and DG-3 was deduced to be a new stereoisomer of **4** at position 10 (**3**). The stereostructure of **3** was determined by both X-ray crystallographic analysis of its dibenzoate and application of the modified Mosher's method to **3**. Here, DG-3 was named 10-*epi*-colletodiol (**3**). DG-1 and -5 were also deduced to be a new natural product, 4,8-dimethyl-1,5-dioxacyclooctane-2,6-dione (**1**) and a known compound, isosclerone (**5**), respectively. This report deals with the isolation, structure elucidation and immunosuppressive activity of these five components newly isolated from *D. grovesii*.

Results and Discussion

The acetone solution of *D. grovesii* IFM4650[4] cultivated on sterilized rice was concentrated *in vacuo* to *ca.* 1/54th of the initial volume. The concentrated solution was partitioned between *n*-hexane and H_2O to afford the *n*-hexane layer and an aqueous suspension. The aqueous suspension was further partitioned between AcOEt and water. The AcOEt layer suppressed by 50% the Con A-induced proliferation of mouse spleen lymphocytes (T-cells) at 10—20 μg/ml, whereas the *n*-hexane and aqueous layers suppressed it less than 50% even at 50 μg/ml. Repeated chromatography of the AcOEt layer afforded DG-1 (**1**), -5 (**5**), -2 (**2**), -4 (**4**), and -3 (**3**), among which **2**, **4**, and **3** were found to be the immunosuppressive principles of this fungus.

The major immunosuppressive principle, DG-2 (**2**), was obtained as colorless needles, $C_{17}H_{20}O_8$, optically inactive. The IR and UV spectra suggested the presence of an oxycarbonyl moiety conjugated with an extended unsaturation system and a hydroxyl group in **2**. The ^{1}H- and ^{13}C-NMR spectral data, including spin-decoupling ^{1}H-NMR and two-dimensional NMR spectra, ^{1}H–^{1}H and ^{13}C–^{1}H shift correlation spectroscopy (COSY), suggested that two olefinic methyls, two methoxyls, two oxygen-bearing methylenes, three olefinic methines of which two existed adjacent to each other in *E*-configuration, five quaternary sp^2 carbons, and three oxycarbonyls were present in **2** (see Table 1). On acetylation with acetic anhydride and pyridine, **2** gave the monoacetate (**6**), suggesting the presence of one $-CH_2OH$ group in **2**. The ^{1}H- and ^{13}C-NMR spectra of **2**, which were assigned with the aid of a ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) NMR experiment, were found to be quite similar to those of a fungal metabolite isolated from *Macrophoma commelinae*, macrophin,[2] having a 3,6-disubstituted 5-hydroxymethyl-4-methoxy-α-pyrone moiety. Comparison of the ^{13}C-NMR spectrum of **6** with that of **2** showed that the signals of α- and β-carbons to the acetoxyl group (C-11, -5) were shifted to δ 55.74 (+0.95) and 114.02 (−3.95), respectively, in accordance with the acetylation shift rule,[5] again suggesting that **2** is quite similar to macrophin. The melting point, MS, UV, IR, ^{1}H- and ^{13}C-NMR spectral data of DG-2 were the same as the corresponding data of macrophin reported in the literature,[2] indicating that DG-2 was identical with macrophin (**2**) (see Experimental and Chart 1). To our knowledge, this is the first time that macrophin (**2**) has been identified as having immunosuppressive activity. As fungal components having similar structure to **2**, an immunosuppressant, multiforisin A (**7**), from *Gelasinospora multiforis*,[1c] and islandic acid (**8**) from *Penicillium islandicum*[6] have so far been isolated (see Table 1).

DG-4 (**4**) was obtained as colorless needles, $C_{14}H_{20}O_6$, $[\alpha]_D^{24}$ +35°. The IR and UV spectra suggested the presence of an oxycarbonyl group conjugated with an α,β-unsaturated system and a hydroxyl group in **4**. The ^{1}H- and ^{13}C-NMR spectral data suggested that four partial structures, *a*: [O]–CH(CH_3)–CH_2–CH(OH)–CH(OH)–

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Table 1. ^{1}H-NMR and ^{13}C-NMR Data for DG-2 (**2**), DG-2 Acetate (**6**), and Macrophin (**2**), δ (ppm) from Tetramethylsilane (TMS) as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	DG-2 (**2**)			DG-2 Acetate (**6**)		Macrophin (**2**)[2]	
	^{1}H-NMR	^{13}C-NMR	HMBC[a] (^{1}H-NMR/^{13}C-NMR)	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
2	—	162.42 (s)	—	—	162.22 (s)	—	162.3 (s)
3	—	109.46 (s)	—	—	109.27 (s)	—	109.6 (s)
4	—	169.29 (s)	—	—	169.08 (s)	—	169.3 (s)
4-OCH_3	4.12 (3H, s)	62.75 (q)	4.12/169.29	4.07 (3H, s)	62.78 (q)	4.03 (3H, s)	62.6 (q)
5	—	117.97 (s)	—	—	114.02 (s)	—	118.1 (s)
6	—	154.10 (s)	—	—	155.41 (s)	—	154.1 (s)
7	7.58 (d, 15.3)	130.36 (d)	7.58/117.97, 125.74, 154.10, 166.22	7.57 (d, 15.3)	130.26 (d)	7.46 (d, 15.2)	130.3 (d)
8	6.82 (d, 15.3)	125.74 (d)	6.82/130.36, 154.10, 166.22	6.84 (d, 15.3)	126.44 (d)	6.64 (d, 15.2)	125.4 (d)
9	—	166.22 (s)	—	—	166.05 (s)	—	166.1 (s)
9-OCH_3	3.82 (3H,s)	52.26 (q)	3.82/166.22	3.83 (3H, s)	52.33 (q)	3.73 (3H, s)	52.1 (q)
10	5.10 (2H,s)	56.02 (t)	5.10/109.46, 162.42, 169.29	5.09 (2H, s)	56.08 (t)	4.99 (2H, s)	55.9 (t)
11	4.61 (2H,s)	54.79 (t)	4.61/117.97, 154.10, 169.29	5.13 (2H, s)	55.74 (t)	4.52 (2H, s)	54.5 (t)
11-OH	—	—	—	—	—	2.31 (br s)	—
11-$OCOCH_3$	—	—	—	—	170.44 (s)	—	—
11-$OCOCH_3$	—	—	—	2.08 (3H, s)	20.80 (q)	—	—
1′	—	166.09 (s)	—	—	166.05 (s)	—	166.0 (s)
2′	5.68 (m)	115.24 (d)	5.68/20.33, 27.46	5.69 (m)	115.25 (d)	5.57 (m)	115.2 (d)
3′	—	158.24 (s)	—	—	158.30 (s)	—	157.9 (s)
4′	1.89 (3H, d, 1.2)	27.46 (q)	1.89/20.33, 115.24, 158.24	1.89 (3H, d, 1.2)	27.49 (q)	1.85 (3H, d, 1.6)	27.2 (q)
5′	2.18 (3H, d, 1.2)	20.33 (q)	2.18/27.46, 115.24, 158.24	2.18 (3H, d, 1.2)	20.36 (q)	2.12 (3H, d, 1.2)	20.1 (q)

a) J_{C-H} for HMBC measurement: 8.0 Hz.

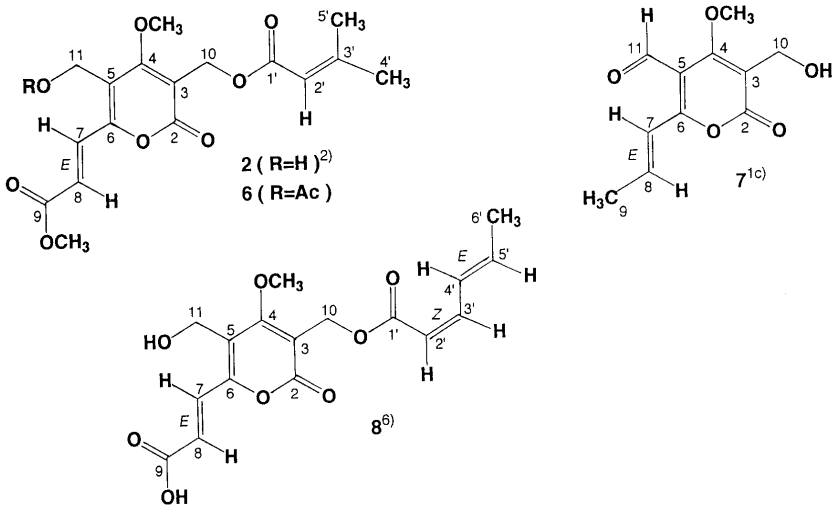


Chart 1. Structures of Macrophin (**2**), Macrophin Acetate (**6**), Multiforisin A (**7**), and Islandic Acid (**8**)

CH=CH–[C], *b*: [O]–CH(CH_3)–CH_2–CH=CH–[C], *c*: O–C(=O)–, and *d*: O–C(=O)–, were present in **4**. These spectroscopic data of **4** were quite similar to those of a fungal metabolite, colletodiol, from *Colletotricum capsici*[3a–d] and *Chaetomium funicola*.[3e] The melting point, specific rotation, and CD, UV, IR, and ^{1}H-NMR spectral data of DG-4 were the same as the corresponding data of colletodiol reported in the literature,[3] indicating that DG-4 is identical with colletodiol (**4**) (see Experimental and Table 2). The absolute stereostructure of colletodiol (**4**) has already been determined as (2*R*,8*R*,10*R*,11*R*),[3d] (see Chart 2). To our knowledge, this is the first time that colletodiol (**4**) has been shown to have immunosuppressive activity.

DG-3 (**3**) was obtained as a pale yellow oil, $C_{14}H_{20}O_6$, $[\alpha]_D^{24}$ −19°. The IR and UV spectra of **3** were quite similar to those of **4**. The ^{1}H- and ^{13}C-NMR spectra of **3** were assigned with the aid of a two-dimensional COSY *via* long-range couplings (COLOC) NMR experiment (see Table 2). All of the signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were quite similar to the corresponding signals in those of **4**, except that the signals at positions 9, 10, and 11 in both spectra of **3** were different from those of **4**, suggesting that **3** is a new stereoisomer of **4**. On benzoylation with benzoyl chloride and pyridine, **4** afforded a monobenzoate (**9**), a colorless resinous substance, and a dibenzoate (**10**), colorless plates. Comparison of the ^{1}H-NMR spectra of **9** and **10** with that of **3** showed that the signal of H-10 was shifted to δ 5.03 (+1.22) in the spectrum of **9**, and the signals of H-10 and -11 were shifted to δ 5.21 (+1.40) and 6.10 (+1.62) in that of **10**, respectively (see Table 2). These results imply that during the reaction the hydroxyl at position 10 in **3** was benzoylated to give **9**, while the hydroxyls at positions 10

Table 2. ^{1}H-NMR and ^{13}C-NMR Data for DG-4 (**4**), Colletodiol (**4**), and DG-3 (**3**), DG-3 Monobenzoate (**9**), and DG-3 Dibenzoate (**10**), δ (ppm) from TMS as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	DG-4 (**4**)		Colletodiol (**4**)[2c]	**3**		
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{1}H-NMR	^{13}C-NMR	COLOC[a] (^{13}C-NMR/^{1}H-NMR)
2	5.31 (dqd, 11.5, 6.4, 3.4)	68.75 (d)	5.30 (dqd, 11.8, 6.0, 4.0)	5.18 (dqd, 11.5, 6.3, 4.1)	69.34 (d)	69.34/1.41
2-CH_3	1.37 (3H, d, 6.4)	20.39 (q)	1.38 (3H, d, 6.0)	1.41 (3H, d, 6.3)	20.32 (q)	20.32/
3	2.23 (ddd, 12.7, 11.5, 11.3)	41.13 (t)	2.21 (ddd, 11.8, 11.8, 11.0)	2.21 (ddd, 12.9, 11.5, 8.8)	39.31 (t)	39.31/1.41, 5.68
	2.52 (dddd, 12.7, 4.9, 3.4, 1.2)		2.50 (dddd, 11.8, 5.2, 4.0, 1.0)	2.61 (ddd, 12.9, 7.3, 4.1)		
4	6.71 (ddd, 16.1, 11.3, 4.9)	144.22 (d)	6.70 (ddd, 16.0, 11.0, 5.2)	6.74 (ddd, 15.7, 8.8, 7.3)	143.75 (d)	143.75/2.61
5	5.73 (dd, 16.1, 1.2)	125.70 (d)	5.72 (dd, 16.0, 1.0)	5.68 (d, 15.7)	125.73 (d)	125.73/2.61
6		165.23 (s)			165.52 (s)	165.52/5.68
8	5.18 (qdd, 6.6, 4.4, 1.8)	68.08 (d)	5.16 (qdd, 6.0, 4.4, 2.0)	4.97 (dqd, 9.4, 6.3, 1.5)	70.20 (d)	70.20/
8-CH_3	1.36 (3H, d, 6.6)	18.15 (q)	1.38 (3H, d, 6.0)	1.31 (3H, d, 6.3)	21.62 (q)	21.62/1.60
9	1.51 (ddd, 15.6, 6.1, 1.8)	36.24 (t)	1.50 (ddd, 15.8, 6.0, 2.0)	1.60 (dd, 15.9, 1.5)	38.31 (t)	38.31/1.31, 4.48
	2.02 (ddd, 15.6, 4.4, 1.8)		2.00 (ddd, 15.8, 4.4, 2.0)	2.39 (ddd, 15.9, 9.4, 7.4)		
10	3.66 (ddd, 9.1, 6.1, 1.8)	71.83 (d)	3.63 (ddd, 9.0, 6.0, 2.0)	3.81 (dd, 7.4, 2.1)	74.73 (d)	74.73/1.60
11	4.07 (ddd, 9.1, 5.7, 1.2)	73.86 (d)	4.06 (ddd, 9.0, 5.5, 1.0)	4.48 (ddd, 4.9, 2.1, 1.9)	75.10 (d)	75.10/6.70
12	6.74 (dd, 15.7, 5.7)	146.54 (d)	6.73 (dd, 16.0, 5.5)	6.70 (dd, 15.7, 4.9)	145.87 (d)	145.87/4.48
13	6.14 (dd, 15.7, 1.2)	123.77 (d)	6.14 (16.0, 1.0)	6.06 (dd, 15.7, 1.9)	122.44 (d)	122.44/4.48, 6.70
14		166.72 (s)			166.48 (s)	166.48/6.06, 6.70

a) J_{C-H} for COLOC measurement: 8.0 Hz.

Position	**9**		**10**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
2	5.18 (ddd, 9.0, 6.5, 4.0)	69.42 (d)	5.17 (m)	69.70 (d)
2-CH_3	1.41 (3H, d, 6.5)	20.39 (q)	1.40 (3H, d, 6.4)	20.43 (q)
3	2.22 (ddd, 14.3, 9.0, 9.0)	39.34 (t)	2.26 (ddd, 13.0, 8.4, 8.4)	38.96 (t)
	2.59 (ddd, 14.3, 7.1, 4.0)		2.63 (ddd, 13.0, 7.5, 4.3)	
4	6.74 (ddd, 15.8, 9.0, 7.1)	143.20 (d)	6.79 (ddd, 15.9, 8.4, 7.5)	143.12 (d)
5	5.73 (d, 15.8)	126.12 (d)	5.77 (d, 15.9)	126.05 (d)
6		165.68 (s)		165.34 (s)
8	5.13 (dq, 9.0, 6.1)	69.95 (d)	5.25 (m)	70.08 (d)
8-CH_3	1.31 (3H, d, 6.1)	21.43 (q)	1.36 (3H, d, 6.1)	21.48 (q)
9	1.72 (d, 15.9)	35.55 (t)	1.88 (d, 15.9)	37.16 (t)
	2.66 (ddd, 15.9, 9.4, 7.8)		2.75 (ddd, 15.9, 9.6, 7.1)	
10	5.03 (br d, 7.8)	78.20 (d)	5.21 (br d, 7.1)	74.12 (d)
11	4.69 (ddd, 4.7, 2.2, 2.1)	72.96 (d)	6.10 (br d, 5.4)	76.08 (d)
12	6.80 (dd, 14.5, 4.7)	145.31 (d)	6.83 (dd, 15.6, 5.4)	141.69 (d)
13	6.13 (dd, 14.5, 2.1)	122.84 (d)	6.04 (dd, 15.6, 2.0)	124.14 (d)
14		166.15 (s)		165.52 (s)
$-COC_6H_5$	7.45 (2H, t, 7.9), 7.58 (tt, 7.9, 1.2), 8.05 (2H, dd, 7.9, 1.2)	128.38, 128.42, 129.65, 129.76, 133.31 (each d)	7.40 (2H, t, 7.8), 7.54 (3H, br t, 8.0), 7.66 (tt, 7.5, 1.3), 7.96 (2H, dd, 8.4, 1.4), 8.16 (2H, dd, 8.5, 1.4)	128.40 (d), 128.66 (3C, d), 129.74 (2C, d), 129.84 (2C, d), 133.26 (d), 133.56 (d)
$-COC_6H_5$		165.26 (s)		165.20 (s), 165.34 (s)

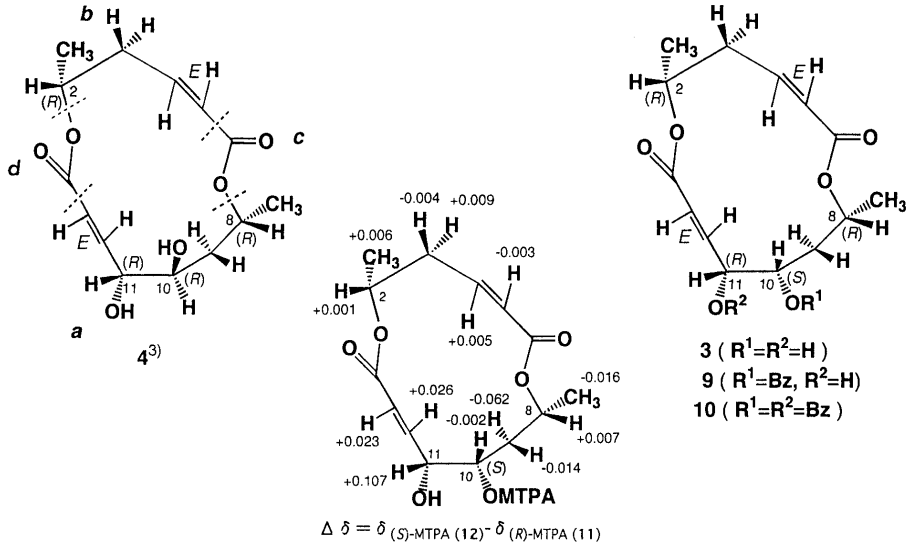


Chart 2. Structures of DG-3 (**3**), DG-3 Monobenzoate (**9**), DG-3 Dibenzoate (**10**), DG-3 (*R*)-MTPA Ester (**11**), DG-3 (*S*)-MTPA Ester (**12**), and DG-4 (**4**)

and 11 in **3** were benzoylated to afford **10**. The relative stereostructure of **10** was solved directly by X-ray crystallographic analysis of **10**, as shown in Fig. 1 (see Experimental). All of the relative configurations in **3** determined by X-ray analysis were the same as the corresponding ones in **4** except for that at position 10. In order to apply the modified Mosher's method[7] to **3**, the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetate of **3** ((*R*)-MTPA ester) (**11**) and the (*S*)-MTPA ester of **3** (**12**) were prepared. The signals of the carbinyl proton at position 10 in **11** and **12** were shifted to δ 5.019 (+1.209) and 5.017 (+1.207), respectively, indicating that during these reactions the hydroxyl group at position 10 in **3** was (*R*)- and (*S*)-MTPA-esterified to give **11** and **12**, respectively. The $\Delta\delta$ values ($\delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$) between **11** and **12** were calculated to be as shown in Table 3 and Chart 2, indicating that the absolute configuration at position 10 in **3** is (*S*). Thus, the absolute stereostructure of **3** was deduced to be (2*R*,8*R*,10*S*,11*R*). We propose to name DG-3 10-*epi*-colletodiol (**3**).

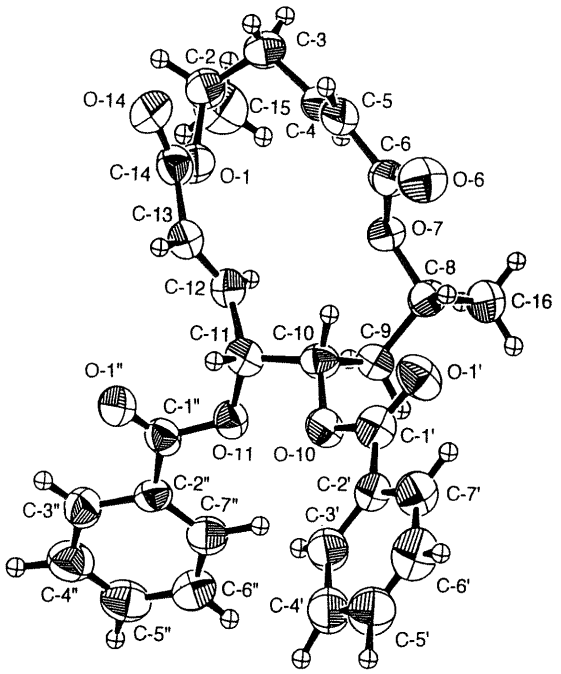
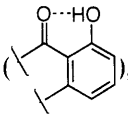


Fig. 1. Relative Stereostructure of DG-3 Dibenzoate (**10**) Obtained by X-Ray Crystallographic Analysis

DG-1 (**1**) was obtained as a colorless amorphous powder, $C_8H_{12}O_4$. The IR spectrum of **1** suggested the presence of an oxycarbonyl moiety in **1**. The ^{1}H- and ^{13}C-NMR spectra of **1** (see Table 4) suggested that four partial structures, *e*: [O]–CH(CH_3)–CH_2–[C] × 2 and *f*: [C]–C(=O)–O–[C] × 2, were present in **1**. Considering that the hydroxyl group was absent in DG-1, DG-1 was supposed to be a cyclic dimer of β-hydroxybutyric acid, namely, 4,8-dimethyl-1,5-dioxacyclooctane-2,6-dione (**1**) (see Chart 3). To our knowledge, this is the first time that **1** has been isolated as a natural product.

DG-5 (**5**) was obtained as colorless needles, $[\alpha]_D^{24}$ +15°. The IR and UV spectra suggested the presence of conjugated carbonyl, hydroxyl, and benzene ring moieties in **5**. The electron impact-MS (EI-MS) afforded an ion peak considered to be the molecular ion at *m/z* 178 ($C_{10}H_{10}O_3^{\cdot+}$). The ^{1}H-NMR spectrum indicated the presence of two partial structures, *g*: [C]–CH_2–CH_2–CH(OH)–[C] and *h*: 2,3-disubstituted phenol in which the phenolic O–H (δ 12.40) is hydrogen-bonded with >C=O at the *peri*-position (), in **5**. These spectroscopic data of **5** were similar to those of isosclerone from *Sclerotinia sclerotiorum*[8] (see Chart 3). The melting point, specific rotation, EI-MS, UV, IR, and ^{1}H-NMR spectral data of DG-5 were the same as the corresponding data of isosclerone reported in the literature,[8] indicating that DG-5 is identical with isosclerone (**5**) (see Experimental and Table 4). Thus, isosclerone (**5**) has been isolated for the first time from *D. grovesii*.

The IC_{50} values of an immunosuppressive α-pyrone, macrophin (**2**), and its acetate (**6**) were calculated to be 0.4 and 0.8 μg/ml against Con A-induced and 0.3 and 0.7 μg/ml against LPS-induced proliferations of mouse spleen lymphocytes, respectively (see Fig. 2), suggesting that the presence of a free hydroxyl group at position 11 is not indispensable for the appearance of immunosup-

Table 3. ^{1}H-NMR Data for 10-*epi*-Colletodiol (**3**)-(*S*)-MTPA Ester (**12**) and -(*R*)-MTPA-Ester (**11**), and $\Delta[\delta(\mathbf{12})-\delta(\mathbf{11})]$, δ (ppm) from TMS as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**12**	**11**	$\Delta[\delta(\mathbf{12})-\delta(\mathbf{11})]$
2	5.182 (dqd, 9.2, 6.4, 3.9)	5.181 (dqd, 9.2, 6.4, 3.9)	+0.001
2-CH_3	1.413 (3H, d, 6.4)	1.407 (3H, d, 6.4)	+0.006
3	2.227 (m)	2.218 (dd, 14.0, 8.0)	+0.009
	2.606 (dd, 15.5, 7.7)	2.610 (m)	−0.004
4	6.733 (ddd, 15.5, 8.8, 7.1)	6.728 (ddd, 15.8, 9.0, 6.9)	+0.005
5	5.694 (d, 15.7)	5.697 (d-like, 15.6)	−0.003
8	5.087 (dqd, 9.6, 6.1, 1.3)	5.080 (dqd, 9.7, 6.3, 1.3)	+0.007
8-CH_3	1.275 (3H, d, 6.3)	1.291 (3H, d, 6.3)	−0.016
9	1.538 (d, 16.1)	1.600 (br d, 14.7)	−0.062
	2.576 (m)	2.590 (m)	−0.014
10	5.017 (dt, 7.3, 1.8)	5.019 (dt, 7.6, 1.4)	−0.002
11	4.594 (br s)	4.487 (br s)	+0.107
12	6.717 (dd, 15.6, 4.7)	6.691 (dd, 15.5, 4.5)	+0.026
13	6.111 (dd, 15.6, 2.1)	6.088 (dd, 15.5, 2.0)	+0.023
$-OCOC(CF_3)(OC\underline{H}_3)C_6H_5$	3.545 (3H, s)	3.558 (3H, s)	
$-OCOC(CF_3)(OCH_3)C_6\underline{H}_5$	7.416—7.530 (5H, m)	7.415—7.523 (5H, m)	

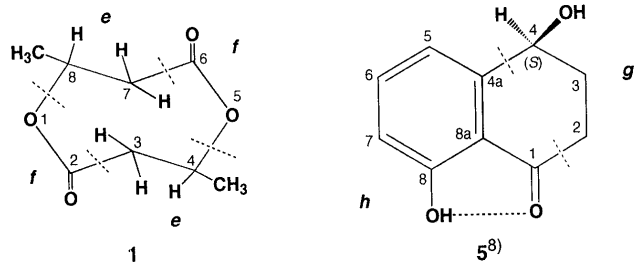


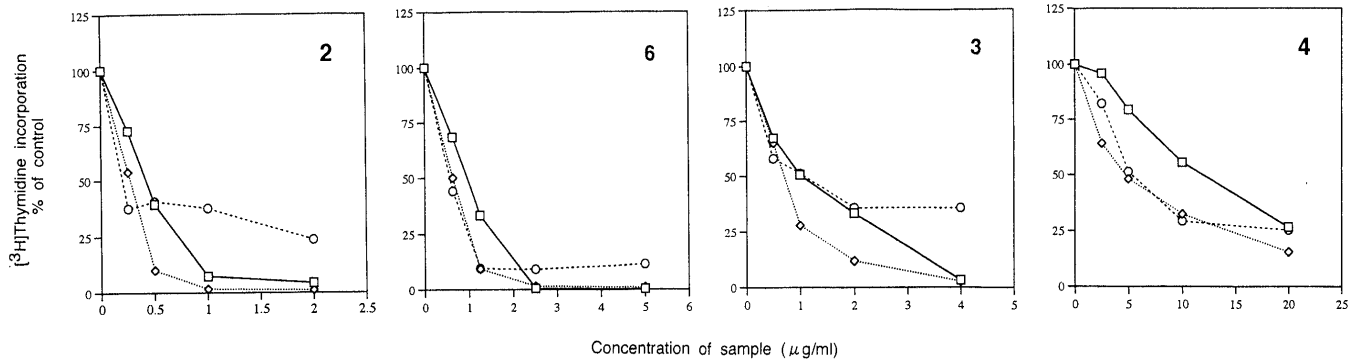
Chart 3. Structures of DG-1 (**1**) and DG-5 (**5**)

pressive activity of **2**. The IC_{50} values of another immunosuppressant, multiforisin A (**7**), have been reported to be 0.6 and 0.6 μg/ml against Con A- and LPS-induced proliferations of mouse spleen lymphocytes, respectively,[1c] while those of dexamethasone (**13**) and azathioprine (**14**), were calculated to be 0.02 and 2.7 μg/ml against Con A-induced and 0.02 and 2.7 μg/ml against LPS-induced proliferations of mouse spleen lymphocytes, respectively. Thus, the immunosuppressive activities of the two α-pyrones, **2** and **7**, are about as strong as those of **13** and **14**. On the other hand, the IC_{50} values of **7** were

Table 4. ^{1}H-NMR and ^{13}C-NMR Data for DG-1 (**1**) and DG-5 (**5**), and ^{1}H-NMR Data for Isosclerone (**5**), δ (ppm) from TMS as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**1**		DG-5 (**5**)			Isosclerone (**5**)[8]
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	HMBC[a] (^{1}H-NMR/^{13}C-NMR)	
1	—	—	—	204.34 (s)	—	—
2	—	169.14 (s)	2.64 (m)	34.57 (t)	2.64/31.20, 67.68, 204.34	2.00—3.15 (2H, m)
			3.00 (m)		3.00/31.20, 67.68, 204.34	
3	2.48 (dd, 15.5, 5.7)	40.74 (t)	2.16 (m)	31.20 (t)	2.16/34.57, 67.68, 145.88, 204.34	2.00—3.15 (2H, m)
	2.61 (dd, 15.5, 7.6)		2.19 (m)		2.19/204.34	
4	5.26 (dqd, 7.6, 6.4, 5.7)	67.58 (d)	4.91 (dd, 5.9, 3.7)	67.68 (d)	4.91/34.57, 115.25, 117.43, 145.88	4.89 (dd, 6.5, 4.0)
4-CH_3	1.27 (3H, d, 6.4)	19.75 (q)	—	—	—	—
4-OH	—	—	—	—	—	1.93 (s)
4a	—	—	—	145.88 (s)	—	—
5	—	—	7.02 (dd, 7.4, 1.8)	117.43 (d)	7.02/67.68, 115.25, 117.75, 137.01	7.00 (dd, 7.5, 2.0)
6	—	169.14 (s)	7.49 (dd, 8.3, 7.4)	137.01 (d)	7.49/145.88, 162.68	7.48 (t, 7.5)
7	2.48 (dd, 15.5, 5.7)	40.74 (t)	6.92 (dd, 8.3, 1.8)	117.75 (d)	6.92/115.25, 117.43, 162.68	6.91 (dd, 7.5, 2.0)
	2.61 (dd, 15.5, 7.6)	—	—	—	—	—
8	5.26 (dqd, 7.6, 6.4, 5.7)	67.58 (d)	—	162.68 (s)	—	—
8-CH_3	1.27 (3H, d, 6.4)	19.75 (q)	—	—	—	—
8-OH	—	—	12.40 (s)	—	—	12.65 (s)
8a	—	—	—	115.25 (s)	—	—

a) J_{C-H} for HMBC measurement: 8.0 Hz.



cpm at 0 μg/ml	
Con A :	84477
LPS :	25589
mitogen-free :	2441

%S.E. of each point			
μg/ml	Con A	LPS	Mitogen-free
control	3.7	4.4	7.3
0.25	4.6	9.8	19.9
0.5	1.0	10.5	26.5
1.0	6.2	31.0	19.6
2.0	29.1	4.9	29.5

cpm at 0 μg/ml	
Con A :	70267
LPS :	22721
mitogen-free :	3707

%S.E. of each point			
μg/ml	Con A	LPS	Mitogen-free
control	2.9	6.7	5.8
0.63	2.7	10.5	21.7
1.25	15.9	12.3	27.1
2.5	14.0	39.1	37.5
5.0	52.0	52.2	1.1

cpm at 0 μg/ml	
Con A :	57712
LPS :	14856
mitogen-free :	592

%S.E. of each point			
μg/ml	Con A	LPS	Mitogen-free
control	8.8	4.0	71.3
0.5	7.6	18.4	25.3
1.0	6.4	13.6	33.7
2.0	0.9	20.1	40.0
4.0	7.0	10.0	32.1

cpm at 0 μg/ml	
Con A :	56259
LPS :	11259
mitogen-free :	1292

%S.E. of each point			
μg/ml	Con A	LPS	Mitogen-free
control	3.6	4.0	5.0
2.5	3.3	6.1	28.0
5.0	7.2	10.5	5.8
10.0	4.8	10.0	11.3
20.0	3.0	9.0	24.0

Fig. 2. Effects of Macrophin (**2**), Macrophin Acetate (**6**), DG-3 (**3**), and DG-4 (**4**) on Mitogen-Induced and Mitogen-Free Proliferation of Mouse Spleen Lymphocytes

—□—, against Con A-induced proliferation (T-cell); ---◇---, against LPS-induced proliferation (B-cell); ---○---, against mitogen-free proliferation. Each point represents the mean of 3 experiments (see %S.E. of each point).

10.0[1c] and 1.0 μg/ml against human KB and HL-60 cells, respectively, and that of **2** was 0.2 μg/ml against human HL-60 cells. A study on the structure–activity relationship of these α-pyrone-type immunosuppressants is in progress. The IC_{50} values of 10-*epi*-colletodiol (**3**) and colletodiol (**4**) were calculated to be 5.0 and 12.0 μg/ml against Con A-induced and 3.8 and 5.0 μg/ml against LPS-induced proliferations of the lymphocytes, respectively (see Fig. 2), suggesting that **3** (10*S*-isomer) is a little more potent than **4** (10*R*-isomer). 4,8-Dimethyl-1,5-dioxacyclooctane-2,6-dione (**1**) and isosclerone (**5**) suppressed both Con A- and LPS-induced proliferations of mouse spleen lymphocytes by less than 50% even at 30 μg/ml.

In summary, the fractionation of the AcOEt layer of the acetone extract of *D. grovesii*, guided by monitoring of the immunosuppressive activity, afforded three active components, namely, an α-pyrone, macrophin (**2**), and two macrolides, colletodiol (**4**) and 10-*epi*-colletodiol (**3**), together with two non-active components, 4,8-dimethyl-1,5-dioxacyclooctane-2,6-dione (**1**) and isosclerone (**5**). The immunosuppressive activity of **2** was more than ten times stronger than that of **3** or **4**.

Experimental

The general procedures for the chemical experiments and other experimental conditions, including those for evaluation of the effect of samples on proliferation of mouse spleen lymphocytes, were the same as described in our previous report.[1c]

Isolation of DG-1 (1), -2 (2), -3 (3), -4 (4), and -5 (5) *Diplogelasinospora grovesii* CAILLEUX IFM4650[4] was cultivated on sterilized rice (200 g/flask × 150) at 25 °C for 37 d. The moldy rice was extracted with acetone (27.0 l) with shaking at room temperature for 6 h, two times, to give an acetone solution (54.0 l), which was concentrated *in vacuo* to give a concentrated solution (*ca.* 1.0 l). The concentrated solution was partitioned between *n*-hexane (1.0 l) and H_2O (300 ml) three times to afford an *n*-hexane layer (after evaporation, 24.9 g) and an aqueous suspension. The aqueous suspension was further partitioned with AcOEt (1.0 l) three times to afford an AcOEt layer (after evaporation, 23.4 g) and an aqueous layer (after evaporation, 79.5 g). The AcOEt layer was subjected to silica gel column chromatography with *n*-hexane–acetone (20 : 1, v/v), (5 : 1), and (1 : 1) to give four fractions, I—IV. Fraction IV (5.48 g) was further chromatographed on a silica gel column with $CHCl_3$–MeOH (100 : 1), (30 : 1), and (10 : 1) to give four fractions IVa—IVd. Fraction IVa (550 mg) was recrystallized from EtOH to afford **1** (99 mg) as needles and a mother liquor. The mother liquor was chromatographed on a silica gel column with C_6H_6–AcOEt (5 : 1), (4 : 1), and (2 : 1) to give three fractions IVaa—IVac. Fraction IVab (215 mg) was then subjected to medium-pressure liquid chromatography (MPLC) on a silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (1 : 2) at a flow rate of 4.0 ml/min to give **5** (10 mg) and **2** (25 mg). Fraction IVc (2.05 g) was further chromatographed on a silica gel column with *n*-hexane–AcOEt (2 : 1) and (1 : 1) to afford three fractions, IVca—IVcc. Fraction IVca (250 mg) was recrystallized from AcOEt to afford **4** (30 mg) as needles. Fraction IVcb (745 mg) was then chromatographed on a silica gel column with *n*-hexane–acetone (1 : 1) and (1 : 2) to afford **3** (700 mg) as a pale yellow oil.

DG-1 (4,8-Dimethyl-1,5-dioxacyclooctane-2,6-dione) (**1**): Colorless amorphous powder from EtOH, mp 117—120 °C, $[\alpha]_D^{25}$ −4.6° (*c* = 1.00, $CHCl_3$), high-resolution FAB-MS (HR-FAB-MS) *m/z*: 173.0811 ($C_8H_{13}O_4$ requires 173.0814 $[(M+H)^+]$). IR $\nu_{mac}^{CHCl_3}$ cm^{-1}: 2975 (C–H), 1735 (C=O).

DG-2 (Macrophin[2]) (**2**): Colorless needles from AcOEt, mp 119—122 °C (lit.[2] 118—121 °C), HR-FAB-MS *m/z*: 353.1237 ($C_{17}H_{21}O_8$ requires 353.1236 $[(M+H)^+]$) (lit.[2] EI-MS *m/z*: 352 (M^+)). UV λ_{max}^{EtOH} nm (log ε): 229 (4.63), 338 (4.03) (lit.[2] 231 (4.52), 340 (4.16)). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3608 (O–H), 2928 (C–H), 1716 (C=O), 1646, 1607 (C=C) (lit.[2] 3490, 1720 (sh), 1701, 1643, 1608).

DG-3 (10-*epi*-Colletodiol) (**3**): Pale yellow oil, $[\alpha]_D^{24}$ −19.1° (*c* = 0.13, $CHCl_3$), HR-FAB-MS *m/z*: 285.1334 ($C_{14}H_{21}O_6$ requires 285.1339 $[(M+H)^+]$). UV λ_{max}^{MeOH} nm (log ε): 210 (3.87). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660 (O–H), 2950 (C–H), 1713 (C=O), 1650, 1600 (C=C). CD (*c* = 0.025, MeOH) Δε (nm): 0 (278), −11.2 (221), −2.4 (210).

Table 5. Fractional Coordinates and Isotropic Thermal Parameters for Non-Hydrogen Atoms of DG-3 Dibenzoate (**10**) with Estimated Standard Deviations in Parentheses

Atom	*x*	*y*	*z*	B_{eq}
C- 2	0.1921(4)	−0.5407(2)	−0.0885(5)	5.6(1)
C- 3	0.2628(4)	−0.5745(2)	−0.1890(5)	5.8(1)
C- 4	0.2139(3)	−0.6138(2)	−0.3036(4)	4.8(1)
C- 5	0.2238(3)	−0.6805(2)	−0.3255(5)	4.7(1)
C- 6	0.1658(3)	−0.7199(2)	−0.4227(5)	4.7(1)
C- 8	0.0132(3)	−0.7230(2)	−0.5350(4)	4.7(1)
C- 9	−0.0747(3)	−0.7282(2)	−0.4458(4)	4.57(10)
C-10	−0.0646(3)	−0.7758(2)	−0.3163(5)	4.42(10)
C-11	−0.0812(3)	−0.7440(2)	−0.1723(5)	4.41(10)
C-12	−0.0101(3)	−0.6907(2)	−0.1380(4)	4.29(10)
C-13	0.0516(3)	−0.6973(2)	−0.0334(5)	4.39(10)
C-14	0.1283(4)	−0.6480(2)	−0.0085(5)	4.7(1)
C-15	0.1487(5)	−0.4770(2)	−0.1460(7)	7.6(2)
C-16	0.0018(4)	−0.6808(3)	−0.6684(5)	6.2(1)
C- 1′	−0.1038(4)	−0.8849(2)	−0.4100(5)	4.9(1)
C- 2′	−0.1695(3)	−0.9435(2)	−0.4014(4)	4.31(10)
C- 3′	−0.2537(4)	−0.9383(2)	−0.3281(5)	5.8(1)
C- 4′	−0.3124(4)	−0.9950(3)	−0.3271(5)	6.6(1)
C- 5′	−0.2857(5)	−1.0550(2)	−0.3920(6)	7.0(2)
C- 6′	−0.2031(5)	−1.0598(2)	−0.4628(6)	6.5(1)
C- 7′	−0.1446(4)	−1.0039(2)	−0.4685(5)	5.7(1)
C- 1″	−0.2099(3)	−0.7015(2)	−0.0389(5)	4.5(1)
C- 2″	−0.3015(3)	−0.6656(2)	−0.0470(5)	4.4(1)
C- 3″	−0.3364(4)	−0.6385(2)	0.0814(5)	5.4(1)
C- 4″	−0.4196(4)	−0.6030(3)	0.0790(6)	6.3(1)
C- 5″	−0.4682(3)	−0.5951(2)	−0.0441(7)	6.1(1)
C- 6″	−0.4354(4)	−0.6223(2)	−0.1722(5)	5.6(1)
C- 7″	−0.3504(3)	−0.6578(2)	−0.1730(5)	4.9(1)
O- 1	0.1120(2)	−0.5872(1)	−0.0692(3)	5.00(7)
O- 6	0.1872(2)	−0.7742(2)	−0.4744(4)	6.25(9)
O- 7	0.0834(2)	−0.6894(1)	−0.4458(3)	4.46(7)
O-10	−0.1293(2)	−0.8327(1)	−0.3248(3)	4.74(7)
O-11	−0.1737(2)	−0.7131(1)	−0.1710(3)	4.26(6)
O-14	0.1980(2)	−0.6616(2)	0.0597(3)	5.54(8)
O- 1′	−0.0340(3)	−0.8847(1)	−0.4826(4)	5.90(8)
O- 1″	−0.1710(2)	−0.7173(2)	0.0685(3)	5.52(8)

DG-4 (=Colletodiol[3]) (**4**): Colorless needles from AcOEt. mp 159—161 °C (lit.[3a] 163—164 °C), $[\alpha]_D^{24}$ +34.9° (*c* = 0.01, $CHCl_3$) (lit.[3a] $[\alpha]_D^{20}$ +36° (*c* = 1.0, $CHCl_3$)), CD (MeOH) Δε (nm): 0 (270), −12.7 (223), 0 (211) (lit.[3b] 0 (270), −13.0 (223), 0 (211)). FAB-MS *m/z*: 285 $[(M(C_{14}H_{20}O_6)+H)^+]$. UV λ_{max}^{MeOH} nm (log ε): 206 (4.11) (lit.[3c] 211 (4.02)). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3670 (O–H), 2920 (C–H), 1718 (C=O), 1650, 1600 (C=C) (lit.[3b] 3400, 1721, 1710, 1657, 1630).

DG-5 (=Isosclerone[8]) (**5**): Colorless needles from C_6H_6, mp 78—79 °C (lit.[8] 74—76 °C), $[\alpha]_D^{24}$ +15.3° (*c* = 0.16, $CHCl_3$) (lit.[8] $[\alpha]_D^{15}$ +19° (*c* = 0.34, $CHCl_3$)), EI-MS *m/z*: 178 $[(M(C_{10}H_{10}O_3))^+]$ (lit.[8] *m/z*: 178 (M^+)). UV λ_{max}^{EtOH} nm (log ε): 215 (4.33), 260 (3.99), 336 (3.72) (lit.[8] 215 (4.26), 260 (4.00), 334 (3.63)). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575 (O–H), 2960 (C–H), 1630 (C=O), 1570 (C_6H_6 ring) (lit.[8] 3620, 2950, 1640, 1580).

Acetylation of DG-2 A solution of DG-2 (**2**) (7 mg) in Ac_2O (200 μl) and pyridine (200 μl) was allowed to stand at room temperature for 3 h, then ice-water was added and the whole was extracted with AcOEt. The AcOEt layer was washed with water and water saturated with NaCl, then evaporated *in vacuo* to give a pale yellow resinous substance (**6**) (5 mg), FAB-MS *m/z*: 433 $[(M(C_{19}H_{22}O_9)+K)^+]$. UV λ_{max}^{EtOH} nm (log ε): 229 (4.26), 335 (3.75). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930 (C–H), 1722 (C=O), 1655, 1610 (C=C).

Benzoylation of DG-3 A solution of DG-3 (**3**) (110 mg) in benzoyl chloride (200 μl) and pyridine (400 μl) was allowed to stand at room temperature for 5 h, then ice-water was added and the whole was extracted with AcOEt. The AcOEt layer was treated in a similar way to that described for the acetylation of **2** to give a resinous residue, which

was chromatographed on a silica gel column to afford the dibenzoate of **3** (**10**) (8 mg), and the monobenzoate of **3** (**9**) (27 mg). DG-3 monobenzoate (**9**), a colorless resinous substance, FAB-MS *m/z*: 389 [$(M(C_{21}H_{24}O_7)+H)^+$]. DG-3 dibenzoate (**10**), colorless plates from $CHCl_3$–MeOH, mp 150—152 °C, HR-FAB-MS *m/z*: 493.1874 ($C_{28}H_{29}O_8$ requires 493.1866 [$(M+H)^+$]). UV λ_{max}^{EtOH} nm (log ε): 205 (sh, 4.35), 229 (4.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920 (C–H), 1720 (C=O), 1650 (C=C), 1600, 1500 (C_6H_6 ring).

X-Ray Crystallographic Analysis of DG-3 Dibenzoate (10) For X-ray crystallographic analysis of **10** [orthorhombic, space group $P2_12_12$ (#18), lattice constants $a=14.273(2)$, $b=19.442(7)$, $c=9.323(1)$ Å, $V=2587.2400$ Å^3, $Z=4$, D_{calcd} 1.264 g/cm^3], the data on 1713 observed reflections [$I>1.50\sigma(I)$] within the range of $0°<2\theta<44.1°$, measured with Mo$K\alpha$ radiation, were solved directly by the SHELXS86 program[9] and the solution was refined by the full-matrix least-squares method with anisotropic and isotropic temperature factors for all non-hydrogen atoms and all hydrogen atoms, respectively, to give a final *R* value of 0.058, including the contributions of all hydrogen atoms in **10**.[10] The final fractional coordinates of all non-hydrogen atoms with estimated standard deviations are listed in Table 5.

(*R*)- and (*S*)-MTPA Esters of DG-3 A solution of **3** (12 mg), (*R*)-MTPA acid (20 mg), and dicyclohexylcarbodiimide (DCC) (18 mg) in pyridine (50 μl) and CH_2Cl_2 (300 μl) was allowed to stand at room temperature for 1 h. The reaction mixture was evaporated *in vacuo* to give a resinous residue, which was purified by chromatography on a silica gel column with *n*-hexane–AcOEt to give the (*R*)-MTPA ester of **3** (**11**) (4 mg) as a pale yellow resinous substance, EI-MS *m/z*: 500 (M^+). UV λ_{max}^{EtOH} nm (log ε): 215 (sh, 4.30), 225 (sh, 3.98). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660 (O–H), 2960 (C–H), 1720 (C=O), 1650 (C=C), 1600, 1500 (C_6H_6 ring). A solution of **3** (19 mg), (*S*)-MTPA acid (32 mg), and DCC (28 mg) in pyridine (80 μl) and CH_2Cl_2 (470 μl) was allowed to stand at room temperature for 1 h. The reaction mixture was treated in the same way as described for the preparation of **11** from **3** to give the (*S*)-MTPA ester of **3** (**12**) (5.5 mg) as a pale yellow resinous substance, EI-MS *m/z*: 500 (M^+). UV λ_{max}^{EtOH} nm (log ε): 215 (sh, 4.22), 225 (sh, 3.91). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660 (O–H), 2920 (C–H), 1720 (C=O), 1650 (C=C), 1600, 1500 (C_6H_6 ring).

Acknowledgements We are grateful to Miss R. Hara of Analysis Center, Chiba University, for HR-FAB-MS and FAB-MS measurements, and Dr. K. Sugawara and Mr. T. Okazaki of Taisho Pharmaceutical Co. Ltd., for cytotoxicity assay. This study was supported in part by a Grant-in-Aid for Scientific Research (No. 09672273) from the Ministry of Education, Science and Culture of Japan.

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