Nine Constituents Including Six Xanthone-Related Compounds Isolated from Two Ascomycetes, *Gelasinospora santi-florii* and *Emericella quadrilineata*, Found in a Screening Study Focused on Immunomodulatory Activity

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Five metabolites tentatively called GS-1 (1)—5 (5) from *Gelasinospora santi-florii*, and four tentatively called EQ-4 (6), EQ-6 (7)—8 (9) together with 1—4 from *Emericella quadrilineata* have been isolated in a screening study on immunomodulatory fungal constituents. Among these nine metabolites, EQ-7 and 8 have been unknown. This time, the structures of GS-4 which has previously been isolated, EQ-7, and -8 have been determined to be (4*R*,4a*S*,9a*R*)-1,9a-dihydronidulalin A (4), (4*S*,4a*R*,9a*R*)-4a-carbomethoxy-1,4,4a,9a-tetrahydro-4,8-dihydroxy-6-methylxanthone (8), and 9-hydroxymicroperfuranone (9), respectively, and the six other metabolites have been identified. On bioassay, a dihydroxanthone, nidulalin A (1), a hexaketide, sordarial (5), and a xanthone, pinselin (7) have displayed significant immunosuppressive activities. The structure–activity relationships of these constituents have also been discussed.

Key words immunosuppressive fungal component; *Gelasinospora santi-florii*; *Emericella quadrilineata*; Ascomycete; xanthone; hexaketide

In our screening program on immunomodulatory constituents from fungi, many immunosuppressive metabolites have already been isolated from various Ascomycetes belonging to *Gelasinospora*, *Diplogelasinospora*, *Microascus*, *Emericella*, *Eupenicillium*, *Chaetomium*, and *Zopfiella* and a Fungi Imperfecti belonging to *Trichurus*.[1)] Successively, the EtOAc extracts of the two Ascomycetes, *Gelasinospora santi-florii* CAILLEUX and *Emericella quadrilineata* (THOM & RAPER) C. R. BENJAMIN showed appreciably suppressive effects on the proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with the mitogens, concanavalin A (Con A), and lipopolysaccharide (LPS). Repeated chromatographic fractionations of the EtOAc extract of *G. santi-florii* IFM4514,[2)] which suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 99.6% at 50 μg/ml, gave five constituents tentatively called GS-1 (**1**)—5 (**5**) [yields (%) of **1**, **2**, **3**, **4**, and **5** from the EtOAc extract: 0.84, 0.14, 0.69, 0.077, and 0.52, respectively].

Meanwhile, the EtOAc extract of *E. quadrilineata* IFM42027,[2)] which suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 96.6% at 50 μg/ml, was treated with *n*-hexane to give a defatted extract, suppressing the Con A-induced proliferation 99.7% at 50 μg/ml. Repeated chromatographic fractionations of an EtOAc layer, which was obtained from partition of the defatted extract with EtOAc–H_2O (3 : 1, v/v), gave eight constituents tentatively called EQ-1—8. The fact that EQ-1, 2, 3, and 5 were identical with **3**, **2**, **4**, and **1**, respectively, on the ^{1}H- and ^{13}C-NMR spectra, TLC behaviors, and specific rotations unified the names EQ-1, 2, 3, and 5 with the names GS-3, 2, 4, and 1, respectively. The eight constituents GS-3 (**3**), 2 (**2**), 4 (**4**), 1 (**1**), and EQ-4 (**6**), 6 (**7**)—8 (**9**) were obtained from *E. quadrilineata* in the following yields [yields (%) of **3**, **2**, **4**, **1**, **6**, **7**, **8**, and **9** from the EtOAc extract: 0.060, 1.3, 0.51, 1.1, 0.77, 0.15, 0.089, and 0.025, respectively]. Among the nine constituents **1**—**9** from the two fungi, **1**, **5**, and **7** showed significantly immunosuppressive activity (IC_{50} value of **1**, **5**, and **7** against the Con A-induced proliferation: 0.15, 6.5, and 5.1 μg/ml, respectively). This paper deals with the structures and immunosuppressive activities of the nine constituents **1**—**9** from the two fungi, among which we briefly reported on the structures and immunosuppressive activities of **1**—**5** in 1998.[3)]

GS-1 and 2 were identified with a dihydroxanthone, nidulalin A (**1**) and a benzophenone, nidulalin B (**2**) from the Ascomycete, *Emericella nidulans* var. *lata*,[4)] by direct comparison with the authentic specimen and comparison with the ^{1}H- and ^{13}C-NMR spectra, respectively. GS-3 was identified with 1-hydroxy-3-methylxanthone (**3**), a xanthone from an Ascomycete, *Anixiella* (=*Gelasinospora*) *micropertusa*,[5)] by the ^{1}H- and ^{13}C-NMR spectra. GS-5 was identified with sordarial (**5**), a hexaketide from some Ascomycetes, *Sordaria macrospora*,[6)] *Gelasinospora heterospora*, and *G. longispora*,[7)] by the ^{1}H- and ^{13}C-NMR spectra and the specific rotation. EQ-4 was identified with the racemic form of microperfuranone (**6**), a furanone from *G. micropertusa*,[5)] by the ^{1}H- and ^{13}C-NMR spectra and the specific rotation ($\pm$0°). EQ-6 was identified with pinselin (**7**), a xanthone from the Ascomycete, *Talaromyces bacillosporus*[8)] by direct comparison with the authentic specimen. To study the structure–activity relationship, **7** was methylated with (trimethylsilyl)diazomethane to give 2-methoxy derivative (**10**), ^{1}H-NMR: δ 4.03 (3H, s).

GS-4 (**4**), obtained as an optically active white powder ($[\alpha]_D^{25}$ −347.2°), gave $C_{16}H_{16}O_6$ as the molecular formula. These physicochemical data and the ^{1}H- and ^{13}C-NMR data including the two dimensional ^{1}H–^{1}H shift correlation (COSY), ^{1}H-detected heteronuclear correlation through multiple quantum coherence (HMQC), and ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) NMR data of **4** indicated that **4** was identical with 1,9a-dihydronidulalin A which was originally isolated from *G. micropertusa*, and

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Table 1. ^{1}H- and ^{13}C-NMR Data for GS-1 (**1**), GS-4 (**4**), EQ-7 (**8**), (*R*)-MTPA Ester of **8** (**11**), and (*S*)-MTPA Ester of **8** (**12**), and $\Delta\delta$ Value ($\delta_{12}-\delta_{11}$) in $CDCl_3$, δ (ppm) from TMS as an Internal Standard

Position	**1**		**4**		**8**		**11**	**12**	$\Delta\delta$ value
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_H	($\delta_{12}-\delta_{11}$)
1	7.34 (dd, 4.5, 2.1)	131.7 (d)	2.64 (m)	24.5 (t)	2.47 (2H, m)	25.6 (t)	2.625 (m)	2.630 (m)	0.005
			2.73 (m)				2.725 (m)	2.725 (m)	0
2	6.40*a)*	126.5 (d)	6.04 (m)	132.5 (d)	5.89 (m)	127.4 (d)	6.053 (m)	6.050 (m)	−0.003
3	6.40*a)*	131.6 (d)	5.86 (m)	123.7 (d)	5.73 (m)	127.3 (d)	5.848 (m)	5.845 (m)	−0.003
4	4.67 (d, 5.5)	65.2 (d)	4.66 (d, 5.9)	66.0 (d)	4.55 (d, 8.9)	69.3 (d)	4.650 (d, 7.8)	4.652 (d, 5.7)	0.002
4a		82.9 (s)		85.2 (s)		82.7 (s)			
$\underline{C}OOCH_3$-4a		168.8 (s)		168.3 (s)		170.7 (s)			
$COO\underline{CH_3}$-4a	3.62 (3H, s)	53.4 (q)	3.62 (3H, s)	52.9 (q)	3.71 (3H, s)	53.4 (q)	3.621 (3H, s)	3.622 (3H, s)	0.001
5	6.33 (br s)	108.3 (d)	6.27 (s)	108.0 (d)	6.42 (s)	108.4 (d)	6.338 (s)	6.339 (s)	0.001
6		151.0 (s)		149.8 (s)		151.1 (s)			
$\underline{CH_3}$-6	2.28 (3H, s)	22.6 (q)	2.26 (3H, s)	22.4 (q)	2.29 (3H, s)	22.6 (q)	2.240 (3H, s)	2.241 (3H, s)	0.001
7	6.40*a)*	111.4 (d)	6.36 (s)	111.2 (d)	6.36 (s)	111.1 (d)	6.250 (s)	6.251 (s)	0.001
8		162.8 (s)		161.3 (s)		162.5 (s)			
O$\underline{H}$-8	12.10 (s)		11.50 (s)		11.30 (s)		11.577 (s)	11.578 (s)	0.001
8a		105.7 (s)		105.1 (s)		103.6 (s)			
9		182.8 (s)		197.7 (s)		197.1 (s)			
9a		127.1 (s)	3.62 (m)	40.3 (d)	3.27 (dd, 10.7, 7.3)	45.2 (d)	3.602 (m)	3.605 (m)	0.003
10a		158.0 (s)		157.6 (s)		159.0 (s)			

Multiplicities and coupling constants (in Hz) in parenseses. *a*) Overlapped.

whose relative configuration between $COOCH_3$-4a and H-9a was elucidated to be *trans* by the differential nuclear Overhauser (DifNOE) experiment in 1998.[5] This time, the absolute structure of GS-4 was finally determined to be (4*R*,4a*S*,9a*R*)-1,9a-dihydronidulalin A (**4**) by the fact that GS-4 was derived from nidulalin A (**1**), whose absolute configurations at positions 4 and 4a were (*R*) and (*S*), respectively,[4] on hydrogenation with $NaBH_4$ in EtOH (this result was briefly reported by us in 1998[3]). The fact that the specific rotation and the circular dichroism (CD) curve of **4** were similar to those of **1** suggested that the absolute configurations at positions 4 and 4a in **4** were important for the appearance of the optical activity of **4** (see Experimental). Compound **4** was later synthesized by Tsuji and colleagues in 1999.[9]

EQ-7 (**8**), obtained as an optically active white powder ($[\alpha]_D^{25}$ +31.4°), gave $C_{16}H_{16}O_6$ as the molecular formula, the same as that of **4**. The ^{1}H- and ^{13}C-NMR data of **8** was similar to those of **4** except that the signals of H-4 [δ 4.55 (d, J=8.9 Hz)], C-4 [δ 69.3 (d)], and C-4a [δ 82.7 (s)] in **8** were different from those of H-4 [δ 4.66 (d, J=5.9 Hz)], C-4 [δ 66.0 (d)], and C-4a [δ 85.2 (s)] in **4**; this suggested that **8** might be a new stereoisomer of **4** (see Table 1). Contrary to the fact that the specific rotation and the CD curve of **4** were similar to those of **1**, the specific rotation and the CD curve of **8** were quite different from those of both **1** and **4**, suggesting that the absolute configurations at positions 4 and 4a in **8** might be different from those in **1** and **4** (see Experimental). On treatment with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)- and (*R*)-MTPA chloride] in pyridine, the hydroxyl at position 4 in **8** was esterified to give (*R*)- and (*S*)-MTPA esters of **8** (**11** and **12**), respectively. To apply the modified Mosher's method[10] to **8**, the $\Delta\delta$ values ($\delta_{12}-\delta_{11}$) were calculated as shown in Table 1, indicating that the absolute configuration at position 4 in **8** was (*S*). In the DifNOE experiment, 1.2, 6.3, and 1.9% of NOEs were observed between H-4 and $COOCH_3$-4a, between H-4 and H-9a, and between $COOCH_3$-4a and H-9a, respectively, indicating that $COOC\underline{H}_3$-4a was located at the *cis* sides of both H-4 and H-9a in **8**. Thus, the absolute configurations at positions 4a and 9a in **8** were (*R*) and (*R*), respectively. Accordingly, EQ-7 was deduced to be (4*S*,4a*R*, 9a*R*)-4a-carbomethoxy-1,4,4a,9a-tetrahydro-4,8-dihydroxy-6-methylxanthone (**8**), as shown in Fig. 1.

EQ-8 (**9**), obtained as a white amorphous powder, gave $C_{17}H_{14}O_4$ as the molecular formula. Comparison of the molecular formula and the ^{1}H- and ^{13}C-NMR data of **9** with those of **6** showed that **9** might be a new monohydroxyl derivative of **6** (see Table 2). The HMBC NMR experiment of **9** showed that the new hydroxyl group might be present at position 9 in **9** (see Fig. 1). Accordingly, EQ-8 was deduced to be 9-hydroxymicroperfuranone (**9**), as shown in Fig. 1. This compound was also racemic as well as **6**, because the specific rotation was ±0°.

The immunosuppressive activities (IC_{50} values) of **1**—**7**, **9**, and **10**, and some known immunosuppressants were calculated against Con-A induced (T cell) and LPS-induced (B cell) proliferations of mouse splenic lymphocytes, as shown in Table 3. This table shows that **1** possessed considerably high immunosuppressive activity, while those of **5** and **7** were moderate. The fact that the immunosuppressive activities of **4** and **8** were lower than that of **1** suggested that the presence of C=C bond between position 1 and 9a in **1** might be important for the appearance of the immunosuppressive activity of **1**. The fact that the activity of **10** was lower than that of **7** also suggested that the presence of the free OH group at position 2 in **7** might be important for the appearance of the activity of **7**. It was already known that the suppressive effects of substituted xanthones against the proliferation of human lymphocytes were ascribable to the positions of substituents on the xanthone nucleus.[11] The immunosuppressive activity of **5** was also already known.[7]

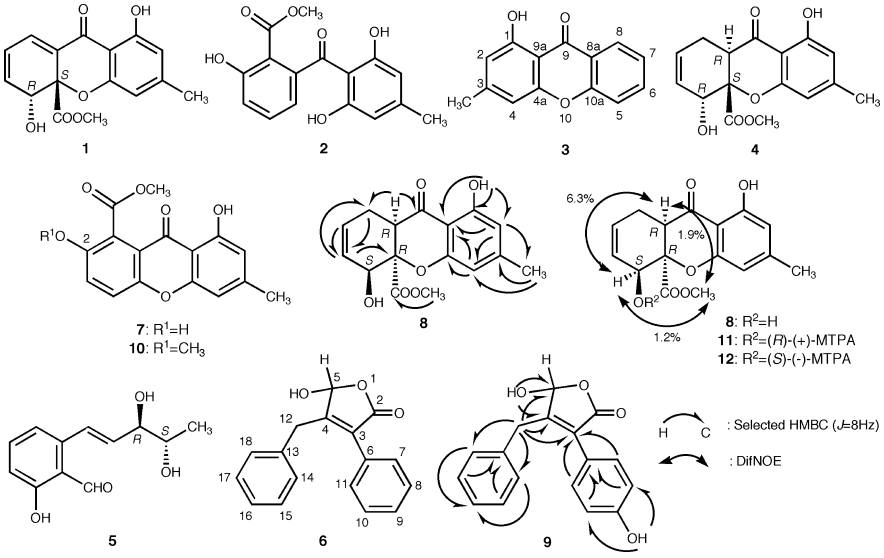

Fig. 1

Table 2. ^{1}H- and ^{13}C-NMR Data for EQ-4 (**6**) and EQ-8 (**9**) in Acetone-d_6, δ (ppm) from TMS as an Internal Standard

Position	**6**		**9**	
	δ_H	δ_C	δ_H	δ_C
2		170.9 (s)		171.2 (s)
3		130.8 (s)		129.8 (s)
4		159.6 (s)		157.5 (s)
5	5.98 (br s)	97.7 (d)	5.91 (d, 7.8)	97.4 (d)
OH-5	6.90 (br s)		6.77 (d, 7.8)	
6		130.2 (s)		121.8 (s)
7	7.54 (br d, 6.7)	129.9 (d)	7.42 (ddd, 9.2, 2.5, 2.5)	131.3 (d)
8	7.46 (m)	129.3 (d)	6.91 (ddd, 9.2, 2.5, 2.5)	116.2 (d)
9	7.43 (m)	129.6 (d)		158.9 (s)
OH-9			8.70 (s)	
10	7.46 (m)	129.3 (d)	6.91 (ddd, 9.2, 2.5, 2.5)	116.2 (d)
11	7.54 (br d, 6.7)	129.9 (d)	7.42 (ddd, 9.2, 2.5, 2.5)	131.3 (d)
12	3.97 (2H, br s)	32.9 (t)	3.83 (d, 15.4), 4.11 (d, 15.4)	32.9 (t)
13		137.5 (s)		137.8 (s)
14	7.30 (m)	129.6 (d)	7.30 (m)	129.6 (d)
15	7.26 (m)	129.7 (d)	7.26 (m)	129.7 (d)
16	7.22 (m)	127.7 (d)	7.24 (m)	127.6 (d)
17	7.26 (m)	129.7 (d)	7.26 (m)	129.7 (d)
18	7.30 (m)	129.6 (d)	7.30 (m)	129.6 (d)

Multiplicities and coupling constants (in Hz) in parentheses.

Table 3. Immunosuppressive Effects of GS-1 (**1**)—5 (**5**), EQ-4 (**6**), and EQ-6 (**7**)—8 (**9**), 2-Methoxy EQ-6 (**10**), and Azathioprine, Cyclosporin A, and Tacrolimus (FK506) on the Con A-Induced and LPS-Induced Proliferations of Mouse Splenic Lymphocytes

Compound	IC_{50} (μg/ml)	
	Con A-induced	LPS-induced
GS-1 (**1**)	0.15	0.06
GS-2 (**2**)	>25	17
GS-3 (**3**)	>25	>25
GS-4 (**4**)	>25	>25
GS-5 (**5**)	6.5	5.1
EQ-4 (**6**)	>25	n.t.
EQ-6 (**7**)	5.1	7.4
EQ-7 (**8**)	>25	>25
EQ-8 (**9**)	>25	n.t.
2-Methoxy EQ-6 (**10**)	>25	>25
Azathioprine	2.7	2.7
Cyclosporin A	0.04	0.07
Tacrolimus (FK506)	1.5×10^{-5}	1.6×10^{-3}

The IC_{50} value of each sample was calculated from the correlation curve between the sample concentration (horizontal axis) and the cell proliferation (vertical axis). The curve of each sample was drawn with 7 points, each of which represented the mean of three experiments on each correlation between 7 different concentrations and cell proliferations. n.t.: not tested.

Experimental

The general procedures for chemical experiments and other experimental conditions, including those for the evaluation of suppressive activity (IC_{50} values) of samples against the proliferation of mouse splenic lymphocytes stimulated with Con A and LPS, were the same as those described in our previous reports. [This method is based on the formation ratio of MTT-formazan from exogenous 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) in lymphocytes].[1)] Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard.

Isolation of GS-1 (1)—5 (5) from *G. santi-florii* IFM4514 *G. santi-florii* IFM4514[2)] was cultivated on sterilized moistened-rice in Roux flasks (200 g/flask×15) at 25 °C for 21 d. The dark brownish moldy-rice was extracted with EtOAc (4.5 l) with shaking at room temperature for 6 h three times to give an EtOAc solution (*ca.* 13.5 l), which gave, after evaporation *in vacuo*, an EtOAc extract (20.0 g). The extract suppressed the Con A-induced proliferation of mouse splenic lymphocytes 99.6% at 50 μg/ml. The EtOAc extract (13.0 g) was subjected to chromatography on a silica gel (Wakogel C-200, Wako) column with *n*-hexane–EtOAc (25 : 25, v/v), *n*-hexane–EtOAc–MeOH (25 : 25 : 1), (25 : 25 : 5), (25 : 25 : 50), and MeOH to give five fractions 1a—e (7.56, 2.44, 1.20, 1.05, 0.10 g), respectively. Fraction 1b (1.80 g) was further chromatographed on a silica gel (C60, Nacalai Tesque) column with *n*-hexane–EtOAc (25 : 25), (25 : 25), *n*-hexane–EtOAc–MeOH (25 : 25 : 1), (25 : 25 : 1), (25 : 25 : 5), (25 : 25 : 50), and MeOH to give seven fractions 2a—g (11, 440, 453, 379, 190, 64, 23 mg), respectively. Fraction 2c (100 mg) was subjected to preparative TLC on silica gel plates (Kieselgel 60, Merck) with $CHCl_3$–acetone (9 : 1) followed by HPLC on an octadecyl silica gel (ODS) column (Pegasil ODS, Senshu) with CH_3CN–H_2O (1 : 1) at a flow rate of 8 ml/min to give **1** (16 mg) and **2** (3 mg). Fraction 1a (7.55 g) was further chromatographed on an ODS (Chromatorex ODS, Fuji-Silysia) column with MeOH–H_2O followed by HPLC on an ODS column with CH_3CN–H_2O to give **4** (10 mg), **3** (90 mg), and **1** (10 mg). Fraction 2e (180 mg) was subjected to preparative TLC on silica gel plates with $CHCl_3$–ace-

tone (9 : 1) to give **5** (50 mg).

Isolation of GS-1 (1)—4 (4), EQ-4 (6), 6 (7), 7 (8), and 8 (9) from *E. quadrilineata* IFM42027 *E. quadrilineata* IFM42027[2] was cultivated on sterilized moistened-rice in Roux flasks (200 g/flask×3) at 25 °C for 23 d. The dark brownish moldy-rice was extracted with EtOAc (0.9 l) with shaking at room temperature for 6 h twice to give an EtOAc extract (4.47 g), which suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 96.6% at 50 μg/ml. The extract was treated with *n*-hexane (9.0 ml) to give a defatted extract as precipitate (2.68 g) and a fatty portion as a supernatant. The defatted extract, which suppressed the Con A-induced proliferation by 99.7% at 50 μg/ml, was partitioned with EtOAc–H_2O (3 : 1) (1.2 l) into an EtOAc layer (2.11 g) and an aqueous suspension. The aqueous suspension was further treated with *n*-BuOH–H_2O (2 : 1) (1.2 l) into an *n*-BuOH layer (162 mg) and an aqueous layer (48 mg). The EtOAc layer was subjected to chromatography on a silica gel (PSQ-100B, Fuji-Silysia) column with *n*-hexane–EtOAc (9 : 1), (9 : 1), (9 : 1—4 : 1), (2 : 1), (2 : 1—1 : 1), (2 : 1—1 : 1), and EtOAc–MeOH (1 : 1) to give seven fractions 1a—g (39, 296, 75, 92, 200, 237, 614 mg), respectively. Fraction 1a was further chromatographed on an ODS (Chromatorex ODS) column with MeOH–H_2O (4 : 1) to give **3** (2.7 mg). Fraction 1c was chromatographed on an ODS sep-pak cartridge (Waters) with MeOH–H_2O (3 : 2) to give **2** (57.7 mg). Fraction 1d was chromatographed on an ODS sep-pak cartridge with MeOH–H_2O (3 : 2), then on an ODS column with MeOH–H_2O (3 : 2) to give **4** (23.0 mg) and **6** (34.5 mg). Fraction 1f was chromatographed on a silica gel (PSQ-100B) column with *n*-hexane–acetone (3 : 1), then on an ODS sep-pak cartridge with MeOH–H_2O (3 : 2), followed by HPLC on an ODS column (Develosil ODS, Nomura) with MeOH–H_2O (1 : 1) to give **1** (50.1 mg), **7** (6.9 mg), **8** (4.0 mg), and **9** (1.1 mg).

GS-1 (**1**) [Nidulalin A]: Yellow prism (lit.[4] orange prism), $[\alpha]_D^{25}$ −493° (*c*=0.50, MeOH) (lit.[4] −426° ($CHCl_3$)). CD (0.0751 mM, MeOH) $\Delta\varepsilon$ (nm): 0 (378), −1.1 (274), −0.045 (243), −0.055 (237), 1.80 (211). The ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ were identical with those of nidulalin A.[4] This compound was directly identified by authentic nidulalin A[4] on a TLC plate (silica gel 60F_{254}, Merck) with $CHCl_3$–acetone (10 : 1) (*Rf*: 0.56) and on an HPLC ODS column (Pegasil ODS, Senshu) with CH_3CN–H_2O (1 : 1) at a flow rate of 0.8 ml/min (t_R: 8.87 min).

GS-2 (**2**) [Nidulalin B]: Yellow amorphous powder (lit.[4] colorless needles), HR-FAB-MS *m/z* 302.0773 (Calcd for $C_{16}H_{14}O_6$ (M^+): 302.0790). The ^{1}H- and ^{13}C-NMR data in $CDCl_3$ were identical with those of nidulalin B.[4]

GS-3 (**3**) [1-Hydroxy-3-methylxanthone]: Yellow powder (lit.[5] yellow needles), HR-FAB-MS *m/z* 226.0621 (Calcd for $C_{14}H_{10}O_3$ (M^+): 226.0630). The ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ were identical with those of 1-hydroxy-3-methylxanthone from *G. micropertusa*.[5]

GS-4 (**4**) [(4*R*,4a*S*,9a*R*)-1,9a-Dihydronidulalin A]: White powder (lit.[5] white amorphous solid), mp 145 °C from EtOH (lit.[5] 144—146 °C), $[\alpha]_D^{25}$ −347.2° (*c*=0.83, MeOH) (lit.[5] −431° (MeOH)), HR-FAB-MS *m/z* 305.1030 (Calcd for $C_{16}H_{17}O_6$ [$(M+H)^+$]: 305.1025) (lit.[5] 305.1041). CD (0.495 mM, MeOH) $\Delta\varepsilon$ (nm): +0.67 (378), −6.77 (279), +0.019 (243), −0.20 (237), +12.3 (211). The ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ (see Table 1) were identical with those of 1,9a-dihydronidulalin A from *G. micropertusa*.[5]

GS-5 (**5**) [Sordarial]: Pale yellow needles (lit.[7] pale yellow needles), mp 90.5 °C (lit.[7] 90.5—92.0 °C). $[\alpha]_D^{25}$ +22° (*c*=0.37, MeOH) (lit.[7] +22° (MeOH)). The ^{1}H- and ^{13}C-NMR spectra in CD_3OD were identical with those of sordarial from *G. heterospora* and *G. longispora*.[7]

EQ-4 (**6**) [Microperfuranone]: Colorless plates (lit.[5] colorless plates), $[\alpha]_D^{25}$ ±0° (*c*=1.48, MeOH) (lit.[5] −6.8° (MeOH)), HR-FAB-MS *m/z* 267.1001 (Calcd for $C_{17}H_{15}O_3$ [$(M+H)^+$]: 267.1021) (lit.[5] 267.1011). The ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ were identical with authentic microperfuranone.[5]

EQ-6 (**7**) [Pinselin]: Yellow powder (lit.[8] yellow prism), HR-FAB-MS *m/z* 300.0644 (Calcd for $C_{16}H_{12}O_6$ (M^+): 300.0634). The ^{1}H-NMR spectrum in acetone-d_6 was identical with that of pinselin isolated from *T. bacillosporus*.[8] This compound was directly identified with authentic pinselin on a TLC plate (silica gel 60F_{254}, Merck) with *n*-hexane–acetone (1 : 1) (*Rf*: 0.40).

EQ-7 (**8**) [(4*S*,4a*R*,9a*R*)-4a-Carbomethoxy-1,4,4a,9a-tetrahydro-4,8-dihydroxy-6-methylxanthone]: White powder, $[\alpha]_D^{25}$ +31.4° (*c*=0.087, MeOH), HR-FAB-MS *m/z* 305.1000 (Calcd for $C_{16}H_{17}O_6$ [$(M+H)^+$]: 305.1025). UV λ_{max}^{MeOH} nm (log ε): 280 (4.77), 348 (4.17). IR ν_{max}^{KBr} cm^{-1}: 3477, 2918, 1729, 1646, 1560, 1458, 1362, 1281, 1200, 1083. CD (0.131 mM, MeOH) $\Delta\varepsilon$ (nm): −1.84 (314), +8.64 (278), −6.20 (238), +44.2 (220), +28.0 (211), +61.9 (205).

EQ-8 (**9**) [9-Hydroxymicroperfuranone]: White powder, $[\alpha]_D^{25}$ ±0° (*c*= 0.030, MeOH), HR-FAB-MS *m/z* 283.0964 (Calcd for $C_{17}H_{15}O_4$ [$(M+H)^+$]: 283.0971). UV λ_{max}^{MeOH} nm (log ε): 281 (3.73). IR ν_{max}^{KBr} cm^{-1}: 3448, 2912, 1736, 1655, 1560, 1509, 1458.

Hydrogenation of GS-1 (1) A solution of **1** (3.0 mg) and $NaBH_4$ (3.0 mg) in EtOH (1.0 ml) was stirred at room temperature for 1 min to give a product mixture. The product mixture was purified by preparative TLC on silica gel plates (silica gel 60, Merck) with $CHCl_3$–acetone (9 : 1) to give a product (2.5 mg), which was identical with **4** by the ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ and the CD spectra in MeOH.

Methylation of EQ-6 (7) A 10% solution of $(CH_3)_3SiCHN_2$ in *n*-hexane (200 μl) was added to a solution of **7** (0.7 mg) in MeOH (200 μl) under ice-cooling to prepare a reaction mixture. The reaction mixture was stirred at 3 °C for 1 h to give a product solution, whose solvent was evaporated *in vacuo* to give a yellow powder (**10**) (0.7 mg).

2-Methoxy Derivative of EQ-6 (**10**): Yellow powder, ^{1}H-NMR, δ (ppm) from TMS in $CDCl_3$: 2.41 (3H, s, CH_3-6), 3.90 (3H, s, $COOCH_3$-1), 4.03 (3H, s, CH_3O-2), 6.59 (s, H-7), 6.71 (s, H-5), 7.38 (d, 9.4, H-4), 7.50 (d, 9.4, H-3), 12.1 (s, OH-8).

Formation of (*R*)- and (*S*)-MTPA Esters of EQ-7 (8) (*S*)-MTPA chloride (20 μl) was added to a solution of **8** (0.7 mg) in pyridine (200 μl) to prepare a reaction solution. The reaction solution was stirred at room temperature for 24 h to give a product solution, whose solvent was evaporated *in vacuo* to give a crude product. The crude product was chromatographed on a silica gel column followed on an ODS sep-pak cartridge to afford a white powder (**11**) (0.6 mg).

(*R*)-MTPA chloride (20 μl) was added to a solution of **8** (0.9 mg) in pyridine (200 μl) to prepare a reaction solution. The reaction solution was stirred at room temperature for 24 h to give a product solution, which was treated in the same way as that for the formation of **11** from **8** to afford a white powder (**12**) (0.8 mg).

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References and Notes

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