Isolation of Some Immunosuppressive Components from an Ascomycete, *Gelasinospora multiforis*

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Five new components, named multiforisins A, B, C, D, and E, with immunosuppressive activity were isolated from an Ascomycete, *Gelasinospora multiforis*. Multiforisin A, the main immunosuppressive principle of this fungus, was deduced to be 5-formyl-3-(hydroxymethyl)-4-methoxy-6-(1*E*-propenyl)-α-pyrone. Multiforisins B, C, D, and E were also deduced to be α-pyrone derivatives related to multiforisin A. The IC_{50} values of multiforisins A, B, C, D, E, and dihydro multiforisin A were evaluated against proliferation of mouse spleen lymphocytes stimulated with concanavalin A and lipopolysaccharide.

Key words fungal metabolite; Ascomycete; *Gelasinospora multiforis*; immunosuppressant; multiforisin; α-pyrone (2*H*-pyran-2-one)

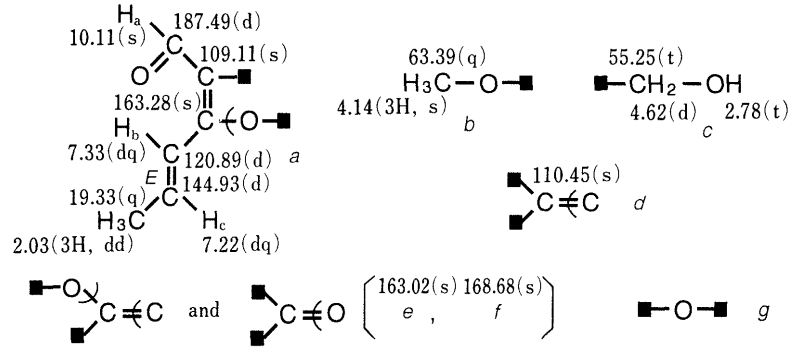
In our screening project to find immunologically active components of fungi, several immunosuppressive components have so far been isolated from some Basidiomycetes, *Lactarius flavidulus*,[1a] *Pisolithus tinctorius*, *Microporus flabelliformis* and *Lenzites betulina*.[1b] Now it has been found that the ethyl acetate extract of cultivated mycelia of an Ascomycete, *Gelasinospora multiforis* CAILLEUX, appreciably suppressed proliferation of mouse spleen lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partition followed by fractionation with repeated chromatography of the extract afforded an α-pyrone (2*H*-pyran-2-one) named multiforisin A (**1**) and four α-pyrones named multiforisins B (**2**), C (**3**), D (**4**), and E (**5**). This report deals with the isolation, structure elucidation and immunosuppressive activity of these new fungal immunosuppressive components.

Results and Discussion

The defatted extract prepared from the AcOEt extract of *G. multiforis* cultivated on sterilized rice suppressed by 82 and 65% the proliferation (blastogenesis) of mouse spleen lymphocytes stimulated with Con A (T-cells) and LPS (B-cells) at 10.0 μg/ml, respectively. The extract was partitioned with AcOEt–H_2O. The AcOEt layer suppressed by 90% the Con A-induced proliferation of lymphocytes, but the aqueous layer suppressed it by only 6%, at 10.0 μg/ml. Repeated chromatography of the AcOEt layer afforded multiforisin A (**1**) as an immunosuppressive component.

Multiforisin A (**1**) was obtained as pale yellow needles, $C_{11}H_{12}O_5$, optically inactive. The UV and IR spectra of **1**, suggested that it contains carbonyl systems conjugated with C=C bonds. These and the ^{1}H- and ^{13}C-NMR spectral data including spin-decoupling ^{1}H-NMR and two-dimensional ^{1}H–^{1}H (^{1}H–^{1}H COSY) and ^{13}C–^{1}H shift correlation (^{13}C–^{1}H COSY) NMR spectra of **1** in $CDCl_3$ (see Table 1) indicated that **1** may be composed of seven partial structures *a*—*g* (see Chart 1). Among them *a* was constructed with the aid of significant cross peaks due to H–C long-range coupling with J_2 and/or J_3 between H_a and C at δ 109.11, between H_a and C at δ 163.28, between H_b and C at δ 163.28, and between H_c and C at δ 163.28 observed in the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) NMR spectrum of **1** in $CDCl_3$, and the coupling constant of H_b with H_c (15.5 Hz) observed in the ^{1}H-NMR spectrum of **1**, indicating that H_b has *E* configuration with respect to H_c.

The HMBC NMR spectrum in $CDCl_3$ further suggested the presence of significant cross peaks between H_3 (in partial structure *b*) and C (*f*), between H_2 (*c*) and C (*f*), between H_2 (*c*) and C (*d*), and between H_2 (*c*) and C (*e*)



^{13}C-NMR and ^{1}H-NMR data : in $CDCl_3$

Chart 1. Partial Structures *a*—*g* for Multiforisin A (**1**)

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Table 1. ^{13}C-NMR and ^{1}H-NMR Data for Multiforisin A(**1**), B(**2**), C(**3**), D(**4**), E(**5**), and Dihydromultiforisin A(**6**), δ (ppm) from TMS in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**1**		**2**		**3**	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2	163.02 (s)		161.53 (s)		164.04 (s)	
3	110.45 (s)		106.17 (s)		105.14 (s)	
4	168.68 (s)		170.60 (s)		168.82 (s)	
4-OMe	63.39 (q)	4.14 (3H, s)	63.09 (q)	4.06 (3H, s)	62.33 (q)	4.06 (3H, s)
5	109.11 (s)		108.80 (s)		110.54 (s)	
6	163.28 (s)		164.40 (s)		160.10 (s)	
7	120.89 (d)	7.33 (dq, 15.5, 1.6)	120.86 (d)	7.34 (dq, 15.4, 1.5)	33.90 (t)	2.46 (dd, 18.1, 9.6) 2.47 (dd, 18.1, 4.9)
8	144.93 (d)	7.22 (dq, 15.5, 7.0)	145.73 (d)	7.26 (dq, 15.4, 6.7)	62.19 (d)	4.31 (qdd, 6.4, 9.6, 4.9)
9	19.33 (q)	2.03 (3H, dd, 7.0, 1.6)	19.40 (q)	2.04 (3H, dd, 6.7, 1.5)	20.75 (q)	1.36 (3H, d, 6.4)
10	55.25 (t)	4.62 (2H, d, 6.7)	56.44 (t)	5.07 (2H, s)	57.08 (t)	5.04 (2H, s)
10-OH		2.78 (t, 6.7)				
10-OAc			170.69 (s)		170.83 (s)	
			20.89 (q)	2.10 (3H, s)	20.97 (q)	2.08 (3H, s)
10-OMe						
11	187.49 (d)	10.11 (s)	187.02 (d)	10.09 (s)	94.25 (d)	5.33 (s)
11-OMe					55.47 (q)	3.49 (3H, s)

Position	**4**		**5**		**6**	
	^{13}C-NMR[a)]	^{1}H-NMR[a)]	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2	162.62 (s)		162.60 (s)		163.85 (s)	
3	105.98 (s)		106.78 (s)		112.18 (s)	
4	170.39 (s)		170.11 (s)		168.23 (s)	
4-OMe	63.14 (q)	4.25 (3H, s)	62.68 (q)	4.18 (3H, s)	63.29 (q)	4.16 (3H, s)
5	109.09 (s)		109.04 (s)		109.59 (s)	
6	163.46 (s)		163.52 (s)		174.50 (s)	
7	121.08 (d)	7.33 (dq, 15.6, 1.6)	121.04 (d)	7.33 (dq, 15.5, 1.5)	33.90 (t)	2.95 (2H, m)
8	145.01 (d)	7.20 (dq, 15.6, 7.0)	145.00 (d)	7.22 (dq, 15.5, 6.8)	21.12 (t)	1.73 (2H, m)
9	19.35 (q)	2.02 (3H, dd, 7.0, 1.6)	19,33 (q)	2.02 (3H, dd, 6.8, 1.5)	13.81 (q)	1.01 (3H, t, 7.3)
10	62.32 (t)	4.56 (2H, s)	63.90 (t)	4.42 (2H, s)	55.25 (t)	4.62 (2H, br s)
10-OH						2.77 (br s)
10-OAc						
10-OMe			58.47 (q)	3.44 (3H, s)		
11	187.80 (d)	10.10 (s)	187.76 (d)	10.11 (s)	187.24 (d)	10.10 (s)
11-OMe						

a) For the monomeric moiety of the molecule.

(see Chart 2). This result indicated that the partial structures, *a*—*g*, together construct a 3,4,5,6-tetrasubstituted α-pyrone (2*H*-pyran-2-one) (**1**), or a 2,3,5,6-tetrasubstituted γ-pyrone (4*H*-pyran-4-one) (**1a**), or a 2,3,4,5-tetrasubstituted furan (**1b** or **1c**), as shown in Chart 2. In **1** and **1a**, *e* is suggested to be present as a lactone carbonyl and a ketone carbonyl, respectively. On the other hand, in **1b** and **1c**, *e* is suggested to be present as an –O–C(|)=C< system. On acetylation with acetic anhydride and sodium acetate, **1** gave a monoacetate (**2**), ^{1}H-NMR ($CDCl_3$): δ 2.10 (3H, s, CH_3CO). It was considered that the hydroxymethyl group *c* was acetylated to give **2**. If multiforisin A is **1a**, or **1b**, or **1c**, multiforisin A monoacetate should be expressed as **2a**, or **2b**, or **2c**, respectively, as shown in Chart 2. In a differential nuclear Overhauser effect (differential NOE) experiment on multiforisin A monoacetate in $CDCl_3$, NOEs were observed between H_a (in partial structure *a*) and H_b (*a*), between H_a (*a*) and H_3 (*b*), and between H_3 (*b*) and H_2 (*c*), amounting to 2.2—3.6, 3.5—5.2, and 3.5—8.9%, respectively (see Chart 2). Thus, structures **1a** and **1c** could be rejected for multiforisin A, because if multiforisin A monoacetate is **2a** or **2c** NOEs should not be observed between H_a (*a*) and H_3 (*b*) or between H_3 (*b*) and H_2 (*c*). All of the significant cross peaks including those between H_3 (in partial structure *b*) and C (*f*), between H_2 (*c*) and C (*f*), between H_2 (*c*) and C (*d*), and between H_2 (*c*) and C (*e*) observed in the HMBC NMR spectrum of multiforisin A were compatible with H–C long-range coupling with J_2 and/or J_3 in the case of **1**, as shown in Chart 2. Meanwhile, in the case of **1b**, the cross peak observed between H_2 (*c*) and C (*f*) in the HMBC NMR spectrum was compatible with H–C long-range coupling with J_4 but not H–C long-range coupling with J_2 and/or J_3 (see Chart 2). Comparison of the ^{13}C-NMR spectrum of multiforisin A monoacetate with that of multiforisin A showed that the signals of α- and β-carbons to the acetoxyl group (C-10 and -3) in the case of the acetate of **1** (**2**) were shifted to δ 56.44 (+1.19) and 106.17 (−4.28), respectively,

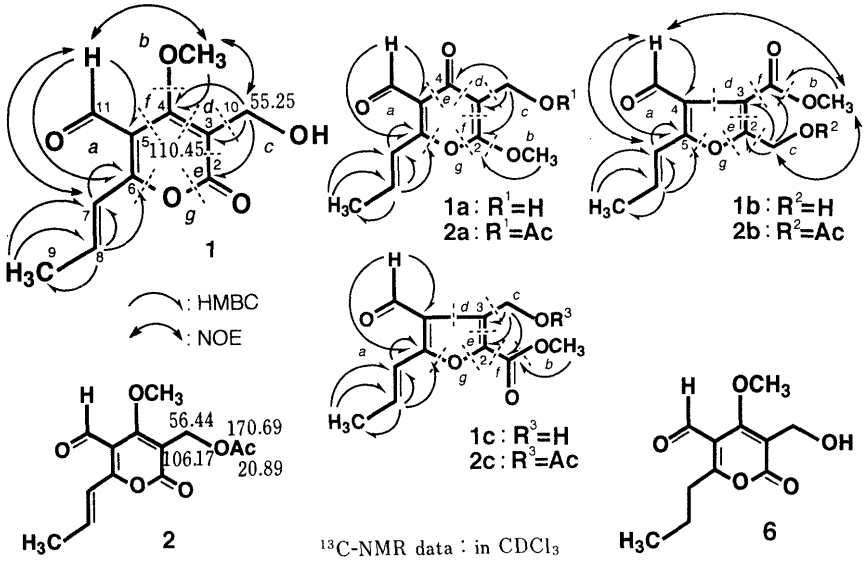


Chart 2

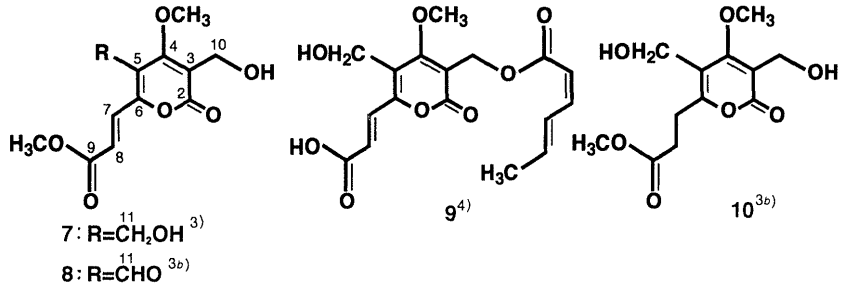


Chart 3. Structures of Rosellisin (**7**), Rosellisin Aldehyde (**8**), Islandic Acid (**9**), and Dihydrorosellisin (**10**)

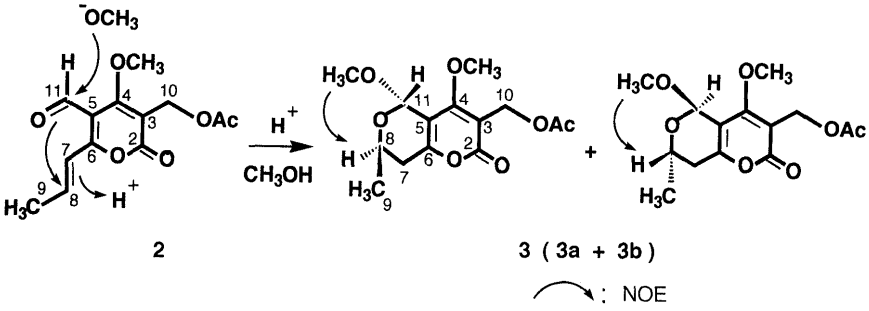


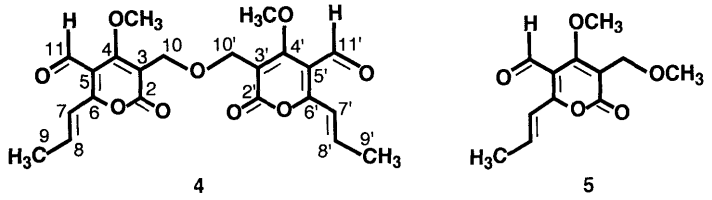
Chart 4. Possible Mechanism for Formation of Multiforisin C [**3**(**3a**+**3b**)] from Multiforisin B (**2**)

in accordance with the acetylation shift rule.[2] On the other hand, the acetylation shifts of the signals of α- and β-carbons to the acetoxyl group would be δ 56.44 (+1.19) and 161.53 (only −1.49), respectively, in the case of the acetate of **1b** (**2b**). Accordingly, the structure **1b** could be finally rejected, and the structure of multiforisin A was deduced to be 5-formyl-3-hydroxymethyl-4-methoxy-6-(1*E*-propenyl)-α-pyrone (**1**). On catalytic hydrogenation of multiforisin A, the conjugated double bond at position 7 was hydrogenated to afford a colorless dihydro derivative (**6**), ^{1}H-NMR ($CDCl_3$): δ 1.73 and 2.95 (each 2H, m), the signals at δ 7.22 and 7.33 (each 1H, dq) disappeared (see Table 1, Chart 2). As fungal metabolites having similar structures to **1**, two antibiotics, rosellisin (**7**)[3] and rosellisin aldehyde (**8**),[3b] from *Hypomyces rosellus*, and an antitumour substance, islandic acid (**9**), from *Penicillium islandicum*,[4] have so far been isolated (see Chart 3). The ^{1}H- and ^{13}C-NMR spectral data of **1** and **6** partly resembled those of **7**, **8**, **9**, and a dihydro derivative of **7** (**10**)[3b] (see Chart 3) reported in the literature.

Multiforisin B (**2**) was obtained as pale yellow needles, $C_{13}H_{14}O_6$. The ^{1}H- and ^{13}C-NMR spectra of multiforisin B were similar to those of multiforisin A acetate (**2**). Direct comparison of multiforisin B with multiforisin A acetate proved that this compound is identical with multiforisin A acetate (**2**) (Chart 2).

Multiforisin C (**3**) was obtained as pale yellow needles, $C_{14}H_{18}O_7$, optically inactive. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **2** showed that three partial structures, *h*: >CH–O–, *i*: –OCH_3, and *j*: –CH(CH_3)–CH_2–, are present at positions 5 and 6 in **3**. The significant cross peaks observed between H (in partial

structure *h*) and C (*i*), between H (*h*) and CH (*j*), between H (*h*) and C-6, between H_3 (*i*) and CH (*h*), and between CH_2 (*j*) and C-6 in the HMBC NMR spectrum suggest that the three partial structures *h*, *i* and *j* are linked together to form bonds from C-5 to -6 in the following mode: (C-5) –$CH(OCH_3)$–O–$CH(CH_3)$–CH_2–(C-6). Thus, the structure of multiforisin C was deduced to be 5,6-disubstituted 3-acetoxymethyl-4-methoxy-α-pyrone (**3**), as shown in Chart 4. The ^1H- and ^{13}C-NMR spectral data of **3** (see Table 1) and the fact that **3** is optically inactive suggested that **3** is a racemic mixture of two enantiomeric isomers (**3a** and **3b**) at positions 8 and 11. The racemic mixture was supposed to have been formed non-enzymatically from **2**, through protonation at the C=C bond at position 7 and cyclization to C-8 from the carbonyl oxygen, with concerted incorporation of the methoxyl group at C-11. Relative configurations at positions 8 and 11 of **3a** and **3b** were accordingly considered to be as shown in Chart 4, because 2.0—2.2% NOE was observed between the signal of H-8 and that of OCH_3 attached to C-11 in the



4 5

Chart 5

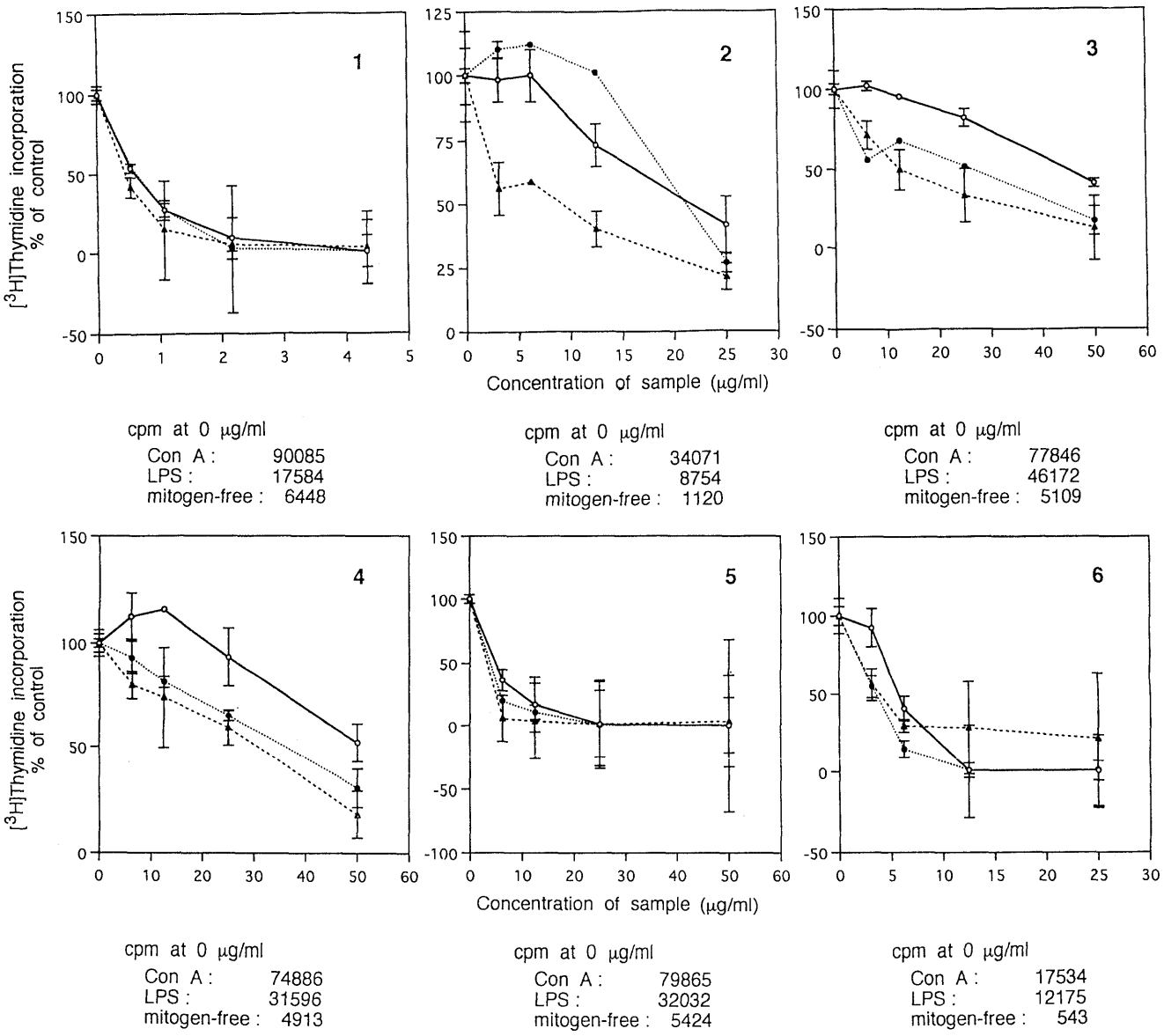


Fig. 1. Effects of Multiforisins A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and Dihydromultiforisin A (**6**) on Mitogen-Induced and Mitogen-Free Proliferation of Mouse Spleen Lymphocytes

—○—, against Con A-induced proliferation (T-cell); ···●···, against LPS-induced proliferation (B-cell); ---▲---, against mitogen-free proliferation. Each point represents the mean±S.E. of 3 experiments.

differential NOE spectrum of **3**. The UV spectrum of **3**, UV_{max}^{MeOH} nm (log ε): 207 (4.14), 295 (3.85), resembled that of α-pyrone, UV_{max}^{MeOH} nm (log ε): 215 (3.28), 289 (3.65).

Multiforisin D (**4**) was obtained as a pale yellow powder, $C_{22}H_{22}O_9$, optically inactive. Comparison of the ^{1}H- and ^{13}C-NMR spectra of multiforisin D with those of **1**, indicated that all of the signals of **4** were the same as those of **1** except that the signal of H_2-10 was changed to δ 4.56 (s) from 4.62 (d, J=6.7 Hz), the signal of OH-10 had disappeared, and the signals of C-10 and -3 were shifted to δ 62.32 (+7.07) and 105.98 (−4.47), respectively (see Table 1). Multiforisin D was thus deduced to have the structure **4** (in Chart 5), which was considered to have been non-enzymatically formed from two molecules of **1** through proton-catalyzed dehydrative condensation between OH-10 of two molecules of **1**.

Multiforisin E (**5**) was obtained as a pale yellow oil, $C_{12}H_{14}O_5$. Comparison of the ^{1}H- and ^{13}C-NMR spectra of multiforisin E with those of **1** indicated that all of the signals of **5** were similar to those of **1** except that the signal of a methoxyl newly appeared at δ 3.44 (3H, s) and 58.47 (q) instead of the signal of OH-10, the signal of H_2-10 was changed to δ 4.42 (s) from 4.62 (d, J=6.7 Hz), and the signals of C-10 and -3 were shifted to δ 63.90 (+8.65) and 106.78 (−3.67), respectively. These results indicated that multiforisin E is 10-methoxymultiforisin A (**5**), as shown in Chart 5.

The IC_{50} values of **1**—**6**, were calculated to be 0.6, 24, 44, >50, 5, and 13 μg/ml against Con A-induced proliferation and 0.6, 22, 27, 37, 4, and 9 μg/ml against LPS-induced proliferation of mouse spleen lymphocytes, respectively (see Fig. 1). These facts indicated that the presence of a hydroxyl at position 10 is very important for the appearance of immunosuppressive activity of **1**, while replacement of the hydroxyl with a methoxyl or dihydrogenation of the C=C double bond at position 7 slightly decreases the immunosuppressive activity, and replacement of the hydroxyl with an acetoxyl or dehydrative condensation with another molecule of **1** at position 10 greatly decreases the immunosuppressive activity. The IC_{50} value of **1** was found to be 10 μg/ml against human KB cells. Therefore, **1** displays immunosuppressive activity at a considerably lower concentration than that at which **1** shows cytotoxicity.

Experimental

The IR spectra were recorded with a Hitachi IR 260-10, the UV spectra with a Hitachi U-3200, the high-resolution FAB-MS (HRFAB-MS) spectra with a JEOL JMS-HX110, and the ^{1}H- and ^{13}C-NMR spectra with a JEOL JNM-A500 at 500 and 125.65 MHz, respectively. Other experimental conditions, including those for evaluation of the effects of samples on proliferation of mouse spleen lymphocytes, were as described in our previous report.[1a)]

Isolation of Multiforisin A (1) *Gelasinospora multiforis* CAILLEUX IFM4498[5)] was cultivated on sterilized rice[6)] (200 g/flask × 100) at 25 °C for 22 d. The moldy rice was extracted with AcOEt (30.0 l) with shaking at room temperature for 5 h two times to give a crude extract (167.0 g), which was then defatted with *n*-hexane (0.6 l). The defatted extract (112.2 g) was partitioned with AcOEt–H_2O (1 : 1, v/v) (4.5 l) into the AcOEt layer (74.0 g) and the aqueous layer (27.0 g). The AcOEt layer was subjected to silica gel column chromatography to give fractions I—V. Fraction III eluted with *n*-hexane–acetone (2 : 1) (32.9 g) was further chromatographed repeatedly on a silica gel column, and then subjected to medium-pressure liquid chromatography (MPLC) on a silica gel column (22 mm i.d. × 100 mm) with C_6H_6–AcOEt (3 : 1) at a flow rate of 5.0 ml/min to give **1** (997 mg), which was recrystallized from *n*-hexane–AcOEt to afford needles.

Isolation of Multiforisins B (2), C (3), D (4), and E (5) The AcOEt extract (355 g) obtained from the moldy rice (200 g/flask × 210) was partitioned with AcOEt–H_2O (1 : 1) (2.8 l) into the AcOEt layer (141.8 g) and the aqueous layer (67.5 g). The AcOEt layer was subjected to silica gel column chromatography to give fractions I′—V′. Among them, fractions III′ and IV′ were obtained as the former and the latter eluates with C_6H_6–AcOEt (1 : 1), respectively. Fraction III′ (18.0 g) was further chromatographed on a silica gel column to afford five fractions III′a—e. Fraction III′c eluted with *n*-hexane–acetone (4 : 1) (8.46 g) was further chromatographed on a silica gel column, and then subjected to MPLC on a silica gel column with *n*-hexane–acetone (3 : 1) to give **2** (2.03 g), which was recrystallized from *n*-hexane–AcOEt to afford needles. Fraction III′d eluted with *n*-hexane–acetone (2 : 1) (1.26 g) was further chromatographed repeatedly on a silica gel column and then subjected to MPLC on an octadecyl silica gel (ODS) column (22 mm i.d. × 100 mm) with MeOH–H_2O (2 : 3) at a flow rate of 4.8 ml/min to give **3** (48.8 mg), which was recrystallized from *n*-hexane–acetone to afford needles. Fraction IV′ (30.0 g) was chromatographed on a silica gel column repeatedly to give four fractions IV′a—d. Among them, fractions IV′b and c were obtained as the former and the latter eluates with $CHCl_3$–MeOH (50 : 1), respectively. Fraction IV′b (198 mg) was further chromatographed on a silica gel column with *n*-hexane–AcOEt (2 : 1), and subjected to MPLC on an ODS column with MeOH–H_2O (3 : 2) to give **5** (an oil, 8 mg). Fraction IV′c (6.09 g) was further chromatographed on a silica gel column to afford five fractions IV′ca—ce. Fraction IV′cb eluted with *n*-hexane–AcOEt (1 : 1) (693 mg) was further chromatographed on a silica gel column with $CHCl_3$–MeOH (40 : 1) to give **4** (28.6 mg), which was purified with *n*-hexane–AcOEt to afford an amorphous powder. Fraction IV′cc eluted with *n*-hexane–AcOEt (1 : 4) (3.57 g) was further subjected to MPLC on a silica gel column as described above for the isolation of **1**, to give **1** (2.09 g), which was recrystallized from *n*-hexane–AcOEt to afford needles.

Multiforisin A (**1**): Pale yellow needles, mp 81.5—82.5 °C (from *n*-hexane–AcOEt). $[\alpha]_D^{26}$ 0.0° (c=0.51, $CHCl_3$). HRFAB-MS m/z Calcd for $C_{11}H_{13}O_5$ [$(M+H)^+$]: 225.0763. Found: 225.0757. UV_{max}^{MeOH} nm (log ε): 218 (4.19), 232 (4.11), 260 (4.08), 283 (sh, 3.88), 294 (sh, 3.78), 338 (3.94). IR_{max}^{KBr} cm^{-1}: 3480 (O–H), 1715, 1690 (C=O), 1640, 1595, 1540 (C=C), 1350, 1265, 1020 (C–O), 910, 800, 770 (C–H).

Multiforisin B (**2**): Pale yellow needles, mp 78.0—79.0 °C (from *n*-hexane–AcOEt). This product was identical with multiforisin A acetate described below (**2**), in terms of mixed melting point, ^{1}H- and ^{13}C-NMR spectra ($CDCl_3$), and TLC behavior [plate: Merck Kieselgel 60F254, solvent: C_6H_6–AcOEt (1 : 1) and *n*-hexane–acetone (1 : 1), reagent: 10% H_2SO_4].

Multiforisin C (**3**): Pale yellow needles, mp 134—135 °C (from *n*-hexane–acetone). $[\alpha]_D^{19}$ 0.0° (c=0.14, MeOH). HRFAB-MS m/z Calcd for $C_{14}H_{18}O_7K$ [$(M+K)^+$]: 337.0689. Found: 337.0696. UV_{max}^{MeOH} nm (log ε): 207 (4.14), 295 (3.85). $IR_{max}^{CHCl_3}$ cm^{-1}: 1730, 1710 (C=O), 1650, 1595, 1570 (C=C), 1365 (C–O).

Multiforisin D (**4**): Pale yellow amorphous powder. $[\alpha]_D^{24}$ 0.0° (c=0.04, $CHCl_3$). HRFAB-MS m/z Calcd for $C_{22}H_{22}O_9K$ [$(M+K)^+$]: 469.0901. Found: 469.0908. UV_{max}^{MeOH} nm (log ε): 210 (3.97), 228 (3.88), 262 (3.72), 282 (sh, 3.65), 293 (sh, 3.59), 323 (3.54). $IR_{max}^{CHCl_3}$ cm^{-1}: 1725, 1680 (C=O), 1630, 1590, 1525 (C=C), 1440, 1370, 1340 (C–O).

Multiforisin E (**5**): Pale yellow oil. HRFAB-MS m/z Calcd for $C_{12}H_{15}O_5$ [$(M+H)^+$]: 239.0919. Found: 239.0910. UV_{max}^{MeOH} nm (log ε): 213 (4.06), 232 (3.94), 260 (3.84), 282 (sh, 3.72), 292 (sh, 3.68), 330 (3.67). $IR_{max}^{CHCl_3}$ cm^{-1}: 1715, 1680 (C=O), 1625, 1595 (C=C), 1440, 1380, 1338 (C–O).

Acetylation of Multiforisin A Sodium acetate (9.0 mg) was added to a solution of **1** (7.4 mg) in acetic anhydride (6.0 ml) to prepare a suspension, which was stirred at room temperature for 16.5 h. The reaction mixture was diluted with ice-water and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated *in vacuo* to give a crude product, which was purified through a silica gel column (6 mm i.d. × 80 mm) with C_6H_6–AcOEt (3 : 1) to furnish multiforisin A acetate (5.0 mg), which was recrystallized from *n*-hexane–AcOEt to afford pale yellow needles. This product was identical with **2**, mp 78.0—79.0 °C. HRFAB-MS m/z Calcd for $C_{13}H_{15}O_6$ [$(M+H)^+$]: 267.0869. Found: 267.0862. UV_{max}^{MeOH} nm (log ε): 217 (4.16), 233 (4.12), 261 (4.13), 279 (sh, 4.01), 291 (sh, 3.90), 338 (3.94). $IR_{max}^{CHCl_3}$ cm^{-1}: 1775, 1750 (sh), 1720 (C=O), 1655, 1630, 1520

(C=C), 1380, 1360 (C–O).

Formation of Dihydromultiforisin A (6) A solution of **1** (25.0 mg) in EtOH (1.0 ml) was added to a suspension of 5% palladium in carbon (6.6 mg) in EtOH (1.0 ml) activated under hydrogen gas in advance, and stirred under hydrogen gas for 10 min at room temperature. After removal of the catalyst, the solution was evaporated *in vacuo* to give a product mixture (16.1 mg), which was subjected to MPLC on a silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (3:2) at a flow rate of 4.3 ml/min, followed by recrystallization from *n*-hexane–acetone to afford colorless fine needles of dihydromultiforisin A (**6**) (8.6 mg), mp 65.5—66.5 °C. HRFAB-MS *m/z* Calcd for $C_{11}H_{15}O_5$ $[(M+H)^+]$: 227.0919. Found: 227.0912. UV_{max}^{MeOH} nm (log ε): 211 (3.98), 228 (3.95), 270 (4.15).

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