Immunomodulatory Constituents from an Ascomycete, *Emericella aurantio-brunnea*

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Fractionation monitored by the immunomodulatory activity of the AcOEt extract of an Ascomycete, *Emericella aurantio-brunnea*, afforded two known fungal sesterterpenes, variecolin (1) and variecolactone (2), two new variecolin congeners named variecoacetals A (3) and B (4), and a new sesquiterpenetriol diester named emeremophiline (5), as the immunosuppressive constituents of this fungus. The absolute configuration of 1, which was previously not determined, was determined to be (2*S*,3*S*,6*R*,10*S*,11*R*,14*S*,15*R*,16*S*) from the NMR spectral data of the (6*R*,7*R*)-dimethyl-1,3,5-trioxacycloheptyl derivative of 1 (7). The absolute configurations of the other variecolin congeners, 2—4, and variecolol (6) are also proposed from biosynthetic considerations.

Key words fungal metabolite; *Emericella aurantio-brunnea*; immunosuppressant; variecolin congener; absolute configuration; Ascomycete

Recently, several 2-pyrones, multiforisins A—I[1a,b] (from *Gelasinospora multiforis*, *G. heterospora*, and *G. longispora*), macrophin[1c] (from *Diplogelasinospora grovesii*), a macrocyclic sesterterpene, kobiin, three 2-furanones, kobifuranones A—C[1d] (from *Gelasinospora kobi*), a hexaketide, sordarial[1b] (from *G. heterospora* and *G. longispora*), two anthraquinones, questin and rubrocristin, two octaketides, cladosporin and cladosporin 8-*O*-methylether, and two dioxopiperazines, tardioxopiperazines A and B[1e] (from *Microascus tardifaciens*), have been isolated as immunosuppressive constituents in our screening program on immunomodulatory components from fungi. We have also found that the AcOEt extract of an Ascomycete, *Emericella aurantio-brunnea* appreciably suppressed the proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partition followed by repeated chromatographic fractionation of the extract guided by immunosuppressive activity afforded five constituents tentatively named EA-1 (**1**)—-5 (**5**), as the immunosuppressive features of this fungus. This report deals with the structures and immunosuppressive activities of these constituents recently isolated from *E. aurantio-brunnea.*

Results and Discussion

The AcOEt extract of *E.aurantio-brunnea* IFM42008[2] cultivated on sterilized rice medium suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 99% at 50 μg/ml. The AcOEt extract was partitioned with *n*-hexane and water into an *n*-hexane layer and an aqueous suspension. The aqueous suspension was further partitioned with AcOEt and water into an AcOEt layer and an aqueous layer [yields (%) of the *n*-hexane, AcOEt, and aqueous layers, after evaporation of the solvents, from the AcOEt extract: 72.4, 22.1, and 1.0, respectively]. The *n*-hexane, AcOEt, and aqueous layers had immunosuppressive activity of 99, 85, and −9% at 2.5 μg/ml, respectively. Repeated chromatographic fractionation of the *n*-hexane layer guided by the immunosuppressive activity afforded five constituents tentatively named EA-1—-5 (**1**—**5**) as the immunosuppressive constituents of this fungus [yields (%) of **1**—**5** from the AcOEt extract: 3.66, 0.28, 0.79, 0.24, and 0.21, respectively].

EA-1 (**1**), $C_{25}H_{36}O_2$, was obtained as optically active colorless prisms, whose UV spectrum gave absorptions due to >C=C—C=O and >C=C< systems. The ^{1}H- and ^{13}C-NMR data including spin-decoupling ^{1}H-NMR, and 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 showed the similarity of EA-1 to a tetracyclic (rings A—D) fungal sesterterpene, variecolin (**1**), which was isolated from a Fungi Imperfecti *Aspergillus variecolor* as an angiotensin II receptor binding inhibitor by Hensens *et al.*,[3] and later isolated also from an Ascomycete *Emericella purpurea* together with its congeners, valiecolactone (**2**) and valiecolol (**6**), by Kawai and his co-workers.[4] Direct comparison of EA-1 with an authentic sample of variecolin showed that EA-1 was identical with variecolin (**1**).

On the stereostructure of variecolin (**1**) and its congeners variecolactone and variecolol, their relative configurations have already been elucidated,[3,4] but the absolute configurations has not yet been determined. Namely, it is known that the absolute structure of variecolin is expressed as **1** [(2*S*,3*S*,6*R*,10*S*,11*R*,14*S*,15*R*,16*S*)-configuration] or **1a** [(2*R*,3*R*,6*S*,10*R*,11*S*,14*R*,15*S*,16*R*)-configuration] (see Chart 1). In order to decide the absolute configuration, **1** was treated with (2*R*,3*R*)-(−)-butane-2,3-diol[5] to give an optically active product (**7**), $C_{29}H_{44}O_3$, whose IR spectrum suggested the absence of any OH group and UV spectrum gave only the end absorption due to an isolated >C=C< system. Comparison of the ^{1}H- and ^{13}C-NMR data of **7** with those of **1** (in $CDCl_3$) indicated the appearance of six new fragments, $\underline{CH_3}CH<$ [δ_H/δ_C, 1.09 (3H, d, 6.6)/18.55 (q)], $\underline{CH_3}CH<$ [1.11 (3H, d, 6.6)/18.59 (q)], $CH_3\underline{CH}(O–)CH<$ [3.62 (1H, dq, 7.3, 6.6)/73.58 (d)], $CH_3\underline{CH}(O–)CH<$ [3.99 (1H, dq, 7.3, 6.6)/77.39 (d)], –O$\underline{CH}$(O–)– [5.69 (1H, s)/106.70 (d)], and –(–O)$\underline{C}$(O–)– [121.72 (s)], instead of just the expected disappearance of –$\underline{CH}$=O at position 20 [9.16 (1H, s)/193.04 (d)] and >$\underline{C}$=O at position 5 [220.74 (s)] (see Table 1). These data suggested that the glycol group of (2*R*,3*R*)-(−)-butane-

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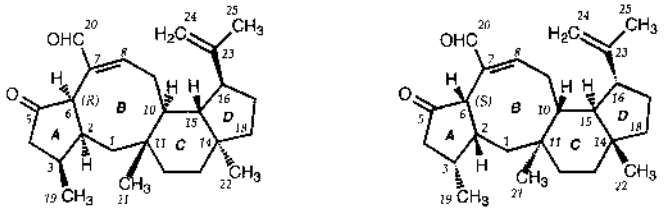
Dedicated to the memory of Dr. Kyosuke Tsuda.

2,3-diol reacted with not only the –CH=O group but also the >C=O group of **1** to form a hexacyclic (rings A—F) product possessing two new rings E (five-membered) and F (seven-membered), that is, a (6*R*,7*R*)-dimethyl-1,3,5-trioxacycloheptyl derivative of **1** (**7**), during this reaction (see Chart 2). From a nuclear Overhauser effect (NOE) experiment, an NOE was significantly observed between H-6 and H-3′ in the (2*R*,3*R*)-2,3-dioxybutane group of **7**, indicating that both H-6 and the (2*R*,3*R*)-2,3-dioxybutane group were present on the same side of the plane of ring E in **7**. Furthermore, the HMBC NMR data supported also the structure of **7**, as shown in Chart 2.

Considering the rings E and F were five- and seven-membered respectively, it was found from careful investigation with molecular models that only four conformations I—IV were possible for rings E and F of **7**. Namely, conformation I or II was possible for **7** if this compound possessed a (6*R*)-type configuration structure in which the configuration at position 6 was (*R*), and conformation III or IV was possible for **7** if this compound possessed a (6*S*)-type configuration structure in which that was (*S*) (see Chart 2). Here, it was expected from molecular models that a significant NOE would be observed between H-6 and H-3′, between H-6 and CH_3-1′, between H-6 and H-2′, and between H-6 and CH_3-4′ in the case of conformations I, II, III, and IV, respectively, as shown in Chart 2. In the NOE experiment on **7**, an NOE was not observed between H-6 and CH_3-1′, between H-6 and H-2′, or between H-6 and CH_3-4′, but was observed of 6—9% between H-6 and H-3′, indicating that **7** had conformation I. Thus, the configuration at 6 in **7** was elucidated to be (*R*). Accordingly, the absolute structure of variecolin was decided not to be **1a**, but to be **1** [(2*S*,3*S*,6*R*,10*S*,11*R*,14*S*,15*R*,16*S*) configuration] (see Chart 1). Considering the biosynthetic fact that **2** was isolated together with **1** from *E. aurantiobrunnea* as mentioned below, and both **2** and **6** were formerly isolated together with **1** from *E. purpurea*,[4] the absolute configuration of the two variecolin congeners, variecolactone (**2**)



1 **1a**

Chart 1

Table 1. ^{1}H-NMR and ^{13}C-NMR Data for EA-1 (**1**), (6*R*,7*R*)-Dimethyl-1,3,5-Trioxacycloheptyl Derivative of **1** (**7**), and Dihydro Derivative of **1** (**6**) in $CDCl_3$

Position	**1**		**7**		**6**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.20 (br d, 14.3), 1.53 (dd, 14.3, 11.9)	42.19 (t)	1.00 (dd, 14.4, 1.2), 1.64 (dd, 14.4, 12.5)	41.37 (t)	1.01 (dd, 14.4, 1.3), 1.76 (dd, 14.4, 12.5)	41.37 (t)
2	2.62 (m)	39.22 (d)	2.70 (br td, 11.8, 5.8)	39.45 (d)	2.86 (m)	39.57 (d)
3	2.38 (m)	34.71 (d)	2.10 (m)	36.72 (d)	2.00 (m)	38.55 (d)
4	2.38 (m), 2.50 (dd, 18.6, 8.3)	46.26 (t)	1.90 (m), 2.10 (m)	43.46 (t)	1.90 (m), 2.40 (m)	45.16 (t)
5		220.74 (s)		121.72 (s)		119.68 (s)
6	3.55 (d, 10.3)	50.34 (d)	3.19 (br d, 11.5)	55.74 (d)	3.38 (br d, 9.5)	53.66 (d)
7		139.82 (s)		135.55 (s)		135.53 (s)
8	6.89 (dd, 5.1, 1.1)	160.76 (d)	5.85 (dd, 6.6, 3.9)	128.11 (d)	5.45 (br s)	120.59 (d)
9	2.19 (m), 2.83 (br d, 17.0)	31.51 (t)	1.90 (m), 2.45 (dt, 19.3, 3.4)	29.11 (t)	2.00 (m), 2.42 (br d, 19.5)	28.85 (t)
10	2.19 (m)	40.62 (d)	2.10 (m)	38.46 (d)	1.93 (m)	38.63 (d)
11		39.10 (s)		38.78 (s)		38.72 (s)
12	1.01 (dt, 13.8, 2.6), 1.85 (td, 13.8, 4.4)	35.42 (t)	0.93 (ddd, 13.7, 3.7, 2.7), 1.95 (m)	34.54 (t)	0.93 (dt, 13.7, 3.4), 2.00 (m)	34.57 (t)
13	1.42 (2H, m)	35.21 (t)	1.50 (2H, m)	35.35 (t)	1.45 (2H, m)	35.36 (t)
14		43.26 (s)		43.48 (s)		43.44 (s)
15	1.42 (m)	48.63 (d)	1.50 (m)	48.38 (d)	1.45 (m)	48.45 (d)
16	2.38 (m)	48.42 (d)	2.41 (td, 11.0, 5.1)	47.98 (d)	2.38 (td, 11.0, 5.5)	48.13 (d)
17	1.20 (m), 1.95 (m)	29.89 (t)	1.35 (m), 2.00 (m)	30.38 (t)	1.34 (m), 2.00 (m)	30.24 (t)
18	1.20 (m), 1.42 (m)	39.81 (t)	1.25 (m), 1.45 (m)	40.10 (t)	1.23 (m), 1.45 (m)	40.01 (t)
19	0.77 (3H, d, 7.3)	15.78 (q)	0.76 (3H, d, 7.6)	15.71 (q)	0.81 (3H, d, 7.3)	15.45 (q)
20	9.16 (s)	193.04 (d)	5.69 (s)	106.70 (d)	4.45 (br d, 11.8), 4.62 (br d, 11.8)	73.44 (t)
21	0.91 (3H, s)	21.80 (q)	0.90 (3H, s)	22.01 (q)	0.90 (3H, s)	21.98 (q)
22	0.81 (3H, s)	18.04 (q)	0.87 (3H, s)	18.15 (q)	0.85 (3H, s)	18.13 (q)
23		150.34 (s)		151.08 (s)		151.11 (s)
24	4.61 (br t, 1.5), 4.70 (br d, 1.5)	110.66 (t)	4.61 (dd, 2.3, 1.3), 4.71 (d, 2.3)	109.64 (t)	4.58 (dd, 2.0, 1.5), 4.69 (br d, 2.0)	109.60 (t)
25	1.69 (3H, br s)	19.05 (q)	1.70 (3H, s)	19.62 (q)	1.68 (3H, br s)	19.40 (q)
1′			1.11 (3H, d, 6.6)	18.59 (q)		
2′			3.99 (dq, 7.3, 6.6)	77.39 (d)		
3′			3.62 (dq, 7.3, 6.6)	73.58 (d)		
4′			1.09 (3H, d, 6.6)	18.55 (q)		

δ (ppm) from TMS as an internal standard [coupling constants (Hz) in parentheses].

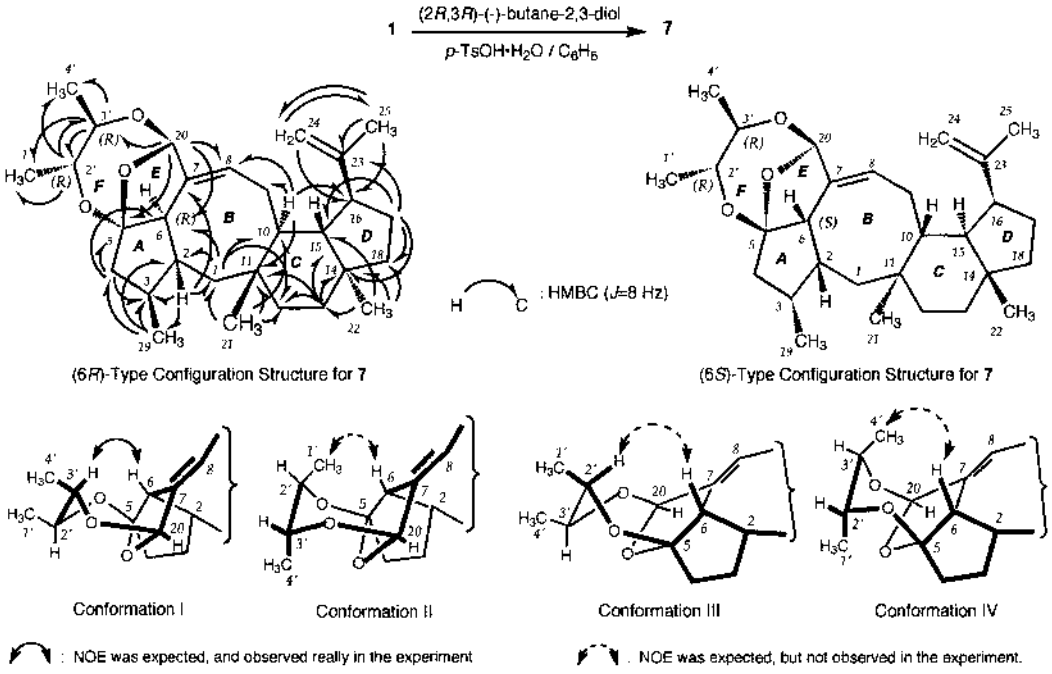


Chart 2

and variecolol (**6**), are proposed to be (2*S*,3*S*,5*R*,6*R*,10*S*, 11*R*,14*S*,15*R*,16*S*) (Chart 2). Similarly to Kawai and his coworkers,[4] EA-1 (**1**) was treated with $NaBH_4$ to give a dihydro derivative which was identical with an authentic sample of variecolol (**6**) (see Chart 3).

EA-2 (**2**), $C_{25}H_{36}O_3$, was obtained as colorless prisms. The physicochemical and spectral data of EA-2 showed a similarity to variecolactone.[4] Direct comparison of EA-2 with an authentic sample of variecolactone indicated that EA-2 was identical with variecolactone (**2**).

EA-3 (**3**), $C_{27}H_{42}O_3$, was obtained as a colorless amorphous solid, whose UV spectrum gave only end absorption. Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** suggested that the hemiacetal group –(H<u>O</u>)<u>C</u>(O–)– at position **5** [δ_H, 3.57 (1H, br s), δ_C, 115.24 (s)] was substituted with an acetal group –(H$_3$<u>C</u>O)<u>C</u>(O–)– [δ_H/δ_C, 3.38 (3H, s)/50.91 (q), 123.18 (s)], and the lactone –O–<u>C</u>=O at position 20 [170.68 (s)] was substituted with an acetal –O–<u>C</u>H–O<u>C</u>H$_3$ [5.26 (1H, s)/108.91 (d), 3.42 (3H, s)/54.94 (q)] (see Table 2). Significant NOEs of 5% were observed between H-2 and H-6, of 3% between H-6 and OCH_3-5, and of 1% between OCH_3-5 and OCH_3-20 on the NOE experiment of **3**, suggesting the possibility that the configurations at positions 5 and 20 in **3** were (*R*) and (*S*), respectively. Finally, considering the biosynthetic fact that **3** was isolated together with **1** from *E. aurantio-brunnea*, the absolute configuration of EA-3 (**3**) was proposed to be (2*S*,3*S*,5*R*,6*R*,10*S*, 11*R*,14*S*,15*R*,16*S*,20*S*), as shown in Chart 3.

EA-4 (**4**), $C_{28}H_{44}O_3$, was obtained as a colorless amorphous solid, whose UV spectrum gave only end absorption. Comparison of the ^{1}H- and ^{13}C-NMR data of **4** with those of **3** suggested that the acetal –(H$_3$CO)C(O–)– at position 5 was substituted with a different acetal –(H$_3$<u>C</u>H$_2$<u>C</u>O)<u>C</u>(O–)– [δ_H/δ_C, 1.24 (3H, t, J=7.3 Hz)/15.63 (q), 3.59, 3.78 (each 1H, dq, J=9.0, 7.3 Hz)/58.87 (t), δ_C, 122.94 (s)] (see Table 2). Significant NOEs were observed of 6% between H-2 and H-6, and of 1—2% between H-6 and O<u>C</u>H$_2$CH$_3$-5 on the NOE experiment of **4**, suggesting the possibility that the configuration at position 5 in **4** was (*R*). Though the NOE between O<u>C</u>H$_2$CH$_3$-5 and OCH_3-20 was not clearly observed in the experiment, the fact that the ^{1}H- and ^{13}C-NMR signals of <u>C</u>H-20 [δ_H/δ_C, 5.28 (1H, s)/108.85 (d)] and O<u>C</u>H$_3$-20 [3.42 (3H, s)/54.75 (q)] of **4** were quite similar to those of <u>C</u>H-20 [5.26 (1H, s)/108.91 (d)] and O<u>C</u>H$_3$-20 [3.42 (3H, s)/54.94 (q)] of **3** suggested that the configuration at position 20 in **4** was the same to that in **3** (see Table 2). Finally, considering the biosynthetic fact that **4** was isolated together with **1** from *E. aurantio-brunnea*, the absolute configuration of EA-4 (**4**) is also proposed to be (2*S*,3*S*,5*R*,6*R*,10*S*,11*R*,14*S*,15*R*,16*S*, 20*S*), as shown in Chart 3. Now, we propose the names variecoacetals A (**3**) and B (**4**) for EA-3 and EA-4, respectively.

EA-5 (**5**), was obtained as a colorless amorphous solid. The ^{13}C-NMR spectral data showed the presence of six methyls, namely, one <u>C</u>H$_3$–CH$_2$– (δ_C, 11.88), two <u>C</u>H$_3$–CH< (10.40, 20.09), and three <u>C</u>H$_3$–C– (12.40, 20.89, 22.75), four methylenes, namely, two C–<u>C</u>H$_2$–C (29.94, 44.96), one C–<u>C</u>H$_2$–O– (63.63) and one <u>C</u>H$_2$=C (114.37), nine methines, namely, two C–<u>C</u>H–C (34.94, 41.01), one C–<u>C</u>H–O (68.67) and six C–<u>C</u>H=C (114.89, 124.07, 130.69, 133.88, 149.13, 150.79), and eight quaternary carbons, namely, one C–<u>C</u>–C (36.33) and one C–<u>C</u>–O (76.48), three C=<u>C</u>–C (131.54, 147.58, 163.20), two –O–<u>C</u>=O (167.07, 170.40), and one >C=O (198.40) in **5**. These ^{13}C-NMR data were quite compatible with the ^{1}H-NMR data (see Table 3). All of these fragments were connected by the aid of the COSY and the HMBC NMR data to construct an eremophilane-type sesquiterpenetriol diester (**5**). This structure was also supported by the presence of a molecular ion peak at *m/z* 456 in the electron impact (EI)-MS spectrum. As fungal eremophilane-type metabolites, phomenone (**8**) from *Phoma exigua* var. *non oxydabilis*,[6] and other compounds[7] have already

Table 2. ^{1}H-NMR and ^{13}C-NMR Data for EA-2 (**2**), EA-3 (**3**) and EA-4 (**4**) in $CDCl_3$

Position	**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.09 (br d, 14.6), 1.52 (m)	40.84 (t)	0.99 (br d, 13.5), 1.66 (m)	41.05 (t)	1.02 (br d, 12.7), 1.69 (br t, 12.7)	41.12 (t)
2	2.78 (m)	39.78 (d)	2.61 (m)	38.94 (d)	2.65 (m)	38.80 (d)
3	2.25 (m)	37.94 (d)	2.20 (m)	38.23 (d)	2.11 (br t, 11.4)	38.27 (d)
4	2.12 (m), 2.24 (m)	44.85 (t)	2.00 (2H, m)	41.69 (t)	2.01 (2H, m)	42.29 (t)
5		115.24 (s)		123.18 (s)		122.94 (s)
5-OH	3.57 (br s)					
5-OMe			3.38 (3H, s)	50.91 (q)		
5-OEt					1.24 (3H, t, 7.3)	15.63 (q)
					3.59 (dq, 9.0, 7.3), 3.78 (dq, 9.0, 7.3)	58.87 (t)
6	3.60 (d, 10.7)	51.73 (d)	3.49 (d, 11.4)	52.32 (d)	3.48 (br d, 8.6)	52.36 (d)
7		125.12 (s)		136.23 (s)		136.35 (s)
8	6.98 (dd, 6.7, 4.3)	144.64 (d)	5.77 (dd, 5.8, 2.9)	128.72 (d)	5.79 (dd, 6.0, 3.2)	128.65 (d)
9	2.15 (m), 2.78 (m)	29.74 (t)	1.90 (m), 2.49 (br d, 19.8)	29.08 (t)	1.92 (m), 2.49 (br d, 19.5)	29.13 (t)
10	2.15 (m)	38.65 (d)	2.15 (m)	38.32 (d)	2.16 (m)	38.27 (d)
11		38.96 (s)		38.76 (s)		38.80 (s)
12	1.00 (dt, 12.2, 3.1), 1.97 (m)	34.35 (t)	0.90 (dt, 13.6, 3.3), 1.97 (m)	34.41 (t)	0.93 (m), 1.95 (m)	34.40 (t)
13	1.50 (2H, m)	35.10 (t)	1.50 (2H, m)	35.31 (t)	1.46 (2H, m)	35.35 (t)
14		43.40 (s)		43.42 (s)		43.45 (s)
15	1.52 (m)	47.94 (d)	1.45 (m)	48.29 (d)	1.48 (m)	48.31 (d)
16	2.39 (td, 11.2, 5.5)	48.10 (d)	2.29 (td, 11.2, 5.3)	47.82 (d)	2.35 (td, 11.0, 5.3)	47.62 (d)
17	1.37 (m), 2.00 (m)	29.98 (t)	1.37 (m), 2.00 (m)	30.23 (t)	1.30 (m), 1.96 (m)	30.30 (t)
18	1.25 (m), 1.45 (m)	39.86 (t)	1.20 (m), 1.45 (m)	40.01 (t)	1.20 (m), 1.42 (m)	40.09 (t)
19	0.69 (3H, d, 7.4)	15.92 (q)	0.74 (3H, d, 7.6)	15.58 (q)	0.76 (3H, d, 6.6)	15.63 (q)
20		170.68 (s)	5.26 (s)	108.91 (d)	5.28 (s)	108.85 (d)
20-OMe			3.42 (3H, s)	54.94 (q)	3.42 (3H, s)	54.75 (q)
21	0.91 (3H, s)	21.75 (q)	0.87 (3H, s)	21.92 (q)	0.90 (3H, s)	21.91 (q)
22	0.86 (3H, s)	18.14 (q)	0.78 (3H, s)	18.06 (q)	0.83 (3H, s)	18.15 (q)
23		150.48 (s)		150.64 (s)		150.69 (s)
24	4.63 (br s), 4.71 (br s)	110.42 (t)	4.52 (d, 1.6), 4.62 (d, 1.6)	109.83 (t)	4.54 (d, 2.1), 4.64 (d, 2.1)	109.79 (t)
25	1.70 (3H, s)	19.28 (q)	1.63 (3H, s)	19.46 (q)	1.65 (3H, s)	19.50 (q)

δ (ppm) from TMS as an internal standard [coupling constants (Hz) in parentheses].

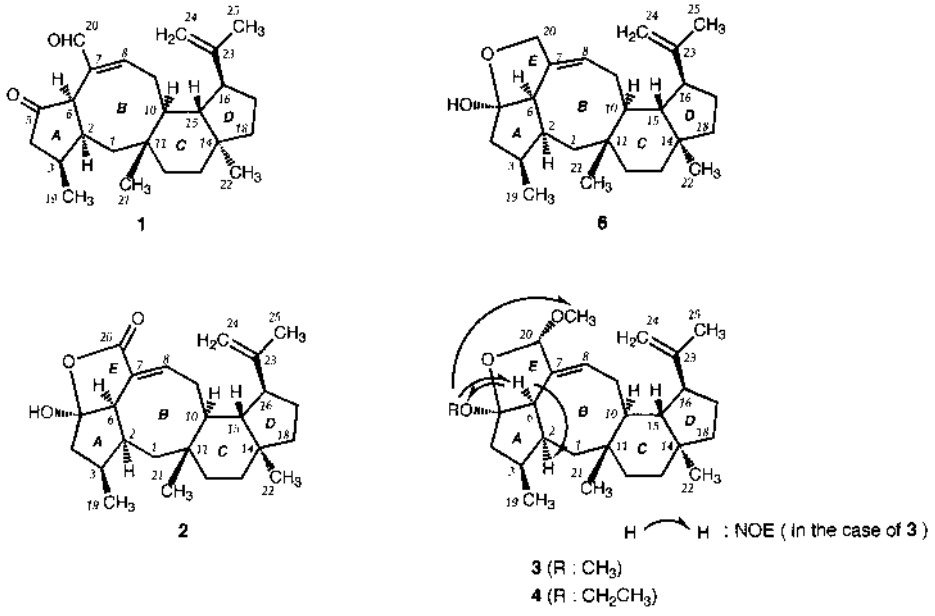
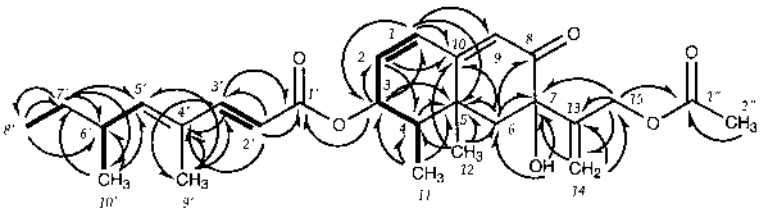


Chart 3

been isolated. The ^{1}H-NMR data of the eremophilane-type skeleton moiety of EA-5 (**5**) were similar to those of **8**[6] (see Table 3). To our knowledge, the structure of **5** has hitherto been unknown. But at this time we were not successful in elucidating of the stereostructure of EA-5 because this compound was unstable and rapidly decomposed. We propose to



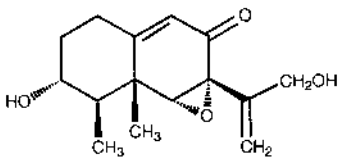


Chart 4

Table 3. ^{1}H-NMR and ^{13}C-NMR Data for EA-5 (**5**)

Position	**5**	
	δ_H	δ_C
1	6.38 (d, 9.7)	130.69 (d)
2	6.35 (dd, 9.7, 5.1)	133.88 (d)
3	5.45 (t, 5.1)	68.67 (d)
4	1.95 (m)	41.01 (d)
5		36.33 (s)
6	2.00 (d, 14.9), 2.25 (d, 14.9)	44.96 (t)
7		76.48 (s)
7-OH	2.70 (br s)	
8		198.40 (s)
9	5.95 (s)	124.07 (d)
10		163.20 (s)
11	1.08 (3H, d, 7.1)	10.40 (q)
12	1.50 (3H, s)	22.75 (q)
13		147.58 (s)
14	5.35 (s), 5.48 (s)	114.37 (t)
15	4.58 (2H, br s)	63.63 (t)
1′		167.07 (s)
2′	5.78 (d, 15.7)	114.89 (d)
3′	7.30 (d, 15.7)	150.79 (d)
4′		131.54 (s)
5′	5.68 (br d, 9.8)	149.13 (d)
6′	2.45 (m)	34.94 (d)
7′	1.35 (m), 1.40 (m)	29.94 (t)
8′	0.85 (3H, t, 7.3)	11.88 (q)
9′	1.80 (3H, d, 1.2)	12.40 (q)
10′	1.00 (3H, d, 6.6)	20.09 (q)
1″		170.40 (s)
2″	2.10 (3H, s)	20.89 (q)

δ (ppm) from TMS as an internal standard in $CDCl_3$ [coupling constants [Hz] in parentheses].

Table 4. Immunosuppressive Effects of EA-1 (**1**)—EA-5 (**5**), Dihydro Derivative of **1** (**6**), and (6*R*,7*R*)-Dimethyl-1,3,5-Trioxacycloheptyl Derivative of **1** (**7**), and Azathioprine, Cyclosporin A, and FK506 on the Con A-Induced and LPS-Induced Proliferation of Mouse Splenic Lymphocytes

Compound	IC_{50} (μg/ml)	
	Con A-induced	LPS-induced
1	0.4	0.1
2	8.0	2.5
3	4.5	1.5
4	6.5	2.2
5	2.0	Not tested
6	1.7	0.6
7	12.0	4.0
Azathioprine	2.7	2.7
Cyclosporin A	0.04	0.07
FK506 (tacrolimus)	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 3 experiments on each correlation between 7 different concentrations and cell proliferations.

call EA-5 emeremophiline (**5**).

The immunosuppressive activities (IC_{50} values) of **1**—**7** were calculated against Con A-(T cells) and LPS-induced (B-cells) proliferations of mouse splenic lymphocytes, as shown in Table 4. Among these five natural metabolites **1**—**5** from *E. aurantio-brunnea*, **1** which was obtained in the largest quantities from the fungus showed the highest immunosuppressive activity, indicating that **1** was the main immunosuppressive constituent of this fungus. The fact that **1** which possessed both the ketone group at position 5 and the conjugated aldehyde group at position 20 displayed higher immunosuppressive activity than other variecolin related compounds **2**—**4**, **6**, and **7** which lost both the ketone group and the conjugated aldehyde group indicated that the existence of both the ketone group at position 5 and the conjugated aldehyde group at position 20 in **1** might be very important for the appearance of immunosuppressive activity of **1**. The immunosuppressive activity of **1** seemed to be comparatively high, because it was higher than that of azathioprine, though lower than those of cyclosporin A and FK506 (tacrolimus). To our knowledge, this is the first time that **1** and **2** have been isolated as immunosuppressive constituents from a natural source.

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 report [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].[1b] Investigation on the stereostructure of **7** used molecular models, HGS Type-C (Maruzen Co.). Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard in $CDCl_3$.

Isolation of EA-1 (1)—-5 (5) from *E. aurantio-brunnea* *E. aurantio-brunnea* IFM42008[2] was cultivated on sterilized rice (200 g/flask×3) at 25 °C for 23 d. The moldy rice was extracted with AcOEt (900 ml) with shaking at room temperature for 6 h two times to give an AcOEt solution (1.80 l), which gave, after evaporation *in vacuo*, an AcOEt extract (2.90 g). The AcOEt extract was partitioned with *n*-hexane–H_2O (1 : 1, v/v) (240 ml) into an *n*-hexane layer (after evaporation *in vacuo*, 2.10 g), and an aqueous suspension which was further partitioned with AcOEt (120 ml) into an AcOEt layer (0.64 g) and an aqueous layer (0.03 g). The *n*-hexane, AcOEt, and aqueous layers suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 99, 85, and −9% at 2.5 μg/ml, respectively. The *n*-hexane layer was subjected to chromatography on a silica gel column with *n*-hexane–AcOEt (20 : 1), (15 : 1), (5 : 1), (1 : 1), and MeOH to give seven fractions: I—VII (154, 120, 24, 315, 53, 7, and 29 mg, respectively). Fractions I—VII suppressed the Con A-induced proliferation of the lymphocytes by 2, −6, −3, 100, 10, 1, and −2% at 2.5 μg/ml, respectively. Fraction IV was recrystallized from CH_3CN to give **1** (80 mg), and the mother liquor was chromatographed on an HPLC octadecyl silica gel (ODS) column with 90% CH_3CN at a flow rate of 8 ml/min to afford **1** (26 mg) and **2** (8 mg). Fraction V was chromatographed on a silica gel column with *n*-hexane–AcOEt (5 : 1), (1 : 1), and MeOH to give four fractions: Va—Vd (15, 14, 19, and 13 mg, respectively). Fractions Va—Vd suppressed the Con A-induced proliferation of the lymphocytes by −16, −25, 54, and 31% at 2.5 μg/ml, respectively. Fraction Vc was further chromatographed on an HPLC ODS column with 90% CH_3CN at a flow rate of 8 ml/min to afford **5** (6 mg). Fraction I was also chromatographed on a silica gel column with *n*-hexane–AcOEt (100 : 1) and MeOH to give three fractions: Ia—Ic (49, 45, and 59 mg, respectively). Fraction Ib was further chromatographed on an HPLC ODS column with CH_3CN at a flow rate of 8 ml/min to afford **3** (23 mg) and **4** (7 mg).

EA-1 (**1**) (Variecolin): Colorless prisms from CH_3CN, mp 160.5—161.5 °C. $[\alpha]_D^{19}$ −110.5° (c=0.50, CH_3CN) [lit.[3] −11.5°, the authentic sample provided by Dr. Kawai gave −84.4°]. High resolution (HR)-FAB-MS *m*/*z*: 369.2779 (Calcd for $C_{25}H_{37}O_2$ [$(M+H)^+$]: 369.2794). IR (KBr) cm^{-1}: 2940, 1737, 1684, 1626, 1455, 1377, 1227, 887. UV λ_{max} (MeOH) nm (log ε): 200 (end absorp., 3.90), 240 (3.74). Circular dichroism (CD) (0.54 mM, MeOH) $\Delta\varepsilon$ (nm): +4.3 (295), −3.7 (234), −2.2 (220). This compound was identical with an authentic sample of variecolin (**1**)[4] in terms of ^{1}H-NMR spectra and TLC behavior [Kieselgel 60F_{254} (Merck), *n*-hexane–AcOEt (2 : 1); RP18 F_{254}S (Merck), CH_3CN].

EA-2 (**2**) (Variecolactone): Colorless prisms from CH_3CN, mp 253—255 °C (lit.[4] 249—251 °C). $[\alpha]_D^{24}$ +87.4° (c=0.19, CH_3CN). Electron impact (EI)-MS *m*/*z* (%): 384 (20, M^+), 366 (21), 323 (12) [lit.[4] 384 (21, M^+), 369 (7), 366 (8)]. HR-FAB-MS *m*/*z*: 385.2747 (Calcd for $C_{25}H_{37}O_3$ [$(M+H)^+$]: 385.2743). IR (KBr) cm^{-1}: 3391, 2912, 1729, 1662, 1438, 1384, 1243, 961, 882. UV λ_{max} (MeOH) nm (log ε): 200 (end absorp., 3.97), 231 (3.96) [lit.[4] 232 (3.78)]. This compound was identical with an authentic sample of variecolactone (**2**) in terms of ^{1}H- and ^{13}C-NMR spectra and TLC behavior [Kieselgel 60F_{254} (Merck), *n*-hexane–AcOEt (2 : 1)].

EA-3 (**3**) (Variecoacetal A): Colorless amorphous solid, $[\alpha]_D^{25}$ +101.4° (c=0.14, CH_3CN). EI-MS *m*/*z* (%): 414 (29, M^+), 399 (10), 383 (100), 367 (40), 355 (41). HR-EI-MS *m*/*z*: 414.3147 (Calcd for $C_{27}H_{42}O_3$: 414.3134). IR (KBr) cm^{-1}: 2938, 1642, 1456, 1379, 1323, 1090, 1019, 969. UV λ_{max} (MeOH) nm (log ε): 205 (end absorp., 4.12).

EA-4 (**4**) (Variecoacetal B): Colorless amorphous solid, $[\alpha]_D^{25}$ +81.5° (c=0.13, CH_3CN). EI-MS *m*/*z* (%): 428 (18, M^+), 413 (10), 396 (100), 381 (38), 367 (49). HR-EI-MS *m*/*z*: 428.3270 (Calcd for $C_{28}H_{44}O_3$: 428.3290). IR (KBr) cm^{-1}: 2938, 1638, 1460, 1382, 1320, 1062, 1015, 968. UV λ_{max} (MeOH) nm (log ε): 205 (end absorp., 4.11).

EA-5 (**5**) (Emeremophiline): Colorless amorphous solid, EI-MS *m*/*z* (%): 456 (2, M^+), 397 (6), 228 (16), 211 (14), 152 (100).

Formation of the (6*R*,7*R*)-Dimethyl-1,3,5-Trioxacycloheptyl Derivative of EA-1 (7) from EA-1 (1) To a solution of **1** (103 mg) and *p*-toluenesulfonic acid monohydrate (TsOH·H_2O) (20 mg) in benzene (16 ml), (2*R*,3*R*)-(−)-butane-2,3-diol (800 μl) was added. The reaction mixture was stirred at room temperature for 19 h. The reaction mixture was neutralized with a saturated $NaHCO_3$ solution under ice-cooling. The organic layer was washed with water, and then with a saturated NaCl solution. After evaporation *in vacuo*, the organic layer was subjected to chromatography on a silica gel C-60 column with *n*-hexane–AcOEt (15 : 1) to give **7** (30 mg) as a colorless oil, $[\alpha]_D^{28}$ +92.0° (c=0.2, CH_3CN). EI-MS *m*/*z* (%): 440 (90, M^+), 425 (4), 396 (8), 368 (14), 352 (100). HR-FAB-MS *m*/*z*: 441.3356 (Calcd for $C_{29}H_{45}O_3$ [$(M+H)^+$]: 441.3369. IR (KBr) cm^{-1}: 2937, 1646, 1449, 1379, 1305, 1230, 1123, 1056. UV λ_{max} (MeOH) nm (log ε): 205 (end absorp., 4.08).

Formation of Variecolol (6) from EA-1 (1) A suspension of $NaBH_4$ (4 mg) in EtOH (400 μl) was added to a solution of **1** (22 mg) in EtOH (750 μl). The reaction mixture was stirred at room temperature for 30 min. After addition of a saturated NH_4Cl solution (2 ml) under ice-cooling, the reaction mixture was treated as usual to give a crude product which was purified on an HPLC ODS column with CH_3CN–H_2O (9 : 1) to afford **6** (13 mg) as a colorless amorphous powder, $[\alpha]_D^{24}$ +137.1° (c=0.14, CH_3CN), EI-MS *m*/*z* (%): 370 (9, M^+), 352 (100), 337 (13), 309 (10). UV λ_{max} (MeOH) nm: end absorp. only. This compound was identical with an authentic sample of variecolol (**6**) in terms of ^{1}H- and ^{13}C-NMR spectra.

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References and Notes

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