Four New Immunosuppressive Components, Kobiin and Kobifuranones A, B, and C, from an Ascomycete, *Gelasinospora kobi*

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A new sesterterpenetriol named kobiin and three new 2-furanones named kobifuranones A, B, and C were isolated from an Ascomycete, *Gelasinospora kobi*. Kobiin, the main immunosuppressive principle of this fungus, possesses a bicyclic skeleton of five- and fifteen-membered rings. Kobifuranones A, B, and C were supposed to be metabolites formed from a common intermediate biosynthesized through the acetate–malonate pathway. The immunosuppressive activity of kobiin and kobifuranones A, B, and C was evaluated in a system of mouse spleen lymphocytes stimulated to proliferate with concanavalin A and lipopolysaccharide.

Key words fungal metabolite; Ascomycete; *Gelasinospora kobi*; immunosuppressant; sesterterpenetriol; 2-furanone

In our screening program on immunomodulatory fungal components, several immunosuppressive compounds have so far been isolated from some Basidiomycetes, *Lactarius flavidulus*,[1a] *Pisolithus tinctorius*, *Microporus flabelliformis*, and *Lenzites betulina*,[1b] and an Ascomycete, *Gelasinospora multiforis*.[1c] Recently, it was found that the AcOEt extract of cultivated mycelia of another Ascomycete, *Gelasinospora kobi* CAILLEUX, appreciably suppressed proliferation (blastogenesis) of mouse spleen lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partition followed by repeated chromatography to fractionate the extract afforded a new sesterterpenetriol named kobiin (**1**), and three new 2-furanones named kobifuranones A (**2**), B (**3**), and C (**4**) as the immunosuppressive principles of this fungus. This report deals with the isolation, structure elucidation and immunosuppressive activity of these new fungal immunosuppressive components.[2]

Results and Discussion

The AcOEt extract of *G. kobi* IFM4650[3] cultivated on sterilized rice suppressed by 50% the proliferation of mouse spleen lymphocytes stimulated with Con A (T-cells) at 29 μg/ml. After having been defatted with *n*-hexane, the extract was partitioned with AcOEt–methanolic H_2O into the AcOEt layer and aqueous layer. The AcOEt layer suppressed by 50% the Con A-induced proliferation of the lymphocytes at 19 μg/ml, while the aqueous layer suppressed it by 50% at 45 μg/ml. Repeated chromatography of the AcOEt layer afforded kobiin (**1**), and kobifuranones A (**2**), B (**3**), and C (**4**), as immunosuppressive components, and a known compound, *p*-hydroxybenzaldehyde (**5**), which showed no immunosuppressive activity [yield (%) from the AcOEt layer, **1**: 0.12, **2**: 0.23, **3**: 0.070, **4**: 0.015, and **5**: 0.32].

Kobiin (**1**) was obtained as a pale yellow oil, $C_{25}H_{40}O_3$, and was optically active. The IR and UV spectra suggested the presence of OH groups and conjugated C=C bonds in **1**. Compound **1** gave a triacetate (**6**) on acetylation with acetic anhydride and pyridine. The ^{1}H- and ^{13}C-NMR spectra, including spin-decoupling ^{1}H-NMR and two-dimensional ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and ^{13}C–^{1}H COSY NMR spectra, of **1** and **6** indicated that **1** was a new sesterterpenetriol having one primary and two secondary OH groups and composed of four partial structures *a*—*d*. The whole structure of kobiin without its stereochemistry was constructed from the partial structures *a*—*d* with the aid of the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) NMR (Table 1) and the differential nuclear Overhauser effect (DifNOE) NMR data. The constructed plane structure of **1** (Chart 1) possessed a skeleton of a five-membered ring (ring A) condensed with a fifteen-membered one (ring B), having a primary hydroxyl at position 23 and two secondary hydroxyls at positions 2 and 10, an isopropyl at position 1, two methyls at positions 4 and 12, and four C=C bonds at positions 6, 8, 12, and 16(25) (*exo* methylene), respectively. A DifNOE experiment on **1** revealed significant NOEs as depicted in Chart 1, but no NOE was observed between H-9 and H_2-23, or between H-13 and H_3-24. These data indicated that the configurations of the C=C bonds at positions 6, 8, and 12 in **1** may be *Z*, *E*, and *E*, respectively (Table 1, Chart 1).

Comparison of the ^{1}H-NMR spectrum of the triacetate (**6**) with that of **1** showed that the carbinyl protons at positions 2, 10, and 23 were shifted to δ 5.25 (+0.97), 5.74 (+1.11), and 4.67 (2H, +0.36, +0.74), respectively, and comparison of the ^{13}C-NMR spectrum of **6** with that of **1** showed that the signals of the α-, β-, and β-carbons to the acetoxyl at position 2 (C-2, -1, -3) and those of the α- and β-carbons to the acetoxyl at position 23 (C-23, -8) were shifted (Table 1) in accordance with the acetylation shift rule,[4] supporting the presence of hydroxyls at positions 2, 10, and 23 in **1**. In a DifNOE experiment on **6**, significant NOEs were observed between H-19 (δ1.80) and H-2 (5.25), and between H-19 and H-5 (3.07), but no NOE was observed between H-19 and H-1, or between H-5 and H_3-22 (0.90), suggesting that the relative configurations of H-1 to H-2, of H-1 to H-5, and of H-5 to CH_3-4 are *E*, *E*, and *E* in the ring A moiety in **1**, respectively, as shown in Chart 1.

On treatment with *tert*-butylacetyl chloride and pyridine, **1** afforded a mono-*tert*-butylacetate (**7**), whose ^{1}H-NMR showed that only the primary hydroxyl at position 23 in **1** was *tert*-butylacetylated (see Experimental). It has already been proved that the modified Mosher's method[5] is effective to determine the absolute configurations of

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Table 1. ^{1}H-NMR and ^{13}C-NMR Data for Kobiin (**1**) and Kobiin Triacetate (**6**)

Position	**1**			**6**		
	^{1}H-NMR	^{13}C-NMR	HMBC[a] correlation	^{1}H-NMR	^{13}C-NMR	HMBC[a] correlation
1	1.49 (br t, 11.9)	56.50 (d)	H-1/C-4, -5	1.67 (td, 11.9, 2.0)	54.74 (d)	H-1/C-2, -3, -4
2	4.28 (td, 4.1, 2.1)	73.86 (d)	H-2/C-5	5.25 (td, 5.2, 2.0)	77.10 (d)	H-2/C-4, -5
3	1.68 (2H, m)	47.55 (t)	H_2-3/C-4, -22	1.70 (2H, m)	45.93 (t)	H_2-3/C-1, -2, -4, -11, -18, -22
4	—	44.86 (s)	—	—	44.74 (s)	—
5	3.02 (t, 11.7)	46.16 (d)	H-5/C-1, -4, -6, -7, -18, -19, -22	3.07 (t, 11.9)	46.86 (d)	H-5/C-1, -4, -6, -7, -18, -22
6	5.46 (t, 11.7)	135.81 (d)	H-6/C-1, -5, -8	5.48 (t, 11.9)	136.15 (d)	H-6/C-1, -8
7	5.92 (d, 11.7)	128.90 (d)	H-7/C-5, -6, -9, -23	5.84 (d, 11.9)	128.35 (d)	H-7/C-5, -9
8	—	139.43 (s)	—	—	135.49 (s)	—
9	5.74 (d, 7.9)	132.13 (d)	H-9/C-7, -10, -11	5.73 (d, 7.9)	129.34 (d)	H-9/C-7, -23
10	4.63 (ddd, 7.9, 5.8, 3.8)	67.15 (d)	H-10/	5.74 (td, 9.1, 2.8)	69.20 (d)	H-10/
11	2.18 (dd, 12.4, 10.3)	46.68 (t)	H-11a (δ 2.18)/C-10, -12, -13, -24	2.34 (dd, 14.0, 9.0)	44.46 (t)	H-11a (δ 2.34)/C-9, -10, -12, -13, -24
	2.49 (br d, 12.4)		H-11b (δ 2.49)/C-12	2.45 (br d, 14.0)		H-11b (δ 2.45)/C-9, -10, -12
12	—	130.55 (s)	—	—	129.80 (s)	—
13	5.21 (t, 6.1)	127.44 (d)	H-13/C-11, -14, -24	5.28 (t, 6.8)	128.25 (d)	H-13/C-11, -24
14	2.20 (2H, m)	27.17 (t)	H_2-14/C-12, -13, -16	2.20 (2H, m)	28.47 (t)	H_2-14/C-13
15	2.05 (m),	35.01 (t)	H-15a (δ 2.05)/C-13, -14, -16, -17, -25	2.13 (m)	35.22 (t)	H-15a (δ 2.13)/C-16, -25
	2.23 (m)		H-15b (δ 2.23)/C-13, -16, -25	2.18 (m)		H-15b (δ 2.18)/C-13, -16
16	—	150.70 (s)	—	—	151.10 (s)	—
17	1.91 (2H, m)	31.88 (t)	H_2-17/C-15, -16, -18, -25	1.89 (2H, m)	32.08 (t)	H_2-17/C-16, -18, -25
18	1.26 (m)	40.75 (t)	H-18a (δ 1.26)/C-4, -17	1.31 (td, 12.7, 5.3)	42.10 (t)	H-18a (δ 1.31)/
	1.49 (m)		H-18b (δ 1.49)/C-4, -17	1.49 (td, 12.7, 5.3)		H-18b (δ 1.49)/C-17
19	1.80 (m)	27.94 (d)	H-19/C-1, -20, -21	1.80 (m)	27.99 (d)	H-19/C-1, -2, -5, -20, -21
20	1.00 (3H, d, 6.8)	22.47 (q)	H_3-20/C-1, -19, -21	0.91, (3H, d, 6.9)	21.92 (q)	H_3-20/C-1, -19, -21
21	1.02 (3H, d, 6.8)	22.28 (q)	H_3-21/C-1, -19, -20	0.98 (3H, d, 6.9)	21.70 (q)	H_3-21/C-1, -19, -20
22	0.85 (3H, s)	24.96 (q)	H_3-22/C-3, -4, -18	0.90 (3H, s)	24.35 (q)	H_3-22/C-3, -4, -18
23	3.93 (d, 12.2)	62.27 (t)	H-23a (δ 3.93)/C-7, -8, -9	4.67 (2H, s)	62.89 (t)	H_2-23/C-7, -8, -9
	4.31 (d, 12.2)		H-23b (δ 4.31)/C-7, -8, -9			
24	1.66 (3H, s)	17.66 (q)	H_3-24/C-11, -12, -13	1.70 (3H, s)	17.19 (q)	H_3-24/C-11, -12, -13
25	4.72 (br s)	108.92 (t)	H-25a (δ 4.72)/C-15, -17	4.69 (br s)	109.72 (t)	H-25a (δ 4.69)/C-15, -17
	4.74 (br s)		H-25b (δ 4.74)/C-15, -17	4.74 (br s)		H-25b (δ 4.74)/C-15, -17

δ (ppm) from tetramethylsilane (TMS) as an interal standard in $CDCl_3$ [coupling constants (Hz) in parentheses]. *a*) J_{C-H} for HMBC measurement: 8.0 Hz.

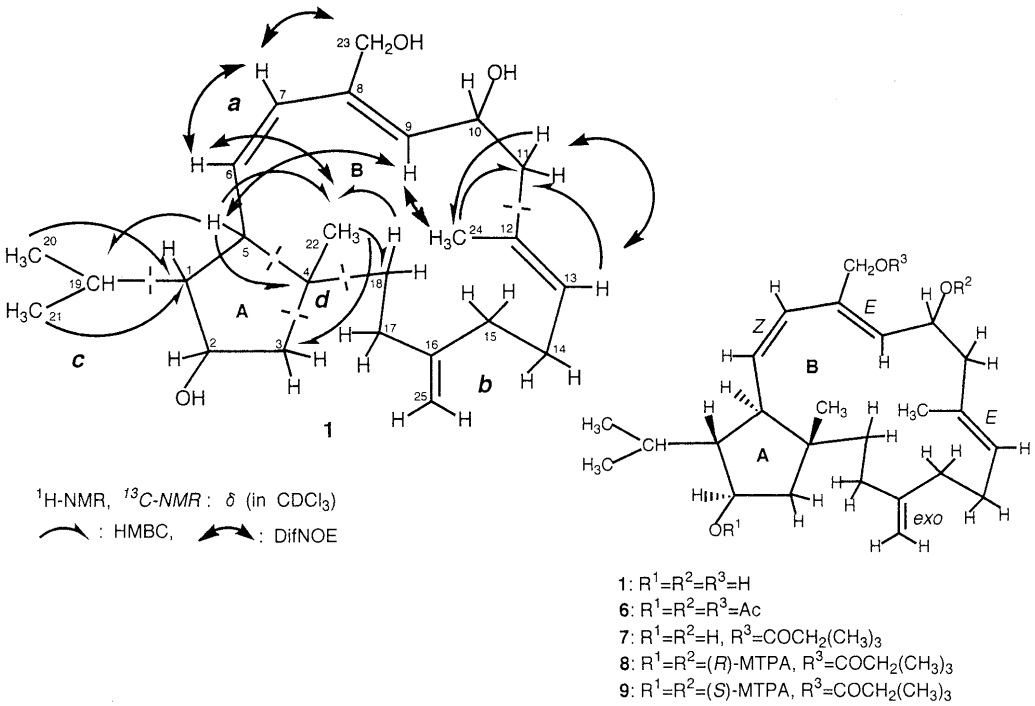


Chart 1. Plane Structure of Kobiin (**1**) and Relative Stereostructures (in Part) of **1** and Its Derivatives **6**—**9**

sec-hydroxyl groups in an oligohydroxylated compound.[6] In order to apply the modified Mosher's method to **7**, the di-(*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetate (di-(*R*)-MTPA ester) (**8**) and di-(*S*)-MTPA ester (**9**) were prepared from **7**. Comparison of the ^{1}H-NMR spectra of **8** and **9** with that of **7** indicated that both secondary hydroxyls at positions 2 and 10 in **7** were esterified to give **8** and **9**. The $\Delta\delta$ values ($\delta_{(S)\text{-}(-)\text{-MTPA}} - \delta_{(R)\text{-}(+)\text{-MTPA}}$) between **8** and **9** were calculated as shown in Table 2, indicating that the absolute configurations at positions 2 and 10 in **1** are (*R*) and (*S*), respectively. Accordingly, kobiin was deduced to be a new bicyclic sesterterpenetriol containing (1*R*,2*R*,4*R*,5*R*,10*S*) configurations (**1**), as shown in Chart 1. Considering the structure of kobiin (**1**), a possible biogenetic pathway is presented in Chart 2. Recently, a fungal metabolite, proliferin (**10**), having a similar structure to **1**, was isolated from *Fusarium proliferatum*[7] (Chart 2).

Kobifuranone A (**2**) was obtained as a pale yellow oil, $C_{11}H_{16}O_3$, which was optically active. In the IR spectrum, significant absorptions existed at 3600, 1760, and 1600 cm^{-1}, suggesting the presence of OH, strained OC=O, and conjugated C=C groups in **2**, respectively. From the ^{1}H- and ^{13}C-NMR data of **2** including the HMBC and DifNOE NMR data (Table 3, Chart 3), three partial structures *e*—*g* were identified, and the structure of kobifuranone A was concluded to be 4,5-dihydro-3-(1-hydroxyethyl)-4-((1*E*,3*E*)-pentadienyl)-2(3*H*)-furanone (**2**) (see Chart 3).

Kobifuranone B (**3**) was obtained as a yellow oil, $C_{11}H_{14}O_3$, which was optically active. The IR spectrum suggested the presence of OH, strained OC=O, and conjugated C=C in **3**. From the ^{1}H- and ^{13}C-NMR data of **3** (Table 3, Chart 3), six partial structures *h*—*m* were identified, and in a similar way to that described for

Table 2. ^{1}H-NMR Data for Kobiin Mono-*tert*-butylacetate-(*S*)-MTPA Ester (**9**) and -(*R*)-MTPA Ester (**8**), and Δ [δ(**9**)—δ(**8**)] Values

Position	**9**	**8**	Δ [δ(**9**)—δ(**8**)]
1	1.685 (ddd, 11.8, 10.3, 4.4)	1.667[a]	+0.008
2	5.102 (br t, 6.6)	5.002 (br t, 6.2)	+0.100
3	2.176 (2H, m)	2.086 (2H, m)	+0.110
5	3.015 (t, 11.8)	3.031 (t, 11.8)	−0.016
6	5.481 (t, 11.8)	5.487 (t, 11.8)	−0.006
7	5.771 (d, 11.8)	5.770 (d, 11.8)	+0.001
9	5.701 (d, 9.0)	5.684 (d, 9.0)	+0.017
10	5.969 (t, 9.0)	5.977 (td, 8.7, 3.0)	−0.008
11	2.238 (t, 12.2)	2.427 (t, 12.5)	−0.189
	2.362 (br d, 12.2)	2.536 (br d, 12.5)	−0.174
13	5.546 (t, 5.1)	5.478 (t, 4.2)	+0.068
14	1.886 (m)	1.821 (m)	+0.065
	1.924 (dd, 15.0, 5.5)	1.854 (dd, 15.0, 5.5)	+0.070
15	2.097 (m)	2.078 (m)	+0.019
	2.138 (m)	2.104 (m)	+0.034
17	[b]	[b]	
18	[b]	[b]	
19	1.551 (m)	1.670 (m)	−0.119
20	0.796 (3H, d, 6.5)	0.815 (3H, d, 6.3)	−0.019
21	0.826 (3H, d, 6.5)	0.838 (3H, d, 6.3)	−0.012
22	0.890 (3H, s)	0.864 (3H, s)	+0.026
23	4.589 (d, 12.5)	4.511 (d, 12.3)	+0.078
	4.672 (d, 12.5)	4.636 (d, 12.3)	+0.036
24	1.621 (3H, s)	1.667 (3H, s)	−0.046
25	4.667 (br s)	4.565 (br s)	+0.102
	4.711 (br s)	4.627 (br s)	+0.084
$-OCOCH_2C(CH_3)_3$	1.004 (9H, s)	1.003 (9H, s)	+0.001
$-OCOCH_2C(CH_3)_3$	2.176 (s)	2.174 (s)	+0.002
	2.177 (s)	2.177 (s)	0.000
$-OCOC(CF_3)(OCH_3)C_6H_5$	3.483 (3H, s)	3.506 (6H, s)	
	3.501 (3H, s)		
$-OCOC(CF_3)(OCH_3)C_6H_5$	7.282—7.516 (10H, m)	7.283—7.515 (10H, m)	

δ (ppm) from TMS as an internal standard in $CDCl_3$ [coupling corstants (Hz) in parentheses]. *a*) The coupling pattern was not clarified because of overlapping with other signals. *b*) Chemical shift was not determined accurately.

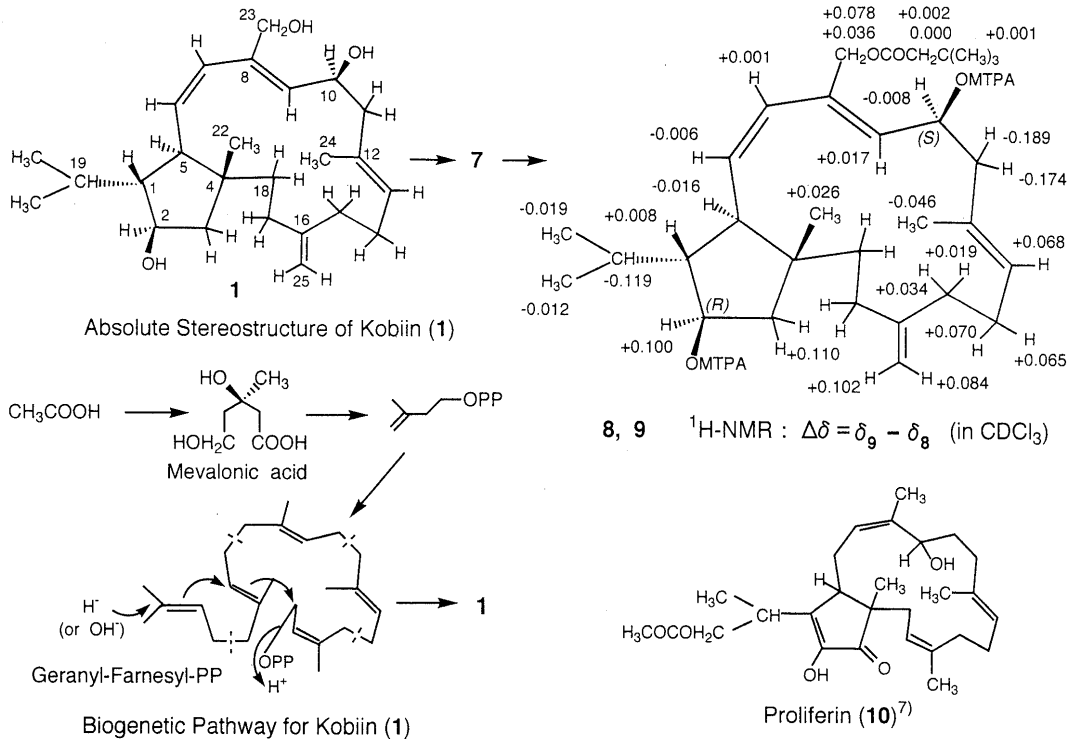


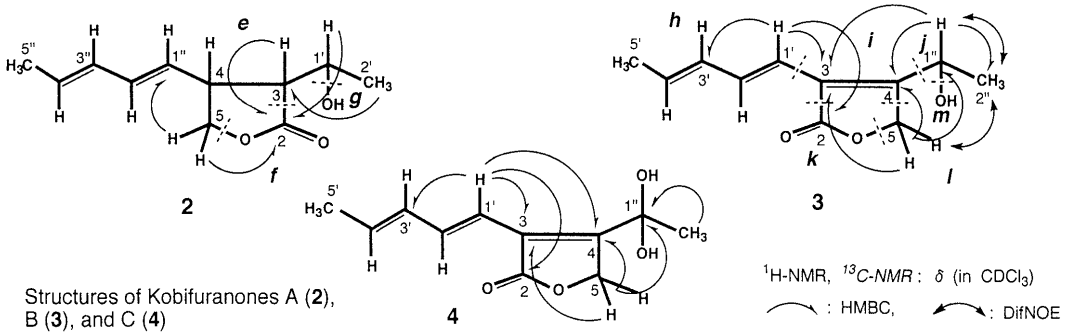
Chart 2

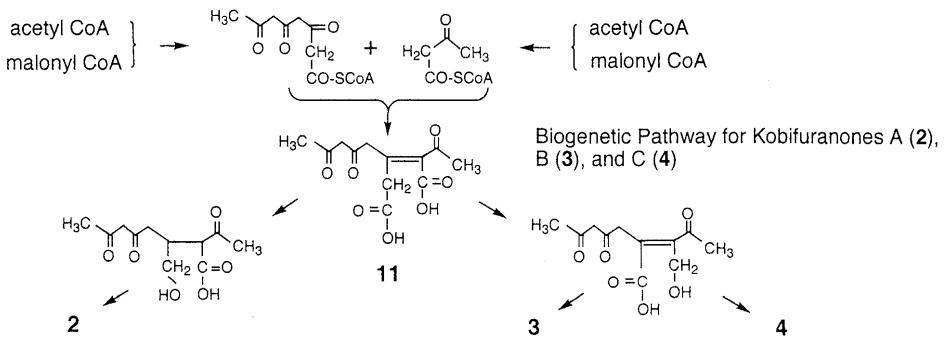
Table 3. ^{1}H-NMR and ^{13}C-NMR Data for Kobifranones A (**2**), B (**3**), and C (**4**)

Position	**2**			**3**		
	^{1}H-NMR	^{13}C-NMR	HMBC[a] correlation	^{1}H-NMR	^{13}C-NMR	HMBC[a] correlation
2	—	177.81 (s)	—	—	171.81 (s)	—
3	2.52 (dd, 8.2, 2.1)	52.09 (d)	H-3/C-2	—	123.14 (s)	—
4	3.35 (dddd, 9.3, 8.9, 8.7, 8.2)	39.59 (d)	H-4/C-2″	—	159.62 (s)	—
5	3.87 (t, 9.3)	70.77 (t)	H-5a (δ 3.87)/C-1″, -2	4.52 (d, 15.0)	56.90 (t)	H-5a (δ 4.52)/C-3, -4, -1″
	4.37 (dd, 9.3, 8.7)		H-5b (δ 4.37)/C-1″, -2	4.76 (d, 15.0)		H-5b (δ 4.76)/C-3, -4, -1″
1′	4.36 (qd, 6.7, 2.1)	65.54 (d)	H-1′/C-2	6.10 (d, 15.7)	116.21 (d)	H-1′/C-2, -3, -3′
2′	1.26 (3H, d, 6.7)	20.48 (q)	H_3-2′/C-3	7.30 (dd, 15.7, 10.6)	136.05 (d)	H-2′/
3′	—	—	—	6.13 (dd, 15.1, 10.6)	131.80 (d)	H-3′/C-4′
4′	—	—	—	5.96 (m)	134.02 (d)	H-4′/C-2′, -3′, -5′
5′	—	—	—	1.82 (3H, d, 7.3)	18.48 (q)	H_3-5′/C-3′, -4′
1″	5.44 (dd, 15.0, 8.9)	127.89 (d)	H-1″/C-2″, -3″	5.12 (q, 6.6)	77.40 (d)	H-1″/C-3, -4, -2″
2″	6.18 (dd, 15.0, 10.4)	133.76 (d)	H-2″/C-4, -3″	1.50 (3H, d, 6.6)	18.61 (q)	H_3-2″/
3″	6.02 (dd, 14.9, 10.4)	130.28 (d)	H-3″/C-4″	—	—	—
4″	5.71 (dq, 14.9, 6.4)	130.38 (d)	H-4″/C-2″, -5″	—	—	—
5″	1.75 (3H, d, 6.4)	18.00 (q)	H_3-5″/C-3″, -4″	—	—	—

Position	**4**		
	^{1}H-NMR	^{13}C-NMR	HMBC[a] Correlation
2	—	169,25 (s)	—
3	—	125.63 (s)	—
4	—	154.12 (s)	—
5	4.59 (d, 11.2)	56.27 (t)	H-5a (δ 4.59)/C-3, -4, -1″
	4.64 (d, 11.2)		H-5b (δ 4.64)/C-3, -4, -1″
1′	6.18 (d, 15.7)	115.89 (d)	H-1′/C-2, -3, -4, -3′
2′	7.36 (dd, 15.7, 10.8)	138.08 (d)	H-2′/
3′	6.14 (dd, 15.1, 10.8)	131.86 (d)	H-3′/C-4′
4′	5.99 (dq, 15.1, 6.8)	135.26 (d)	H-4′/C-2′, -3′, -5′
5′	1.86 (3H, d, 6.8)	18.59 (q)	H_3-5′/C-3′, -4′
1″	—	104.23 (s)	—
2″	1.72 (3H, s)	24.47 (q)	H_3-2″/C-4, -1″
3″	—	—	—
4″	—	—	—
5″	—	—	—

δ (ppm) from TMS as an internal standard in $CDCl_3$ [coupling constants (Hz) in parentheses]. *a*) J_{C-H} for HMBC measurement: 8.0 Hz.





Biogenetic Pathway for Kobifuranones A (**2**), B (**3**), and C (**4**)

Chart 3. Kobifuranones A (**2**), B (**3**), and C (**4**)

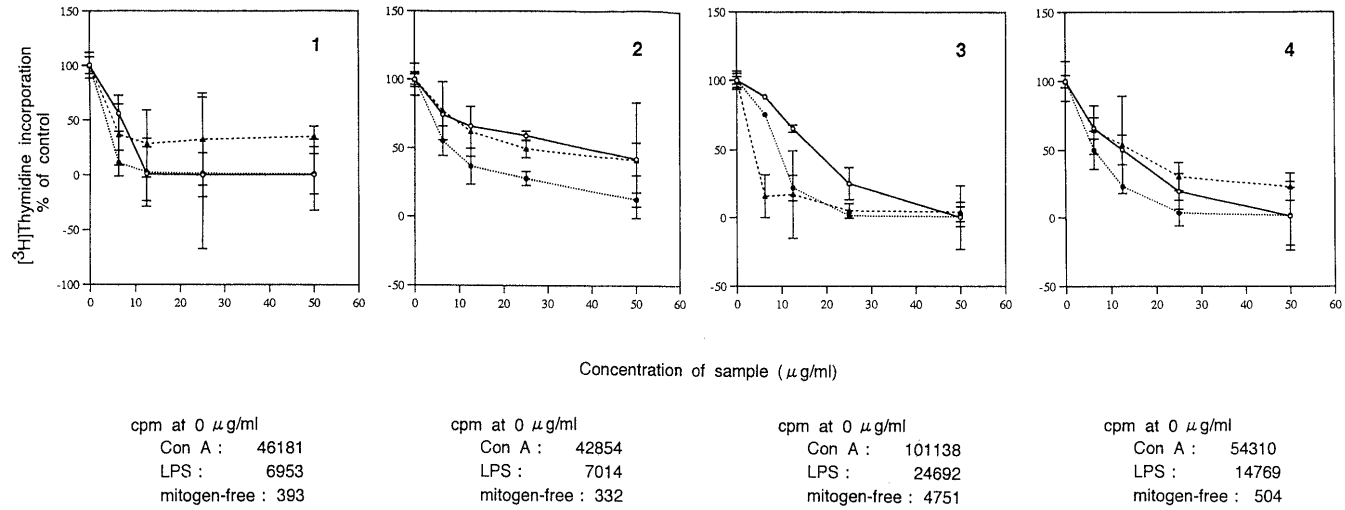


Fig. 1. Effects of Kobiin (**1**), and Kobifuranones A (**2**), B (**3**), and C (**4**) on Mitogen-Induced and Mitogen-Free Proliferation of Mouse Spleen Lymphocytes

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

2, the structure of kobifuranone B was concluded to be 4-(1-hydroxyethyl)-3-((1*E*,3*E*)-pentadienyl)-2(5*H*)-furanone (**3**) (see Chart 3).

Kobifuranone C (**4**) was also obtained as a yellow oil, $C_{11}H_{14}O_4$. The IR spectrum suggested the presence of OH, strained OC=O, and conjugated C=C in **4**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **3** indicated that all of the signals of **4** were quite similar to those of **3** except that the signals due to the H_3C–CH(OH)– group at position 4 in **3** were changed to those of H_3C–$C(OH)_2$– in **4** (Table 3). This fact indicated that the structure of kobifuranone C was 4-(1,1-dihydroxyethyl)-3-((1*E*,3*E*)-pentadienyl)-2(5*H*)-furanone (**4**) (Chart 3). Many fungal metabolites containing a 2-furanone moiety, namely, multicolanic acid, multicolic acid, multicolosic acid, dihydropenicillic acid[8a] and penicillic acid[8b] are considered to be biosynthesized by way of the acetate–malonate pathway.[8] Thus, the three new 2-furanones, kobifuranones A (**2**), B (**3**), and C (**4**), may be biosynthesized from a common intermediate (**11**) formed from two polyketides through the acetate–malonate pathway, as shown in Chart 3.

On chromatographic separation of kobifuranone A (**2**)-containing fraction, a pale yellow powder (**5**) was also isolated together with **2**. The fast atom bombardment-MS (FAB-MS) spectrum suggested that **5** has the molecular formula, $C_7H_6O_2$. The ^{1}H- and ^{13}C-NMR spectra of **5** were identical with those of *p*-hydroxybenzaldehyde (see Experimental), which has been isolated from several microorganisms, namely, *Streptomyces rimosus*[9a] and *Sirodesmium diversum*.[9b] This is the first time that *p*-hydroxybenzaldehyde (**5**) has been isolated from *Gelasinospora kobi*.

The immunosuppressive activities (IC_{50} values) of kobiin (**1**) and its triacetate (**6**) were calculated to be 7.0 and 8.7 μg/ml against Con A-induced proliferation and 3.5 and 3.7 μg/ml against LPS-induced proliferation of mouse spleen lymphocytes, respectively (see Fig. 1), suggesting that the presence of three hydroxyl groups at positions 2, 10, and 23 in **1** is not indispensable for the appearance of immunosuppressive activity. The three-dimensional stereostructure of the whole molecule of **1** might be important for the activity. The IC_{50} values of kobifuranones A (**2**), B (**3**), and C (**4**), were calculated to be 39, 17.5, and 13 μg/ml against Con A-induced proliferation and 8, 9, and 6 μg/ml against LPS-induced proliferation of the lymphocytes, respectively (see Fig. 1). The IC_{50} value of *p*-hydroxybenzaldehyde (**5**) was more than 50 μg/ml against Con A-induced proliferation and 40 μg/ml against LPS-induced proliferation of the lymphocytes. The IC_{50} values of **1** and **3** were found to be 10 and 25 μg/ml against human HL-60 cells. Therefore, both **1** and **3** display immunosuppressive activity against LPS-induced proliferation of lymphocytes (B-cells) at slightly lower concentrations than those at which both **1** and **3** are active.

Experimental

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

Isolation of Kobiin (1), Kobifuranones A (2), B (3), C (4), and *p*-Hydroxybenzaldehyde (5) *Gelasinospora kobi* CAILLEUX IFM4650[3] was cultivated on sterilized rice (200 g/flask × 300) at 25 °C for 22 d. The moldy rice was extracted with AcOEt (90.0 l) with shaking at room temperature for 6 h, twice, to give a crude extract (78.0 g), which was then dissolved in MeOH (300 ml). The MeOH solution was partitioned with a mixture of *n*-hexane (1400 ml) and H_2O (1100 ml) into an *n*-hexane-soluble portion (37.4 g) and an aqueous suspension. The aqueous suspension was partitioned with AcOEt (1400 ml) into an AcOEt layer and an aqueous layer. After evaporation *in vacuo*, the AcOEt layer and the aqueous layer gave the AcOEt-soluble portion (33.0 g) and the aqueous portion (9.2 g), respectively. The AcOEt-soluble portion was subjected to silica gel column chromatography repeatedly with C_6H_6–AcOEt (9 : 2, v/v), (4 : 1), (2 : 1), (1 : 2), (1 : 3) and acetone to give fractions I—VII. Fraction II eluted with C_6H_6–AcOEt (9 : 2) (820 mg) was further chromatographed repeatedly on a silica gel column, and then subjected to medium-pressure liquid chromatography (MPLC) on an octadecyl silica gel (ODS) column (22 mm i.d. × 100 mm) with MeOH–H_2O (1 : 3) at a flow rate of 4.0 ml/min to give **5** (105 mg) and **2** (77 mg). Fraction III eluted with C_6H_6–AcOEt (4 : 1) (546 mg) was

chromatographed on an ODS column and on a silica gel column, and then subjected to MPLC on a silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (1 : 1) at a flow rate of 4.0 ml/min to give **3** (23 mg). Fraction IV eluted with C_6H_6–AcOEt (2 : 1) (2.8 g) was chromatographed three times on silica gel columns, and then subjected to MPLC on a silica gel column (22 mm i.d. × 100 mm) with $CHCl_3$–MeOH (30 : 1) at a flow rate of 6.0 ml/min to give **4** (5 mg). Fraction V eluted with C_6H_6 AcOEt (1 : 2) (2.3 g) was chromatographed repeatedly on silica gel columns, and then subjected to MPLC on a silica gel column (22 mm i.d. × 100 mm) with *n*-hexane–AcOEt (1 : 1) at a flow rate of 4.0 ml/min to give **1** (40 mg).

Kobiin (**1**): Pale yellow oil, $[\alpha]_D^{22}$ +41.2° (c=0.25, $CHCl_3$). HR-FAB-MS m/z: 427.2612 ($C_{25}H_{40}O_3K$ requires 427.2615 $[(M+K)^+]$). UV λ_{max}^{MeOH} nm (log ε): 238.4 (3.69). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3200 (OH), 1640, 1610 (C=C).

Kobifuranone A (**2**): Pale yellow oil, $[\alpha]_D^{22}$ −40.1° (c=0.47, $CHCl_3$). HR-FAB-MS m/z: 197.1170 ($C_{11}H_{17}O_3$ requires 197.1177 $[(M+H)^+]$). UV λ_{max}^{MeOH} nm (log ε): 221.0 (3.69). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1760 (C=O), 1600 (C=C), 1380, 1350, 1150 (C–O).

Kobifuranone B (**3**): Pale yellow oil, $[\alpha]_D^{20}$ +11.0° (c=0.10, $CHCl_3$). EI-MS m/z: 194 $[M^+(C_{11}H_{14}O_3)]$. UV λ_{max}^{MeOH} nm (log ε): 281.4 (4.06). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620 (OH), 1745 (C=O), 1600 (C=C), 1320, 1170 (C–O).

Kobifuranone C (**4**): Pale yellow oil. HR-FAB-MS m/z: 249.0508 ($C_{11}H_{14}O_4K$ requires 249.0529 $[(M+K)^+]$). UV λ_{max}^{MeOH} nm: 290.8. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650 (OH), 1760 (C=O), 1630 (C=C), 1500, 1410, 1160 (C–O).

Compound **5**: Pale yellow powder, mp 115—118 °C (lit.[10] 116 °C). FAB-MS m/z: 123 $[(M(C_7H_6O_2)+H)^+]$. UV λ_{max}^{MeOH} nm (log ε): 221.4 (4.08), 284.6 (4.17), 291.4 (sh, 4.11). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650 (OH), 1685 (C=O), 1605, 1595 (C=C). ^{1}H-NMR δ in $CDCl_3$: 6.56 (1H, br s, OH-4), 6.98 (2H, m, H-3, 5), 7.82 (2H, m, H-2, 6), 9.86 (1H, s, CHO-1). ^{13}C-NMR δ in $CDCl_3$: 116.0 (2C, d, C-3, 5), 129.8 (1C, s, C-1), 132.5 (2C, d, C-2, 6), 191.2 (1C, d, CHO-1). This compound **5** was identical with *p*-hydroxybenzaldehyde.[10]

Acetylation of Kobiin A solution of kobiin (**1**) (14 mg) in Ac_2O (600 μl) and pyridine (1200 μl) was allowed to stand at room temperature for 12 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 vaporated *in vacuo* to give a resinous residue, which was chromatographed on an MPLC silica gel column with *n*-hexane–AcOEt (3 : 1) at a flow rate of 4.0 ml/min to afford the triacetate **6**, as a resinous residue (12 mg).

***tert*-Butylacetate of Kobiin** A solution of kobiin (**1**) (21 mg) in *tert*-butylacetylchloride (100 μl) and pyridine (200 μl) was allowed to stand at room temperature for 5 min, then ice-water was added and the whole was extracted with $CHCl_3$. The $CHCl_3$ layer was treated in the same way as for the acetylation of **1** to give a resinous residue, which was chromatographed on an MPLC silica gel column with *n*-hexane–AcOEt (4 : 1) at a flow rate of 3.5 ml/min to afford the mono-*tert*-butylacetate **7**, as a resinous residue (6.0 mg). Compound **7**, ^{1}H-NMR δ in $CDCl_3$: 1.01 [9H, s, $-OCOCH_2C(C\underline{H}_3)_3$], 2.17 [2H, br s, $-OCOC\underline{H}_2C(CH_3)_3$], 4.32, 4.98 (each 1H, d, J=12.2 Hz, H_2-23).

Di-(*R*)- and Di-(*S*)-MTPA Esters of Mono-*tert*-Butylacetate of Kobiin A solution of mono-*tert*-butylacetate **7** (3.0 mg), (*R*)-MTPA chloride (3.8 mg), dimethylaminopyridine (3.8 mg), and triethylamine (1.4 μl) in dry $CHCl_3$ (200 μl) was allowed to stand at room temperature for 15 h, then 3-(dimethylaminopropyl)amine (3.2 μl) was added, and evaporation of the whole *in vacuo* afforded a resinous residue, which was chromatographed on an MPLC silica gel column with *n*-hexane–AcOEt at a flow rate of 3.8 ml/min to give the di-(*R*)-MTPA ester **8**, as a resinous residue (1.0 mg).

A solution of **7** (3.0 mg), (*S*)-MTPA chloride (3.8 mg), dimethylaminopyridine (3.8 mg), and triethylamine (1.4 μl) in dry $CHCl_3$ (200 μl) was similarly treated, and the product was chromatographed, in the same way as for the preparation of **8** from **7** to give the di-(*S*)-MTPA ester **9**, as a resinous residue (1.0 mg).

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