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Fungal Isobisvertinol, a New Inhibitor of Lipid Droplet Accumulation in Mouse Macrophages

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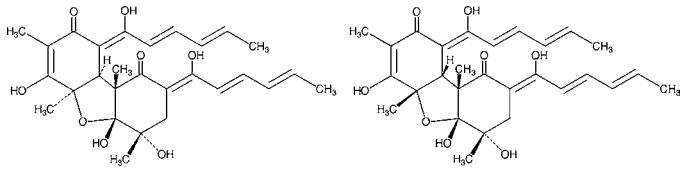
ABSTRACT



Isobisvertinol (1) **Bisvertinol (2)**

New isobisvertinol and known bisvertinol were isolated from the culture broth of *Aspergillus* sp. FKI-1746. Isobisvertinol with the two alkenyl side chains extending in the same direction inhibited lipid droplet accumulation in macrophages, whereas bisvertinol with those extending in the reverse direction had almost no effect on the accumulation.

In the course of screening for inhibitors of lipid droplet accumulation in mouse macrophages, we have reported several inhibitors of microbial origin.[1] Our efforts were rewarded with the discovery of a novel compound having a hexahydrodibenzofuran skeleton, designated isobisvertinol (**1**), from the culture broth of *Aspergillus* sp. FKI-1746, which was isolated from a mangrove slurry sample collected at Amamiohshima Island, Kagoshima, Japan.

Structurally related bisvertinol (**2**) and dihydrobisvertinol (**3**), previously isolated from *Verticillium intertextum*,[2] were also obtained from the culture broth; however, their biological activity has not been reported so far. In this study, the isolation and structural elucidation, including the absolute stereochemistry and biological activity of **1**, are described.

The production culture was initiated by transferring 1 mL of the seed culture of strain FKI-1746 into a Jar tank containing 20 L of the production medium (glycerol, 3.0%; oatmeal, 2.0%; dry yeast, 1.0%; KH_2PO_4, 1.0%; Na_2HPO_4, 1.0%; $MgCl_2 \cdot 6H_2O$, 0.5%), and fermentation was carried out at 27 °C with rotation at 210 rpm. After 6 days, the culture broth was treated with ethanol, and the supernatant was recovered by centrifugation and concentrated under reduced pressure. The resulting aqueous layer was extracted twice with an equal volume of ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a dark brown oil (35.5 g). This oil was applied to a silica gel column and eluted stepwise with 100% $CHCl_3$ and 100:1, 50:1, 10:1, and 1:1 (v/v) $CHCl_3-CH_3OH$ solvents (500 mL for each solvent). The 100:1 fraction was concentrated, and the resulting oil was subjected to a second silica gel column and eluted with 100% $CHCl_3$ and 50:1, 25:1, and 10:1 $CHCl_3-CH_3OH$ solvents (50 mL × 6 for each solvent). The second and third fractions of 50:1 were

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(1) (a) Koyama, N.; Nagahiro, T.; Yamaguchi, Y.; Ohshiro, T.; Masuma, R.; Tomoda, H.; Omura, S. *J. Antibiot.* **2005**, *58*, 338−345. (b) Tomoda, H.; Namatame, I.; Si, S.; Kawaguchi, K.; Masuma, R.; Mamikoshi, M.; Omura, S. *J. Antibiot.* **1999**, *52*, 851−856. (c) Namatame, I.; Tomoda, H.; Ishibashi, S.; Omura, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 737−742. (d) Namatame, I.; Tomoda, H.; Matsuda, D.; Tabata, N.; Kobayashi, S.; Omura, S. *Proc. Japan Acad.* **2002**, *78B*, 45−50. (e) Uchida, R.; Kim, Y. P.; Namatame, I.; Tomoda, H.; Omura, S. *J. Antibiot.* **2006**, *59*, 93−97.
(2) Trifonov, L. S.; Hilpert, H.; Floersheim, P.; Dreiding, A. S.; Rest, D. M.; Skrivanova, R.; Hoesch, L. *Tetrahedron* **1986**, *42*, 3157−3179.

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collected and concentrated to give a yellow brown oil (766 mg). The enriched active materials were finally purified with preparative HPLC under the following conditions: column, PEGASIL ODS (Senshu Science i.d. 20 × 250 mm); mobile phase, 55% CH_3CN containing 0.05% trifluoroacetic acid; flow rate, 6 mL/min; detection, UV at 210 nm. Compounds **1**−**3** were eluted at 81, 56, and 68 min, respectively. Each fraction was collected and concentrated to dryness to give yellow amorphous **1** (11.3 mg), **2** (293 mg), and **3** (84.3 mg).

Isobisvertinol (**1**) showed a molecular ion peak at *m/z* 499 $(M + H)^+$ in FAB-MS, and the molecular formula $C_{28}H_{34}O_8$ was assigned on the basis of HRFAB-MS [*m/z* 499.2331 $(M + H)^+$, Δ +0.1 mmu], indicating 12 degrees of unsaturation. IR absorptions at 3415, 1675, and 1621 cm^{-1} indicated the presence of an enolized β-diketone.[3,4]

The ^1H and ^{13}C NMR spectra of **1** showed 29 proton and 28 carbon signals, and the multiplicity of the carbon signals was classified into 6 methyl, 1 methylene, 9 methine, and 12 quaternary carbons by analysis of the HMQC data. The connectivity of proton and carbon atoms was established by HMQC (Table 1). The ^1H and ^{13}C NMR chemical shifts of

Table 1. ^1H (300 MHz) and ^{13}C NMR (75 MHz) Chemical Shifts of **1** (in CD_3OD) and **2** (in $CDCl_3$)

	isobisvertinol (**1**)		bisvertinol (**2**)	
position	δ_C	δ_H	δ_C	δ_H
1	206.3	–	191.7	–
2	104.6	–	104.0	–
3	33.8	2.63 d, 2.86 d	35.8	2.58 d, 2.76 d
4	73.7	–	73.8	–
4a	109.6	–	106.1	–
5	–	–	–	–
5a	81.6	–	79.7	–
6	170.8	–	164.8	–
7	110.6	–	110.2	–
8	193.0	–	191.8	–
9	102.0	–	100.7	–
9a	55.8	3.19 s	53.6	3.64 s
9b	62.8	–	58.4	–
10	170.1	–	179.7	–
11	121.3	6.40 d	120.3	6.14 m
12	140.7	7.24 dd	139.0	7.27 m
13	132.5	6.40 m	130.8	6.25 m
14	138.5	6.16 m	137.0	6.08 m
15	18.9	1.89 d	18.7	1.85 d
16	22.8	1.42 s	22.4	1.28 s
17	25.4	1.39 s	25.5	1.48 s
18	7.5	1.72 s	6.8	1.53 s
19	170.3	–	168.7	–
20	122.0	5.78 d	120.3	6.40 m
21	139.2	7.05 dd	142.9	7.27 m
22	132.9	6.05 m	131.1	6.25 m
23	137.0	6.05 m	140.2	6.20 m
24	18.8	1.82 d	18.9	1.85 d
25	14.7	1.06 s	19.2	1.34 s

1 resembled those of **2**, but the proton signals of H9a, H20, and $H_3$25 were shifted more upfield than those of **2**. The presence of two enolized β-diketones could be deduced from four carbonyl-like carbons (δ 206.3, 193.0, 170.3, and 170.1) and two sp^2 quaternary carbons (δ 104.6 and 102.0) and the chemical shifts (δ 14.97 and 16.62) of two hydroxyl groups observed in acetone-d_6 (data not shown). The similarity in the spectral data of **1** and **2** suggested that they are stereoisomers. The planar structure of **1** was elucidated by comparison of the 2D NMR ($^1H-^1H$ COSY and HMBC) data of **1** and **2**. As shown by the bold lines in Figure 1, two

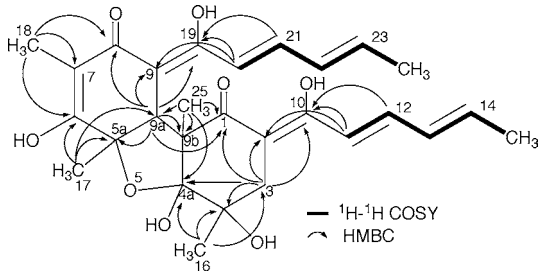


Figure 1. $^1H-^1H$ COSY and HMBC correlations of **1**.

identical partial structures (−CH═CH−CH═CH−CH_3) were determined by $^1H-^1H$ COSY. Furthermore, $^1H-^{13}C$ long-range couplings of ^2J and ^3J were measured in the HMBC spectrum (Figure 1). First, it became clear that the two side chains were fused with the enolized β-diketone overlapping the partial structure (−CH═CH−CH═CH−CH_3) due to the following observations: (1) The cross-peaks from H20 (δ 5.78) and H21 (δ 7.05) to C19 (δ 170.3) and from H20 to C9 (δ 102.0) suggested that C19 of the enolized β-diketone was connected to C20 in one of the partial structures. (2) The cross-peaks from H11 (δ 6.40) and H12 (δ 7.24) to C10 (δ 170.1) and from H11 to C2 (δ 104.6) suggested that C10 of the enolized β-diketone was attached to C11 in the other. Second, it became clear that the hexahydrodibenzofuran skeleton was connected to the two side chains due to the following facts: (1) The cross-peaks from $H_3$18 to C6 (δ 170.8), C7 (δ 110.6), and C8, from $H_3$17 (δ 1.39) to C5a (δ 81.6), C6, and C9a (δ 55.8), and from H9a (δ 3.19) to C8, C9, and C19 showed that a six-membered ring was formed with C5a, C6, C7, C8, C9, and C9a, as shown in Figure 1, and one side chain was connected to C9 of the ring. (2) The cross-peaks from $H_3$25 (δ 1.06) to C1, C4a (δ 109.6), and C9b (δ 62.8), from $H_3$16 (δ 1.42) to C3 (δ 33.8), C4 (δ), and C4a, and from $H_2$3 to C1, C2, C4, C4a, and C10 suggested that another six-membered ring was formed with C1, C2, C3, C4, C4a, and C9b, as shown in Figure 1, and the other side chain was attached to C2 of the second six-membered ring. Taking the degree of unsaturation and the molecular formula into consideration, a furan ring should

(3) Andrade, R.; Ayer, W. A.; Mebe, P. P. *Can. J. Chem.* **1992**, *70*, 2526−2535.

(4) Isobisvertinol (**1**): yellow amorphous; $[\alpha]^{26}_D$ −400.8° (*c* 0.01, MeOH); IR (KBr) υ_{max} 3415, 2981, 2937, 2837, 2873, 1675, 1621, 1554 cm^{-1}; UV (MeOH) λ_{max} 232 (ε 24 179), 273 (ε 26 577), 310 (ε 23 467), 359 (ε 33 840); ^1H and ^{13}C NMR data (Table 1); FAB-MS *m/z* 499 $(M + H)^+$; HRFAB-MS *m/z* 499.2331 ($(M + H)^+$; calcd for $C_{28}H_{34}O_8$, 499.2332); CD (MeOH) $\lambda_{extremum}$ (Δε) 354 (−23), 274 (+42), A = −65.

be formed as a residual unit. Cross-peaks were observed from H9a to C5a and C9b and from $H_3$25 to C9a, 9b, and C4a, and the chemical shift value of C4a corresponded to that of a hemiketal carbon, thus revealing the presence of a hexahydrodibenzofuran skeleton fused with the two side chains. Taken together, the structure of isobisvertinol was elucidated as **1**, which is the same planar structure as **2**. Furthermore, it was reported that the position of enolization of β-diketone for **2** and **3** was arbitrarily chosen because it was unable to be defined by spectral data: a carbonyl group is on C10; an enol group of β-diketone is on C1; another carbonyl group is on C19; and another enol group of β-diketone is on C8.[2] In this study, the position of β-diketone was confirmed by an HMBC experiment. As for **2**, cross-peaks were observed from $H_3$25 to C1 (δ 191.7) and from $H_3$18 to C8 (δ 191.8) but not from $H_3$25 to C10 (δ 179.7) and from $H_3$18 to C19 (δ 168.7), suggesting that a carbonyl group is on C1 and an enol group of β-diketone is on C10 and that another carbonyl group is on C8 and another enol group is on C19. As for **3**, cross-peaks were observed from $H_3$25 to C1 (δ 181.7) and from $H_3$18 to C8 (δ 191.9) but not from $H_3$25 to C10 (δ 199.3) and from $H_3$18 to C19 (δ 168.4), suggesting that a carbonyl group is on C10 and an enol group is on C1 and that another carbonyl group is on C8 and another enol group is on C19.

The relative stereochemistry of **1** was studied. As shown in Figure 2A, NOEs were observed between H9a and H17

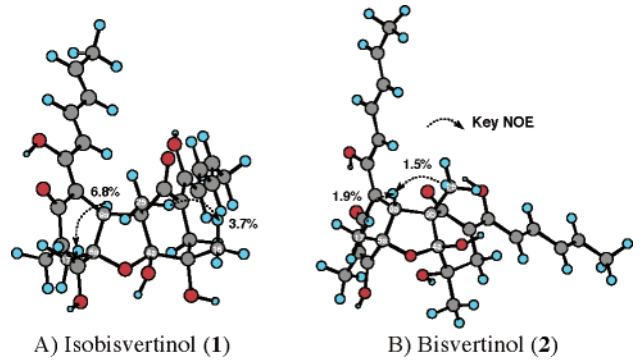


Figure 2. Key NOE correlations for **1** and **2**.

(6.8%) but not between H9a and H25. Furthermore, NOEs were observed between H25 and H16 (3.7%). On the other hand, the NOEs of **2** were observed between H17 and H9a (1.9%) and between H25 and H9a (1.5%) but not between H25 and H16 (Figure 2B). Furthermore, the proton signals of two alkenyl side chains for **2** were almost overlapped, whereas those for **1** were separated in the ^{1}H NMR (Table 1). The UV spectrum of **1** was drastically shifted,[4,5] mainly due to the orientation of two alkenyl side chains corresponding to chromophores. These results indicated that the C9a/C9b ring juncture of **1** between the left and right halves was different from that of **2**. Therefore, we concluded that **1** is a stereoisomer of **2** on C5a and C9a. This was supported by the fact that the proton chemical shifts of H9a, H20, and H25 were shifted more upfield than those of **2** due to the anisotropic effect of C1 and C8 carbonyl groups. Regarding the stereochemistry of the C4a hydroxyl group, it should be on the same side of H25 due to the enhanced stability of a *cis* 5-6 ring junction over a *trans* 5-6 ring junction as previously reported by Andrade et al.; [3] moreover, the stereochemistry of bisvertinolone, the C-3 carbonyl derivative of **2**, was also reported to have the same configuration from biosynthetic study.[6] Thus, the relative chemistry of **1** was defined as shown in Figure 2A.

Furthermore, **1** showed a negative Cotton effect at 354 nm ($\Delta\epsilon$ −23) and 274 nm ($\Delta\epsilon$ +42) in the CD spectrum. As previously reported,[3,5] **2** also showed a negative Cotton effect at 442 nm ($\Delta\epsilon$ −39) and 372 nm ($\Delta\epsilon$ +33). This result indicated that the two chromophores were oriented in a counterclockwise fashion.[7] Thus, the absolute stereochemistry of **1** was assigned as 4*S*, 4a*R*, 5a*R*, 9a*S*, and 9b*S*. The stereoviews of **1** and **2** are displayed in Figures 3A and 3B,

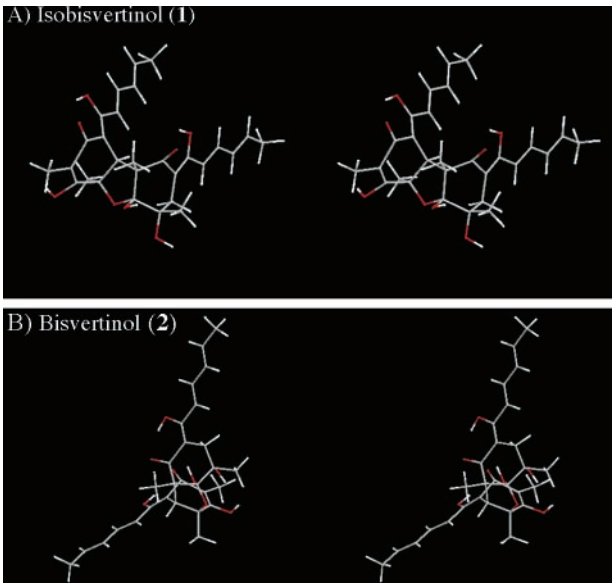


Figure 3. Stereoviews of **1** and **2**.

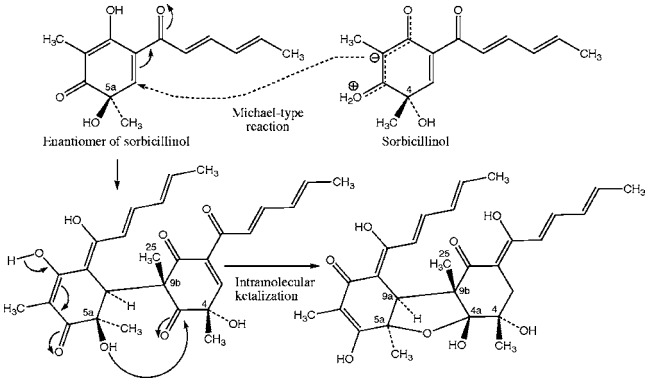
respectively, revealing that the two alkenyl side chains of **1** extended in a similar direction, whereas those of **2** extended in the opposite direction.

Hypothetical biosynthesis of **1** is proposed as shown in Scheme 1. Assuming that **1** is generated through a route

(5) Bisvertinol (**2**): yellow amorphous; $[\alpha]^{26}_D$ −3052.8° (*c* 0.01, MeOH); IR (KBr) υ_{max} 3428, 3066, 2927, 2859, 1621, 1558, 1525 cm^{-1}; UV (MeOH) λ_{max} 234 (ϵ 15 412), 273 (ϵ 20 462), 301 (ϵ 18 238), 403 (ϵ 25 965); ^{1}H and ^{13}C NMR data (Table 1); FAB-MS *m/z* 499 (M + H)$^+$; HRFAB-MS *m/z* 499.2319 ((M + H)$^+$; calcd for $C_{28}H_{34}O_8$, 499.2332); CD (MeOH) $\lambda_{extremum}$ ($\Delta\epsilon$) 442 (−39), 372 (+33), A = −72.

(6) Abe, N.; Arakawa, T.; Hirota, A. *Chem. Commun.* **2002**, 204−205.
(7) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy−Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

Scheme 1. Hypothetical Biosynthesis of Isobisvertinol (**1**)



parallel with bisvertinolone,[6] **1** is considered to be biosynthesized via a heterodimeric ''Michael-type" reaction of an enantiomer of sorbicillinol and sorbicillinol. On the other hand, **2** might be generated via a homodimeric reaction of two sorbicillinols. As reported, it seemed that the initial nucleophilic addition occurs from each side of the hydroxyl group at C4 and C5a, and the subsequent intramolecular ketalization occurs from the opposite side of $H_3$4 and $H_3$25. Furthermore, hypothesizing the case that the acceptor is sorbicillinol and the donor is an enantiomer of sorbicillinol, another isomer of **2** on C4a and C9b is proposed. However, this isomer would show a positive Cotton effect due to the orientation of two chromophores in a clockwise fashion. Thus, the biosynthetic pathway of **1** is the most likely. Further studies on the culture condition are needed to establish the production of all isomers.

An assay for CE and TG syntheses in mouse macrophages was carried out by the method described previously.[1] Compound **1** inhibited the syntheses of CE and TG, the main constituents of lipid droplets in macrophages, with IC_{50} values of 2.5 and 4.0 μM, respectively. On the other hand, **2** and **3** showed almost no effect even at 10 μM. Notably, **1** showed much higher activity than **2**, suggesting that the stereochemical difference is important for binding to a target molecule and eliciting this activity. The inhibition site of **1** on CE and TG syntheses in macrophages is under investigation.

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Supporting Information Available: Experimental spectral data of isobisvertinol (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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