COMMUNICATION

ChemComm
www.rsc.org/chemcomm

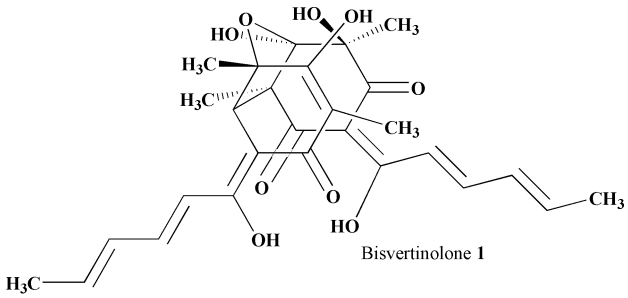
# The biosynthesis of bisvertinolone: evidence for oxosorbicillinol as a direct precursor

**Naoki Abe,* Tadaharu Arakawa and Akira Hirota**
*School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan. E-mail: abe@fns1.u-shizuoka-ken.ac.jp; Fax: +81-54-264-5099; Tel: +81-54-264-5555*

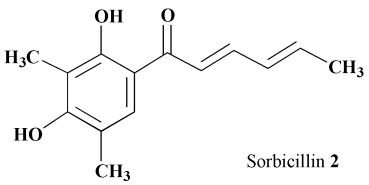
***Received (in Cambridge, UK) 18th October 2001, Accepted 5th December 2001***
***First published as an Advance Article on the web 7th January 2002***

**Biosynthetic incorporation of labeled sodium acetate into oxosorbicillinol in *Trichoderma* sp. USF-2690 suggests that oxosorbicillinol is derived from six acetate units, and subsequent bioconversion of the labeled oxosorbicillinol to bisvertinolone in the fermentation of the strain suggests that bisvertinolone is biosynthesized from oxosorbicillinol and sorbicillinol in a Michael-type reaction.**

Bisvertinolone[1] **1** is a member of the family of bisorbicillinoids, comprised of dimeric sorbicillin-related natural products.[2] Several compounds of this group exhibit interesting biologic




Bisvertinolone **1**




Sorbicillin **2**

activities.[3–7] Bisvertinolone **1** is reported to be a β-1,6-glucan biosynthesis inhibitor[8] and a potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger.[4] Bisorbicillinoids have a complex structure and their biosynthesis is of interest to many investigators.[1,5,9–12] Total synthesis was recently attempted.[13–16] A biogenesis of bisvertinol, 3-dihydrobisvertinolone, was proposed by Trifonov *et al*. in 1986.[1] They suggested that bisvertinol is biosynthesized from a sorbicillinol-derived epoxide and a 1-hydrosorbicillinol by an $S_N2$-type reaction with inversion. Furthermore, bisvertinolone **1** should have the same stereochemistry as the biogenetically related bisvertinol. Andrade[17] and Kontani[8] have each proposed revised stereochemistries for **1**. Our elucidation of the biosynthesis of **1**, as reported in the sequel, supports the previous speculative proposal of Andrede; 4*R*, 4a*R*, 5a*S*, 9a*R*, and 9b*R*. [17]

From the culture broth of *Trichoderma* sp. USF-2690, we found 11 related-compounds, including bisvertinolone **1**,[4] oxosorbicillinol **3**,[7] and sorbicillinol **4**.[11] The discovery of oxosorbicillinol **3** and sorbicillinol **4** from the bisvertinolone-producing strain led us to hypothesize that there might be a biosynthetic route from **3** and **4** to **1** *via* Michael-type reaction, as previously proposed by Nicolaou for trichodimerol.[2] We used $^{13}$C-labeled intermediates to provide evidence for this biosynthetic route.

*Trichoderma* sp. USF-2690 was cultivated on a reciprocal shaker at 30 °C for 3 d.†A mixture of 10 mg [1-$^{13}$C] labeled sodium acetate (SA) and 10 mg of non-labeled SA were added to each flask, and then fermented with reciprocal shaking at 30 °C for three additional days. The filtered broth (2 L) was extracted with ethyl acetate (1 L × 2) at pH 3. The organic extract, concentrated *in vacuo*, was purified on a Sephadex LH-20 column (methanol). The fraction including **1** was re-chromatographed using medium-pressure liquid chromatography under the following conditions: support, YMC-ODS-AQ 120-S50; solvent, acetonitrile–$H_2O$ (1 : 1, containing 0.1% trifluoroacetic acid); detection, UV at 370 nm. Labeled bisorbicillinol **1** was obtained (35.1 mg). The experiment gave **1** ([1-$^{13}$C]-SA-**1**) with 12 $^{13}$C-enriched carbon signals (C-1, C-3, C-4a, C-6, C-8, C-9a, C-10, C-12, C-14, C-19, C-21, and C-23) in the $^{13}$C-NMR spectrum.‡ The results of this experiment revealed that bisvertinolone **1** was derived from 12 acetate units and [1-$^{13}$C] SA was incorporated at all of the expected carbons.

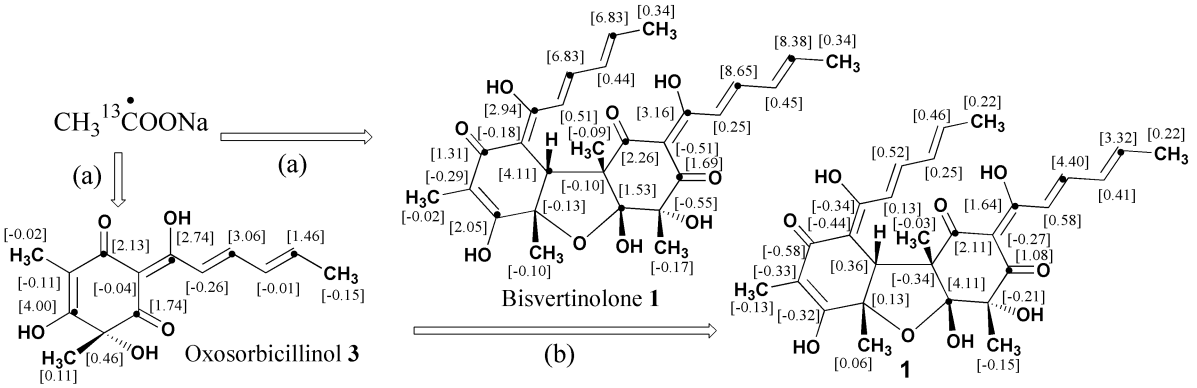
A feeding experiment to provide oxosorbicillinol **3** from [1-$^{13}$C] SA was performed under the following procedure. The fungus was fermented for 48 h.† A mixture (20 mg) of [1-$^{13}$C]-labeled SA and non-labeled SA (1 : 1) was added to each flask, and then fermented for an additional 42 h. The resulting filtrate (1 L) was extracted with ethyl acetate at pH 3. The concentrate was applied to a Sephadex LH-20 column and eluted with methanol. The subsequent chromatography of the crude oxosorbicillinol on high pressure liquid chromatography§ yielded 1.0 mg of pure **3** ([1-$^{13}$C]-SA-**3**). This experiment was repeated several times to prepare 10.0 mg of pure [1-$^{13}$C]-SA-**3**. The $^{13}$C-NMR spectrum of the [1-$^{13}$C]-SA-**3** demonstrated that the $^{13}$C atoms of [1-$^{13}$C] SA were apparently incorporated at C-1, C-3, C-5, C-1′, C-3′, and C-5′. The incorporation pattern suggested that the six acetates, combined in the classical head-to-tail manner of fatty acids and polyketides, formed **3**.

Labeled oxosorbicillinol ([1-$^{13}$C]-SA-**3**), obtained from the feeding experiment above, was applied to the subsequent experiment. The incorporation study employing [1-$^{13}$C]-SA-**3** as a precursor of bioconversion was achieved in the following manner. The fungus was inoculated in a 0.5-L flask containing 100 mL of the medium from a preserved slant and cultured for 2 d.† Then, 10 mg of [1-$^{13}$C]-SA-**3** was added to the flask. Additional incubation was continued for 5 h. The filtrate was extracted with an equal volume of ethyl acetate. The organic extract was subjected to preparative HPLC,§ to give 4.8 mg of bisvertinolone **1**. The $^{13}$C-NMR spectrum of **1**, obtained from the feeding experiment, showed only 6 $^{13}$C-enhriched peaks at $\delta_C$ 199.8 (C-1), 196.4 (C-3), 185.6 (C-10), 148.5 (C-12), 144.1 (C-14), and 104.2 (C-4a). The labeling pattern in [1-$^{13}$C]-SA-**3** was retained in the labeled bisvertinolone **1**; C-1, C-3, C-10, C-12, C-14, and C-4a of the labeled **1** originated from C-3, C-1, C-1′, C-3′, C-5′, and C-5 of [1-$^{13}$C]-SA-**3**. When compared to the $^{13}$C-NMR spectrum of [1-$^{13}$C]-SA-**1**, the effective $^{13}$C-enrichments at C-9a, C-6, C-8, C-19, C-21, and C-23 were not observed; therefore, oxosorbicillinol **3** was established to be one of two direct precursors in bisvertinolone biosynthesis (Scheme 1).

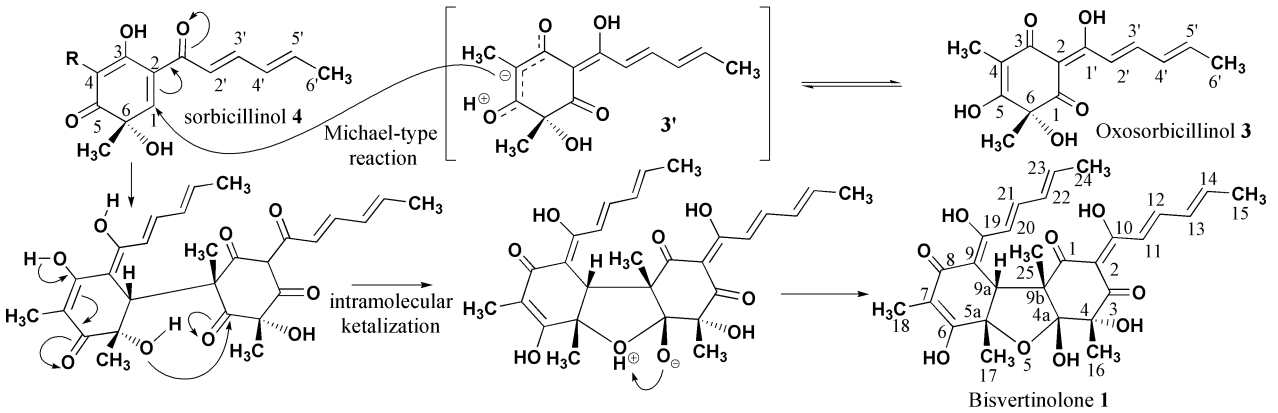
From the same fungus, we recently found a highly reactive quinol, sorbicillinol **4**, which was postulated to be a key intermediate in bisorbicillinoid biosynthesis, and determined the absolute structure of **4**. The significance of sorbicillinol **4** in the biosynthesis was supported by chemical conversions of **4** to

DOI: 10.1039/b109505f




**Scheme 1** Labeling patterns of bivertinolone **1** and oxosorbicillinol **3** incorporating (a) [1-$^{13}$C] acetate and (b) labeled **3**. The numbers in brackets are the number of fold increase in the height of the $^{13}$C resonance relative to unlabeled **1** or **3**, with correction by standardizing the two samples using the mean peak height for all unlabelled carbons. The $^{13}$C-NMR measurement of labeled **3** in experiment (a) and the incorporation study of labeled **3** in experiment (b) were performed by the use of the different samples of labeled **3**, prepared individually.




**Scheme 2** Proposed biosynthetic pathway for bisvertinolone.

bisorbicillinol and trichodimerol.[11] In the case of bisvertinolone, we hypothesized that a carbanion **3′**, formed from oxosorbicillinol **3**, as a nucleophile attacks the C-1 position of sorbicillinol **4** in a Michael-type reaction, followed by intramolecular ketalization between 6-OH of **4** and C-5 of **3** (Scheme 2). Thus, because of the *S*-configuration at C-6 of **4**, it seemed that the initial nucleophilic addition occurring between the *re*-face at C-4 of **3** and the *si*-face at C-1 of **4** caused the subsequent intramolecular ketalization. This reaction produced the most protable sterochemistry of **1**, corresponding to 4*R*, 4a*R*, 5a*S*, 9a*R*, and 9b*R*, which was reported by Andrade *et al*.[17]

Further studies on the bisvertinolone biosynthesis using labeled sorbicillinol **4** are under investigation.

This work was supported in part by a grant-in-aid for Scientific Research (C) (No. 12660100) to A. H. from the Ministry of Education, Science, Sports and Culture of Japan.

## Notes and references

† The fermentation broth was obtained from 10 0.5-L flasks each containing 100 mL of the following medium: 2.0% glucose, 0.05% polypeptone, 0.2% yeast extract, 0.1% $KH_2PO_4$, 0.1% $MgSO_4{\cdot}7H_2O$, and 0.1% trace salt mixture at pH 7.

‡ Bisvertinolone **1**: $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$ 199.8 (s, C-1), 196.7 (s, C-3), 191.2 (s, C-8), 185.8 (s, C-10), 170.0 (s, C-19), 164.2 (s, C-6), 148.4 (d, C-12), 144.2 (d, C-14), 139.6 (d, C-21), 137.5 (d, C-23), 131.1 (d, C-22), 131.0 (d, C-13), 121.9 (d, C-11), 120.0 (d, C-20), 110.7 (s, C-7), 107.2 (s, C-2), 104.0 (s, C-4a), 99.8 (s, C-9), 79.9 (s, C-5a), 79.5 (s, C-4), 59.8 (s, C-9b), 54.4 (d, C-9a), 25.5 (q, C-17), 22.8 (q, C-16), 19.1 (q, C-25), 18.7 (q, C-15), 18.7(q, C-24), 6.9 (q, C-18).

§ A preparative HPLC system under the following conditions: column, Capcell pak $C_{18}$ SG120 ($\phi$ 15 × 250 mm, Shiseido, Japan); solvent system, $H_2O$–$CH_3CN$–$CF_3COOH$ (6:4:0.1 for oxosorbicillinol **3** or 1:1:0.1 for bisvertinolone **1**); flow rate, 10.0 ml min$^{-1}$; detection, 370 nm.


1 L. S. Trifonov, H. Hilpert, P. Floersheim, A. S. Dreiding, D. M. Rast, R. Skrivanova and L. Hoesch, *Tetrahedron*, 1986, **42**, 3157.
2 K. C. Nicolaou, R. Jautelat, G. Vassilikogiannakis, P. S. Baran and K. B. Simonsen, *Chem. Eur. J.*, 1999, **5**, 3651.
3 G. A. Warr, J. A. Veitch, A. W. Walsh, G. A. Hesler, D. M. Pirnik, J. E. Lett, P.-F. M. Lin, I. A. Medina, K. D. McBrien, S. Forenza, J. M. Clark and K. S. Lam, *J. Antibiot.*, 1996, **49**, 234.
4 N. Abe, T. Murata and A. Hirota, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 661.
5 N. Abe, T. Murata and A. Hirota, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 2120.
6 N. Abe, T. Murata, K. Yamamoto and A. Hirota, *Tetrahedron Lett.*, 1999, **40**, 5203.
7 N. Abe, K. Yamamoto and A. Hirota, *Biosci. Biotechnol. Biochem.*, 2000, **64**, 620.
8 M. Kontani, Y. Sakagami and S. Marumo, *Tetrahedron Lett.*, 1994, **35**, 2577.
9 O. Shirota, V. Pathak, C. F. Hossain, S. Sekita, K. Takataori and M. Satake, *J. Chem. Soc., Perkin Trans. 1*, 1997, 2961.
10 S. Sperry, G. J. Samuels and P. Crews, *J. Org. Chem.*, 1998, **63**, 10011.
11 N. Abe, O. Sugimoto, K-i. Tanji and A. Hirota, *J. Am. Chem. Soc.*, 2000, **122**, 12606.
12 N. Abe, K. Yamamoto, T. Arakawa and A. Hirota, *Chem. Commun.*, 2001, 23.
13 D. Barnes-Seeman and E. J. Corey, *Org. Lett.*, 1999, **1**, 1503.
14 K. C. Nicolaou, K. B. Simonsen, G. Vassilikogiannakis, P. S. Baran, V. P. Vidali, E. N. Pitsinos and E. A. Couladouros, *Angew. Chem., Int. Ed.*, 1999, **38**, 3555.
15 K. C. Nicolaou, G. Vassilikogiannakis, K. B. Simonsen, P. S. Baran, Y.-L. Zhong, V. P. Vidali, E. N. Pitsinos and E. A. Couladouros, *J. Am. Chem. Soc.*, 2000, **122**, 3071.
16 L. H. Pettus, R. D. V. D. Water and T. R. R. Pettus, *Org. Lett.*, 2001, **3**, 905.
17 R. Andrade, W. A. Ayer and P. P. Mebe, *Can. J. Chem.*, 1992, **70**, 2526.