Identification of the Quinol Metabolite "Sorbicillinol", a Key Intermediate Postulated in Bisorbicillinoid Biosynthesis

Naoki Abe,* Osamu Sugimoto, Ken-ichi Tanji, and Akira Hirota

School of Food and Nutritional Sciences
University of Shizuoka
52-1 Yada, Shizuoka 422-8526, Japan

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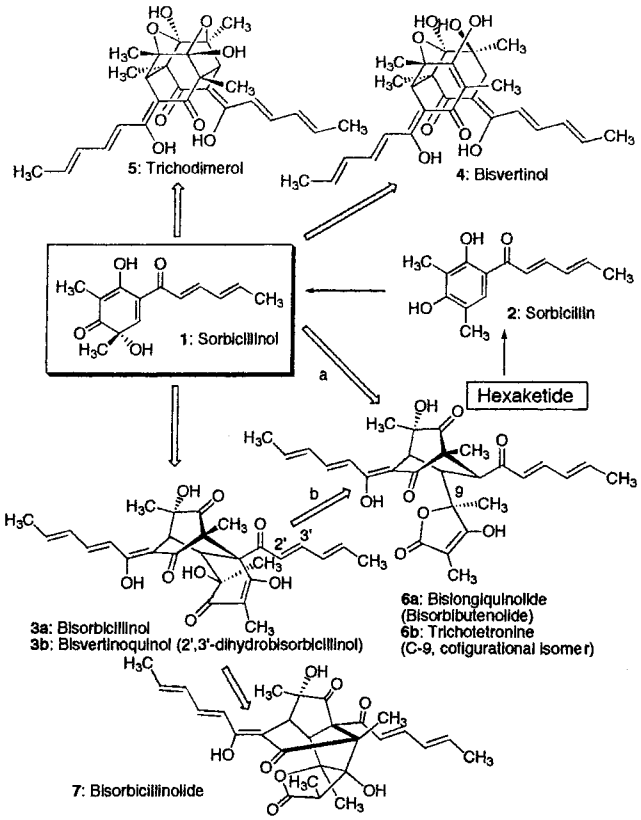
The "Bisorbicillinoids"[1] were recently defined by Nicolaou et al. to designate a group of dimeric sorbicillin[2]-related natural products, the diverse structures[3] and interesting biological activities[4] of which were reported by several groups. It is known that a few fungal genera (*Trichoderma*, *Verticillium*, and *Penicillium*) produce bisorbicillinoids, and their biosynthesis is of interest to many investigators. Dreiding et al. postulated a hypothetical biosynthetic scheme from sorbicillin (**2**) to bisvertinoquinol (**3b**) and/or to bisvertinol (**4**) in 1981,[3a] 1983,[3c] and 1986.[3d] Biogenesis of trichotetronine (**6b**) and bislongiquinolide (**6a**) were reported by Satake et al.[3h] and Crews et al.,[3k] respectively. We also proposed a different biosynthetic route to bislongiquinolide (bisorbibutenolide **6a**) and bisorbicillinolide (**7**) in 1998.[3j] All of these biosynthetic pathways, postulated by independent investigators, proposed a common key intermediate, quinol **1** (Scheme 1), which can exist in tautomeric forms. Corey et al. synthesized trichodimerol (**5**) by way of the quinol intermediate (*S*)-**1**.[5] On the other hand, Nicolaou and co-workers generated quinol intermediate (±)-**1** in biomimetic total synthesis of bisorbicillinol (**3a**), bislongiquinolide (bisorbibutenolide, **6a**), and **5**, on the basis of our proposed biosynthetic route.[3j,6] In these synthetic studies, dimerization of quinol intermediate **1** occurred spontaneously under the reactive conditions and no evidence for the postulated intermediate was obtained. Therefore, quinol intermediate **1** seemed to be highly reactive and difficult to isolate.

We have isolated 10 sorbicillin-related compounds, which can scavenge a free radical species, 1,1-diphenyl-2-picrylhydrazyl (DPPH).[3i,j,m,l] A continuous, careful HPLC analysis of bisorbicillinoids produced by *Trichoderma* sp. USF-2690 indicated that an unstable metabolite accumulated in the early stages of the fermentation. The compound was highly sensitive to concentration and solvent extraction, and quickly changed into other compounds. We thought that the unknown was likely to be the quinol intermediate **1**, hence we tried to determine its structure.

Scheme 1. Summary of the Proposed Biosynthetic Relationship between Bisorbicillinoids and Postulated Common Precursor



[a] References 3h and 3k. [b] Reference 3j.

The strain was cultivated on a reciprocal shaker at 30 °C;[7] the products were monitored by HPLC analysis,[8] and an unidentified metabolite at 4.3 min was detected (Metabolite X, Figure 1A). The area of the peak gradually reduced with production of bisorbicillinoids. Solvent extraction caused rapid disappearance of the peak of X, while a peak at 16.8 min, which was consistent with the retention time of bisorbicillinol (**3a**), appeared in the HPLC analysis. In addition, concentration to dryness, including lyophilization, increased the peak at 22.7 min for trichodimerol (**5**), with concomitant decrease of peak X. These results strongly suggested Metabolite X was the biosynthetic common precursor **1**.

A hundred milliliters of filtered 3-day-cultivated broth was concentrated in vacuo to 20 mL. The concentrated aqueous solution was applied to a Sephadex LH-20 column, using H_2O as an eluent, to give 20 mL of the desired fraction with 75%

* Corresponding author. E-mail: abe@fns1.u-shizuoka-ken.ac.jp.

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(7) The fermentation broth was given from a 0.5-L flask containing 100 mL of each of the following: 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.

(8) A 10-μL aliquot of the filtered fermentation broth was directly injected into an analytical HPLC system under the following conditions: column Capcell pak C_{18} SG120 (ϕ 4.6 × 150 mm, Shiseido, Japan); solvent system, 0.15% KH_2PO_4 (pH 3.5) solution (solvent A) and CH_3CN (solvent B), a gradient program made up linear segments with 40% of solvent B (from 0 to 10 min), from 40 to 60% of solvent B (from 10 to 20 min) and with 60% of solvent B (from 20 to 25 min); flow rate, 1.0 mL/min; detection, 370 nm.

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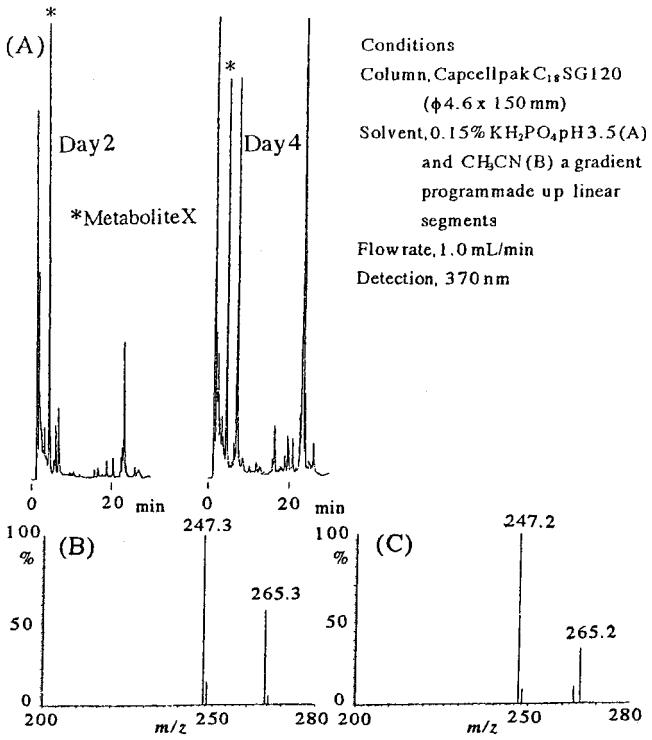
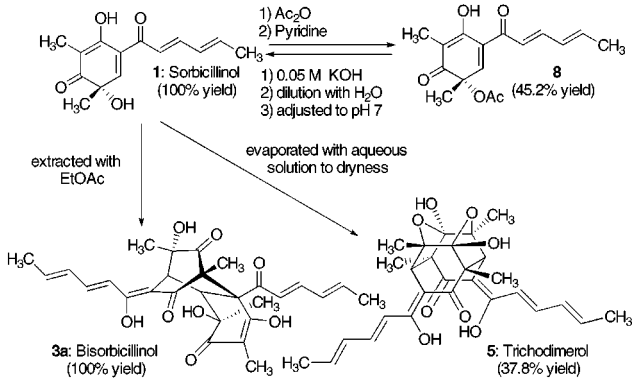


Figure 1. (A) HPLC profile of the products of *Trichoderma* sp. USF-2690 at day 2 and day 4 and LC-ESI-MS (negative) spectra of (B) Metabolite X and (C) a main product from α-acetoxy dienone (**8**) treated with 0.05 M KOH.

purity of Metabolite X, by HPLC analysis. LC-ESI-MS (negative) analysis[9] for the fraction gave *m*/*z* 247 as a $(M - 1)^-$ ion peak of X (Figure 1B) eluted at 8.5 min under these conditions. The postulated precursor **1** was expected to have a molecular weight of 248; therefore, the LC-ESI-MS (negative) analysis supported the conclusion that Metabolite X was compound **1**.

To confirm the hypothesis experimentally, the aqueous LH-20 fraction containing Metabolite X was treated with acetic anhydride for 1 h, and then pyridine was added to the reaction mixture, to yield a yellowish compound (45.2% yield).[10] The ^{1}H NMR spectrum of the derivative was consistent with that expected for α-acetoxy dienone **8** (Scheme 2), which had been reported as an intermediate of total syntheses of bisorbicillinol (**3a**)[6b] and trichodimerol (**5**).[5] According to Corey's method,[5] we prepared synthetic α-acetoxy dienone (**8**), and the identity of

Scheme 2. Chemical Conversion for Structural Determination of Sorbicillinol (**1**)



both compounds was established by direct comparison of ^{1}H NMR spectra, R_f values on TLC, and t_r values in HPLC analyses.

Further investigation using a chiral HPLC column[11] afforded significant information regarding stereochemistry of the α-acetoxy dienone (**8**). The synthetic α-acetoxy dienone (**8**) gave two separable peaks in equal parts, which were assigned to the *S*-isomer (t_r = 9.6 min) and *R*-isomer (t_r = 11.2 min),[5] while the one derived from naturally occurring Metabolite X revealed just one peak coinciding with the *S*-isomer, consistent with the reported absolute stereochemistry of bisorbicillinoids except trichotetronine (**6b**).

In the next step, we tried to generate compound **1** from the synthetic α-acetoxy dienone (**8**). We expected that it would be stable in dilute neutral solution because we recognized that Metabolite X could exist in the fungal culture broth at pH 6−8 for several days or in aqueous LH-20 fraction at pH 7 for several weeks. The synthetic acetate (**8**) was treated with 0.05 M KOH aqueous solution[12] followed by dilution with distilled water and neutralization. The LC-ESI-MS (negative) analysis[9] of the resulting solution showed *m*/*z* 247 as a $(M - 1)^-$ ion peak (Figure 1C) for the only major peak eluted at 8.5 min, which was completely identified with that of Metabolite X. In addition, evaporation in vacuo to dryness and EtOAc extraction at pH 3.5 of the resulting solution gave the same products as those derived from Metabolite X.

The chemical conversions shown in Scheme 2 indicate that Metabolite X is identical with compound **1**, "sorbicillinol"; therefore, the results reported here provide the first unambiguous evidence for the real intermediate **1** in bisorbicillinoids biosynthesis, in answer to a long-outstanding question.

We have definite evidence that **3a** and **5** are naturally occurring compounds produced by the fungus, and this will be reported elsewhere.

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(9) A Shiseido NANOSPACE SI-1 HPLC system was linked to a ThermoQuest LCQ LC mass spectrometer operating in the ESI negative ion mode. A Shiseido Capcell pak C_{18} SG120 (ϕ 4.6 × 150 mm) column was used at a flow rate of 0.5 mL/min with a solvent system of acetonitrile−0.5% CH_3COOH (4:6).

(10) Ten milliliters of the aqueous Metabolite X fraction was added to 55 mL of acetic anhydride with stirring for 1 h at room temperature. After the reaction mixture turned into a pale yellow homogeneous solution, 55 mL of pyridine was added and the mixture was then stirred for 1 h. The resulting solution was poured into 2 L of distilled water, extracted with 400 mL of $CHCl_3$, and dried over Na_2SO_4. Evaporation of the solvent followed by preparative TLC (Merck Art. No. 13794, *n*-hexane/EtOAc 1:1) gave 2.3 mg of 2,6-dimethyl-6-acetoxy-4-(2,4-hexadienoyl)-3-hydroxy-2,4-cyclohexadien-1-one (**8**).

(11) A 10-μL aliquot of 1 mg/mL of each sample was injected into an analytical HPLC system under the following conditions: column CHIRALPAK AD (ϕ 4.6 × 150 mm, Daisel Chemical Ind., Ltd., Japan); solvent system, *n*-hexane/*i*-PrOH/MeOH 80:15:5 cotaining 0.1% TFA; flow rate, 0.5 mL/min; detection, 370 nm.

(12) Two milligrams of α-acetoxy dienone (**8**) was dissolved in 2.0 mL of 0.05 M KOH aqueous solution and then stirred for 6 h at room temperature. The reaction mixture was diluted with 30 mL of distilled water. The resulting solution was adjusted to pH 7 with 0.1 N HCl aqueous solution.