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The biosynthesis of sorbicillinoids in *Trichoderma* sp. USF-2690: prospect for the existence of a common precursor to sorbicillinol and 5-epihydroxyvertinolide, a new sorbicillinoid member

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Abstract

Biosynthetic feeding studies of [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$]-labeled sodium acetates into 5-epihydroxyvertinolide, a new sorbicillinoid, gives an incorporation pattern that proves the γ-lactone ring formation associated with a ring cleavage reaction of the precursor, a potential intermediate of sorbicillinol biosynthesis.

Keywords: 5-Epihydroxyvertinolide; *Trichoderma* sp. USF-2690; Sorbicillinoids; Polyketide; Biosynthesis; Bisorbicillinoids

'Sorbicillinoids'[1] and 'bisorbicillinoids'[2] are terms used for the group of hexaketide compounds with a sorbyl chain, for example, sorbicillins,[3–5] vertinolides,[6,7] sorbicillinols,[5,8–10] and sohirnones,[11] and all dimeric sorbicillinoid-derived natural products possessing complex structures. Several fungal genera, including *Trichoderma*, *Penicillium*, *Verticillium*, *Aspergillus*, and *Paecilomyces*, produce sorbicillinoids and bisorbicillinoids. Recently, several groups reported new types of sorbicillinoids, such as rezishanones A–D,[11] sorbicillactones A and B,[12] a sorbicillinoid urea,[13] trichodermanones A–D,[14] and trisorbicillinone A.[15] Several compounds belonging to both groups exhibit the following important biological activities: trichodimerol, inhibits lipopolysaccharide-induced production of tumor necrosis factor-α in human monocytes;[16] bisvertinolone, inhibits β-1,6-glucan biosynthesis in fungi;[17] bisorbicillinol, an antioxidant with a new type of radical scavenging mechanism;[18] sorbicillactones A and B, with antileukemic, antiviral, and neuroprotective activities;[12] and isobisvertinol, inhibits lipid droplet accumulation in mouse macrophages.[19]

Since 1981, the biogenesis of sorbicillinoids and successive bisorbicillinolides was thought to drive from sorbicillin **1**.[3] We recently reported the existence of a key intermediate of the sorbicillinoid–bisorbicillinoid biosynthesis, sorbicillinol **2**, in *Trichoderma* sp. USF-2690 as well as the biosynthetic routes for sorbicillinoids (sorbicillin) and bisorbicillinoids (trichodimerol, bisorbicillinol, bisorbibutenolide, bisorbicillinolide, and bisvertinolone) from **2** based on the findings from stepwise ^{13}C incorporation.[1,9,10,20,21] In these studies, the rapidly increasing production of sorbicillinol **2** from an early stage of the fermentation, shown by HPLC monitoring at 370 nm, suggested that **2** is the true origin of sorbicillinoid–bisorbicillinoid biosynthesis, whereas tiny amounts of sorbicillin **1**, which is chemically

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more stable than sorbicillinol **2**, were detected in the mycelial cake as well as in the broth.[9] If **1** was the precursor of **2**, **1** would more likely exist in large quantity in the culture. The high level incorporation of ^{13}C-labeled **2** into **1** also supported the idea that **2** is the true origin.[1] We therefore hypothesized that **2** is biosynthesized via a hexaketide ring formation by the Claisen reaction (Scheme 1). In aromatic polyketide biosyntheses, the aldol and Claisen reactions are important mechanisms for achieving carbon–carbon bond formation. In feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$]-labeled sodium acetates (SAs), **2** gave an isotope pattern consistent with an origin generated from a Claisen-type precursor **3** via an intermediate **4**.[1] The hypothetical precursor **3** leads to the presence of 5-hydroxyvertinolide **5**, which is expected by the biosynthesis of bisorbibutenolide via bisorbicillinol.[20] 5-Hydroxyvertinolide **5** was reported by Andrade et al. in 1997, but their attempts to reisolate **5** were unsuccessful.[7] Here, we establish our hypothesis as shown in Scheme 1, and report the presence of **5** expected in the culture broth and the results of ^{13}C-labeling experiments of **5**.

Trichoderma sp. USF-2690 was precultured on a reciprocal shaker at 30 °C for 2 d.[22] [1-^{13}C]-SA (50 mg), [2-^{13}C]-SA (50 mg), or [1,2-$^{13}C_2$]-SA (20 mg) were added to each flask, and the mixture was fermented with reciprocal shaking at 30 °C for 24 h. In an independent experiment, broth cultivated for 3 d without additional SA was prepared as a standard. Each culture broth, derived from the above three conditions, was individually treated as follows. Filtered broth (3 L) was extracted with chloroform (1 L × 3) at pH 3. The combined organic extract, evaporated in vacuo, was applied to a Sephadex LH-20 column, using methanol as an eluent, to give a fraction containing a compound with the same λ_{max} values in its UV spectrum as 5-hydroxyvertinolide **5**, by HPLC analysis using a diode array detector (DAD).[23] The resulting fraction was rechromatographed using a semi-preparative Capcell pak C_{18} SG120 (∅ 15 × 250 mm, Shiseido); solvent system, acetonitrile/0.15% KH_2PO_4 (pH 3.5); flow rate, 8.8 mL/min; detection, UV at 280 nm, to yield **5′** (2.1 mg), [1-^{13}C]-SA-**5′** (2.1 mg), [2-^{13}C]-SA-**5′** (1.3 mg), and [1,2-$^{13}C_2$]-SA-**5′** (3.2 mg).

The structure of **5′** was elucidated spectroscopically.[24] Compound **5′** was obtained as a colorless amorphous powder and formulated as $C_{14}H_{18}O_5$ from HRFAB-MS data. The IR spectrum showed the characteristic absorption bands at 3409 cm^{-1} (–OH) and 1726 and 1660 cm^{-1} (=CO). The ^{13}C NMR and HSQC spectra of **5′** comprised only 14 carbon signals; two methyls (δ 6.4 and 19.8, each singlet in ^{1}H NMR), one methyl (δ 18.8, doublet in ^{1}H NMR), one sp^3 methylene (δ 42.3), four olefinic methines (δ 129.1, 131.3, 141.1, and 143.9), three carbonyl-like quaternary carbons (δ 173.9, 176.3, and 198.7), and other quaternary carbons (δ 71.3, 84.7, and 97.1). The ^{1}H NMR spectrum revealed the presence of two singlet methyl groups (δ 1.49 and 1.63), an (*E*,*E*)-1,3-pentadienyl moiety (δ 1.85, 6.12, 6.20–6.30 (2H), and 7.19), and a three-spin system (δ 2.54, 2.84, and 4.30). The structure was further elucidated through interpretation of the HMBC experiments on **5′** (Fig. 1). A 3-hydroxy-2,4-dimethyl-2-buteno-4-olide moiety was confirmed by the following data; cross peaks between 2-CH_3 (δ_H 1.63) and three carbons C-1

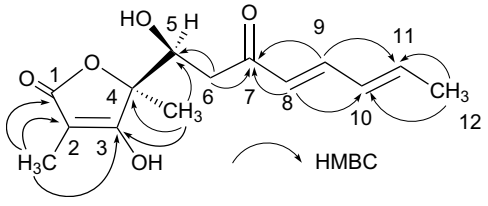
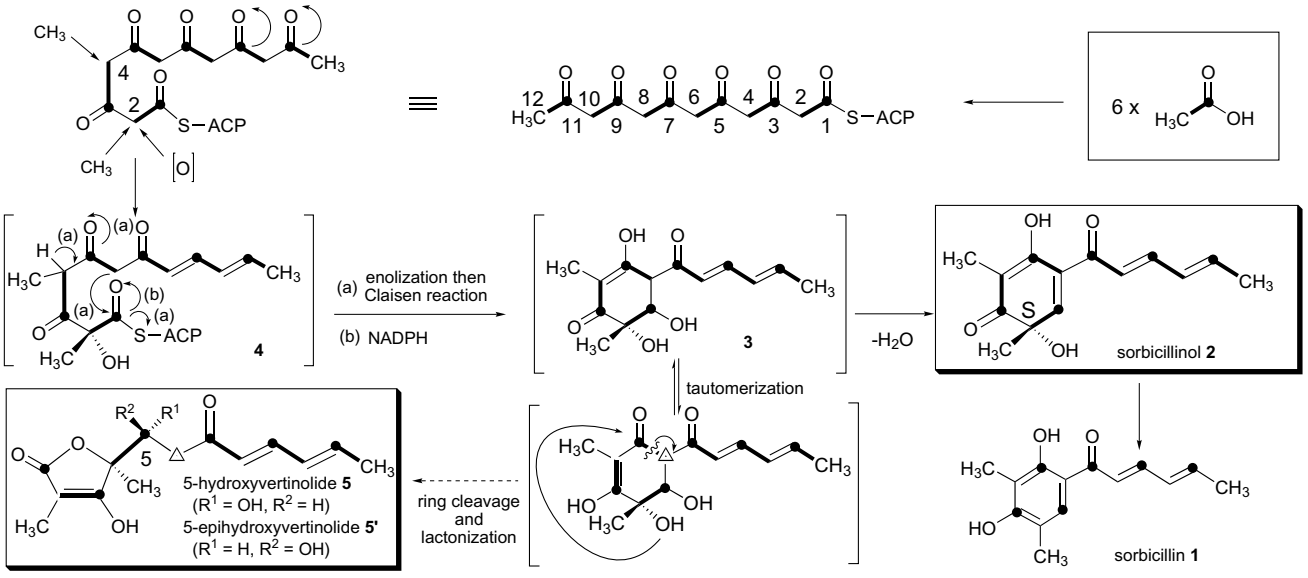


Fig. 1. Summary of the HMBC results for compound **5′**.



Scheme 1. Proposed biosynthetic mechanism for the formation of ^{13}C-labeled sorbicillinol **2** and 5-epihydroxyvertinolide **5′**.

(δ_C 173.9), C-2 (δ_C 97.1), and C-3 (δ_C 176.3) and between 4-CH_3 (δ_H 1.49) and C-3 and C-4 (δ_C 84.7). A 1-hydroxy-3-oxo-(*E*,*E*)-4,6-octadienyl side chain from C-5 to C-12 bound to C-4 was deduced on the basis of a cross peak between 4-CH_3 and C-5 (δ_C 71.3). Consequently, compound **5′** had the planar structure depicted in Figure 1, which is the same as that of 5-hydroxyvertinolide (**5**). Despite this, the optical rotation value ($[\alpha]_D^{23}$ −18.3) and the chemical shifts and coupling constants of 6-H_a (δ 2.54, dd, $J = 2.4$ and 16.1 Hz) and 6-H_b (δ 2.84, dd, $J = 9.8$ and 16.1 Hz) were obviously different from those of **5** ($[\alpha]_D$ −64; 6-H_a: δ 2.77, dd, $J = 10$ and 16 Hz, 6-H_b: δ 3.15, dd, $J = 2$ and 16 Hz). These findings indicate that **5′** is an epimer of **5**. In other words, compounds **5′** and **5** are distinguished by differences in their relative stereochemistry at C-5. The absolute configuration at C-5 of **5′** was elucidated by the modified Mosher's method.[25,26] To protect 3-OH, **5′** was treated with diazomethane, and 3-OCH_3-**5′** (**5′a**) was afforded.[6,27] The (*R*)-(+)- and (*S*)-(−)-2-methy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters at 5-OH of **5′a** (**5′b** and **5′c**) were prepared and, in ^{1}H NMR spectra, the differences of the chemical shifts ($\Delta\delta$) between **5′b** and **5′c** are summarized in Figure 2. The protons with positive sign ($\Delta\delta > 0$) were located on the left side of the MTPA plane and ones with negative ($\Delta\delta < 0$) on the right side. This result indicated that the absolute configuration at C-5 of **5′a** was (*S*). Another chiral center at C-4 was expected to be (*S*)-configuration through the possible biosynthetic pathway as shown in Scheme 1. For the aspects mentioned above, it was suggested that the structure of **5′** was (−)-(4*S*)-3-hydroxy-4-[(1*S*)-1-hydroxy-3-oxo-(*E*,*E*)-4,6-octadienyl]-2,4-dimethyl-2-buten-4-olide. The new sorbicillinoid **5′** was designated as 5-epihydroxyvertinolide.

Further studies were performed with 5-epihydroxyvertinolide (**5′**) using ^{13}C-labeling experiments. The first incorporation study using [1-^{13}C]-SA demonstrated that **5′** was labeled at C-1, C-3, C-5, C-7, C-9, and C-11 and the second one using [2-^{13}C]-SA gave ^{13}C-enriched **5′** at C-2, C-4, C-6, C-8, C-10, and C-12, showing the expected ^{13}C{^{1}H} NMR signals. The further incorporation study using [1,2-$^{13}C_2$]-SA was performed according to the protocol described above. The resulting [1,2-$^{13}C_2$]-SA-**5′** gave the ^{13}C{^{1}H} NMR spectrum showing pairs of doublets derived from ^{13}C–^{13}C coupling of intact [1,2-$^{13}C_2$]-SA. In the spectrum, five pairs of doublets were observed: C-2/C-3 ($J =$ 78.2 Hz), C-4/C-5 ($J = 41.4$ Hz), C-7/C-8 ($J = 55.0$ Hz), C-9/C-10 ($J = 54.6$ Hz), and C-11/C-12 ($J = 42.6$ Hz). At the same time, there were two singlets at δ 42.3 (C-6) and 173.9 (C-1) enhanced by ^{13}C. The results of these experiments supported our hypothesis that a carbon–carbon bond break occurred in the biosynthetic route for **5′**. As shown in Scheme 1, this was a clear evidence of the presence of intermediate **3**, which was expected to arise from an unidentified polyketide biosynthetic pathway with an oxidative process.

Further studies to search for the existence of intermediate **3** are in progress.

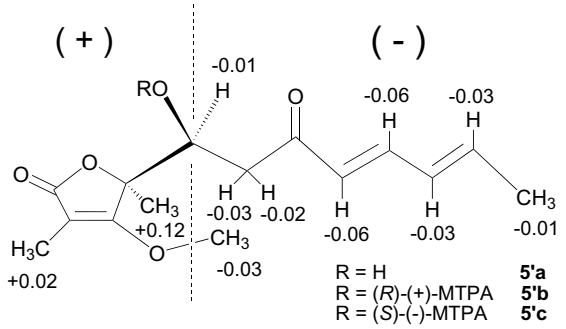


Fig. 2. Application of the modified Mosher's method for **5′a**: in ^{1}H NMR spectra the differences of the chemical shifts [$\Delta\delta$(ppm) = $\delta_S(-) - \delta_R(+)$] between the *R*-(+)-MTPA and *S*-(−)-MTPA esters (**5′b** and **5′c**) of **5′a**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.11.121.

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22. The fungus on a potato-dextrose-agar slant was inoculated into 0.5-L shake flasks 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 7.0, and cultured on a reciprocal shaker at 30 °C for 2 d. One milliliter of the seed culture was inoculated into each of the 30 0.5-L shake flasks containing 100 mL of the previously described medium.
23. A 10-μL aliquot of 1 mg/mL of each sample was injected into an analytical HPLC system under the following conditions: column Capcell pak C_{18} SG120 (∅ 4.6 × 150 mm, Shiseido); solvent system, acetonitrile/H_2O 2:8 containing 0.1% TFA; flow rate, 1 mL/min.
24. 5-Epihydroxyvetinolide (**5′**). Colorless amorphous powder, $[\alpha]_D^{23}$ −18.3 (*c* 0.213, CH_3OH); IR ν_{max} (ATR) cm^{-1}: 3409, 2925, 2855, 1726, 1660, 1631, 1592, 1301, 1063, 1023; HRFAB-MS *m*/*z* 267.1224 $[(M+H)^+$, 267.1232 for $C_{14}H_{19}O_5]$; ^1H NMR (400 MHz, acetone-d_6): δ 1.49 (3H, s, H_3-4-CH_3), 1.63 (3H, s, H_3-2-CH_3), 1.85 (3H, dd, $J = 2.4$, 4.9 Hz, 12-H_3), 2.54 (1H, dd, $J = 2.4$ and 16.1 Hz, 6-H_a), 2.84 (1H, dd, $J = 9.8$ and 16.1 Hz, 6-H_b), 4.30 (1H, dd, $J = 2.4$ and 9.8 Hz, 5-H), 6.12 (1H, d, $J = 15.4$ Hz, 8-H), 6.20–6.30 (2H, m, H-10, H-11), 7.19 (1H, m, 9-H); ^{13}C NMR (100 MHz, acetone-d_6): δ 6.4 (q, 2-CH_3), 18.8 (q, C-12), 19.8 (q, 4-CH_3), 42.3 (t, C-6), 71.3 (d, C-5), 84.7 (s, C-4), 97.1 (s, C-2), 129.1 (d, C-8), 131.3 (d, C-10), 141.1 (d, C-11), 143.9 (d, C-9), 173.9 (s, C-1), 176.3 (s, C-3), 198.7 (s, C-7).
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27. 3-*O*-Methyl derivative **5′a**. Compound **5′** (7.3 mg) in 14.0 mL of CH_3OH–diethylether (1:1) was treated with excess 2% diethylether solution of diazomethane at room temperature for 1 h, and then the solvent was evaporated in vacuo. The mixture of methylation products was purified by using preparative TLC (Merck No. 13794) with a solvent system of *n*-hexane–ethyl acetate (1:1, twice), to yield 2.3 mg of **5′a**. HRESI-MS *m*/*z* 303.12231 $[(M+Na)^+$, 303.12084 for $C_{15}H_{20}NaO_5]$; ^1H NMR (400 MHz, acetone-d_6): δ 1.44 (3H, s, H_3-4-CH_3), 1.86 (3H, br d, $J = 4.4$ Hz, 12-H_3), 1.95 (3H, s, H_3-2-CH_3), 2.52 (1H, dd, $J = 2.7$ and 16.0 Hz, 6-H_a), 2.77 (1H, dd, $J = 8.8$ and 16.0 Hz, 6-H_b), 4.18 (3H, s, H_3-3-OCH_3), 4.20–4.30 (2H, m, 5-H and 5-OH), 6.12 (1H, d, $J = 15.6$ Hz, 8-H), 6.20–6.35 (2H, m, H-10, H-11), 7.16 (1H, m, 9-H).