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Bisorbibetanone, a Novel Oxidized Sorbicillin Dimer, with 1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging Activity from a Fungus

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Abstract: We isolated a novel oxidized sorbicillin dimer (1) as yellowish amorphous powder from the fermentation broth of *Trichoderma* sp. USF-2690 strain isolated from a soil sample. The structure of the compound was determined by elucidation of spectroscopic evidence. The compound was revealed to possess a new carbon skeleton in the group of oxidized sorbicillin dimers and was named bisorbibetanone. The stereostructure of bisorbibetanone was analyzed on the basis of the spectral data. In 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, bisorbibetanone gave an ED_{50} value of 62.5 μM after standing for 30 min.

Keywords: polyketide; fungi; stereochemistry; dimers

Recently, free radicals have been known to mediate a wide range of diseases such as atherosclerosis,[1] ischemia reperfusion injury,[2] inflammation,[3] carcinogenesis,[4] and rheumatoid arthritis.[5] Free radical scavengers are expected to be effective preventive and/or therapeutic agents through the decrease in free radical levels. In the course of our screening program to detect free radical scavengers with the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH),[6] we have already reported seven active yellowish compounds, namely sorbicillin, bisorbicillinol, demethyltrichodimerol, bisorbibutenolide (bislongiquinolide[7]), bisorbicillinolide, bisvertinolone, and trichodimerol, from the fermentation broth of a *Trichoderma* sp. USF-2690 strain isolated from a soil sample collected in Shizuoka City, Shizuoka, Japan.[8, 9] Our continuing investigation to find sorbicillin-related radical scavengers from the strain resulted in the isolation of a novel active compound, named bisorbibetanone (1), which had an unknown carbon skeleton in the group of oxidized sorbicillin dimers (Figure 1).

The fungal strain classified as *Trichoderma* sp. USF-2690 was cultivated on a reciprocal shaker for 12 days at 30°C.[10] The filtered broth (2.7 liters) adjusted to pH 3.0 with HCl was extracted with the same volume of

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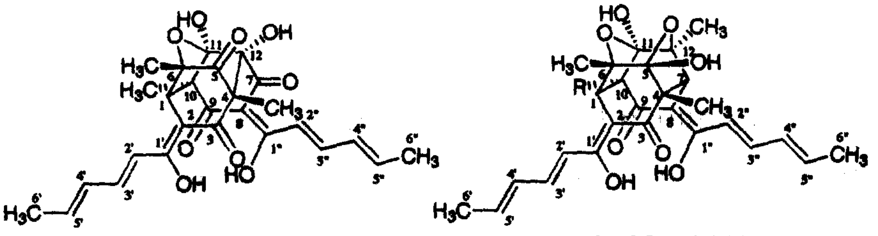

Bisorbibetanone (1)

R = H: Demethyltrichodimerol (2)
R = CH_3: Trichodimerol (3)

Figure 1. Structures of Bisorbibetanone (1), Demethyltrichodimerol (2), and Trichodimerol (3)

ethyl acetate. The organic extract (1.50 g) concentrated *in vacuo* was applied to a Sephadex LH-20 column, using CH_3OH as the eluent. The desired fraction (80.2 mg), including an active compound (1) was obtained. The fraction was concentrated *in vacuo* and then purified with reversed-phase MPLC.[11] Finally, 4.7 mg of 1 was yielded.

The molecular formula of the compound 1, $[\alpha]^{26}_{D}$ -561° (*c* 0.1, in CH_3OH); given as yellowish amorphous powder, was established as $C_{27}H_{28}O_9$ on the basis of HRFAB-MS [*m/z* 497.1890 $(M+H)^+$, 497.1812 for $C_{27}H_{29}O_9$]. The UV and visible spectra, λ_{max}nm (ε, CH_3OH): 261.5 (11,600), 346 (sh 23,200), 362 (25,200), 377 (sh 23,800), and 397 (sh 15,600), were similar to those of demethyltrichodimerol (2)[8] and trichodimerol (3).[12] The IR spectrum of 1 showed absorption bands, ν_{max} (KBr)cm^{-1}: 3430, 1750, 1605, 1550, 1395, 1245, and 1000.

The ^1H- and ^{13}C-NMR spectra of 1 in $CDCl_3$ exhibited 28 proton and 27 carbon signals.[13] Analyses of the ^1H-, ^{13}C-NMR, and HSQC spectral data led to the existence of five methyl containing three singlet methyls, an sp^3 methine, eight sp^2 methine, and thirteen quaternary carbons. The chemical shift of quaternary carbons suggested that 1 had two oxygenated carbons at δ_C 79.2 and 84.8 and a hemiacetal carbon at δ_C 103.4. The ^1H-^1H COSY spectrum of 1 revealed the presence of two (*E,E*)-1,3-pentadienyl moieties consisting of two methyls and eight sp^2 methines, which assigned the sequence from C-2' to C-6' and from C-2" to C-6".

Further structural elucidation was done through interpretation of the HMBC experiment of 1. The cross peaks between 4-CH_3 (δ_H 1.29) and C-3 (δ_C 195.2), C-4 (δ_C 67.1), C-5 (δ_C 197.3) and C-12 (δ_C 84.8), between 6-CH_3 (δ_H 1.58) and C-1 (δ_C 53.1), C-5, and C-6 (δ_C 79.2), and between 10-CH_3 (δ_H 1.71) and C-1, C-9 (δ_C 200.4), C-10 (δ_C 57.5) and C-11 (δ_C 103.4) gave the partial structure 4 (Figure 2).

The advanced partial structure 5 shown in Figure 2 was constructed through useful $^2J_{C\text{-}H}$ or $^3J_{C\text{-}H}$ correlation between 1-H, 12-OH, and 1'-OH and carbons in the HMBC spectrum of 1. The cross peaks between 1-H (δ_H 3.34) and C-2 (δ_C 106.2), C-3, C-5, C-6, C-10, C-11, C-1' (δ_C 173.0), 6-CH_3 (δ_C 19.0), and 10-CH_3 (δ_C 18.1) unambiguously proved the connection from C-1 to C-3 via C-2, and the cross peaks between 1'-OH (δ_H 14.88) and C-2, C-1', and C-2' (δ_C 117.9) indicated that the (*E,E*)-1,3-pentadienyl moiety from C-2' to C-6' bonded to C-2 via C-1' with a hydroxyl group. The hydroxyl group at δ_H 4.88, which gave the cross peaks with δ_C 103.4 (C-11), 84.8 (C-12), and 189.9 (C-7) in the HMBC spectrum of 1, was assigned as 12-OH, because the ^{13}C chemical shift at C-11 ascribed C-11 to be an acetal carbon centered between C-10 and C-12. From the cross peaks between 1"-OH (δ_H 18.51) and C-8 (δ_C 108.9), C-1" (δ_C 188.0), and C-2" (δ_C 121.9) the presence of an enol-sorbyl system, possessing a strong hydrogen bond (6, Figure 2) was deduced. The sequence of HO-(C-11)-

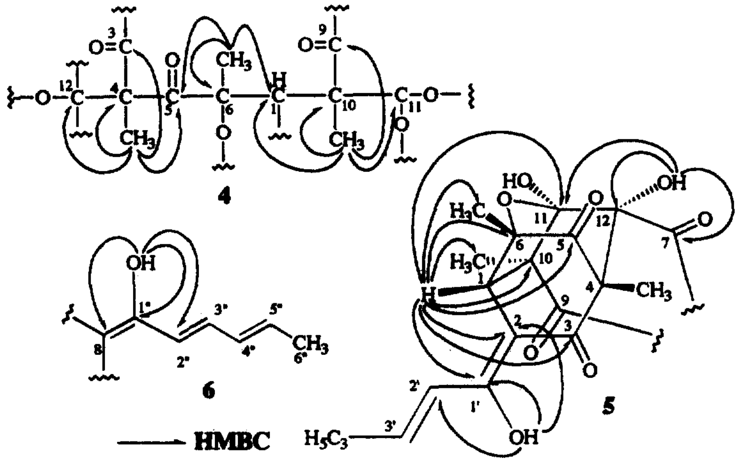

Figure 2. Summary of the HMBC Result for Bisorbibetanone (1)

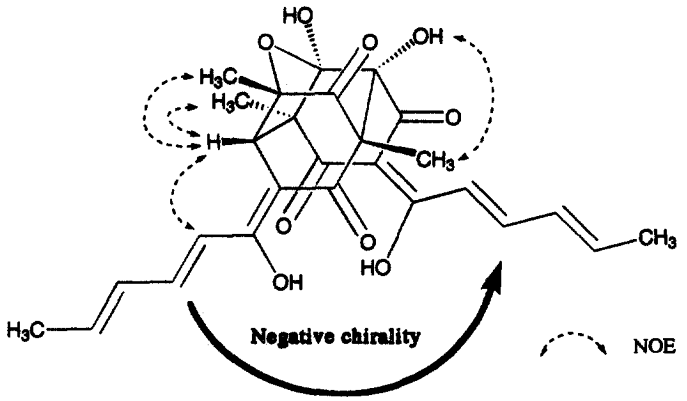

Figure 3. Stereochemistry Based on the NOESY and CD spectra for Bisorbibetanone (1)

O-(C-6) was given through the formula of 1, and C-8 was located between C-7 and C-9.

The relative stereochemistry was elucidated by NOESY experiment as shown in Figure 3. The NOEs between 1-H and 6-CH_3, 10-CH_3, and 2'-H and between 12-OH and 4-CH_3 were observed, which suggested the relative configuration of 1 was 1R*, 4R*, 6S*, 10R*, 11R*, and 12R*.

The absolute stereochemistry of 1 was revealed by analysis of the CD spectrum.[13] The spectrum was depicted by a curve with a negative first maximum value at 390.6 nm ($\Delta\varepsilon$ -12.5) and a positive second maximum value at 346.6 nm ($\Delta\varepsilon$ +13.8), resulting from the chiral exciton coupling between two enol-sorbyl chromophores. The negative chirality from the spectrum of 1 confirmed the structure of 1 (1R, 4R, 6S, 10R, 11R, and 12R).

Finally, the structure of 1 was confirmed as shown in Figure 1. Compound 1 was a novel compound, which presented a new carbon skeleton in the group of oxidized sorbicillin dimers, designated bisorbibetanone. We thought that bisorbibetanone might be biosynthesized through a bisvertinolone-type compound,[14] which was composed of sorbicillin and 5-demethylated sorbicillin units. In addition to the biosynthetic route for demethyltrichodimerol (**2**) and trichodimerol (**3**), which might be biosynthesized through bisvertinolone-type compounds, the structure of bisorbibetanone (**1**) suggested the existence of another route for the introduction of a bond between C-4 and C-12 of bisvertinolone-type compounds.

Bisorbibetanone (**1**) and α-tocopherol were submitted to the assay system with DPPH.[9] The ethanol solution of a sample (2 ml) was mixed with a 0.5 mM DPPH ethanol solution (1 ml) and 0.1 M acetate buffer (pH 5.5; 2 ml). After standing for 30 min, the absorbance of the mixture at 517 nm was measured. The ED_{50} value was determined as the concentration of each sample required to give 50% of the absorbance shown by a blank test. Bisorbibetanone (**1**) and α-tocopherol revealed ED_{50} values of 62.5 and 17.0 μM, respectively.

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10. The fermentation broth was given from eighteen 0.5-liter flasks each containing 150 ml of the following medium: 2.0% glucose and 0.5% polypeptone at pH 5.6.
11. Reversed-phase MPLC: support, YMC-ODS-AQ 120-s50 (25ϕ x 350 mm); solvent system, acetonitrile-H_2O [1:1, including 0.1% trifluoroacetic acid]; detection, UV (370 nm).
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