

Microbial Transformation of Curvularin

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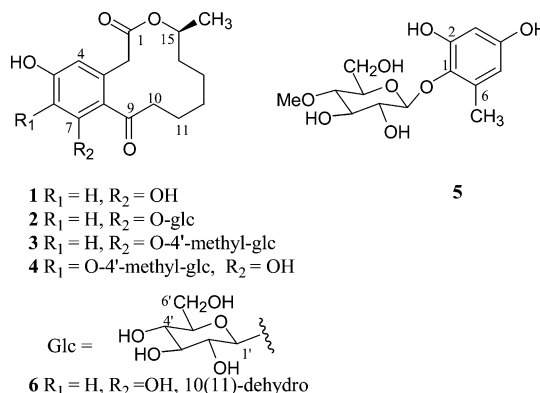
Received March 1, 2005

The microbiological transformation of curvularin (**1**) with *Beauveria bassiana* ATCC 7159 afforded three new metabolites identified as curvularin-7-*O*- β -D-glucopyranoside (**2**), curvularin-4'-*O*-methyl-7-*O*- β -D-glucopyranoside (**3**), and 6-hydroxycurvularin-4'-*O*-methyl-6-*O*- β -D-glucopyranoside (**4**) resulting from hydroxylation, glucosidation, and methylglucosidation of the substrate. Isolation of 6-methyl-2,4-dihydroxyphenyl-4'-*O*-methyl-1-*O*- β -D-glucopyranoside (**5**) from the fermentation broth of *B. bassiana* ATCC 7159 without any added substrate suggested that hydroxylase, glucosyl transferase, and methylase are constitutive enzymes of this organism.

Curvularins are macrocyclic lactones produced by a number of fungi of the genera *Curvularia*,¹ *Penicillium*,² and *Alternaria*³ and have been reported to possess a variety of biological activities, including phytotoxicity,³ cytotoxicity toward sea urchin embryogenesis,^{2a} inhibition of cell division,^{2a} inhibition of expression of human inducible nitric oxide synthase,⁴ and growth-promoting activity in farm animals.⁵ They have also been reported to cause hepatic necrosis.⁶ Our recent isolation of several curvularins with moderate cytotoxicity from two Sonoran desert microorganisms,⁷ together with interesting biological activities associated with these macrolides, prompted us to obtain further curvularin analogues for structure–activity relationship studies. Although microbial biotransformation of the related zearalenone group of macrolides is known,⁸ this is the first report of the biotransformation of curvularin-type microbial macrolides.

Of the eight fungal strains screened, all except *Cunninghamella elegans* ATCC 9245 were able to completely deplete and transform curvularin (**1**). However, *Beauveria bassiana* ATCC 7159 was selected because the TLC analysis indicated that it was able to biotransform **1** into three products compared with six other strains (*Absidia coerulea* MR 27, *Cunninghamella blakesleeana* ATCC 8688a, *Cunninghamella echinulata* ATCC 9244, *C. echinulata* NRRL 3655, *Mucor mucedo* UI 4605, and *Streptomyces griseus* ATCC 13273), which resulted in the formation of only one biotransformation product. The preparative-scale incubation of **1** with *B. bassiana* for 7 days afforded three polar products, **2–4**, identified as due to glucosidation, methylglucosidation, and hydroxylation of **1**. *B. bassiana* (Clavicipitaceae), also known as sugar icing fungus, has previously been used in the biotransformation of more than 300 different substrates,⁹ resulting in hydroxylation,¹⁰ hydrolysis,¹¹ methylglucosidation,¹² and oxidation.¹³

The molecular formula, C₂₂H₃₀O₁₀, of the most polar product (**2**) determined by its HRFABMS, together with the presence of three OH signals at δ_H 4.60, 4.42, and 4.33 in its ¹H NMR spectrum, and five CH signals at δ_C 102.3, 78.2, 77.8, 74.6, and 71.3 and one CH₂ signal at δ 62.6 in its DEPT spectrum, suggested it to be an *O*-glucoside derivative of curvularin. Compared with **1**, only one low field D₂O-exchangeable signal (δ_H 8.96) was observed for



2, indicating that the newly introduced glucose moiety must be attached to one of the phenolic hydroxyls of **1**. The correlation of the anomeric 1'-H signal at δ_H 4.94 to C-7 signal at δ_C 157.4 in the HMBC spectrum of **2** revealed that the glucose moiety was located at C-7. The above data together with the detailed analysis of its 2D NMR spectra including the HMBC spectrum (Figure 1) confirmed its structure as curvularin-7-*O*- β -D-glucopyranoside (**2**). The HRFABMS of the second product (**3**) revealed the molecular formula C₂₃H₃₂O₁₀, suggesting that it contains an additional CH₂ compared with that of **2**. ¹H and ¹³C NMR spectra of **3** were similar to those of **2** except for the signals due to the sugar moiety. Only two aliphatic OH signals (at δ_H 4.64 and 4.49) were observed in its ¹H NMR spectrum compared with three OH signals for compound **2**. The NMR signals at δ_H 3.55 (3H) and δ_C 60.5 suggested the presence of an OMe group in **3**, and strong HMBC correlation of the signal at δ_H 3.55 to the signal at δ_C 80.1 (C-4') indicated that this OMe is attached to C-4' of the sugar. Therefore, this compound was identified as curvularin-4'-*O*-methyl-7-*O*- β -D-glucopyranoside (**3**).

The HRFABMS of the third biotransformation product (**4**) was consistent with the molecular formula C₂₃H₃₂O₁₁,

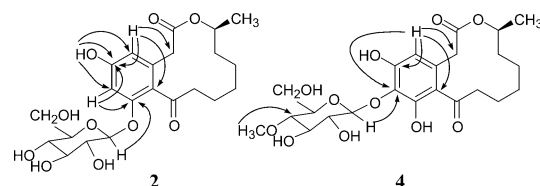


Figure 1. Selected HMBC correlations of **2** and **4**.

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indicating the presence of an additional oxygen compared with **3**, which suggested that a hydroxyl group and a glucose molecule were introduced to curvularin (**1**). Comparison of ^1H and ^{13}C NMR data with those of **3** suggested that compound **4** contained a 4'-*O*-methylglucose moiety. In its ^1H NMR spectrum, **4** had only one aromatic proton signal (at δ 6.44) compared with two aromatic proton signals for both **2** and **3**. This signal at δ_{H} 6.44 of **4** had a strong HMBC correlation to the signal at δ_{C} 39.2 (C-2) (Figure 1), suggesting that the newly introduced hydroxyl group is at C-6, allowing this biotransformation product to be identified as 6-hydroxycurvularin-4'-*O*-methyl-6-*O*- β -D-glucopyranoside (**4**).

In an attempt to determine the nature of the enzymes of *B. bassiana* ATCC 7159 involved in the biotransformation of **1** to above glucosidation, methylglucosidation, and hydroxylation products, **2**–**4**, we investigated the fermentation broth of this fungus in the absence of any added substrate. TLC investigation of the EtOAc extract of the fermentation broth indicated the presence of a major metabolite, and this was isolated by preparative TLC and identified as 6-methyl-2,4-dihydroxyphenyl 4'-*O*-methyl-1-*O*- β -D-glucopyranoside (**5**). Although this is the first report of **5** as a metabolite of *B. bassiana*, it has previously been isolated from the entomogenous deuteromycete, *Beauveria amorphae*,¹⁴ and the presence of novel 4'-*O*-methylglucose derivatives of aromatic compounds in dried silkworm larva stiffened due to *B. bassiana* infection has recently been reported.¹⁵ Occurrence of **5** in the fermentation broth of *B. bassiana* ATCC 7159 suggests that this strain is capable of hydroxylation and 4'-*O*-methylglucosidation even in the absence of the substrate, indicating that hydroxylase, glucosyltransferase, and methylase in these biotransformation processes are not inducible enzymes, but constitutive enzymes. Compounds **2**–**4** were tested for their cytotoxicity against several cancer cell lines [NCI-H460 (nonsmall cell lung), MCF-7 (breast), and SF-268 (CNS glioma)] using the MTT assay⁷ and were found to be inactive at concentrations up to 10 $\mu\text{g/mL}$.

Experimental Section

General Experimental Procedures. Melting points were determined on a Gallenkamp micromelting point apparatus and were uncorrected. Optical rotations were measured in MeOH with a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer in KBr disks and UV spectra in MeOH on a Shimadzu UV-1601 spectrometer. 1D and 2D NMR spectra were recorded in d_6 -acetone on a Bruker DRX-500 instrument at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. The chemical shift values (δ) are given in parts per million (ppm) and the coupling constants are given in hertz. Abbreviations for NMR signals are as follows: s = singlet, d = doublet, t = triplet, m = multiplet. High-resolution FABMS was obtained with a JEOL HX110A mass spectrometer.

Curvularin (1). Dehydrocurvularin (**6**) (49.6 mg), obtained from an unidentified *Penicillium* sp.,^{7a} was dissolved in EtOAc (2.0 mL) and subjected to catalytic hydrogenation with 10% Pd–C for 3 h at 25 °C. Filtration followed by evaporation of EtOAc afforded **1** (49.8 mg) with ^1H and ^{13}C NMR and mass spectral data identical to those reported in the literature.^{2a}

Culture and Biotransformation Procedures. Screening scale biotransformation of **1** by *B. bassiana* ATCC 7159 was carried out in a 125-mL flask containing 50 mL of potato dextrose broth (PDB, Difco, Plymouth, MN). The flask was placed in a rotary shaker at 150 rpm at 25 °C. After 3 days, the fermentation broth turned red, and to this was added **1** (0.1 mL of a solution of 10 mg/mL in acetone). After substrate administration, the flask was maintained under the same

conditions for an additional 7 days. Culture control consisted of fermentation broth of *B. bassiana* ATCC 7159 without the substrate but with the same volume of acetone, and the substrate control consisted of sterile PDB medium with the same amount of a solution of **1** in acetone. Both controls were incubated under the same conditions. A preparative scale experiment was carried out in 30 $\times$ 250 mL flasks, each containing 100 mL of PDB under the same circumstances and conditions as the small scale fermentation. A total of 40 mg of **1** was used (1.33 mg/flask). For isolation of **5**, *B. bassiana* ATCC 7159 was cultivated under the same conditions, but without the substrate in 10 $\times$ 250 mL flasks, each containing 100 mL of PDB.

Extraction, Isolation, and Identification of the Metabolites. The cultures were filtered, and the combined filtrate (1860 mL) was neutralized with aqueous NaOH and extracted with EtOAc (3 $\times$ 1200 mL). Evaporation of EtOAc under reduced pressure yielded a dark brown solid (129.8 mg), which was subjected to gel filtration on Sephadex LH-20 (3.0 g) and eluted with hexane–acetone (10:1) (160 mL), hexane–acetone (2:1) (120 mL), hexane–acetone (1:1) (160 mL), acetone (80 mL), and MeOH (20 mL). Twenty-seven fractions (20 mL each) were collected and combined on the basis of their TLC profiles, yielding combined fractions A (14.7 mg), B (20.6 mg), and C (75.7 mg). Further separation of fraction C (75.0 mg) by preparative TLC (12% MeOH–dichloromethane) furnished **2** (6.7 mg, 16.8%), **3** (9.1 mg, 22.8%), and **4** (2.7 mg, 6.8%). Compound **5** (8.3 mg, 16.6%) was isolated by preparative TLC (12% MeOH in CH_2Cl_2) of the EtOAc extract (50.1 mg) of a culture (1000 mL) of *B. bassiana* ATCC 7159.

Curvularin-7-*O*- β -D-glucopyranoside (2): Colorless crystalline powder; mp 155–156 °C; $[\alpha]_{\text{D}}^{25}$ –45.7° (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (3.74), 218 (4.03) nm; IR (KBr) ν_{max} 3379, 2928, 1705, 1609, 1458, 1312, 1265, 1173, 1076, 1034, and 806 cm^{-1} ; ^1H NMR (d_6 -acetone, 500 MHz) δ 8.96 (1H, s, OH-5), 6.68 (1H, d, J = 2.1 Hz, H-6), 6.51 (1H, d, J = 2.1 Hz, H-4), 4.97 (1H, m, H-15), 4.94 (1H, d, J = 7.6 Hz, H-1'), 4.60 (1H, d, J = 4.7 Hz, OH-2'), 4.42 (1H, d, J = 4.0 Hz, OH-3'), 4.33 (1H, d, J = 4.4 Hz, OH-4'), 3.89 (1H, m, H-6'), 3.72 (2H, m, H-2), 3.69 (1H, m, H-6'), 3.51 (1H, m, H-3'), 3.49 (1H, m, H-5'), 3.42 (1H, m, H-2'), 3.39 (1H, m, H-4'), 3.15 (1H, m, H-10), 2.86 (1H, m, H-10), 1.79 (1H, m, H-11), 1.65 (1H, m, H-14), 1.44 (1H, m, H-13), 1.43 (1H, m, H-12), 1.41 (1H, m, H-14), 1.35 (1H, m, H-11), 1.27 (1H, m, H-13), 1.26 (1H, m, H-12), 1.13 (1H, d, J = 6.3 Hz, CH_3 -15); ^{13}C NMR (d_6 -acetone, 125 MHz) δ 207.0 (C, C-9), 171.0 (C, C-1), 159.8 (C, C-5), 157.4 (C, C-7), 135.5 (C, C-3), 124.7 (C, C-8), 113.9 (CH, C-4), 102.7 (CH, C-6), 102.3 (CH, C-1'), 78.2 (CH, C-5'), 77.8 (CH, C-3'), 74.6 (CH, C-2'), 72.8 (CH, C-15), 71.3 (CH, C-4'), 62.6 (CH_2 , C-6'), 44.0 (CH_2 , C-10), 39.1 (CH_2 , C-2), 33.4 (CH_2 , C-14), 27.8 (CH_2 , C-12), 24.7 (CH_2 , C-13), 23.4 (CH_2 , C-11), 20.7 (CH_3 , CH_3 -15); HRFABMS m/z 455.1905 $[\text{M} + 1]^+$ (calcd for $\text{C}_{22}\text{H}_{31}\text{O}_{10}$ 455.1917).

Curvularin-4'-*O*-methyl-7-*O*- β -D-glucopyranoside (3): Colorless crystalline powder; mp 123–124 °C; $[\alpha]_{\text{D}}^{25}$ –40.1° (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 269 (3.63), 219 (3.94) nm; IR (KBr) ν_{max} 3422, 2925, 1728, 1659, 1616, 1312, 1273, 1180, 1087, 1045, and 806 cm^{-1} ; ^1H NMR (d_6 -acetone, 500 MHz) δ 8.93 (1H, s, OH-5), 6.66 (1H, d, J = 2.2 Hz, H-6), 6.51 (1H, d, J = 2.2 Hz, H-4), 4.97 (1H, m, H-15), 4.92 (1H, d, J = 7.8 Hz, H-1'), 4.64 (1H, d, J = 4.9 Hz, OH-2'), 4.49 (1H, d, J = 4.5 Hz, OH-3'), 3.80 (1H, m, H-6'), 3.72 (2H, m, H-2), 3.67 (1H, m, H-6'), 3.61 (1H, m, H-3'), 3.55 (3H, s, OCH_3 -4'), 3.46 (1H, m, H-5'), 3.45 (1H, m, H-2'), 3.18 (1H, t, J = 9.3 Hz, H-4'), 3.12 (1H, m, H-10), 2.86 (1H, m, H-10), 1.79 (1H, m, H-11), 1.63 (1H, m, H-14), 1.45 (1H, m, H-13), 1.43 (1H, m, H-12), 1.37 (1H, m, H-14), 1.26 (1H, m, H-11), 1.25 (1H, m, H-12), 1.24 (1H, m, H-13), 1.13 (3H, d, J = 6.3 Hz, CH_3 -15); ^{13}C NMR (d_6 -acetone, 125 MHz) δ 207.0 (C, C-9), 171.0 (C, C-1), 159.8 (C, C-5), 157.3 (C, C-7), 135.5 (C, C-3), 124.7 (C, C-8), 113.9 (CH, C-4), 102.6 (CH, C-6), 102.0 (CH, C-1'), 80.1 (CH, C-4'), 78.2 (CH, C-3'), 77.0 (CH, C-5'), 74.8 (CH, C-2'), 72.8 (CH, C-15), 62.1 (CH_2 , C-6'), 60.5 (CH_3 , OCH_3 -4'), 44.0 (CH_2 , C-10), 39.1 (CH_2 , C-2), 33.4 (CH_2 , C-14), 27.8 (CH_2 , C-12), 24.7 (CH_2 , C-13),

23.2 (CH₂, C-11), 20.7 (CH₃, CH₃-15); HRFABMS *m/z* 469.2075 [M + 1]⁺ (calcd for C₂₃H₃₃O₁₀ 469.2074).

6-Hydroxycurvularin-4'-O-methyl-6-O-β-D-glucopyranoside (4): Colorless crystalline powder; mp 127–128 °C; [α]_D²⁵ –17.8° (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 270 (3.74), 226 (4.06) nm; IR (KBr) ν_{max} 3422, 2936, 1713, 1663, 1589, 1454, 1358, 1258, 1200, 1076, and 806 cm⁻¹; ¹H NMR (*d*₆-acetone, 500 MHz) δ 6.44 (1H, s, H-4), 4.93 (1H, m, H-15), 4.64 (1H, d, *J* = 8.0 Hz, H-1'), 3.88 (1H, m, H-6'), 3.72 (2H, m, H-2), 3.71 (1H, m, H-6'), 3.66 (1H, t, *J* = 9.0 Hz, H-3'), 3.55 (3H, s, OCH₃-4'), 3.54 (1H, m, H-2'), 3.51 (1H, m, H-5'), 3.28 (1H, t, *J* = 9.0 Hz, H-4'), 3.05 (1H, m, H-10), 2.81 (1H, m, H-10), 1.77 (1H, m, H-11), 1.60 (1H, m, H-14), 1.51 (1H, m, H-11), 1.45 (1H, m, H-13), 1.44 (1H, m, H-14), 1.43 (1H, m, H-12), 1.27 (1H, m, H-12), 1.25 (1H, m, H-13), 1.12 (3H, d, *J* = 6.3 Hz, CH₃-15); ¹³C NMR (*d*₆-acetone, 125 MHz) δ 205.6 (C, C-9), 170.9 (C, C-1), 151.8 (C, C-5), 149.6 (C, C-7), 133.1 (C, C-6), 132.3 (C, C-3), 121.7 (C, C-8), 112.5 (CH, C-4), 107.6 (CH, C-1'), 79.7 (CH, C-4'), 77.7 (CH, C-5'), 77.1 (CH, C-3'), 74.8 (CH, C-2'), 72.6 (CH, C-15), 61.5 (CH₂, C-6'), 60.7 (CH₃, OCH₃-4'), 43.9 (CH₂, C-10), 39.2 (CH₂, C-2), 33.0 (CH₂, C-14), 27.5 (CH₂, C-12), 24.7 (CH₂, C-13), 23.2 (CH₂, C-11), 20.6 (CH₃, CH₃-15); HRFABMS *m/z* 485.2047 [M + 1]⁺ (calcd for C₂₃H₃₃O₁₁ 485.2023).

6-Methyl-2,4-dihydroxyphenyl-4'-O-methyl-1-O-β-D-glucopyranoside (5): Colorless amorphous solid; ¹H and ¹³C NMR spectral data were consistent with those reported in the literature;⁵ APCIMS (+) ve mode *m/z* 317 [M + 1]⁺.

Acknowledgment. This work was supported by the National Cancer Institute Grant No. 1R01CA90265-01A1, and this support is gratefully acknowledged. We also thank Professor John P. N. Rosazza (University of Iowa) for providing the fungal strains used in this study.

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NP0580309