# Microbial Transformation of Curvularin

Jixun Zhan and A. A. Leslie Gunatilaka*

*Southwest Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, College of Agriculture and Life Sciences, The University of Arizona, 250 East Valencia Road, Tucson, Arizona 85706-6800*

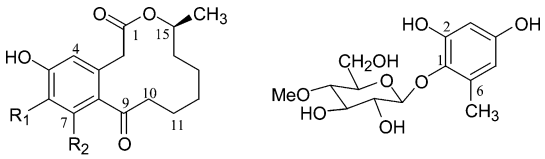
*Received March 1, 2005*

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.

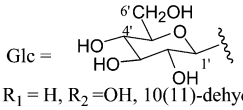
Curvularins are macrocyclic lactones produced by a number of fungi of the genera *Curvularia*,[1] *Penicillium*,[2] and *Alternaria*[3] and have been reported to possess a variety of biological activities, including phytotoxicity,[3] cytotoxicity toward sea urchin embryogenesis,[2a] inhibition of cell 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 coerulia* 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, $C_{22}H_{30}O_{10}$, of the most polar product (**2**) determined by its HRFABMS, together with the presence of three OH signals at $\delta_H$ 4.60, 4.42, and 4.33 in its $^1$H NMR spectrum, and five CH signals at $\delta_c$ 102.3, 78.2, 77.8, 74.6, and 71.3 and one $CH_2$ signal at $\delta$ 62.6 in its DEPT spectrum, suggested it to be an *O*-glucoside derivative of curvularin. Compared with **1**, only one low field $D_2O$-exchangeable signal ($\delta_H$ 8.96) was observed for



**1** $R_1$ = H, $R_2$ = OH
**2** $R_1$ = H, $R_2$ = O-glc
**3** $R_1$ = H, $R_2$ = O-4'-methyl-glc
**4** $R_1$ = O-4'-methyl-glc, $R_2$ = OH

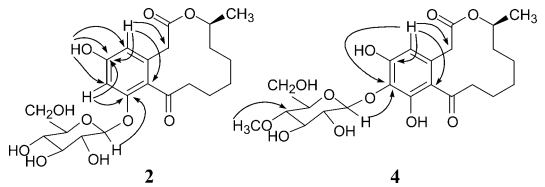


**6** $R_1$ = H, $R_2$ =OH, 10(11)-dehydro

**5**

**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 $\delta_H$ 4.94 to C-7 signal at $\delta_C$ 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 $C_{23}H_{32}O_{10}$, suggesting that it contains an additional $CH_2$ compared with that of **2**. $^1$H and $^{13}$C 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 $\delta_H$ 4.64 and 4.49) were observed in its $^1$H NMR spectrum compared with three OH signals for compound **2**. The NMR signals at $\delta_H$ 3.55 (3H) and $\delta_C$ 60.5 suggested the presence of an OMe group in **3**, and strong HMBC correlation of the signal at $\delta_H$ 3.55 to the signal at $\delta_C$ 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 $C_{23}H_{32}O_{11}$,



**2** **4**

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

* To whom correspondence should be addressed. Tel: (520) 741-1691. Fax: (520) 741-1468. E-mail: leslieg@ag.arizona.edu.

10.1021/np0580309 CCC: $30.25 
Published on Web 07/13/2005

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 $^1$H and $^{13}$C NMR data with those of **3** suggested that compound **4** contained a 4′-*O*-methylglucose moiety. In its $^1$H NMR spectrum, **4** had only one aromatic proton signal (at $\delta$ 6.44) compared with two aromatic proton signals for both **2** and **3**. This signal at $\delta_H$ 6.44 of **4** had a strong HMBC correlation to the signal at $\delta_C$ 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*-$\beta$-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*-$\beta$-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 amorpha*,[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 assay[7] and were found to be inactive at concentrations up to 10 $\mu$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 $^1$H NMR and 125 MHz for $^{13}$C NMR. The chemical shift values ($\delta$) 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 $^1$H 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 × 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 × 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 × 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*-$\beta$-D-glucopyranoside (2):** Colorless crystalline powder; mp 155−156 °C; $[\alpha]^{25}_D$ −45.7° (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 270 (3.74), 218 (4.03) nm; IR (KBr) $\nu_{max}$ 3379, 2928, 1705, 1609, 1458, 1312, 1265, 1173, 1076, 1034, and 806 cm$^{-1}$; $^1$H NMR ($d_6$-acetone, 500 MHz) $\delta$ 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) $\delta$ 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 $[M + 1]^+$ (calcd for $C_{22}H_{31}O_{10}$ 455.1917).

**Curvularin-4′-*O*-methyl-7-*O*-$\beta$-D-glucopyranoside (3):** Colorless crystalline powder; mp 123−124 °C; $[\alpha]^{25}_D$ −40.1° (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 269 (3.63), 219 (3.94) nm; IR (KBr) $\nu_{max}$ 3422, 2925, 1728, 1659, 1616, 1312, 1273, 1180, 1087, 1045, and 806 cm$^{-1}$; $^1$H NMR ($d_6$-acetone, 500 MHz) $\delta$ 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) $\delta$ 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 ($CH_2$, C-11), 20.7 ($CH_3$, $CH_3$-15); HRFABMS *m/z* 469.2075 $[M + 1]^+$ (calcd for $C_{23}H_{33}O_{10}$ 469.2074).

**6-Hydroxycurvularin-4′-*O*-methyl-6-*O*-β-D-glucopyranoside (4):** Colorless crystalline powder; mp 127−128 °C; $[\alpha]^{25}_D$ −17.8° (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 270 (3.74), 226 (4.06) nm; IR (KBr) $\nu_{max}$ 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, $OCH_3$-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, $CH_3$-15); $^{13}C$ 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 ($CH_2$, C-6′), 60.7 ($CH_3$, $OCH_3$-4′), 43.9 ($CH_2$, C-10), 39.2 ($CH_2$, C-2), 33.0 ($CH_2$, C-14), 27.5 ($CH_2$, C-12), 24.7 ($CH_2$, C-13), 23.2 ($CH_2$, C-11), 20.6 ($CH_3$, $CH_3$-15); HRFABMS *m/z* 485.2047 $[M + 1]^+$ (calcd for $C_{23}H_{33}O_{11}$ 485.2023).

**6-Methyl-2,4-dihydroxyphenyl-4′-*O*-methyl-1-*O*-β-D-glucopyranoside (5):** Colorless amorphous solid; $^1H$ and $^{13}C$ 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. 1RO1CA90265-01A1, 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.

## References and Notes

(1) Musgrave, O. C. *J. Chem. Soc.* **1956**, 4301−4305.
(2) (a) Kobayashi, A.; Hino, T.; Yata, S.; Itoh, T. J.; Sato, H.; Kawazu, K. *Agric. Biol. Chem.* **1988**, *52*, 3119−3123. (b) Vesonder, R. F.; Ciegler, A.; Fennell, D.; Tjarks, L. W.; Jensen, A. H. *J. Environ. Sci. Health, Part B* **1976**, *B11*, 289−297.
(3) Robeson, D. J.; Strobel, G. A. *Z. Naturforsch. C: Biosci.* **1981**, *36C*, 1081−1083.
(4) Yao, Y.; Hausding, M.; Erkel, G.; Anke, T.; Förstermann, U.; Kleinert, H. *Mol. Pharmacol.* **2003**, *63*, 383−391.
(5) Commercial Solvent Corp. Br. Patent GB 1,114,954, 1968.
(6) Rout, N.; Nanda, B. K.; Gangopadhyaya, S. *Indian J. Pathol. Microbiol*. **1989**, *32*, 1−6.
(7) (a) Zhan, J.; Wijeratne, E. M. K.; Seliga, C. J.; Pierson, E. E.; Pierson, L. S., III; VanEtten, H. D.; Gunatilaka, A. A. L. *J. Antibiot.* **2004**, *57*, 341−344. (b) He, J.; Wijeratne, E. M. K.; Bashyal, B. P.; Zhan, J.; Seliga, C. J.; Liu, M. X.; Pierson, E. E.; Pierson, L. S., III; VanEtten, H. D.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2004**, *67*, 1985−1991.
(8) El-Sharkawy, S.; Abul-Hajj, Y. J. *J. Org. Chem*. **1988**, *53*, 515−519.
(9) Holland, H. L.; Morris, T. A.; Nava, P. J.; Zabic, M. *Tetrahedron* **1999**, *55*, 7441−7460.
(10) (a) Haufe, G.; Wölker, D.; Fröhlich, R. *J. Org. Chem*. **2002**, *67*, 3022−3028. (b) Vigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447−1458. (c) Hu, Y.; Ziffer, H.; Li, G.; Yeh, H. J. C. *Bioorg. Chem.* **1992**, *20*, 148−154.
(11) Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1993**, *58*, 5533−5536.
(12) Olivo, H. F.; Peeples, T. L.; Rios, M.; Velazquez, F.; Kim, J.; Narang, S. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 97−105.
(13) Holland, H. L.; Brown, F. M.; Johnson, D. V.; Kerridge, A.; Mayne, B.; Turner C. D.; van Vliet, A. J. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 249−256.
(14) Hu, F.; Schmidt, K.; Stoyanova, S.; Li, Z.; Gräfe, U.; Hamburger, M. *Planta Med.* **2002**, *68*, 64−65.
(15) Kikuchi, H.; Takahashi, N.; Oshima, Y. *Tetrahedron Lett.* **2004**, *45*, 367−370.

NP0580309