New Curvularin-Type Metabolites from the Hybrid Strain ME 0005 Derived from *Penicillium Citreo-viride* B. IFO 4692 and 6200

Sheng LAI, Yoshikazu SHIZURI, Shosuke YAMAMURA,* Kazuaki KAWAI,† and Hideyuki FURUKAWA†
Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223
† Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya 468
(Received October 24, 1990)

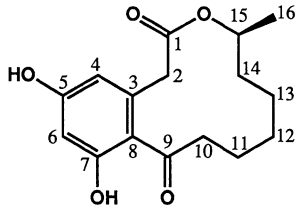
Synopsis. Four new curvularin-type metabolites (11-α-methoxycurvularin, 11-β-methoxycurvularin, 11,12-dihydroxycurvularin, and 12-hydroxy-10,11-*trans*-dehydrocurvularin) have been isolated from the mycelium of the hybrid strain ME 0005 derived from *Penicillium citreo-viride* B. IFO 4692 and 6200. And β-hydroxycurvularin, the biologically active principle from *Alternaria tomato,* was identified as 11-β-hydroxycurvularin.

Curvularin-type macrolides are 12-membered ring keto lactones containing a fused 1,3-dihydroxybenzene ring. Because of an interesting macrocyclic skeleton adopting some flexible conformations and novel biological properties as described later, curvularin (**1**) and related macrolides have been receiving much attention of synthetic chemists[1] since the first isolation of curvularin and α,β-dehydrocurvularin (**2**) from *Curvularia* species.[2] These macrolides have also been found later in *Penicillium gilmanii*[3] and in *Alternaria* species.[4,5] Quite recently, six novel curvularin-type metabolites have been isolated by us from a hybrid strain ME 0005 derived from *Penicillium citreo-viride* B. IFO 4692 and 6200.[6]

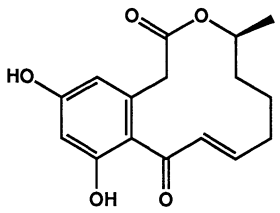
Last decades, Hyeon et al.[7] isolated β-hydroxycurvularin and α,β-dehydrocurvularin as sporulation-suppressing factors from *Alternaria tomato* and recently Kobayashi et al.[8] also reported the isolation of β-hydroxycurvularin, β-methoxycurvularin[9] as well as curvularin and α,β-dehydrocurvularin as unique spindle poisons from *Penicillium* sp. 511. β-Hydroxycurvularin[7] was idenfied as 11-β-hydroxycurvularin (**3**) by comparison of its spectroscopic data, particularly ^{1}H NMR spectrum, with those of 11-β-hydroxycurvularin and 11-α-hydroxycurvularin (**4**),[10] but no stereochemical evidence of the compound β-methoxycurvularin[8,9] has been revealed. In the continuation of our study on the metabolites of the hybrid strain ME 0005, a mixture of methoxycurvularins was also obtained. It was, therefore, considered desirable to make a detailed investigation, which led to the isolation of 11-β-methoxycurvularin (**5**) and 11-α-methoxycurvularin (**6**) together with 11,12-dihydroxycurvularin (**7**) and 12-hydroxy-10,11-*trans*-dehydrocurvularin (**8**) as follows.

According to essentially the same procedure as described in the previous papers,[6] the EtOAc extract was chromatographed on silica gel (Katayama Chemicals, Type 60) using a gradient solvent of MeOH–$CHCl_3$ (1—10%). Each fraction was further separated by repeated preparative TLC and/or HPLC to give four new compounds (**5**, **6**, **7**, and **8**), in addition to the known metabolites *cis*-dehydrocurvularin, citreofuran, and 12-oxocurvularin (see Experimental).

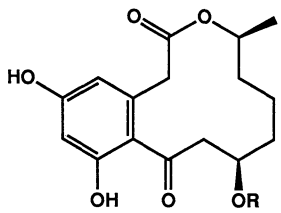
Compounds **5** and **6** are quite similar to each other in their ^{1}H NMR spectral data. Both have a methoxyl



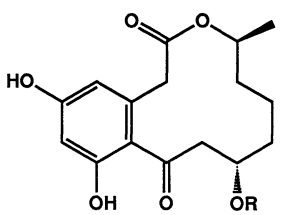
1



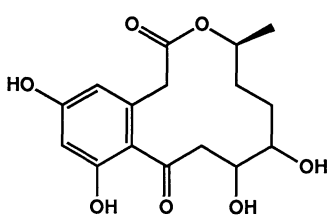
2



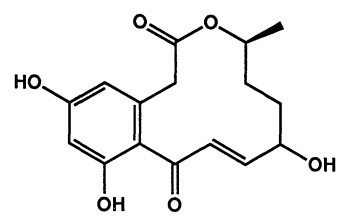
3 (R= H)
5 (R= Me)



4 (R= H)
6 (R= Me)



7



8

Scheme 1.

Table 1. Comparison of ^{1}H NMR Data of 11-Methoxycurvularins with 11-Hydroxycurvularins[a]

	11-β-Hydroxycurvularin (**3**)	11-α-Hydroxycurvularin (**4**)	11-β-Methoxycurvularin (**5**)	11-α-Methoxycurvularin (**6**)
H-10	*δ 3.63 (dd, J=13.7, 3.2)	* 3.23 (2H, br. d, J=6.8)[b]	* 3.88 (dd, J=13.3, 2.5)	* 3.38 (dd, J=14.2, 2.9)
	2.86 (dd, J=13.7, 10.3)		2.60 (dd, J=13.3, 10.1)	3.04 (dd, J=14.2, 10.3)
	** 3.48 (dd, J=13.6, 2.6)	3.25 (dd, J=14.2, 3.4)	3.64 (br. dd)	3.37 (dd, J=14.2, 2.9)
	2.85(overlapped)	3.03 (dd, J=14.2, 9.8)	2.56 (br. dd)	2.90 (dd, J=14.2, 10.2)
H-11	* 4.08 (1H, m)	4.09 (1H, m)	3.64 (1H, m)	3.66 (1H, m)
	** 4.02 (1H, m)	3.94 (1H, m)	3.62 (1H, m)	3.55 (1H, m)
H-15	* 4.87 (1H, m)	4.98 (1H, m)	4.81 (1H, m)	4.96 (1H, m)
	** 4.78 (1H, m)	4.90 (1H, m)	4.76 (1H, m)	4.90 (1H, m)

a) J values were given in Hz. * in CD_3OD; ** in acetone-d_6. b) This J value is an apparent one, because of the close similarity in the chemical shifts.

group [δ=3.33 (3H, s) for **5** and δ=3.25 (3H, s), for **6**] in the spectra which resemble broadly those of 11-hydroxycurvularins (**3** and **4**).[6] They also share the same mass spectrum, showing a molecular ion at m/z 322, and fragment ions at m/z 290, 203, 195, 177, 167, and 150, as that of α,β-dehydrocurvularin.[2] The formation of the prominent ion at m/z 290 (M^+—MeOH) and the ion at m/z 203 requires the presence of the methoxyl group at C-11. Thus, the structures of both **5** and **6** were deduced as 11-methoxycurvularins and were confirmed by chemical transformation of α,β-dehydrocurvularin (**2**) to these two stereoisomers. When treated on SiO_2 with MeOH–EtOAc (1 : 1) at room temperature for 5 d, α,β-dehydrocurvularin was slowly converted into a mixture of **5** and **6** in the ratio of 2 : 3[11] as a colorless oil, which was separated by HPLC to afford two pure 11-methoxycurvularins (**5** and **6**) which were identical with the two natural compounds, respectively. Finally, the stereostructures of the two isomers were determined by comparing their ^{1}H NMR data with those of 11-β-hydroxycurvularin and 11-α-hydroxycurvularin as shown in Table 1, wherein the chemical shifts of the protons at C-10 of the formers are quite different while those of the latters are close or identical to each other.

Compounds **7** and **8** show close similarities in their mass spectra, except for different molecular ions (m/z 324 for **7** and m/z 306 for **8**). Both show a predominent peak at m/z 288 originated from dehydration and the fragment peaks (m/z 203, 195, 177, 167, and 150) characteristic of α,β-dehydrocurvularin[2] and 11-methoxycurvularins (**5** and **6**), strongly suggesting that there should be an olefinic group adjacent to the aromatic carbonyl group or a hydroxyl group at C-11-position. The ^{1}H NMR spectra of **7** and **8** are also similar to each other and similar to curvularin-type compounds[2,6] including 11-methoxycurvularins in spite of somewhat differences as follows. Compound **7** shows two NMR signals at δ=5.25 (1H, m) and 5.46 (1H, m) in addition to δ=4.97 (1H, m), coupled with the molecular ion at m/z 324 in its mass spectrum, indicating the presence of two hydroxyl groups in the molecule. Irradiation of the multiplet at δ=5.25 collapsed the doublet at δ=3.46 (2H, d, J=8.8 Hz) into a singlet and caused partial collapse of the signal at δ=5.46, suggesting that the two hydroxyl groups are adjacent to each other and one should be located at C-11, the other at C-12. Thus, **7** was unambiguously determined as 11,12-dihydroxycurvularin.[12] As for compound **8**, the most significant difference between **7** and **8** is the virtual absence of two adjacent hydroxyl groups in the latter, but instead **8** has one hydroxyl group adjacent to a *trans*-1,2-disubstituted double bond (δ=6.61, 1H, dd, J=15.6, 1.0 Hz; δ=6.68, 1H, dd, J=15.6, 4.4 Hz). Irradiation of the multiplet at δ=4.54 collapsed the olefinc protons to doublets (J=15.6 Hz) and caused changes at δ=2.17 (1H, m) and 1.82 (1H, m). Therefore, compound **8** was unambiguously determined as 12-hydroxy-10,11-*trans*-dehydrocurvularin.[12]

Experimental

Melting points were determined on a Mitamura Riken apparatus and uncorrected. Optical rotations were determined with a JASCO DIP-360 polarimeter. IR spectra were taken on a JASCO A-202 spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard unless otherwise noted. Mass spectra were measured on a Hitachi M-80 GC-MS spectrometer, operating with an ionization energy at 70 eV.

Incubation. Polished rice (600 g) in deionized water (ca. 1600 ml) was cooked using an electric cooker (99 °C, ca. 20 min) and transfered into two Erlenmyer flasks (5 l), which were pasteurized at 120 °C for 20 min at 2 atm. After inoculated with a suspension of the mycelium of the hybrid strain ME 0005 in sterilized water, the rice was incubated stationarily at 25 °C for 23 d and extracted with acetone.

Isolation and Separation. The acetone extract suspended in water (500 ml) was extracted with EtOAc (200 ml×5). The EtOAc extract (dark brown syrup, 4.85 g) was chromatographed on silica gel (300 g, Katayama Chemicals, Type 60) using $CHCl_3$ (2000 ml) and then a gradient solvent of MeOH–$CHCl_3$ (1% MeOH–$CHCl_3$ 2000 ml; 2—10% MeOH–$CHCl_3$ 500 ml for each). Elution with 3—4% MeOH–$CHCl_3$ afforded a dark brown oil, which was separated by repeated preparative TLC (Kieselgel 60 PF_{254}) using hexane–EtOAc (2 : 1), $CHCl_3$–acetone (2 : 1), and $CHCl_3$–MeOH (10 : 1), and then by preparative TLC (Kieselgel 60 F_{254}) using $CHCl_3$–MeOH [(15 : 1)×2], to afford compound **7** (0.02%) in addition to the known compounds *cis*-dehydrocurvularin (0.15%) and citreofuran (0.26%).[6] Elution with 6—7% MeOH–$CHCl_3$ gave a dark brown oil. When subjected to preparative TLC (Kieselgel 60 PF_{254}), developed with $CHCl_3$–acetone (2 : 1),

$CHCl_3$–MeOH (15 : 1), hexane–EtOAc (1 : 1) and then subjected to preparative TLC (Kieselgel 60 F_{254}) using $CHCl_3$–MeOH [(15 : 1)×2], the oil afforded compound **8** (0.15%) and the known 12-oxocurvularin (0.27%),[6] together with a mixture of **5** and **6** in the ratio of 2 : 1[11] as a colorless oil (16.25 mg, 0.34%). The mixture was further separated by HPLC (Unisil Q PH; ϕ 6.0×250 mm) using MeOH–H_2O (3 : 7) (flow rate: 0.5 ml min^{-1}; Detector: UV 250 nm), giving rise to compounds **5** (4.4 mg) and **6** (2.5 g).[13]

Conversion of α,β-Dehydrocurvularin (2) into 11-Methoxycurvularins. α,β-Dehydrocurvularin (**2**) (23.88 mg) in MeOH–EtOAc (1 : 1) (12 ml) was absorbed on SiO_2 (5 g) at room temp for 5 d, and then concentrated to dryness. The residue was subjected to preparative TLC (Kieselgel F_{254}), using $CHCl_3$–MeOH (10 : 1) and then hexane–acetone (2 : 1), to give a mixture of **5** and **6** in the ratio of 2 : 3[11] as a colorless oil (9.56 mg, 36%, c.y. 70%), which afforded compounds 11-β-methoxycurvularin (**5**) and 11-α-methoxycurvularin (**6**) when subjected to HPLC under the same conditions as mentioned above.

Compound 5 (11-β-Methoxycurvularin). Colorless oil; $[\alpha]_D^{23}$ −21.3° (*c* 0.22, EtOH); IR (film) 3300, 2935, 1700, 1656, 1608, 1590, 1460, 1265, 1165, 1132, 1080, 1020, 848, and 760 cm^{-1}; ^1H NMR δ=1.21 (3H, d, *J*=6.35 Hz), 1.4—1.8 (6H, complex), 3.06 (1H, dd, *J*=15.14, 7.81 Hz), 3.27 (1H, dd, *J*=15.14, 4.88 Hz), 3.33 (3H, s), 3.64 (1H, d, *J*=16.30 Hz), 3.81 (1H, m), 3.91 (1H, d, *J*=16.30 Hz), 4.98 (1H, m), 6.17 (1H, d, *J*=2.20 Hz), 6.30 (1H, d, *J*=2.20 Hz), 6.75 (1H, br. s), and 8.50 (1H, br. s); ^1H NMR (CD_3OD) δ=1.12 (3H, d, *J*=6.16 Hz), 1.2—1.7 (6H, complex), 2.60 (1H, dd, *J*=13.25, 10.10 Hz), 3.36 (3H, s), 3.58 (1H, d, *J*=15.40 Hz), 3.64 (1H, m), 3.88 (1H, dd, *J*=13.25, 2.50 Hz), 3.94 (1H, d, *J*=15.40 Hz), 4.81 (1H, m), 6.20 (1H, d, *J*=2.20 Hz), and 6.27 (1H, d, *J*=2.20 Hz); MS (*m/z*) 322 (M^+), 290 (M^+−MeOH, base peak), 203, 195, 177, 167, and 150. Found: *m/z* 290.1156. Calcd for $C_{16}H_{18}O_5$: M^+−MeOH, 290.1153.

Compound 6 (11-α-Methoxycurvularin). Colorless oil; $[\alpha]_D^{26}$ −4.2° (*c* 0.24, EtOH); IR (film) 3300, 2935, 1700, 1656, 1608, 1588, 1460, 1270, 1162, 1080, 986, 845, and 755 cm^{-1}; ^1H NMR δ=1.25 (3H, d, *J*=6.34 Hz), 1.4—1.95 (6H, complex), 3.12 (1H, dd, *J*=13.67, 8.31 Hz), 3.25 (3H, s), 3.34 (1H, dd, *J*=13.67, 4.88 Hz), 3.59 (1H, d, *J*=15.87 Hz), 3.77 (1H, m), 3.95 (1H, d, *J*=15.87 Hz), 5.14 (1H, m), 5.98 (1H, d, *J*=2.20 Hz), 6.36 (1H, d, *J*=2.20 Hz), 6.95 (1H, br. s), and 8.97 (1H, br. s); ^1H NMR (CD_3OD) δ=1.11 (3H, d, *J*=6.35 Hz), 1.44 (4H, m), 1.64 (2H, m), 3.04 (1H, dd, *J*=14.16, 10.26 Hz), 3.32 (3H, s), 3.38 (1H, dd, *J*=14.16, 2.93 Hz), 3.60 (1H, d, *J*=15.14 Hz), 3.66 (1H, m), 3.95 (1H, d, *J*=15.14 Hz), 4.96 (1H, m), 6.22 (1H, d, *J*=2.44 Hz), and 6.28 (1H, d, *J*=2.44 Hz); MS (*m/z*) 322 (M^+), 290 (M^+−MeOH, base peak), 203, 195, 177, 167, and 150. Found: *m/z* 290.1151. Calcd for $C_{16}H_{18}O_5$: M^+−MeOH, 290.1152.

Compound 7 (11,12-Dihydroxycurvularin). Brownish needles; mp 168—170°C (from $CHCl_3$–MeOH). $[\alpha]_D^{25}$ +69.3° (*c* 0.224, EtOH). IR (film) 3350, 1700, 1638, 1610, and 1590 cm^{-1}; ^1H NMR (CD_3OD) δ=1.14 (3H, d, *J*=6.35 Hz), 1.58 (1H, m), 1.63 (1H, m), 2.00 (1H, m), 2.27 (1H, m), 3.46 (2H, d, *J*=8.8 Hz), 3.50 (2H, s), 4.97 (1H, m), 5.25 (1H, m), 5.46 (1H, m), 6.24 (1H, d, *J*=1.95 Hz), 6.26 (1H, d, *J*=1.95 Hz). MS (*m/z*) 324 (M^+), 288 (M^+−2×H_2O, base peak), 203, 195, 177, 167, and 150. Found: *m/z* 288.0945. Calcd for $C_{16}H_{16}O_5$: M^+−2×H_2O, 288.0996.

Compound 8 (12-Hydroxy-10,11-*trans*-dehydrocurvularin). Yellowish powder, $[\alpha]_D^{26}$ −49.5° (*c* 0.86, EtOH). IR (film) 3350, 1705, 1638, 1610, and 1590 cm^{-1}. ^1H NMR (CD_3OD) δ=1.30 (1H, d, *J*=6.35 Hz), 1.70 (1H, m), 1.82 (1H, m), 2.00 (1H, m), 2.17 (1H, m), 3.48 (1H, d, *J*=15.38 Hz), 3.61 (1H, d, *J*=15.38 Hz), 4.54 (1H, m), 4.96 (1H, m), 6.38 (1H, d, *J*=1.95 Hz), 6.43 (1H, d, *J*=1.95 Hz), 6.61 (1H, dd, *J*=15.62, 0.98 Hz), 6.68 (1H, dd, *J*=15.62, 4.40 Hz). MS (*m/z*) 306 (M^+), 288 (M^+−H_2O, base peak), 203, 195, 177, 167, and 150. Found: *m/z* 288.0985. Calcd for $C_{16}H_{16}O_5$: M^+−H_2O, 288.0996.

References

1) R. H. Boeckman and M. Goldstein, "The Total Synthesis of Natural Products," ed by J. W. ApSimon, Wiley-Interscience, New York (1988), Vol. VII, pp. 20—23, and many references cited therein.

2) a) O. C. Musgrave, *J. Chem. Soc.*, **1956**, 4301; *ibid.*, **1957**, 1104; b) A. J. Birch, O. C. Musgrave, R. W. Rickards, and H. Smith, *ibid.*, **1959**, 3146; c) H. D. Munro, O. C. Musgrave, and R. Templeton, *ibid.*, **1967**, 947.

3) H. Raistrick and F. A. H. Rice, *J. Chem. Soc. C*, **1971**, 3069.

4) D. J. Robeson and G. A. Strobel, *Z. Naturforsch., C, Biosci.*, **36C**, 1081 (1981).

5) D. J. Robeson, G. A. Strobel, and R. N. Strange, *J. Nat. Prod.*, **48**, 139 (1985).

6) S. Lai, Y. Shizuri, S. Yamamura, K. Kawai, Y. Terada, and H. Furukawa, *Tetrahedron Lett.*, **30**, 2241 (1989); *Chem. Lett.*, **1990**, 589.

7) S. B. Hyeon, A. Ozaki, A. Suzuki, and S. Tamura, *Agric. Biol. Chem.*, **40**, 1663 (1976).

8) a) A. Kobayashi, T. Hino, K. Uneyama, and K. Kawazu, 27th Symposium on the Chemistry of Natural Products, Hiroshima, October 1985, Abstr., pp. 343—350; b) A. Kobayashi, S. Yata, T. Hino, and K. Kawazu, *Agric. Biol. Chem.*, **51**, 2857 (1987); c) A. Kobayashi, T. Hino, S. Yata, T. J. Itoh, and H. Sato, *ibid.*, **52**, 3119 (1988).

9) Expected to be identical with one of our 11-methoxycurvularins, but the compound β-methoxycurvularin is quite different from both 11-α-methoxycurvularin and 11-β-methoxycurvularin in both the optical rotation property and ^1H NMR data. As ^1H NMR spectrum of α-methoxycurvularin was recorded in $CDCl_3$ with a JEOL FX 100,[8] we also carefully measured ^1H NMR spectra of 11-α-methoxycurvularin and 11-β-methoxycurvularin in $CDCl_3$ on a JEOL FX 90A NMR spectrometer. These data are in accordance with those reported already in the Experimental, while they are still quite different from that of β-methoxycurvularin cited in the Ref. 8.

10) In order to establish the stereostructure of β-hydroxycurvularin, ^1H NMR spectra of 11-α-hydroxycurvularin and 11-β-hydroxycurvularin were also measured using acetone-d_6. The spectra are quite similar to each other except for the following points. 11-α-hydroxycurvularin: δ=3.25 (1H, dd, *J*=14.2, 3.4 Hz) (H-10β), 3.03 (1H, dd, *J*=14.2, 9.8 Hz) (H-10α), 3.94 (1H, m) (H-11β); 11-β-hydroxycurvularin: δ=3.48 (1H, dd, *J*=13.6, 2.6 Hz) (H-10α), 2.85 (overlapped with the signal of H_2O) (H-10β), and 4.02 (1H, m) (H-α). The spectral data of 11-β-hydroxycurvularin were indistinguishable from those of β-hydroxycurvularin.[7] Thus the latter was identified as 11-β-hydroxycurvularin.[6]

11) Based on the integration heights of their methoxyl groups in ^1H NMR spectra and supported by the calculated results according to the optical rotation of 11-β-methoxycurvularin (**5**, $[\alpha]_D^{23}$=−21.3°) and 11-α-methoxycurvularin (**6**, $[\alpha]_D^{26}$−4.2°): **5** : **6**=1.85 : 1 in the natural mixture ($[\alpha]_D^{25}$−15.3°), while **5** : **6**=0.8 : 1 in the synthetic mixture ($[\alpha]_D^{27}$−11.8°).

12) The stereostructures of **7** and **8** have not yet been determined because of the flexible conformations of these new compounds.

13) 11-Methoxycurvularins are very unstable and easily dehydrated to give α,β-dehydrocurvularin during separation on preparative TLC plates or HPLC column.