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NOVEL CURVULARIN-TYPE METABOLITES OF A HYBRID STRAIN ME 0005 DERIVED FROM PENICILLIUM CITREO-VIRIDE B. IFO 6200 AND 4692

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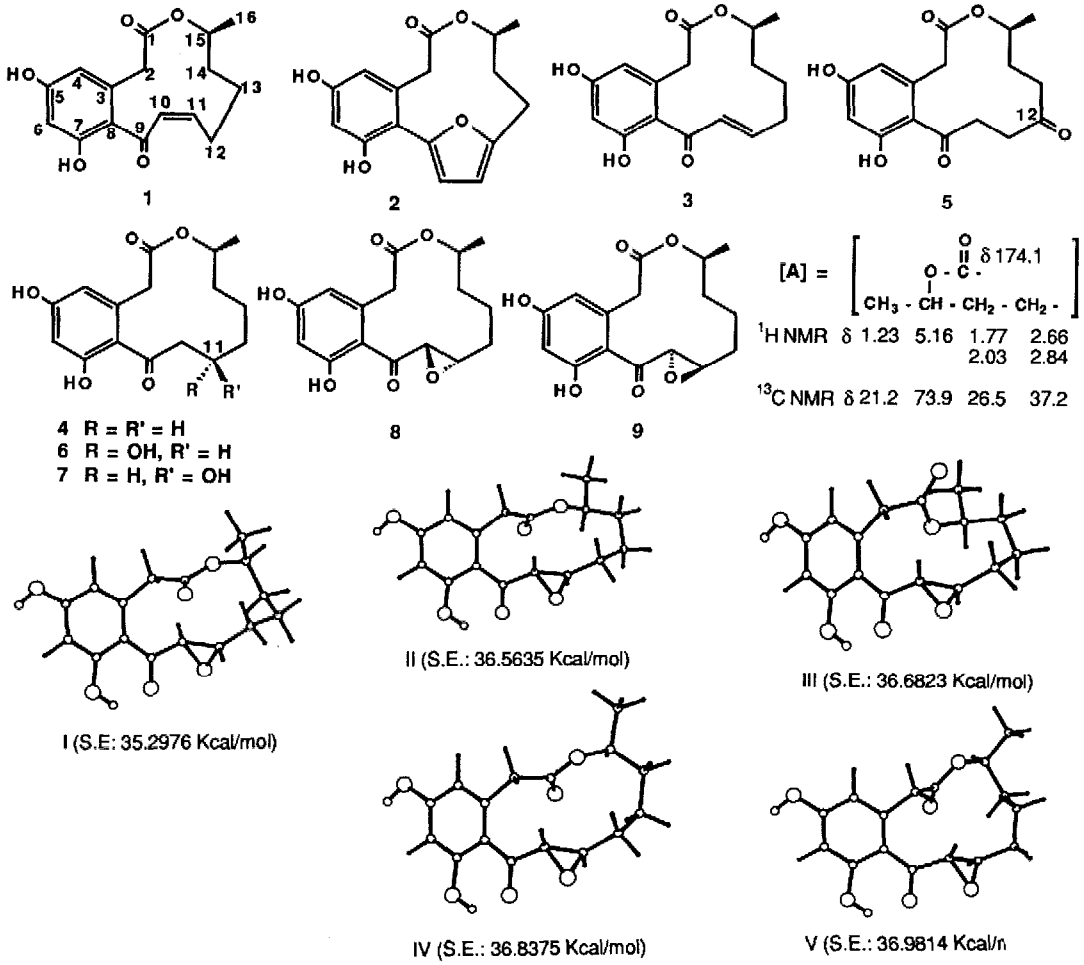
Summary: In addition to curvularin and dehydrocurvularin, several new metabolites have been isolated from the mycelium of a hybrid strain ME 0005 derived from Penicillium citreo-viride B. IFO 6200 and 4692, and their stereostructures have also been elucidated on the basis of some chemical evidence together with spectral data.

In connection with citreoviridin, a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme system, a number of novel metabolites have been isolated from the mycelium of P. citreo-viride B. Particularly, citreoviridin and pyrones have been mainly produced by P. citreo-viride B. (IFO 6200).[1] In the case of another strain IFO 4692, however, citreoviranol and related phenols have been obtained as main products.[2] In the light of these results, more than ten hybrid strains have been produced by means of cell fusion technique using two different strains of IFO 6200 and 4692.[3] Among them, the hybrid strain ME 0005 was used for the present study, as follows.

According to essentially the same procedure as described in the previous papers,[1,2] the polished rice (600 g), which was inoculated with a suspension of mycelium of the hybrid strain ME 0005 in a sterilized water, was incubated stationarily at 25 °C for 23 days and extracted with acetone and then with EtOAc. The combined extracts were partitioned between EtOAc and water. The EtOAc extract was chromatographed on silica gel using a gradient solvent of MeOH - $CHCl_3$ (1 - 50%). Elution with $CHCl_3$ - MeOH (100 : 3 - 4) afforded a dark brown oil, which was separated by repeated preparative TLC (Kieselgel PF_{254}) using hexane - EtOAc (5 : 1), hexane - EtOAc (2 : 1), C_6H_6 - acetone (5 : 1), $CHCl_3$ - MeOH (15 : 1), $CHCl_3$ - acetone (2 : 1) and then $CHCl_3$ - MeOH (10 : 1) to afford cis-dehydrocurvularin (**1**) and citreofuran (**2**) in 0.15 and 0.26% yields,[4] respectively, in addition to the known trans-dehydrocurvularin (**3**) (16.5%).[5] Elution with $CHCl_3$ - MeOH (100 : 4 - 5) afforded the known curvularin (**4**)[5] in 2.6% yield.[4] The dark brown oil eluted with $CHCl_3$ - MeOH (100 : 5 - 6) was also separated by repeated preparative TLC (Kieselgel PF_{254}) using $CHCl_3$ - MeOH (10 : 1), hexane - acetone (1 : 1), $CHCl_3$ - MeOH (15 : 1), hexane - acetone (2 : 1), hexane - EtOAc - MeOH (7 : 2 : 1) and then C_6H_6 - MeOH (4 : 1) to give 12-oxocurvularin (**5**) in 0.27% yield.[4] Furthermore, elution with $CHCl_3$ - MeOH (100 : 9 - 10) gave a dark brown oil which was separated by repeated preparative TLC (Kieselgel PF_{254}) using $CHCl_3$ - MeOH (10 : 1) and then hexane - CH_2Cl_2 - EtOAc - MeOH (3 : 3 : 3 : 1) to afford a pale yellow solid, which was



further separated by preparative HPLC (Develosil ODS-5; ϕ10 mm x 250 mm) using CH_3CN - H_2O (7 : 3) and then MeOH - H_2O (1 : 1) to afford 11-α-hydroxycurvularin (**6**) and 11-β-hydroxycurvularin (**7**) in 0.19 and 0.28% yields,[4] respectively. The spectral data of the new metabolites are shown below.

cis-Dehydrocurvularin (**1**) as an amorphous powder: $C_{16}H_{18}O_5$ [m/z 290.1161(M^+)]; $[\alpha]_D^{22}$ +7.3° (c 0.78, EtOH); UV (EtOH) λ_{max} (log ε) 228 nm(4.02), 292(3.62), 312(3.64); IR ($CHCl_3$) 3310br., 1720, 1695, 1590br.cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.16(3H, d, J = 6.6 Hz), 1.57(2H, m), 1.80(2H, m), 2.32(2H, m), 3.82(1H, d, J = 18 Hz), 3.88(1H, d, J = 18 Hz), 4.96(1H, m), 5.91(1H, ddd, J = 6.6, 6.6, 12.5 Hz), 6.22(1H, d, J = 1.5 Hz), 6.34(1H, d, J = 1.5 Hz), 6.38(1H, d, J = 12.5 Hz), 6.55(1H, br.s, OH), 12.4(1H, br.s, OH).

Citreofuran (**2**): mp 203 - 205 °C; $C_{16}H_{16}O_5$ [m/z 288.1015(M^+)]; $[\alpha]_D^{27}$ +112° (c 0.18, EtOH); UV (EtOH) λ_{max} (log ε) 210 nm(4.21), 220(4.20), 256(3.91), 287(3.74); IR ($CHCl_3$) 3370br., 1705, 1620, 1590 cm^{-1}; ^1H NMR (CD_3OD): δ 1.23(3H, d, J = 6.4 Hz), 1.77(1H, m), 2.03(1H, m), 2.66(1H, ddd, J = 2.9, 11.5, 15 Hz), 2.84(1H, ddd, J = 2.9, 5.9, 15 Hz), 3.10(1H, d, J = 14.4 Hz), 3.20(1H, d, J = 14.4 Hz), 5.16(1H, m), 6.09(1H, d, J = 3.2 Hz), 6.21(1H, d, J =

3.2 Hz), 6.30(1H, d, J = 2.4 Hz), 6.34(1H, d, J = 2.4 Hz); ^{13}C NMR (CD_3OD): δ 21.2(q), 26.5 (t), 37.2(t), 42.5(t), 73.9(d), 102.5(d), 107.5(d), 111.0(d), 111.8(s), 112.2(d), 139.7(s), 148.7(s), 156.0(s), 157.3(s), 159.5(s), 174.1(s).

12-Oxocurvularin (**5**) as an amorphous powder: $C_{16}H_{18}O_6$ [m/z 306.1082(M^+)]; $[\alpha]_D^{29}$ -43.5° (c 0.47, EtOH); UV (EtOH) λ_{max} (log ε) 222 nm(3.99), 272(3.69), 299(3.60); IR (film) 3300br., 1710, 1608, 1590 cm^{-1}; ^1H NMR (CD_3OD): δ 1.13(3H, d, J = 6.4 Hz), 1.72(1H, m), 2.04(1H, m), 2.25 (1H, ddd, J = 2.9, 7.8, 16.6 Hz), 2.66(1H, m), 2.73(1H, ddd, J = 2.3, 9.8, 16.6 Hz), 3.01 (2H, m), 3.42(1H, m), 3.54(1H, d, J = 14.9 Hz), 3.64(1H, d, J = 14.9 Hz), 4.94(1H, m), 6.20 (1H, d, J = 2.4 Hz), 6.28(1H, d, J = 2.4 Hz); ^{13}C NMR (CD_3OD): δ 19.8(q), 30.5(t), 38.3(t), 38.8(t), 39.7(t), 40.4(t), 71.8(d), 102.4(d), 111.4(d), 120.6(s), 137.1(s), 158.7(s), 161.2 (s), 172.4(s), 208.3(s), 212.5(s).

11-α-Hydroxycurvularin (**6**): mp 150 - 152 °C; $C_{16}H_{20}O_6$ [m/z 308.1242(M^+)]; $[\alpha]_D^{26}$ -29.4° (c 0.33, EtOH); UV (EtOH) λ_{max} (log ε) 222 nm(3.98), 274(3.78), 307(3.69); IR (film) 3300br., 1700, 1605, 1585 cm^{-1}; ^1H NMR (CD_3OD): δ 1.13(3H, d, J = 6.3 Hz), 1.45(4H, m), 1.61(1H, m), 1.68(1H, m), 3.23(2H, d, J = 6.8 Hz), 3.59(1H, d, J = 15.6 Hz), 3.97(1H, d, J = 15.6 Hz), 4.09(1H, m), 4.98(1H, m), 6.21(1H, d, J = 2.2 Hz), 6.28(1H, d, J = 2.2 Hz).

11-β-Hydroxycurvularin (**7**): mp 138 - 140 °C; $C_{16}H_{20}O_6$ [m/z 308.1220(M^+)]; $[\alpha]_D^{24}$ -10.9° (c 0.19, EtOH); UV (EtOH) λ_{max} (log ε) 223 nm(3.98), 275(3.71), 306(3.66); IR (film) 3300br., 1700, 1605, 1585 cm^{-1}; ^1H NMR (CD_3OD): δ 1.16(3H, d, J = 5.9 Hz), 1.32(2H, m), 1.48(2H, m), 1.62(2H, m), 2.96(1H, dd, J = 9.0, 13.7 Hz), 3.62(1H, dd, J = 5.1, 13.7 Hz), 3.67(1H, d, J = 15.6 Hz), 3.98(1H, d, J = 15.6 Hz), 4.08(1H, m), 4.87(1H, m), 6.24(1H, d, J = 2.2 Hz), 6.28(1H, d, J = 2.2 Hz).

cis-Dehydrocurvularin (**1**) has the same molecular formula $C_{16}H_{18}O_5$ as that of the known trans-dehydrocurvularin (**3**),[5] and their spectral data are quite similar to each other except for the following points: the coupling constant (J = 12.5 Hz) of C_{10}-H (δ 6.38) in **1** indicates the presence of the cis-1,2-disubstituted double bond, whereas **3** has the corresponding doublet at δ 6.51 (J = 15.6 Hz). On catalytic hydrogenation (10% Pd-C, MeOH), both **1** and **3** were readily converted into curvularin (**4**).[5]

Citreofuran (**2**) has an isolated methylene group (δ 3.10 and 3.20) and a partial structure [A], in addition to the tetrasubstituted phenolic ring, as seen in dehydrocurvularins (**1** and **3**). Furthermore, a 1,4-disubstituted furan ring is included in its structure (δ 6.09 and 6.21; δ 107.5, 111.0, 148.7 and 156.0).[6] From these data, the novel structure of citreofuran is assumed to be **2**. This is also confirmed by chemical correlation with 12-oxocurvularin (**5**), whose structure is discussed below.

12-Oxocurvularin (**5**) also has the same partial structure [A] and an isolated methylene group (δ 3.54 and 3.64), as seen in citreofuran (**2**). However, some remarkable differences are observed particularly in their ^1H and ^{13}C NMR spectra, indicating that the former has two ketonic CO groups (δ 208.3 and 212.5) and an CH_2CH_2 group (δ 2.66, 3.42 and 3.01) as compared with citreofuran (**2**) which contains the 1,4-disubstituted furan ring. Accordingly, the structure of 12-oxocurvularin must be represented by **5**. Finally, when treated with CsOH in benzene under argon (refluxing temp., 3 h), **5** was readily converted into **2** in 71% yield.

11-α-Hydroxycurvularin (**6**) is quite similar to 11-β-hydroxycurvularin (**7**) in their spectral data . Furthermore, their ^1H NMR spectra are also similar to that of curvularin (**4**) except for the following points: both **6** and **7** have a secondary OH group which is located at C_{11}-position [**6**: δ 3.23(C_{10}-H) and 4.09(C_{11}-H); **7**: δ 2.96 and 3.62(C_{10}-H) and

4.08(C_{11}-H)]. Finally, their stereostructures (**6** and **7**) were unambiguously determined by chemical transformation of trans-dehydrocurvularin (**3**) to these two stereoisomers.

On oxidation with 35% H_2O_2 - Na_2CO_3 (1 equiv.) in MeOH (-28 - 3 °C, 2 h), trans-dehydrocurvularin (**3**) was readily converted into two epoxides (**8** and **9**)[7] in 17 and 41% yields, respectively. The former (**8**) was further treated with $Cr(OAc)_2$ in EtOH under argon (room temp., 30 min) to afford 11-α-hydroxycurvularin (**6**) in 38% yield. In the case of the epoxide (**9**), the corresponding 11-β-hydroxy compound (**7**) was also obtained in 50% yield. At this stage, the stereochemistry at C_{11}-position in both **6** and **7** remains undecided. Fortunately, however, the stereochemistry of these epoxides (**8** and **9**) could be determined on the basis of their ^1H NMR spectral data coupled with molecular mechanics calculations successively using Still's RINGMAKER and BAKMOD programs based on Allinger's MM2 and then MMP2, indicating that the epoxide (**8**) adopts only one stable conformer [I]. In the case of the epoxide (**9**), four conformers [II - V] are mainly present.[8] Thus, the J-values of the NMR signal due to C_{11}-H in **8** are roughly compatible with the calculated ones based on [I] [($J_{C11-H - C12-H}$ = 7.3 Hz (calcd., 9.1 Hz); $J_{C11-H - C12-H'}$ < 1 Hz (calcd., 1.1 Hz)].[9] In the case of **9**, the weighted average J-values were calculated on the basis of relative ratio of each conformer. The J-values so far obtained are also compatible with the observed ones [$J_{C11-H - C12-H}$ = 9.8 Hz (calcd., 10.9 Hz); $J_{C11-H - C12-H'}$ = 4.4 Hz (calcd., 3.3 Hz)].

Interestingly, any curvularin-type metabolite has never been found in the mycelium of P. citeo-viride B. IFO 6200 nor 4692. From a view point of physiological activity, it should be noted that curvularin-type metabolites show remarkable activity against sea urchin embryo cells: they induce barrel-like spindles resulting in inhibition of cell proliferation, as reported by Kobayashi et al.[10] Particularly, citreofuran (**2**) and 12-oxocurvularin (**5**) both are quite attractive not only in physiological properties but also in their structures containing an oxygen function at C_{12}-position.

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7. **8**: $C_{16}H_{18}O_6$ [m/z 306.1093(M^+)]; IR ($CHCl_3$) 3350br., 1710br., 1605, 1590 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.29(3H, d, J = 5.9 Hz), 1.64(2H, m), 1.79(1H, m), 1.88(1H, m), 2.03(1H, m), 2.15(1H, m), 3.10(1H, br.d, J = 7.3 Hz), 3.70(1H, d, J = 18 Hz), 3.96(1H, d, J = 1.5 Hz), 4.23(1H, d, J = 18 Hz), 5.12(1H, m), 6.26(1H, d, J = 2.9 Hz), 6.30(1H, d, J = 2.9 Hz). **9**: $C_{16}H_{18}O_6$ [m/z 306.1078(M^+)]; IR ($CHCl_3$) 3365br., 1725, 1700, 1605, 1595 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.24(3H, d, J = 6.4 Hz), 1.20(2H, m), 1.56(1H, m), 1.86(2H, m), 2.08(1H, m), 3.24(1H, d, J = 12.7 Hz), 3.29(1H, ddd, J = 2.0, 4.4, 9.8 Hz), 3.75(1H, J = 12.7 Hz), 4.00(1H, d, J = 2.0 Hz), 4.70(1H, m), 6.31(1H, d, J = 2.2 Hz), 6.45(1H, d, J = 2.2 Hz).
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