Citreopyrones, New Metabolites of Two Hybrid Strains, KO 0092 and KO 0141, Derived from the *Penicillium* Species

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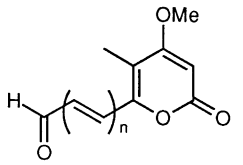
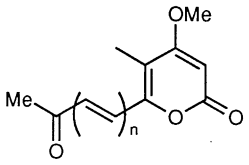
Six new metabolites, citreopyrones A-C and D-F, have been isolated from the mycelium of a hybrid strain KO 0092 derived from *P. citreo-viride* B. IFO 4692 and 6200, and KO 0141 derived from *P. citreo-viride* B. IFO 4692 and *P. pedemontanum* IFO 9583. Their structures have also been elucidated on the basis of their spectral data and some chemical evidence. Citreopyrones A, B, and C inhibited the growth of hypocotyls of lettuce seedlings.

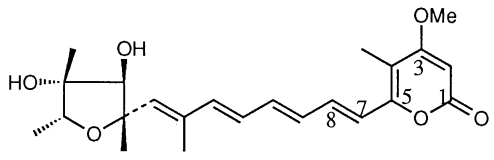
In a series of experiments, we have prepared more than ten hybrid strains by means of a cell fusion technique using two different strains, *Penicillium citreo-viride* B. IFO 4692 and 6200.[1] Some of these hybrid strains produced a number of new interesting metabolites[2] which have not been previously detected in the mycelium of either parent strain. In the light of these results, a new hybrid strain KO 0141 has been produced by means of the cell fusion technique using two different strains of *P. citreo-viride* B. IFO 4692 and *P. pedemontanum* IFO 9583. In this communication we report the isolation and structures of six new metabolites, citreopyrones A-F produced by two hybrid strains KO 0092 (IFO 4692 and 6200) and KO 0141 (IFO 4692 and 9583).



n= 2 citreopyrone A (**1**)
n= 1 citreopyrone B (**2**)
n= 0 citreopyrone C (**3**)



n= 3 citreopyrone D (**4**)
n= 2 citreopyrone E (**5**)
n= 1 citreopyrone F (**6**)



citreoviridin (**7**)

According to essentially the same procedure as described in the previous papers,[2] the EtOAc extract (45.4 g) of a rice medium of KO 0092 (filtered from the mycelium) was chromatographed on silica gel using a gradient solvent of acetone-hexane (1: 5 - 3 : 1). Each fraction was further separated by repeated preparative TLC to afford three new compounds **1**, **2**, and **3** in 0.0022, 0.0024, and 0.0011% yields, respectively. In the case of the hybrid strain KO 0141, the EtOAc extract (14.0 g) was chromatographed on silica gel using a gradient solvent of acetone-hexane (1: 2 - 5 : 1). Each fraction was further separated by repeated preparative TLC to afford three new compounds **4**, **5**, and **6** in 0.025, 0.032, and 0.0071% yields, respectively. Citreoviridin (**7**),[3] a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by a mitocondrial enzyme system, has also been isolated from the mycelia of the hybrid strains KO 0092 and KO 0141. The inhibitory activities of citreopyrones A (**1**), B (**2**), and C (**3**) towards the germination of lettuce seedlings were not observed. These compounds, however, inhibited the growth of hypocotyls of lettuce seedlings 70.1% in **1**, 70.1% in **2** and 58.4% in **3** relative to the control at 37.5 μg/cm^2.

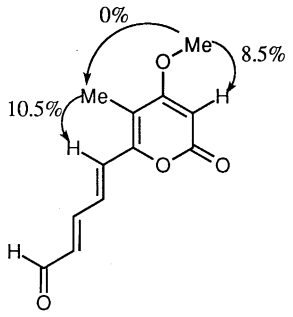
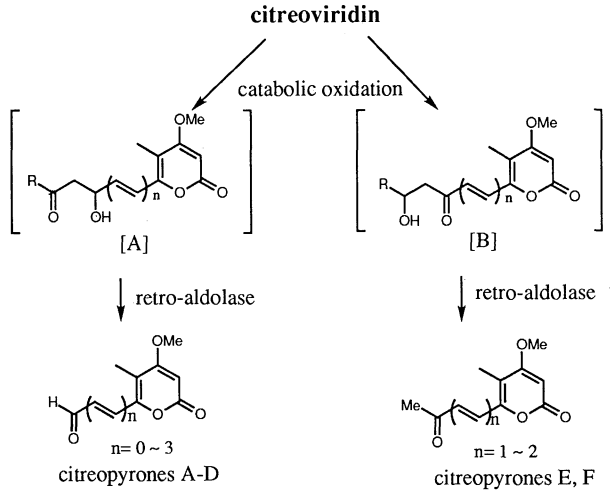


Figure 1. NOE experiments and proposed orientation in C_6D_6 at 35 °C for **1**.

Citreopyrone A (**1**)[4] has a molecular formula of $C_{12}H_{12}O_4$ as determined by the HR-EIMS [*m/z* 220.0708 (M^+), Δ -2.6 mmu] and NMR data in C_6D_6. The presence of an α-pyrone ring in **1** was indicated by the IR spectral data (1720 and 1675 cm^{-1}), which was supported by the observation of the ^{13}C NMR signals at δ 169.0 (s, C3), 161.4 (s, C1), 152.5 (s, C5), 110.8 (s, C4), and 91.0 (d, C2) - all characteristic of an α-pyrone moiety substituted by a methoxy group. This proposed structure was further confirmed by observing HMBC correlations as follows: H2 to C1; H_3CO to C3, $H_3$6 to C3, C4 and C5; H7 to C5, C8 and C9. The ^{1}H NMR signals at δ 9.37 (1H, d, *J*=7.3 Hz), 6.93 (1H, dd, *J*=15.0, 11.4 Hz), 6.30 (1H, dd, *J*=15.4, 11.4 Hz), 6.01 (1H, d, *J*=15.0 Hz), and 5.88 (1H, dd, *J*=15.4, 7.3 Hz) were assigned to the α, β, γ, δ -unsaturated aldehyde. Detailed analysis of the NOE difference experiments in benzene-d_6 at 35 °C of **1** did not allow constraction of a methyl group (δ 1.51) at the β carbon and a methoxy group (δ 2.81) at the γ-carbon on the α-pyrone ring. Irradiation of the methoxy group (δ 2.81) of **1** resulted in 8.5% NOE of the β-proton (δ 5.19), and 0% NOE of the β-methyl group on the α-pyrone ring, irradiation of the methyl group (δ 1.51) of **1** resulted in 10.9% NOE of the olefinic proton (δ 6.01), and 0% NOE of the methoxy group (δ 2.81), indicating that steric repulsion between the methyl and methoxy groups appeared to be an important factor (Figure 1). Eventually, the presence of an α-pyrone ring in **1** was established by



Scheme 1. Mechanism of biosynthesis of simple pyrones.

synthesis.[5] The structures of citreopyrones B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**) were also elucidated on the basis of spectral data.[6-10]

These very simple metabolites are quite interesting from a biogenetic point of view. It is postulated that the mechanism of biosynthesis of these metabolites is a catabolic retro-aldol type reaction. We propose, therefore, that citreoviridin or a similar metabolite is initially oxidized to [A] or [B] type compound which is further subjected to a retro-aldol reaction catalyzed by retro-aldolase to give citreopyrones A-F (Scheme 1).

Detailed biosynthetic studies on citreopyrones A-F (**1-6**) and investigation of the biological activities of citreopyrones A-F (**1-6**) and related synthetic compounds are in progress, and the results will be reported in due course.

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References and Notes

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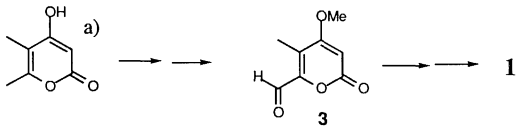
4 Citreopyrone A (**1**) as a yellow powder: $C_{12}H_{12}O_4$ [*m/z* 220.0708 (M^+)]; IR(film) 1720 and 1675 cm^{-1}; ^{1}H-NMR (C_6D_6): δ 9.37 (1H, d, *J*=7.3 Hz), 6.93 (1H, dd, *J*=15.0, 11.4 Hz), 6.30 (1H, dd, *J*=15.4, 11.4 Hz), 6.01 (1H, d, *J*=15.0 Hz), 5.88 (1H, dd, *J*=15.4, 7.3 Hz), 5.19 (1H, s), 2.81 (3H, s) and 1.51 (3H, s). ^{13}C-NMR (C_6D_6): δ 191.9 (d, C11), 169.0 (s, C3), 161.4 (s, C1), 152.5 (s, C5), 148.0 (d, C9), 134.7 (d, C10), 132.1 (d, C8), 127.2 (d, C7), 110.8 (s, C4), 91.0 (d, C2), 55.3 (q, C3-OMe), and 8.84 (q, C6).

5 

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6 Citreopyrone B (**2**) as a yellow powder: $C_{10}H_{10}O_4$ [*m/z* 194.0609 (M^+)]; IR(film) 1725 and 1675 cm^{-1}; ^{1}H-NMR (C_6D_6): δ 9.25 (1H, d, *J*=7.0 Hz), 6.78 (1H, dd, *J*=15.4, 7.0 Hz), 6.42 (1H, d, *J*=15.4 Hz), 5.14 (1H, s), 2.76 (3H, s) and 1.36 (3H, s). ^{13}C-NMR (C_6D_6): δ 191.3 (d, C9), 168.5 (s, C3), 161.1 (s, C1), 151.1 (s, C5), 135.6 (d, C7), 132.2 (d, C8), 114.1 (s, C4), 92.2 (d, C2), 55.5 (q, C3-OMe), and 9.0 (q, C6).

7 Citreopyrone C (**3**) as a yellowish powder: $C_8H_8O_4$ [*m/z* 168.0422 (M^+)]; IR(film) 1740 and 1700 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 9.82 (1H, s), 5.77 (1H, s), 3.90 (3H, s) and 2.31 (3H, s).

8 Citreopyrone D (**4**) as a yellow powder: $C_{15}H_{16}O_4$ [*m/z* 260.1051 (M^+)]; IR(film) 1710 and 1690 cm^{-1}; ^{1}H-NMR (CD_3OD): δ 7.34 (1H, dd, *J*=15.7, 11.4 Hz), 7.16 (1H, dd, *J*=15.0, 10.6 Hz), 6.92 (1H, dd, *J*=14.7, 10.6 Hz), 6.80 (1H, d, *J*=15.0 Hz), 6.72 (1H, dd, *J*=14.7, 11.4 Hz), 6.28 (1H, d, *J*=15.7 Hz), 5.66 (1H, s), 3.90 (3H, s), 2.29 (3H, s) and 2.02 (3H, s).

9 Citreopyrone E (**5**) as a yellow powder: $C_{13}H_{14}O_4$ [*m/z* 234.0881 (M^+)]; IR(film) 1710 and 1690 cm^{-1}; ^{1}H-NMR (CD_3OD): δ 7.42 (1H, dd, *J*=15.5, 10.6 Hz), 7.16 (1H, dd, *J*=15.0, 10.6 Hz), 7.06 (1H, d, *J*=15.0 Hz), 6.40 (1H, d, *J*=15.5 Hz), 5.70 (1H, s), 3.91 (3H, s), 2.32 (3H, s) and 2.02 (3H, s).

10 Citreopyrone F (**6**) as a yellowish powder: $C_{11}H_{12}O_4$ [*m/z* 208.0724 (M^+)]; IR(film) 1715 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.36 (1H, d, *J*=15.3Hz), 7.06 (1H, d, *J*=15.3 Hz), 5.04 (1H, s), 3.87 (3H, s), 2.36 (3H, s) and 2.08 (3H, s).