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Citreoazopyrone, a Novel Metabolite of a Hybrid Strain KO 0011 Derived from *Penicillium citreo-viride* B. IFO 6200 and 4692

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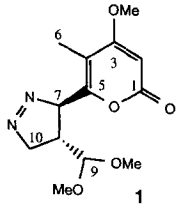
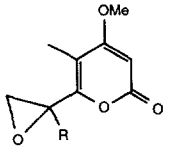
Abstract : A novel metabolite, citreoazopyrone, has been isolated from the mycelium of a hybrid strain KO 0011 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Its stereostructure has also been elucidated on the basis of its spectral data and some chemical evidence. It inhibited the growth of hypocotyls of lettuce seedlings. © 1997 Elsevier Science Ltd.

In a series of experiments, we have achieved 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 present study, the hybrid strain KO 0011 produced the first novel metabolite with an azo functional group named citreoazopyrone **1**[3] in addition to two simple pyrones (**2**, **3**).[4, 5]

According to essentially the same procedure as described in the previous papers,[2] the EtOAc extract (142 g) was chromatographed on silica gel using a gradient solvent of MeOH-$CHCl_3$(1~50%). Elution with $CHCl_3$-MeOH(20:1) afforded a pale yellow powder, which was further separated by preparative TLC using hexane-acetone (4:3) to afford a citreoazopyrone **1** in 0.011% yield, in addition to some simple pyrones. Inhibitory activity of citreoazopyrone towards the germination of lettuce seedlings was not observed. However, the growth of hypocotyls of lettuce seedlings was inhibited 83.5%, relative to the control, by this compound at 12.5 μg/cm^2.



1



2: R= H
3: R= Me

Citreoazopyrone **1** has a molecular formula of $C_{13}H_{18}N_2O_5$ as determined by the HR-EIMS [*m/z* 282.1209 (M^+), Δ -0.4 mmu] in conjunction with ^1H and ^{13}C NMR data. The presence of an azo functional group in **1** was confirmed by the fragment ion 254 (M^+-N_2) in the EI mass spectrum. The presence of an α-pyrone ring in **1** was indicated by the IR spectral data (1715 and 1650 cm^{-1}), which was supported by the observation of the ^{13}C NMR signals at δ ($CDCl_3$) 170.4 (s, C3), 163.3 (s, C1), 153.5 (s, C5), 110.1 (s, C4), and 89.3 (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 C3 and C4; Me (δ 2.15, C6) to C3, C4 and C5; $H_β$10 to C9; MeO (δ 3.83) to C3; MeO (δ 3.29) to C9. The structure of **1** was based

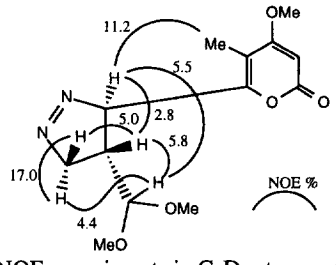


Figure 1. NOE experiments in C_6D_6 at room temp. for **1**

on its spectral data and NOE experiments (Figure 1). Eventually, acetal formation and the presence of an azo functional group in **1** was established by synthesis, which was derived from synthetic citreopyrone B(**4**).[6] The reaction of **4** with an excess of trimethylsilyldiazomethane[7] in benzene-MeOH (1:1) at room temperature for 12 h produced citreoazopyrone **1** in less than 10% yield, but aldehyde, which was consumed in the reaction, was not detected at C9 in **1**. Based on this result, it is deduced an azoaldehyde is easily converted to **1** and a true metabolite must be an aldehyde at C9 in **1**.

Biological studies on citreoazopyrone and related synthetic compounds are in progress, and the results will be reported in due course.

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3. Physical data for citreoazopyrone: a colorless oil; $[\alpha]_D^{25}$ -0.1° (c = 0.5, $CHCl_3$); $C_{13}H_{18}N_2O_5$ [*m/z* 282.1209 (M^+)]; IR(film) 1715, 1650, and 1570 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.59 (1H, ddd, J= 5.13, 2.56, 1.10, H-7), 5.47 (1H, s, H-2), 4.80 (1H, ddd, J= 18.30, 9.33, 2.56, Hβ-10), 4.54 (1H, ddd, J= 18.30, 4.94, 1.10, Hα-10), 4.13 (1H, d, J= 6.23, H-9), 3.83 (3H, s, 3-OMe), 3.29 (6H, s, 9-$(OMe)_2$), 2.65 (1H, dddd, J= 9.33, 6.23, 5.13, 4.94, H-8), and 2.15 (3H, s); ^{13}C-NMR ($CDCl_3$) δ 170.4 (s, C-3), 163.3 (s, C-1), 153.5 (s, C-5), 110.1 (s, C-4), 104.7 (d, C-9), 89.3 (d, C-2), 87.2 (d, C-7), 79.7 (t, C-10), 56.3 (q, 3-OMe), 54.9 (q, 9-OMe), and 53.9 (q, 9-OMe), 38.4 (d, C-8), and 9.4 (q, C-6).
4. Physical data for **2**: 0.0025%; a colorless powder; $C_9H_{10}O_4$ [*m/z* 182.0576 (M^+)]; ^{1}H-NMR ($CDCl_3$) δ 5.52 (1H, s), 3.86 (1H, dd, J= 4.2, 2.4 Hz), 3.85 (3H, s), 3.36 (1H, dd, J= 5.7, 2.4 Hz), 3.06 (1H, dd, J= 5.7, 4.2 Hz), and 2.06 (3H, s).
5. Physical data for **3**: 0.0015%; a colorless powder; $C_{10}H_{12}O_4$ [*m/z* 196.0736 (M^+)]; ^{1}H-NMR ($CDCl_3$) δ 5.50 (1H, s), 3.85 (3H, s), 2.97 (1H, d, J= 5.6 Hz), 2.85 (1H, d, J= 5.6 Hz), 2.00 (3H, s), and 1.61 (3H, s).
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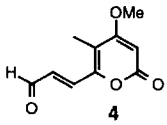

4

Citreopyrone B (**4**) was isolated from the mycelium of a hybrid strain KO 0092 derived from *P. Citreo-viride B.* IFO 6200 and 4692.

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