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Two new metabolites of hybrid strains KO 0201 and 0211 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692

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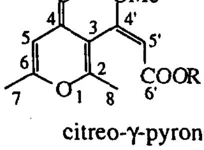
Abstract

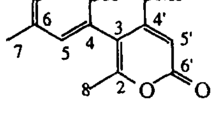
Two new metabolites, citreo-γ-pyrone and citreothiopyrane A, with interesting biological activity have been isolated from the mycelia of hybrid strains KO 0201 and 0211 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Their structures have been elucidated based on their spectral data. In addition, their biological properties are also described. The structure of citreothiolactone previously reported has been revised. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: natural products; *Penicillium citreo-viride*; pyrones; thiopyranes; polyketides.

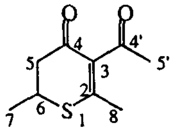
In the series of our experiments, we have already obtained a number of new metabolites of hybrid strains, which were prepared by a cell fusion technique using two different strains, *Penicillium citreo-viride* IFO 6200 and 4692. These new metabolites had a variety of novel structures different from those of the two parent strains (*P. citreo-viride* IFO 6200[1] and 4692[2]).[3] We herein describe the isolation and structural determination of new metabolites from the mycelia of hybrid strains KO 0201 and KO 0211, together with their biological properties.



citreo-γ-pyrone (1) R=H
(3) R=Me



1'



citreothiopyrane A (2)

According to essentially the same procedure as reported in previous papers,[3] two hybrid strains KO 0201 and KO 0211 were prepared by a cell fusion technique using *P. citreo-viride* IFO 6200 and 4692, and then incubated stationarily on rice medium at room temperature for 2 months, respectively; each acetone extract was then partitioned between water and EtOAc.

In the case of KO 0201, the EtOAc extract (93.3 g) was chromatographed on silica gel using a gradient solvent of EtOAc–hexane (50–100%). Elution with EtOAc (100%) gave a brown oil, which was further

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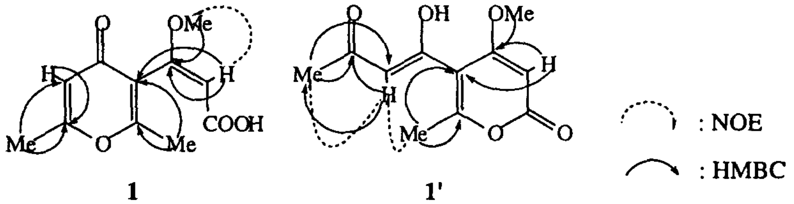
Table 1
The spectral data of citreo-γ-pyrone (**1**) and **1′**, methyl ester of **1** (**3**), and citreopyrone (**6**)

	3		**1**		**1'**		**6**	
Cno	C	H	C	H	C	H	C	H
2	163.6		164.0		162.3		162.7	
3	122.5		122.4		112.0		113.7	
4	176.7		177.0		182.1		190.0	
5	113.7	6.10	113.6	6.11(1H, s)	103.8	5.64(1H, s)	132.7	6.30
6	164.9		163.0		191.4		146.4	6.79
7	19.7	2.20	19.7	2.23(3H, s)	25.0	2.12(3H, s)	18.4	1.97
8	18.0	2.17	18.7	2.18(3H, s	18.1	2.29(3H, s)	18.2	2.17
4'	164.6		165.3		168.4		132.7	
5'	95.8	5.41	95.7	5.40(1H, s)	87.7	5.46(1H, s)	87.5	5.47
6'	166.4		169.3		162.9		161.0	
7'	56.4	3.76	56.5	3.77(3H, s)	56.4	3.80(3H, s)	56.2	3.81
COOMe	51.0	3.59						

^{13}C-NMR (100 MHz), ^{1}H-NMR (400 MHz) δ/ppm in $CDCl_3$.



1 **1'**

Figure 1. NOE and HMBC of citreo-γ-pyrone (**1**) and its structural isomer (**1′**)

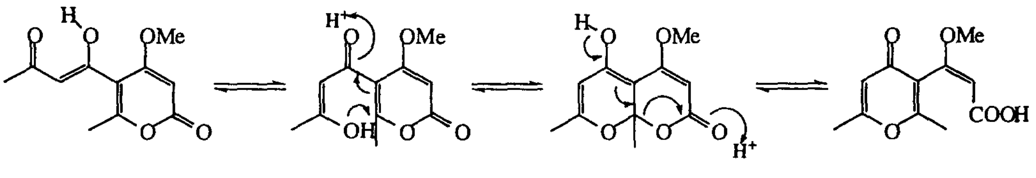
separated using a gradient solvent of MeOH–$CHCl_3$ (3–100%). Elution with MeOH (100%) gave a yellow oil, which was further separated by preparatative TLC [EtOAc:hexane (2:1)] to afford a new metabolite, citreo-γ-pyrone (**1**) as a colorless oil (4.1 mg).

Citreo-γ-pyrone (**1**) has a molecular formula $C_{11}H_{12}O_5$ [HR-EIMS calcd for $C_{11}H_{12}O_5$: 224.0683, found 224.0663], and spectral data IR/cm^{-1} 3400, 1710, 1690, 1660, 1580, 1220. UV max ($CHCl_3$): 230 nm (ε9100). Its NMR spectral data are shown in Table 1, where each NMR signal is reasonably assigned.

Finally, the structure of **1** was determined based on HMQC, HMBC and NOE experiments, as shown in Fig. 1.

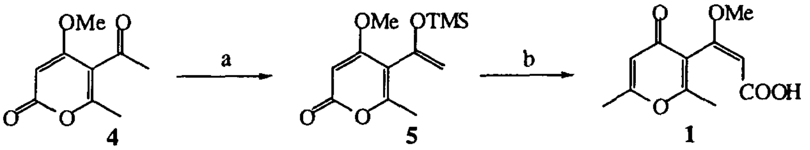
Interestingly, the NMR spectrum of **1** in $CDCl_3$ indicated that **1** was gradually converted to an α-pyrone (**1′**), whose structure was also determined based on HMQC, HMBC and NOE experiments (Table 1 and Fig. 1). The relative ratio of **1**:**1′** was 3:2 the first week, and changed to 1:2 in two weeks. On treatment with trimethylsilyl diazomethane, this mixture (**1**:**1′**=1:2) was converted into citreo-γ-pyrone methyl ester (**3**) as the sole product.

The above experiments indicate that both the γ-pyrone (**1**) and α-pyrone (**1′**) coexist in equilibrium, as shown in Scheme 1.

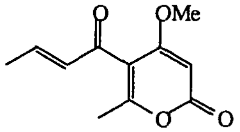


Scheme 1. Equilibrium between **1** and **1′**

In the light of Scheme 1, citreo-γ-pyrone (**1**) was successfully synthesized starting from the known pyrone (**4**) through **5**, as shown in Scheme 2.[5]



Scheme 2. Synthesis of citreo-γ-pyrone (**1**). (a) Et_3SiCl, Et_3N and DABCO in CH_2Cl_2, reflux for 6 h (82%); (b) CH_3COCl and $TiCl_4$ in CH_2Cl_2 at −78°C for 10 min, then H_2O for 30 min at 0°C [23% and **4** (67%)]



citreopyrone(**6**)

In connection with citreopyrone (**6**), a plant growth inhibitor,[4] **1** was expected to show growth inhibitory activity. However, **1** was proved to be a growth-promoting substance. Lettuce seeds germinated in 12 h, in 1 ml water including 0.05% Tween 20, were incubated under white light (10 W). On addition of **1** (1×10^{-3} M), the length of hypocotyl was 4.56±0.13 mm (the control: 3.94±0.13).

In the case of KO 0211, the EtOAc extract (54.8 g) was chromatographed on silica gel using a gradient solvent of EtOAc–hexane (50–100%). Elution with EtOAc:hexane (1:1) gave a yellow oil, which was further separated by using a gradient solvent of EtOAc–hexane (25–100%). Elution with EtOAc:hexane (1:4) gave a yellow oil, which was further separated by repeated preparative TLC using EtOAc:hexane (1:1), and then acetone:hexane (1:1) to afford a new metabolite, citreothiopyrane (**2**) as a colorless oil (11.3 mg): HR-EIMS calcd for $C_9H_{10}O_2S$: 184.0558, found 184.0575. IR cm^{-1} 1700, 1660, 1555, 1435, 1430, 1380, 1360, 1320. $[\alpha]_D^{22}$ +36.3 (c 1.00, in $CHCl_3$). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ/ppm: 201.9 (C-4′), 192.9 (C-4), 161.5 (C-2), 134.5 (C-3), 45.3 (C-5), 36.6 (C-6), 31.7 (C-5′), 22.3 (C-8), 19.5 (C-7). ^1H NMR (270 MHz, $CDCl_3$) δ/ppm: 3.53 (1H, m, C-6), 2.77 (1H, dd, J=9.6, 15.8 Hz, C-5), 2.56 (1H, dd, J=14.1, 15.8 Hz, C-5), 2.36 (3H, s, C-5′), 2.16 (3H, s, C-8), 1.39 (3H, d, J=6.9 Hz, C-7).

The partial structure (C-5~C-7) of **2** was elucidated based on proton homodecoupling experiments. HMBC signals were observed between C-4 and C-5 and between C-8 and both C-2 and C-3. The position of the sulfur atom in **2** was determined by ^{13}C NMR signals of C-2 and C-6. Finally, the acetyl group (C-4′ and C-5′) must be bounded to C-3.

From a biological point of view, the bioassay of **2** was carried out as follows: lettuce seeds germinated in 12 h under white light, in 1 ml water including 0.05% Tween 20, were incubated with **2** under dark conditions at 25°C for 3 h, and the result, as shown in Fig. 2, indicates that **2** is a plant growth inhibitor.

In a previous paper,[6] we reported the isolation and structural determination of citreothiolactone (**7**) found in the culture filtrate of *P. citreo-viride* IFO 6200, as shown in Fig. 3. However, its structure was revised to be **9** renamed citreothiopyrane B, because the spectral data of citreothiopyrane A (**2**) was identical with those of the product (**8**) obtained by treatment of **7** with $AlCl_3$.[6] When treated with $Na_2S\cdot9H_2O$, citreopyrone (**6**) was readily converted into citreothiopyrane B (**9**), as shown in Scheme 3.

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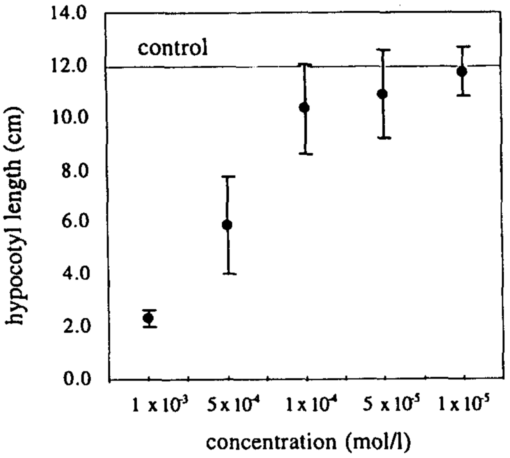
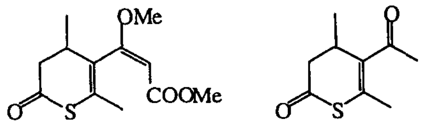
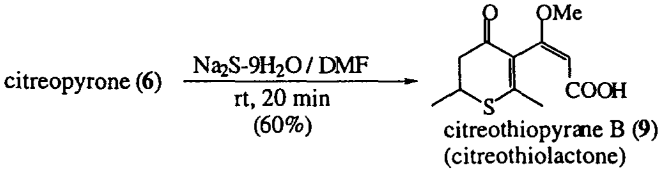


Figure 2. Bioassay of compound **2**



citreothiolactone (**7**) **8**

Figure 3. Previous structure of citreothiolactone



Scheme 3. Synthesis of citreothiopyrane A (**9**) from citreopyrone (**6**)

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