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Metabolites from *Penicillium citreo-viride* B. IFO 4692 and 6200 Hybrid

Seiji KOSEMURA

Summary —— Several interesting metabolites have been isolated from the mycelium of two hybrid strains KO 0011 and KO 0092 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692, and a hybrid strain KO 0141 derived from *P. citreo-viride* B. IFO 4692 and *P. pedemontanum* IFO 9583. Their stereostructures have been elucidated on the basis of their spectral data and some chemical evidence.

Key words: *Penicillium citreo-viride* B., hybrid strain, metabolite, isolation, stereostructure

Introduction

In connection with citreoviridin (**1**), 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 *Penicillium citreo-viride* B. Particularly, citreoviridin (**1**) and related pyrones have been mainly produced by *P. citreo-viride* B. IFO 6200 (Figure 1).[1)-4)] In the case of another strain IFO 4692, however, citreoviranol (**3**) and related phenols have been obtained as main products (Figure 1).[5)] We have achieved more than ten hybrid strains by means of cell fusion technique using two different strains *P. citreo-viride* B. IFO 6200 and 4692. Some of these hybrid strains produced a number of new interesting metabolites, which have not been previously detected in the mycelium of either parent strain. We have been reported the isolation and structure elucidation of twenty high potent antifeeding meroterpenoids (mixed polyketide-terpenoid) against the diamondback moth (*Plutella xylostella*),[6)] citreohybridones A-G (**6-12**), J-L (**13-19**), isocitreohybridones A-C

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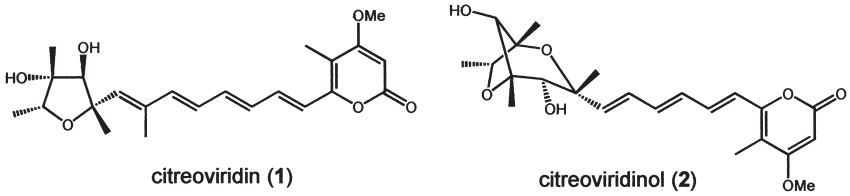
(16-18), G-I (19-21), citreohybriddiones A-C (22-24), citreohybridonol (25) produced by the hybrid strains KO 0031[7)-16)] (Figure 2, 3 and 4), and their absolute configurations.[14),15)] Biosynthetic studies on citreohybridones have been also reported (Scheme 1 and 2).[10),11),13),15)] Three sesquiterpenoid-type metabolites, citreobenzofurans A-C (26-28) have been isolated from the same mycelium of the hybrid strain KO 0031(Figure 5).[9),15)]

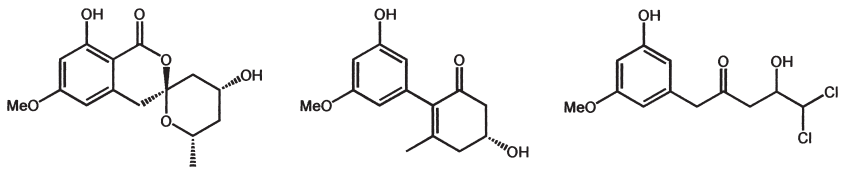
In this communication we wish to report the isolation and structure elucidation of several interesting metabolites produced by three hybrid strains KO 0011, KO 0092 and KO 0141 (IFO 4692 and IFO 9583).

From *P. citreo-viride* B. IFO 6200



citreoviridin (1)　　citreoviridinol (2)

From *P. citreo-viride* B. IFO 4692



citreoviranol (3)　　precitreobiphenyl (4)　　citreovirone (5)

Fig.1

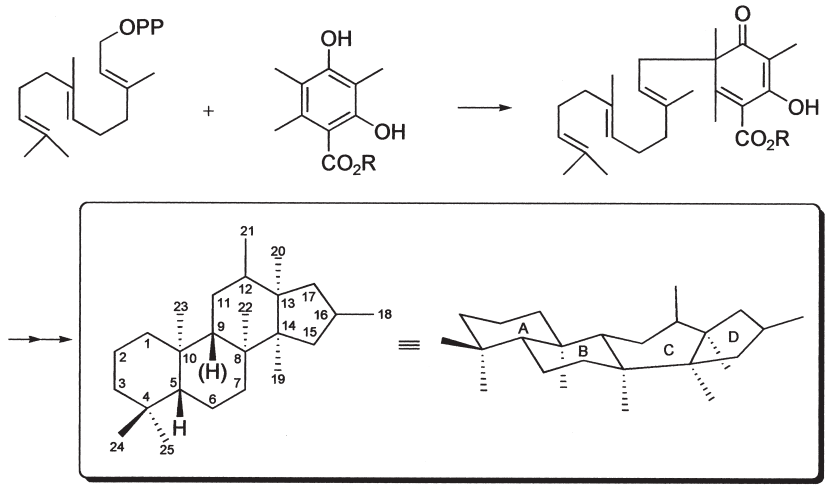


Fig.2　Carbon skeleton for citreohybridones

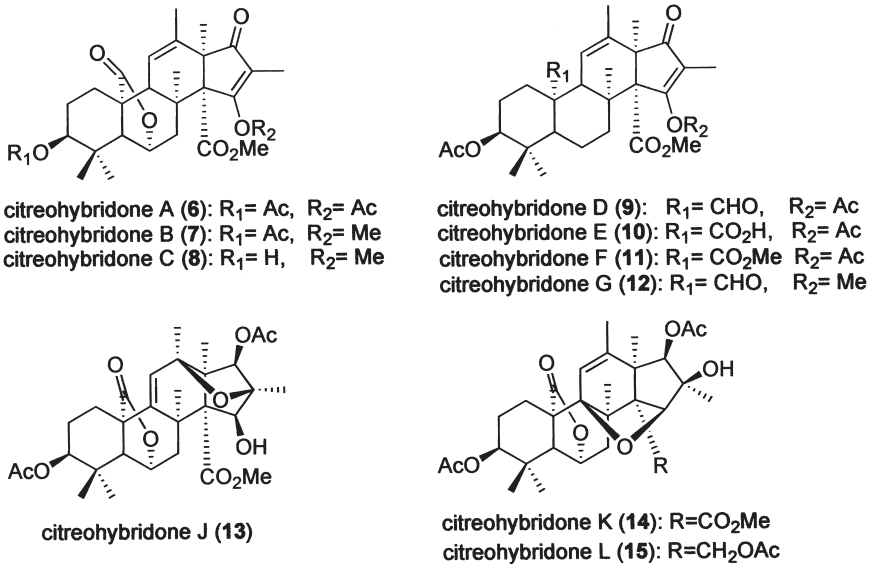


Fig.3 Structures of citreohybridones A-G (6-12) and J-K (13-15).

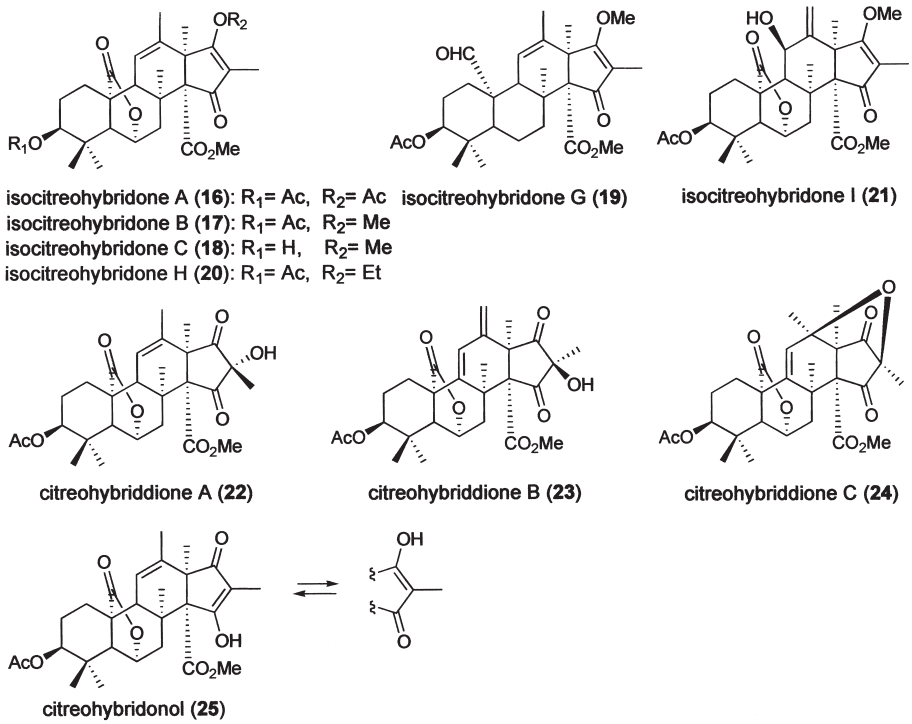
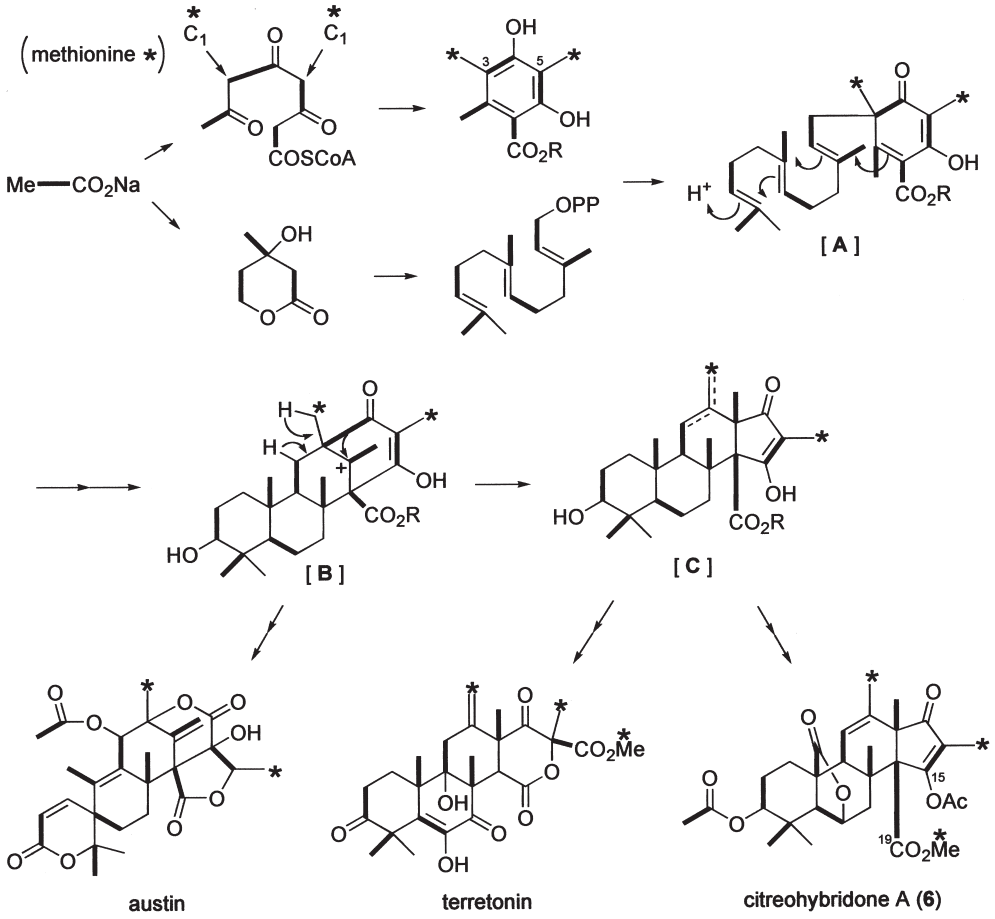
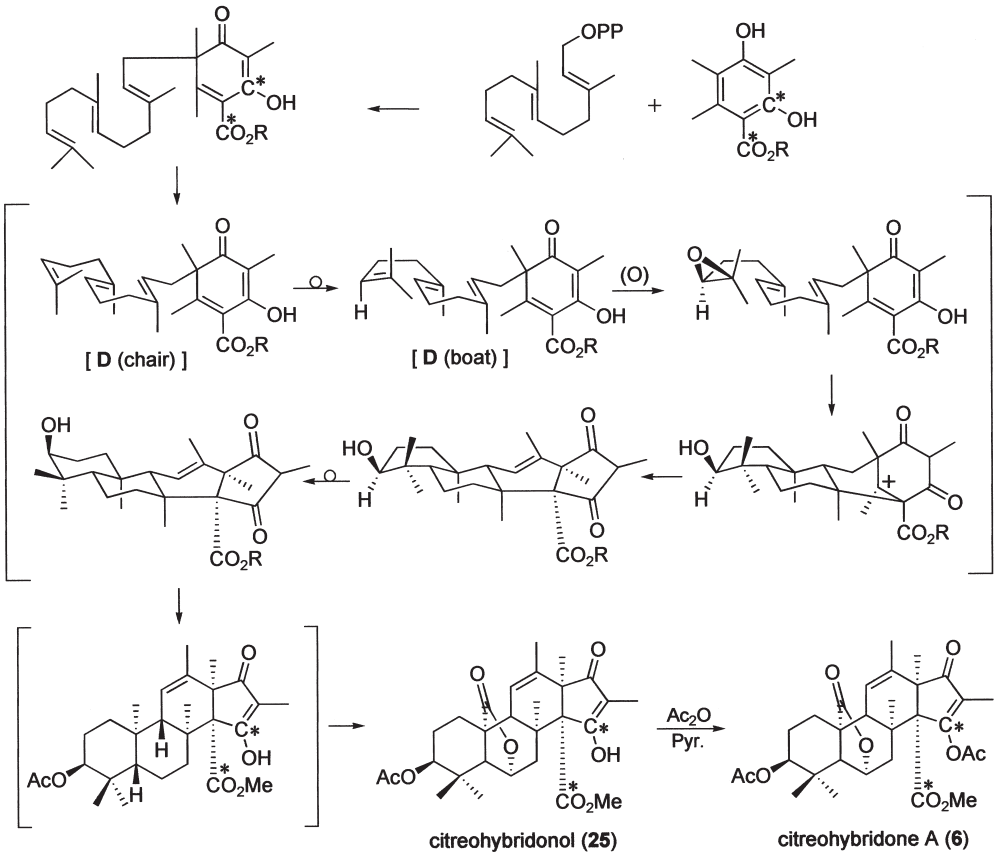


Fig.4 Structures of isocitreohybridones A-C (16-18), G-I (19-21), citreohybriddiones A-C (22-24), and citreohybridonol (25).



Scheme 1　Proposed biosynthetic pathway for meroterpenoids.



Scheme 2 Intact incorporation of ethyl [carboxy-6-$^{13}C_2$]-3, 5-dimethylorsellinate and proposed mechanism for the formation of the axial hydroxyl group on A-ring of citreohybridones.

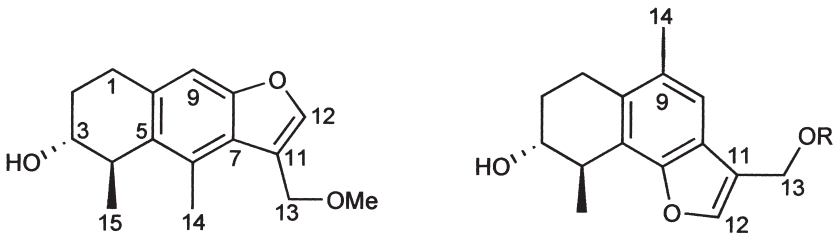


Fig.5 Structures of citreobenzofurans A-C (26-28).

Results and discussion

Metabolites of a hybrid strain KO 0011 (*P. citreo-viride* B. IFO 4692 and 6200)

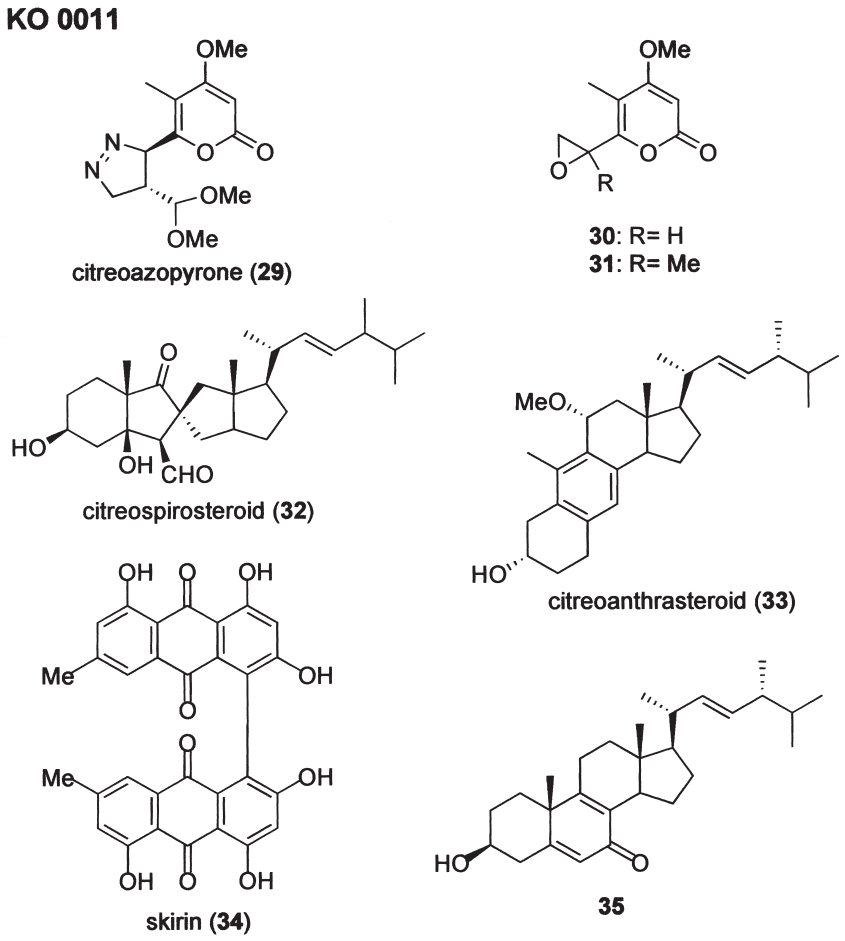


Fig.6 Metabolites of a hybrid strain KO 0011 (*P. citreo-viride* B. IFO 4692 and 6200)

Citreoazopyrone (**29**)[17] has a molecular formula of $C_{13}H_{18}N_2O_5$ as determined by the HR-EIMS [*m/z* 282.1209 (M^+), Δ−0.4mmu] in conjunction with ^1H and ^{13}C NMR data. The presence of an azo functional group in **29** was confirmed by the fragment ion 254 (M^+-N_2) in the EI mass spectrum. The presence of an α-pyrone ring in **29** was confirmed by the IR spectral data (1715 and 1650 cm^{-1}), which was supported by the observation of the ^{13}C NMR signals at δ_C ($CDCl_3$) 170.4 (s, C-3), 163.3 (s, C-1), 153.5 (s, C-5), 110.1 (s, C-4), and 89.3 (d, C-2)—all characteristic of an α-pyrone moiety substituted by a methoxy group. This

proposed structure was further confirmed by observing HMBC correlations as follows: H-2 to C-3 and C-4; Me (δ_H 2.15, C-6) to C-3, C-4 and C-5; H_β-10 to C-9; MeO (δ_H 3.83) to C-3; MeO (δ_H 3.29) to C-9 (Figure 7). The structure of **29** was based on its spectral data and NOE experiments (Figure 8). Eventually, acetal formation and the presence of an azo functional group in **29** was established by synthesis, which was derived from synthetic citreopyrone B (**39**).[18] The reaction of **39** with an excess of trimethylsilyldiazomethane[19] in benzene-MeOH (1:1) at room temperature for 12 h produced citreoazopyrone (**29**) in less than 10% yield, but aldehyde, which was consumed in the reaction, was not detected at C-9 in **29**. Based on this result, it is deduced an azoaldehyde is easily converted to **29**.

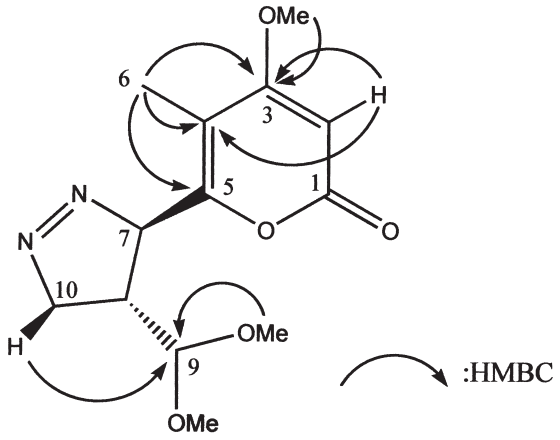


Fig.7 Significant HMBC correlation for citreoazopyrone (**29**)

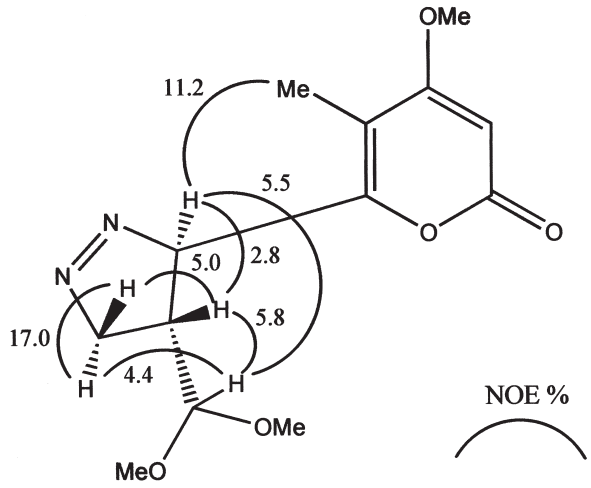


Fig.8 NOE experiments in C_6D_6 at room temp. for citreoazopyrone (**29**)

Citreospirosteroid (**32**)[20] was obtained as a colorless powder; $[\alpha]_D^{24}$+29.3° (c 0.3, $CHCl_3$) and analyzed for $C_{28}H_{44}O_4$ by HR-EIMS [*m/z* 444.3236 (M^+), Δ−0.1mmu]. The IR absorptions at 3350 and 1735 and 1700cm^{-1} suggested the presence of hydroxyl group(s), five membered ketone (δ_C 227.7) and aldehyde (δ_C 208.7), respectively. The ^1H NMR spectrum of **32** showed the presence of an aldehyde proton (δ_H 10.1), one *trans* olefin, and six methyl groups of which four were secondary (δ_H 0.93, 0.92, 0.84, and 0.82), two were tertiary (δ_H 1.09 and 0.60). The ^{13}C NMR spectrum showed the presence of 28 carbons including two oxygenated carbons [a methine (δ_C 68.0, C-3) and a tertiary (δ_C 83.3, C-5)]. The gross structure of **32** was determined by detailed analyses of one and two dimentional NMR spectra. Especially, the spiro ring system of B/C rings was confirmed by observing HMBC correlations as follow: H-6 to C-7, C-8, C-11 and C-14; H-7 to C-6; H_β-11 to C-6, C-8, C-9 and C-12; H-14 to C-6, C-8, C-9, C-13, C-15 and C-18; H_3-19 to C-1, C-5, C-9 and C-10 (Figure 9). The relative stereochemistry of **32** was clarified by the NOE difference spectra (Figure 10). These NOE results suggested that A/B ring junction should be fused *cis*, because of NOE enhancements between H-3 and H-6. Furthermore, as can be seen in Figure 10, NOE enhancements between H-7 and H_β-11, between CH_3-18 and H-7, and between CH_3-18 and H-6 were observed. Thus, the relative structure of citreospirosteroid (**32**) including the side chain is clearly established as depicted in the formula.

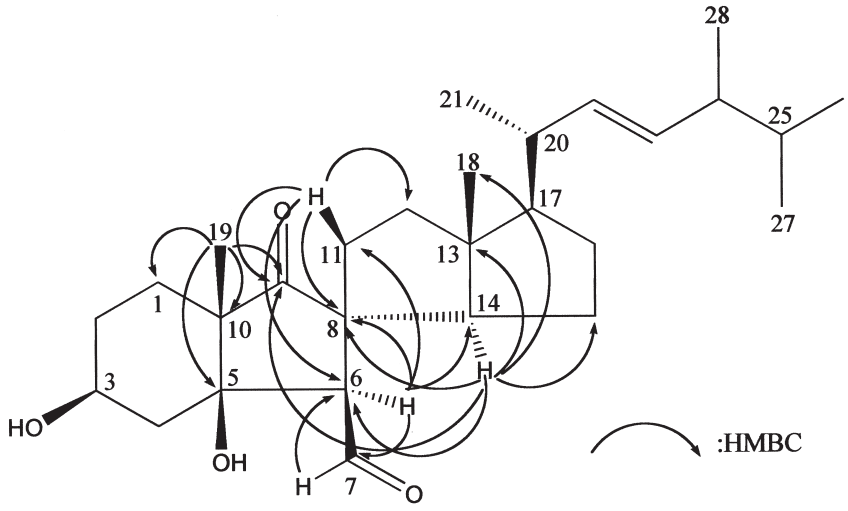


Fig.9 Significant HMBC correlations for citreospirosteroid (**32**)

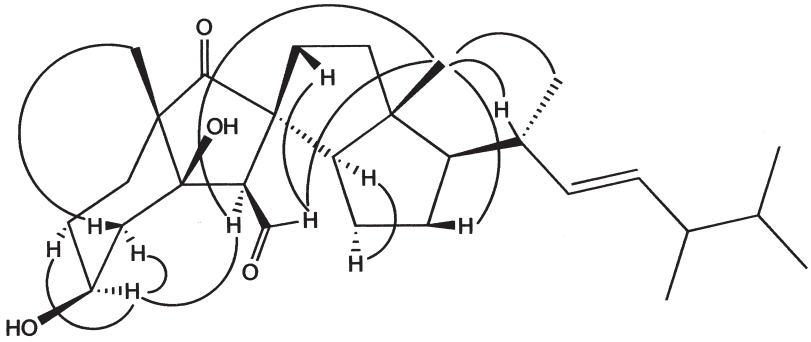
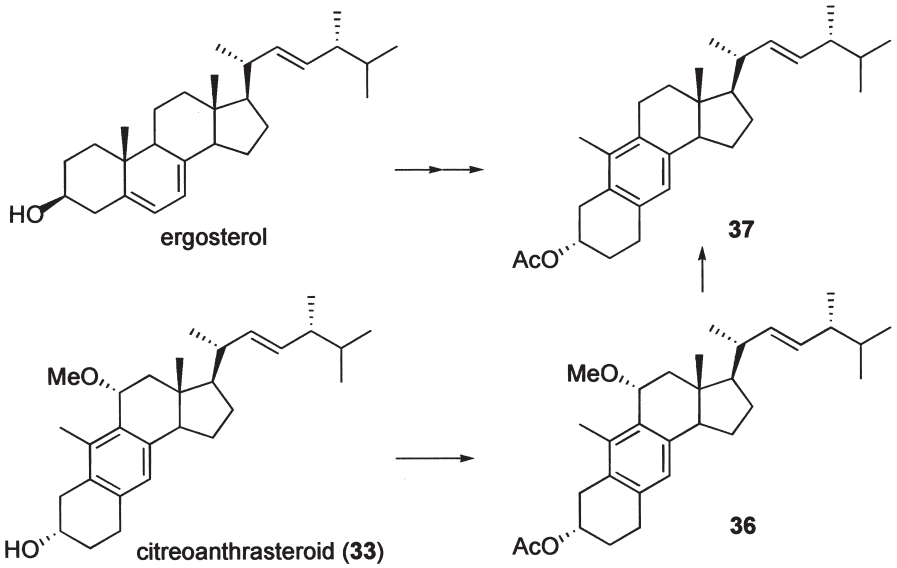


Fig.10 NOE experiments in CD_3OD at room temp. for **32**

Citreoanthrasteroid (**33**)[21] was obtained as a colorless oil; $[\alpha]_D^{22}-0.28^o$ (c 1.31, $CHCl_3$) and analyzed for $C_{29}H_{44}O_2$ by HR-EIMS [*m/z* 424.3304 (M^+), $\Delta-3.4$mmu]. The UV spectrum exhibited the presence of an aromatic ring λ_{max}MeOH 272, ε 300). The IR absorptions at 3400 cm^{-1} suggested the presence of a hydroxyl group. The ^1H NMR spectrum of **33** showed the presence of an aromatic proton (δ_H 6.75), one MeO group (δ_H 3.13), and six methyl groups of which four were secondary (δ_H 1.13, 1.01, 0.93 and 0.92), one was tertiary (δ_H 0.48), and one was olefinic (δ_H 2.22). The ^{13}C NMR spectrum revealed the presence of 29 carbon atoms including eight sp^2 carbons and suggested that **33** had an unusual steroid skeleton with an aromatic ring system. The structure of **33** was based on its spectral data and NOE experiments; furthermore, absolute configuration was determined by comparison with a synthetic anthrasteroid (**37**)[22),23),24)] which was derived from ergosterol. After acetylation of **33**, the reaction of **36** with a large excess of trimethylsilyl iodide (TMSI)[25)] in MeCN at room temperature for 24 h produced the deoxygenated product (**37**) in 89% yield (Scheme 3). The spectral (^1H NMR) and physical properties {$[\alpha]_D^{19}-5.4^o$ (c 0.25, EtOH)} of **37** derived from citreoanthrasteroid (**33**) were compatible with those of the synthetic compound {$[\alpha]_D^{20}-20.0^o$ (c 1.27, EtOH)} which was derived from ergosterol. In the present study, the absolute configuration of citreoanthrasteroid was unambiguously determined to be compatible with that of ergosterol. Compound **33** is the second anthracene-type sterol isolated from a natural source.[26)]



Scheme 3

Metabolites of two hybrid strains KO 0092 (*P. citreo-viride* B. IFO 4692 and 6200) and KO 0141 (*P. citreo-viride* B. IFO 4692 and *P. pedemontanum* IFO 9583)

Citreopyrone A (**38**)[18] has a molecular formula of $C_{12}H_{12}O_4$ as determined by the HR-EIMS [m/z 220.0708 (M^+), Δ−2.6mmu] and NMR data in C_6D_6. The presence of an α-pyrone ring in **38** was indicated by the IR spectral data (1720 and 1675cm^{-1}), which was supported by the observation of the ^{13}C NMR signals at δ_C 169.0 (s, C-3), 161.4 (s, C-1), 152.5 (s, C-5), 110.8 (s, C-4), and 91.0 (d, C-2)—all characteristic of an α-pyrone moiety substituted by a methoxy group. This proposed structure was further confirmed by observing HMBC correlations as follows: H-2 to C-1; H_3CO to C-3, H_3-6 to C-3, C-4 and C-5; H-7 to C-5, C-8 and C-9. The ^1H NMR signals at δ_H 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 **38** did not allow construction of a methyl group (δ_H 1.51) at the β carbon and a methoxy group (δ_H 2.81) at the γ-carbon on the α-pyrone ring. Irradiation of the methoxy group (δ_H 2.81) at the γ-carbon of **38** resulted in 8.5% NOE of the β-proton (δ_H 5.19), and 0% NOE of the β-methyl group on the α-pyrone ring, irradiation of the methyl group (δ_H 1.51) of **38** resulted in 10.9% NOE of the olefinic proton (δ_H 6.01), and 0% NOE of the methoxy group (δ_H 2.81), indicating that steric repulsion between the methyl and methoxy groups appeared to be an important factor (Figure 12). Eventually,

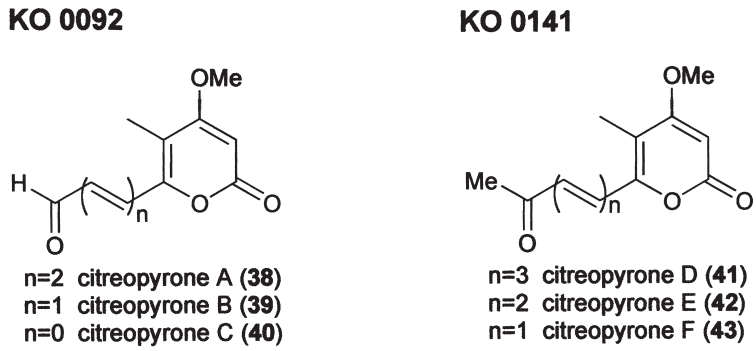


Fig.11 Metabolites of two hybrid strains KO 0092 (*P. citreo-viride* B. IFO 4692 and 6200) and KO 0141 (*P. citreo-viride* B. IFO 4692 and *P. pedemontanum* IFO 9583).

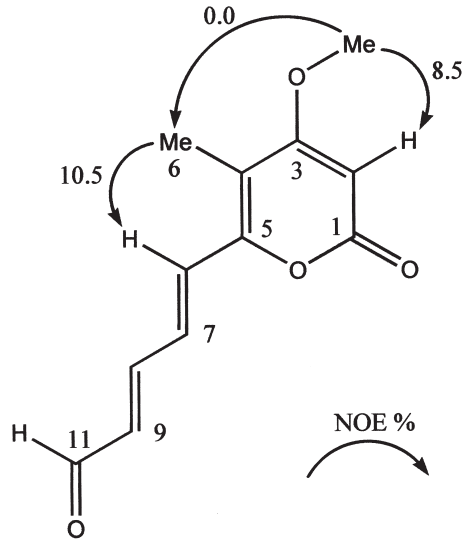
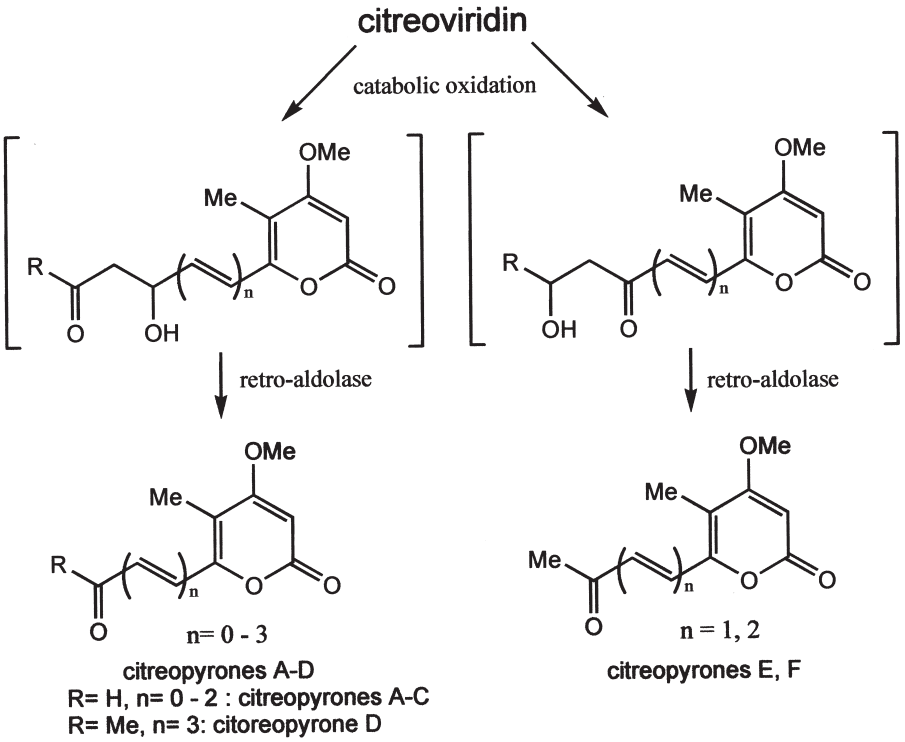


Fig.12 NOE experiments and proposed orientation in C_6D_6 at 35°C for **38**

the presence of an α-pyrone ring in **38** was established by synthesis.[27] The structures of citreopyrones B (**39**), C (**40**), D (**41**), E (**42**), and F (**43**) were also elucidated on the basis of spectral data (shown Experimental).

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 metabolites 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 4).



Scheme 4 Mechanism of biosynthesis of simple pyrones.

Experimental

General.

Melting points were determined on a Mitamura Riken apparatus and uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter and are recorded in units of 10^{-1} deg cm^2g^{-1}. IR spectra were recorded on a JASCO A-202 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer in $CDCl_3$, C_6D_6 or CD_3OD with tetramethylsilane as an internal standard. Coupling constants are give in Hz (s, singlet; d, doublet; t, triplet; q; quartet; m, multiplet), unless otherwise noted. Mass spectra were obtained on a Hitachi M-80 mass spectrometer operating with an ionization energy at 70 eV. Thin layer chromatography was performed using preparative (20 x 20 cm) glass plates coated with a 0.5 mm layer of silica gel (Merck Art. 5744 Kieselgel 60 PF_{254}). UV light of wavelength 254 nm was used to visualize chromatograms.

Cell fusion technique.

Each protoplast corresponding to *Penicillium citreo-viride* B. IFO 6200 and 4692 was prepared

by enzymatic treatment of these two strains, which were incubated on potato sucrose agar (25°C, 7 d), using cellulose, chitinase, pectolyase and sulfatase (30°C, 60 min). And then, these two protoplasts in 0.05 M Ca solution (pH 10.5) were subjected to cell fusion experiments using polyethylene glycol (PEG 6000) as usual and incubated on potato sucrose agar (25°C, 3 d) to give a number of colonies, from which many new hybrid strains including *P. citreo-viride* KO 0011 and KO 0092 were obtained. The hybrid strain KO 0141 was derived from *P. citreo-viride* IFO 4692 and *P. pedemontanum* IFO 9583 in the same manner.

Incubation.

Polished rice (ca. 4.5 Kg) in deionized water (ca. 6 l) was cooked using an electric cooker (99°C, ca. 20 min) and transfered into thirty-five Erlenmyer flasks (3 l), which were pasteurized at 121°C for 20 min at 2.1 atm. After inoculated with a suspension of the mycelium of hybrid strain KO 0011 (KO 0092, KO 0141) in a sterilized water, the rice was incubated stationarily at 25 °C for 60 (30, 30) days and extracted with acetone (160 l).

Isolation and separation.

From KO 0011' rice culture.

The acetone extract suspended in water was extracted with EtOAc. The EtOAc extract (dark brown syrup, 142 g) was chromatographed on silica gel (silica gel 60 K070, 70-230 mesh, Katayama Chemical) using a gradient solvent of MeOH-$CHCl_3$ (1-50%). Elution with $CHCl_3$-MeOH (20:1) afford a yellow powder, which was further separated by preparative TLC using hexane-EtOAc (2:1) to afford citreospirosteroid (**32**; 0.0025%) and the known compound skirin (**34**; 4.2%), the main pigment of the toxic rice fungus *P. islandium*. Further elution with $CHCl_3$-MeOH (24:1) gave a brown oil, which was also separated by preparative TLC using hexane-acetone (3:2) to afford citreoantrasteroid (**33**; 0.007%) and citreoazopyrone (**29**; 0.011%) in addition to some simple pyrones (**30**, **31**) and the known sterol (**35**; 0.015%).

From KO 0092' rice culture.

The acetone extract suspended in water was extracted with EtOAc. The EtOAc extract (dark brown syrup, 45.4 g) was chromatographed on silica gel (silica gel 60 K070, 70-230 mesh, Katayama Chemical) 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, named citreopyrone A (**38**; 0.0022%), B (**39**; 0.0024%), and C (**40;** 0.0011%).

From KO 0141' rice culture.

The acetone extract suspended in water was extracted with EtOAc. The EtOAc extract (dark brown syrup, 14.0 g) was chromatographed on silica gel (silica gel 60 K070, 70-230 mesh, Katayama Chemical) 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, named citreopyrone D (**41**; 0.025%), E (**42**; 0.032%), and F (**43**; 0.0071%).

Physical data for isolated metabolites.

Physical data for citreoazopyrone (29): 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}; ^1H NMR ($CDCl_3$) δ_H 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, H_β -10), 4.54 (1H, ddd, *J*= 18.30, 4.94, 1.10, H_α-10), 4.13 (1H, d, *J*= 6.23, H-9), 3.83 (3H, s, C_3-OMe), 3.29 (6H, s, C_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_C 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, C_3-OMe), 54.9 (q, C_9-OMe), 53.9 (q, C_9-OMe), 38.4 (d, C-8), and 9.4 (q, C-6).

Physical data for 30: a colorless powder: $C_9H_{10}O_4$ [*m/z* 182.0576 (M^+)]; ^1H NMR ($CDCl_3$) δ_H 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).

Physical data for 31: a colorless powder: $C_{10}H_{12}O_4$ [*m/z* 196.0736 (M^+)]; ^1H NMR ($CDCl_3$) δ_H 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).

Physical data for citreospirosteroid (32): a colorless powder: $[\alpha]_D^{24}$ +29.3° (c 0.3, $CHCl_3$); $C_{28}H_{44}O_4$ [*m/z* 444.3236 (M^+)]; IR (film) 3350, 1735, and 1700 cm^{-1}; ^1H NMR (CD_3OD) δ_H 10.1 (1H, d, *J*= 4.8 Hz, H-7), 5.25 (1H, dd, *J*= 15.4, 8.1 Hz, H-23), 5.13 (1H, dd, *J*= 15.4, 8.1 Hz, H-22), 3.73 (1H, m, H-3), 3.00 (1H, ddd, *J*= 14.8, 9.9, 7.7 Hz, H_β-11), 2.78 (1H, d, *J*= 4.8 Hz, H-6), 2.47 (1H, dd, *J*= 13.2, 7.3 Hz, H-14), 2.23 (1H, ddd, *J*= 14.8, 8.8, 1.8 Hz, H_α-11), 2.06 (1H, ddd, *J*= 13.2, 5.1, 1.8 Hz, H_α-4), 2.02 (1H, m, H_α-16), 1.88 (1H, m, H-20), 1.84 (1H, m, H-24), 1.78 (1H, m, H_α-2), 1.63 (1H, m, H_β-16), 1.55-1.15 (9H, complex, $H_{\alpha \text{ and } \beta}$ -1, H_β-2, $H_{\alpha \text{ and } \beta}$ -12, $H_{\alpha \text{ and } \beta}$ -15, H_α-17, H-25), 1.42 (1H, dd, *J*= 13.2, 11.2 Hz, H_β-4), 1.09 (3H, s, H-19), 0.93 (3H, d, *J*= 6.6 Hz, H-21), 0.92 (3H, d, *J*= 6.6 Hz, H-28), 0.84 (3H, d, *J*= 7.1 Hz, H-26 or -27), 0.82 (3H, d, *J*= 7.1 Hz, H-26 or 27), and 0.60 (3H, s, H-18); ^{13}C NMR (CD_3OD) δ_C 227.7 (s, C-9), 208.7 (d, C-7), 135.9 (d, C-22), 134.0 (d, C-23), 83.3 (s, C-5), 68.0 (d, C-3), 67.7 (d, C-14), 56.7 (d, C-6), 54.5 (s, C-13), 53.9 (s, C-8 or -10), 53.7 (s, C-8, or -10), 53.6 (d, C-17), 44.3 (d, C-24), 41.0 (d, C-20), 40.9 (t, C-4), 40.5 (t, C-11), 38.0 (t, C-12), 34.9 (t, C-16), 34.3 (d, C-25), 30.6 (t, C-2), 30.1 (t, C-1), 20.5 (q, C-26 or -27), 20.4 (q, C-26 or -27), 20.1 (q, C-21), 19.6 (t, C-15), 18.2 (q, C-28), 14.5 (q, C-18),

and 14.2 (q, C-19).

Physical data for citreoanthrasteroid (33): a colorless oil; $[\alpha]_D^{22}$ -0.28° (c 1.31, $CHCl_3$); $C_{29}H_{44}O_2$ [*m/z* 424.3304 (M^+)]; UV(λ_{max}^{MeOH}), 272 nm (ε 300); IR (film) 3400 and 1600 cm^{-1}; ^1H NMR (C_6D_6) δ_H 6.75 (1H, s), 5.29 (1H, dd, *J*= 15.1, 7.3 Hz), 5.23 (1H, dd, *J*= 15.1, 7.3 Hz), 4.78 (1H, dd, *J*= 8.1, 5.3 Hz), 3.81 (1H, m), 3.13 (3H, s), 3.06 (1H, dd, *J*= 12.2, 7.3 Hz), 2.83 - 2.77 (2H, complex), 2.62 (1H, m), 2.43 (1H, dd, *J*= 13.2, 8.1 Hz), 2.39 (1H, dd, *J*= 16.6, 8.3 Hz), 2.22 (3H, s), 2.05 (1H, m), 1.97-1.90 (5H, complex), 1.70 (1H, m), 1.62-1.42 (6H, complex), 1.13 (3H, d, *J*= 6.3 Hz), 1.01 (3H, d, *J*= 6.8 Hz), 0.93 (3H, d, *J*= 6.8 Hz), 0.92 (3H, d, *J*= 6.8 Hz), and 0.48 (3H, s); ^{13}C NMR ($CDCl_3$) δ_C 138.9 (s, C-8), 136.3 (s, C-5), 135.4 (d, C-22), 134.8 (s, C-6), 132.2 (d, C-23), 131.4 (s, C-9), 130.5 (s, C-10), 123.5 (d, C-7), 75.1 (d, C-11), 68.1 (d, C-3), 55.9 (d, C-17), 54.6 (q, C_{11}-OMe), 50.2 (d, C-14), 44.8 (s, C-13), 44.3 (t, C-12), 42.9 (d, C-24), 40.5 (d, C-20), 36.5 (t, C-4), 33.1 (d, C-25), 31.1 (t, C-2), 29.0 (t, C-16), 27.5 (t, C-1), 24.0 (t, C-15), 20.9 (q, C-21), 20.0 (q, C-26 or 27), 19.7 (q, C-27 or 26), 17.6 (q, C-28), 15.0 (q, C-19), and 13.4 (q, C-18).

Physical data for 37: $[\alpha]_D^{19}$ -5.4° (c 0.25, EtOH); IR (film) 1735 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$):δ_H 6.65 (1H, s), 5.23 (2H, complex), 3.02 (1H, dd, *J*= 16.5, 5.6 Hz), 2.88-2.80 (2H, complex), 2.76-2.64 (4H, complex), 2.23 (1H, ddd, *J*= 13.2, 7.0, 2.0 Hz), 2.07 (3H, s), 2.05 (3H, s), 2.02 (1H, m), 1.92-1.84 (4H, complex), 1.67 (1H, m), 1.50-1.32 (6H, complex), 1.08 (3H, d, *J*= 6.9 Hz), 0.93 (3H, d, *J*= 6.9 Hz), 0.89 (3H, d, *J*= 6.9 Hz), and 0.59 (3H, s).

Physical data for citreopyrone A (38): a yellow powder; $C_{12}H_{12}O_4$ [*m/z* 220.0708 (M^+)]; IR (film) 1720 and 1675 cm^{-1}; ^1H NMR (C_6D_6) δ_H 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_C 191.9 (d, C-11), 169.0 (s, C-3), 161.4 (s, C-1), 152.5 (s, C-5), 148.0 (d, C-9), 134.7 (d, C-10), 132.1 (d, C-8), 127.2 (d, C-7), 110.8 (s, C-4), 91.0 (d, C-2), 55.3 (q, C_3-OMe), and 8.84 (q, C-6).

Physical data for citreopyrone B (39): a yellow powder; $C_{10}H_{10}O_4$ [*m/z* 194.0609 (M^+)]; IR (film) 1725 and 1675 cm^{-1}; ^1H NMR (C_6D_6) δ_H 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_C 191.3 (d, C-9), 168.5 (s, C-3), 161.1 (s, C-1), 151.1 (s, C-5), 135.6 (d, C-7), 132.2 (d, C-8), 114.1 (s, C-4), 92.2 (d, C-2), 55.5 (q, C_3-OMe), and 9.0 (q, C-6).

Physical data for citreopyrone C (40): a yellowish powder; $C_8H_8O_4$ [*m/z* 168.0422 (M^+)]; IR (film) 1740 and 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 9.82 (1H, s), 5.77 (1H, s), 3.90 (3H, s), and 2.31 (3H, s).

Physical data for citreopyrone D (41): a yellow powder; $C_{15}H_{16}O_4$ [*m/z* 260.1051 (M^+)]; IR (film) 1710 and 1690 cm^{-1}; ^1H NMR (CD_3OD) δ_H 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).

Physical data for citreopyrone E (42): a yellow powder; $C_{13}H_{14}O_4$ [*m/z* 234.0881 (M^+)]; IR (film) 1710 and 1690 cm^{-1}; ^1H NMR (CD_3OD) δ_H 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).

Physical data for citreopyrone F (43): a yellowish powder; $C_{11}H_{12}O_4$ [*m/z* 208.0724 (M^+)]; IR (film) 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 7.36 (1H, d, *J*= 15.3 Hz), 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).

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