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Citreohybriddiones A and B and Related Terpenoids, New Metabolites of a Hybrid Strain KO 0031 Derived from *Penicillium citreo-viride* B. IFO 6200 and 4692

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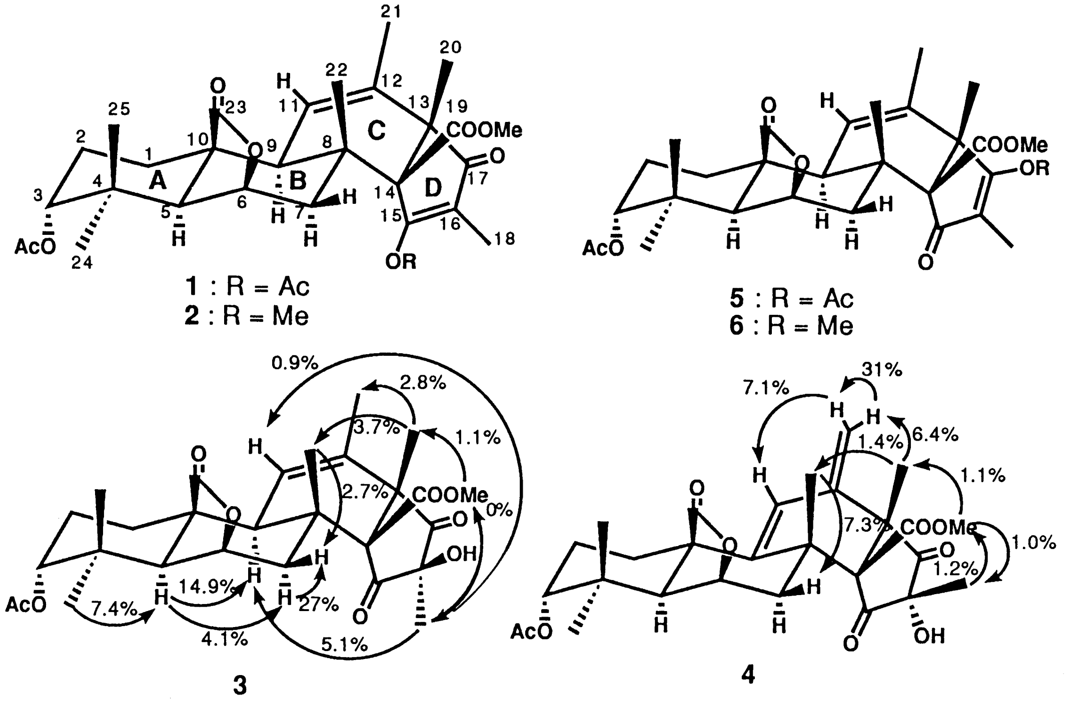
Four new high potent antifeeding substances, citreohybriddiones A and B and isocitreohybridones A and B, have been isolated from the mycelium of a hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Their stereostructures have also been elucidated on the basis of their spectral data and some chemical evidence.

In connection with citreoviridin, citreoviral and related compounds,[1] as reported in the previous paper,[2] more than ten hybrid strains have been produced by means of cell fusion technique [3] using two different strains of IFO 6200 and 4692, and two metabolites, citreohybridones A(**1**) and B(**2**), which have cytotoxicity against HeLa cell, were obtained from the mycelium of the hybrid strain KO 0031.[4] In this communication we wish to report the isolation and structure determination of four new high potent antifeeding terpenoid-type metabolites against *Plutella xylostella*, citreohybriddiones A(**3**) and B(**4**) and isocitreohybridones A(**5**) and B(**6**) produced by the hybrid strain KO 0031, as follows.

According to essentially the same procedure as described in the previous papers,[5] the polished rice (ca. 4.5 kg), which was inoculated with a suspension of mycelium of the hybrid strain KO 0031 in a sterilized water, was incubated stationarily at 25 °C for 30 days and extracted with acetone and then with EtOAc. The combined extracts were partitioned between EtOAc and water. The EtOAc extract (64.2 g) was directly chromatograhed on silica gel (600 g, silica gel 60 K070, 70-230 mesh, Katayama Chemical). After elution of higher fatty acids and their esters with $CHCl_3$, further elution with $CHCl_3$-MeOH(10:1) afforded a pale yellow oil(6.06 g), which was further separated by repeated preparative TLC(Kieselgel PF_{254}) using acetone-$CHCl_3$(1:20-30), acetone-hexane(1:1.5-2.0), and then EtOAc-benzene (1:2-3) to give four new terpenoids, named citreohybriddione A(**3**) and citreohybriddione B(**4**) [**3**; 10.1 mg, **4**; 19.7 mg] and isocitreohybridones A(**5**) and B(**6**) [**5**; 24.1 mg, **6**; 34.1 mg]. The physical data of citreohybriddiones A and B are shown below.

Citreohybriddione A(**3**) as a colorless oil: $[\alpha]_D^{21}$ -142.2° (c 1.00, $CHCl_3$); $C_{28}H_{36}O_9$ [m/z 516.2365(M^+)] ; IR (film) 3500 , 1770 , 1755 , 1735 , and 1240 cm^{-1} ; δ (C_6D_6) 0.70 (3H, s, C_{25}-H_3), 0.73 (3H, s, C_{24}-H_3), 0.98 (1H, ddd, J=13.5, 11.5, 7.2 Hz, C_1-Hα), 1.27 (3H, s, C_{20}-H_3), 1.36 (3H, s, C_{18}-H_3), 1.54 (3H, dd, J= 2.7, 1.3 Hz, C_{21}-H_3), 1.62 (3H, s, C_3-OAc), 1.67 (2H, m, C_2-H_2), 1.69 (3H, s, C_{22}-H_3), 1.89 (1H, s, C_5-H), 2.12 (1H, ddd, J= 13.5, 4.9, 2.3 Hz, C_1-Hβ) , 2.43 (1H, dq, J= 2.7, 2.7 Hz, C_9-Hα), 2.69 (1H, br.s, C_{16}-OH), 2.86 (1H, dd, J= 14.4, 4.3 Hz, C_7-Hβ), 2.95 (3H, s, C_{19}-OMe), 3.12 (1H, d, J= 14.4 Hz, C_7-Hα), 4.40 (1H, d, J= 4.3 Hz, C_6-Hα), 4.65 (1H, dd, J= 2.8, 2.8 Hz, C_3-H), and 5.66 (1H, s, C_{11}-H) ; δ($CDCl_3$) 18.75 (q), 18.93 (q), 20.06 (q), 20.85 (q), 21.25 (t), 22.01 (t), 22.31 (q), 24.49 (q), 26.36 (q), 34.40 (s), 35.16 (t), 39.74 (s), 43.67 (s), 52.53 (q), 53.05 (d), 55.59 (d), 59.40 (s), 74.12 (s), 75.88 (d), 76.24 (s), 77.41 (d), 128.83 (d), 135.37 (s), 167.78 (s), 169.93 (s), 178.25 (s), 210.77 (s), and 212.79 (s).

Citreohybriddione B(**4**) as a colorless oil : $[\alpha]_D^{21}$ +125.8° (c 1.00, $CHCl_3$) ; $C_{28}H_{34}O_9$ [m/z 514.2201 (M^+)] ; IR(film) 3480, 1775, 1755, 1735, and 1245 cm^{-1} ; $\delta(C_6D_6)$ 0.78 (3H, s, C_{25}-H_3), 0.92 (3H, s, C_{24}-H_3), 1.42 (3H, s, C_{18}-H_3), 1.52 (3H, s, C_{20}-H_3), 1.59 (1H, ddd, J= 12.7, 9.8, 2.4 Hz, C_1-Hα), 1.69 (3H, s, C_{22}-H_3), 1.78 (2H, m, C_2-H_2), 1.83 (3H, s, C_3-OAc), 2.07 (1H, ddd, J= 12.7, 2.0, 2.0 Hz, C_1-Hβ), 2.17 (1H, s, C_{16}-OH), 2.54 (1H,dd, J= 14.7, 3.4 Hz, C_7-Hβ), 2.68 (1H, s, C_5-Hα), 2.86 (1H, d, J= 14.7 Hz, C_7-Hα), 2.93 (3H, s, C_{19}-OMe), 4.46 (1H, dd, J= 3.4, 1.5 Hz, C_6-Hα), 4.70 (1H, dd, J= 1.5, 1.5 Hz, C_3-Hβ), 5.02 (1H, s, C_{21}-H), 5.25 (1H, s, C_{21}-H), and 5.99 (1H, s, C_{11}-H) ; $\delta(CDCl_3)$ 20.20 (t), 20.68 (q), 20.88 (q), 21.95 (q), 22.11 (t), 22.28 (q), 26.38 (q), 30.68 (q), 34.57 (s), 37.84 (t), 40.31 (s), 46.32 (d), 47.40 (s), 52.10 (q), 58.65 (s), 71.43 (s), 72.20 (s), 75.83 (d), 77.58 (d), 118.92 (t), 125.19 (d), 140.50 (s), 147.28 (s), 167.31 (s), 170.22 (s), 177.59 (s), 207.74 (s), and 210.66 (s).



1 : R = Ac
2 : R = Me

5 : R = Ac
6 : R = Me

3

4

Fig. 1.

As judged from their detailed ^1H-NMR decoupling experiments, probably, citreohybriddiones A(**3**) and B(**4**) have the same carbon skeleton. On the basis of ^1H-^{13}C COSY spectra of **3** and **4** and detailed low power selective decoupling experiments of **4** in benzene-D_6, citreohybriddiones A and B have two CO groups and a tertiary OH group on D ring. Irradiation of the C_{13}-methyl group (δ 1.52) of **4** enhanced each height of the carbon signals at 12-position (δ 141.18), 13-position (δ 58.70), 14-position (δ 72.45), and 17-position (δ 207.62), and irradiation of the C_{16}-methyl group (δ 1.42) of **4** also enhanced each height of the carbon signals at 15-position (δ 210.76), 16-position (δ 71.63) and 17-position (δ 207.62). Other rings of citreohybriddione A(**3**) are structurally quite similar to those of citreohybridones A and B. On the other hand, citreohybriddione B(**4**) has an exomethylene group (δ 5.02, 5.25) on C ring and no proton at C_9-position. Finally the stereochemistry of citreohybriddiones A(**3**) and B(**4**), especially the configuration at C_{16}-position, was elucidated by the NOE difference experiments in benzene-D_6. Irradiation of the C_{16}-methyl group (δ 1.36) of **3** resulted in 5.1% NOE of the C_9-proton (δ 2.43) , 0.9% NOE of the C_{11}-proton (δ 5.66) , and 0% NOE of the C_{19}- methoxyl group (δ 2.95), irradiation of the C_5-proton (δ 1.89) resulted in 4.1% NOE of the C_7-α-axial proton (δ 3.12), and 14.9% NOE of the C_9-proton, and irradiation of the C_{19}-methoxyl group resulted in 1.1% NOE of the C_{13}-methyl group (δ 1.27) and 0% NOE of the C_{16}-methyl group, thereby, indicating that the C_{16}-methyl group is in an α configuration. Whereas, irradiation of the C_{16}-methyl group (δ 1.42) of **4** resulted in 1.2% NOE of the C_{19}-methoxyl group (δ 2.93), and irradiation of C_{19}-methoxyl group also resulted in 1.0% NOE of the C_{16}-methyl group and 1.1% NOE of the C_{13}-methyl group, establishing that the C_{16} methyl group of **4** is in a β configuration. Other NOE interactions are shown in Fig.1.

The spectral data of citreohybridones A and B and isocitreohybridones A and B,[6,7] of which the structure of citreohybridone A(**1**) has been elucidated by means of an X-ray crystallographic analysis, are quite similar to one another, particularly in their ^{1}H-NMR spectra. In fact, citreohybridone A was subjected to hydrolysis with 20% aq H_2SO_4 -MeOH-$CHCl_3$ (1: 3: 3) (60-70 °C , 1 h)[8] followed by acetylation with Ac_2O- pyridine (room temp , overnight) to give the isocitreohybridone A(**5**) and citreohybridone A(**1**) in 15 and 76% yields, respectively. Furthermore, on methylation with $TMSCHN_2$ in MeOH-benzene (room temp., 10 min), the hydrolysis compound derived from citreohybridone A(**1**) was readily converted into isocitreohybridone B(**6**) and citreohybridone B(**2**) in 47 and 40% yields, respectively. Of these new metabolites, particularly, both **2** and **6** have higher antifeedant activity against *Plutella xylostella*, as compared with the other metabolites. Further studies on biological properties of these compounds are in progress.

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2) S. Lai, Y. Shizuri, S. Yamamura, K. Kawai, Y. Terada, and H. Furukawa, Tetrahedron Lett., **30**, 2241 (1989).

3) 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 days), using cellulase, 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 days) to give a number of colonies, from which many new hybrid strains including *Penicillium citreo-viride* ME 0004 and 0005 were obtained. These experiments were reported briefly (H. Furukawa, K. Kawai, M. Niwa, M. Yogo, S. Yamamura, and Y. Shizuri, 109th National Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1988, Abst., No. 4FF 1-6.).

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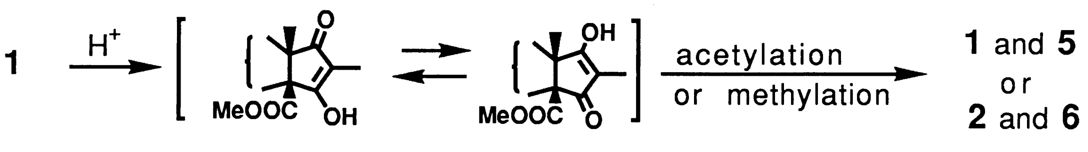
6) Isocitreohybridone A(**5**) as a colorless oil : $[\alpha]_D^{19.6}$ +22.6° (c 1.0, $CHCl_3$) ; m/z 482.2278 [$C_{30}H_{38}O_9$ (M^+) - AcOH] ; IR(film) 1770, 1740, 1710, 1670, 1245, 1225, 1175, and 1135 cm^{-1} ; $\delta(C_6D_6)$ 0.73 (3H, s, C_{25}-H_3), 0.76 (3H, s, C_{24}-H_3), 1.19 (1H, ddd, J=13.4, 13.4, 6.2 Hz, C_1-Hα), 1.25 (3H, s, C_{20}-H_3), 1.58 (3H, s, C_{17}-OAc), 1.62 (3H, s, C_{18}-H_3), 1.65 (3H, s, C_3-OAc), 1.67 (2H, m, C_2-H_2), 1.69 (3H, dd, J=2.4, 1.6 Hz, C_{21}-H_3), 1.80 (3H, s, C_{22}-H_3), 1.99 (1H, s, C_5-H), 2.26 (1H, ddd, J=14.3, 4.8, 2.0 Hz, C_1-Hβ), 2.39 (1H, dq, J=2.4, 2.4 Hz, C_9-H), 2.77 (1H, dd, J=14.3, 4.4 Hz, C_7-Hβ), 3.25 (3H, s, C_{19}-OMe), 3.70 (1H, d, J=14.4 Hz, C_7-Hα), 4.46 (1H, d, J=4.4 Hz, C_6-H), 4.69 (1H, dd, J=3.3, 1.8 Hz, C_3-H), and 5.91 (1H, s, C_{11}-H) ; $\delta(CDCl_3)$ 7.91 (q), 17.61 (q), 20.67 (q), 20.98 (t), 21.04 (q), 21.13 (q), 22.07 (t), 22.33 (q), 25.23 (q), 26.27 (q), 34.30 (s), 36.51 (t), 43.61 (s), 43.96 (s), 51.54 (d), 51.72 (q), 52.74 (s), 54.49 (d), 72.39 (s), 76.05 (d), 77.67 (d), 124.97 (d), 125.82 (s), 137.00 (s), 164.96 (s), 169.19 (s), 170.17 (s), 174.18 (s), 179.05 (s), and 203.90 (s).

7) Isocitreohybridone B(**6**) as colorless prisms : mp 263 - 264.5 °C in a sealed tube (from benzene - hexane) ; $[\alpha]_D^{19.6}$ +51.1° (c 1.0, $CHCl_3$) ; $C_{29}H_{38}O_8$ [m/z 514.2548(M^+)] ; IR(film) 1765, 1740, 1690, 1620, and 1240 cm^{-1} ; $\delta(C_6D_6)$ 0.74 (3H, s, C_{25}-H_3), 0.77 (3H, s, C_{24}-H_3), 1.25 (1H, ddd, J=13.6, 13.6, 5.7 Hz, C_1-Hα), 1.32 (3H, s, C_{20}-H_3), 1.65 (2H, m, C_2-H_2), 1.72 (3H, s, C_3-OAc), 1.77 (3H, s, C_{18}-H_3), 1.80 (3H, s, C_{22}-H_3), 1.83 (3H, dd, J=2.0, 1.5 Hz, C_{21}-H_3), 2.05 (1H, s, C_5-H), 2.29 (1H, ddd, J=13.6, 3.3, 3.3 Hz, C_1-Hβ), 2.49 (1H, dq, J=2.6, 2.6 Hz, C_9-H), 2.81 (1H, dd, J=14.3, 4.5 Hz, C_7-Hβ), 3.21 (3H, s, C_{17}-OMe), 3.25 (3H, s, C_{19}-OMe), 3.85 (1H, d, J=14.3 Hz, C_7-Hα), 4.50 (1H, d, J=4.5 Hz, C_6-H), 4.70 (1H, dd, J=2.0, 1.0 Hz, C_3-H), and 5.89 (1H, s, C_{11}-H) ; $\delta(CDCl_3)$ 8.36 (q), 17.98 (q), 20.95 (t), 21.01 (q), 21.06 (q), 22.09 (t), 22.34 (q), 24.79(q), 26.24 (q), 34.28 (s), 36.57 (t), 43.34 (s), 43.60 (s), 51.33 (d), 51.50 (q), 52.87 (s), 54.39 (d), 59.58 (q), 71.19 (s), 76.15 (d), 77.92 (d), 111.11 (s), 123.78 (d), 138.75 (s), 169.96 (s), 170.10 (s), 179.21 (s), 182.89 (s), and 203.80 (s).

8) Hydrolysis product derived from citreohybridone A : IR(film) 3200(br), 1770, 1740, and 1620 cm^{-1}



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