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Isolation and Biosynthetic Pathway for Citreohybridones from the Hybrid Strain KO 0031 Derived from *Penicillium* Species

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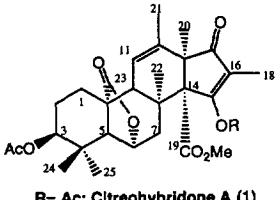
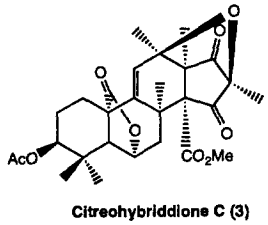
Abstract : Five new metabolites, citreohybridones D - G and isocitreohybridone G, have been isolated from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Their stereostructures have been also elucidated on the basis of their spectral data and some chemical evidence, and a biosynthetic pathway for citreohybridones is proposed.

As described in the previous papers,[1-5] we have succeeded in isolating several new highly potent antifeeding metabolites, citreohybridones A (**1**) and B (**2**) and citreohybriddione C (**3**) and others, against *Plutella xylostella* from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Recently, Omura et al. isolated andrastins A, B and C (**4**)[6], new protein farnesyltransferase inhibitors, from *Penicillium* sp. FO-3929 and determined their absolute configuration. Interestingly, it is clear that these metabolites must be precursors of citreohybridones.[3,4] In view of the biological significance of these unique meroterpenoids (mixed polyketide-terpenoid)[7-13] we further examined the metabolites in the mycelium of the hybrid strain KO 0031, incubated stationarily at room temperature for 14 days. In this communication we wish to report the isolation[14] and structural elucidation of some new meroterpenoids , and consideration of a proposed biosynthetic pathway for citreohybridones.

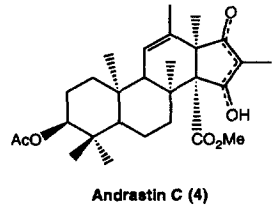
Citreohybridone D (**5**), $[\alpha]_D^{23}$ -80.2° (c= 1.0, $CHCl_3$), $C_{30}H_{40}O_8$ [*m/z* 528.2700 (M^+)] showed ^1H NMR and ^{13}C NMR data similar to those of citreohybridone A (**1**). The ^{13}C NMR signal at δ 76.71(d, C-6) for the methine carbon bearing an oxygen atom in **1** was replaced by a signal at δ 33.1 (t, methylene carbon) in **5**, suggesting that the lactone ring of **1** was opened; moreover, **5** has an aldehyde group [υ_{max} 1715 cm^{-1}, δc 204.7 (d), δH 10.1 (1H,s)], suggesting that **5** is a precursor of **1**. The structure of **5** was based on its spectral data[15] and 2D NMR experiments (COSY, HMQC, HMBC, NOESY).



R= Ac; Citreohybridone A (1)
R= Me; Citreohybridone B (2)

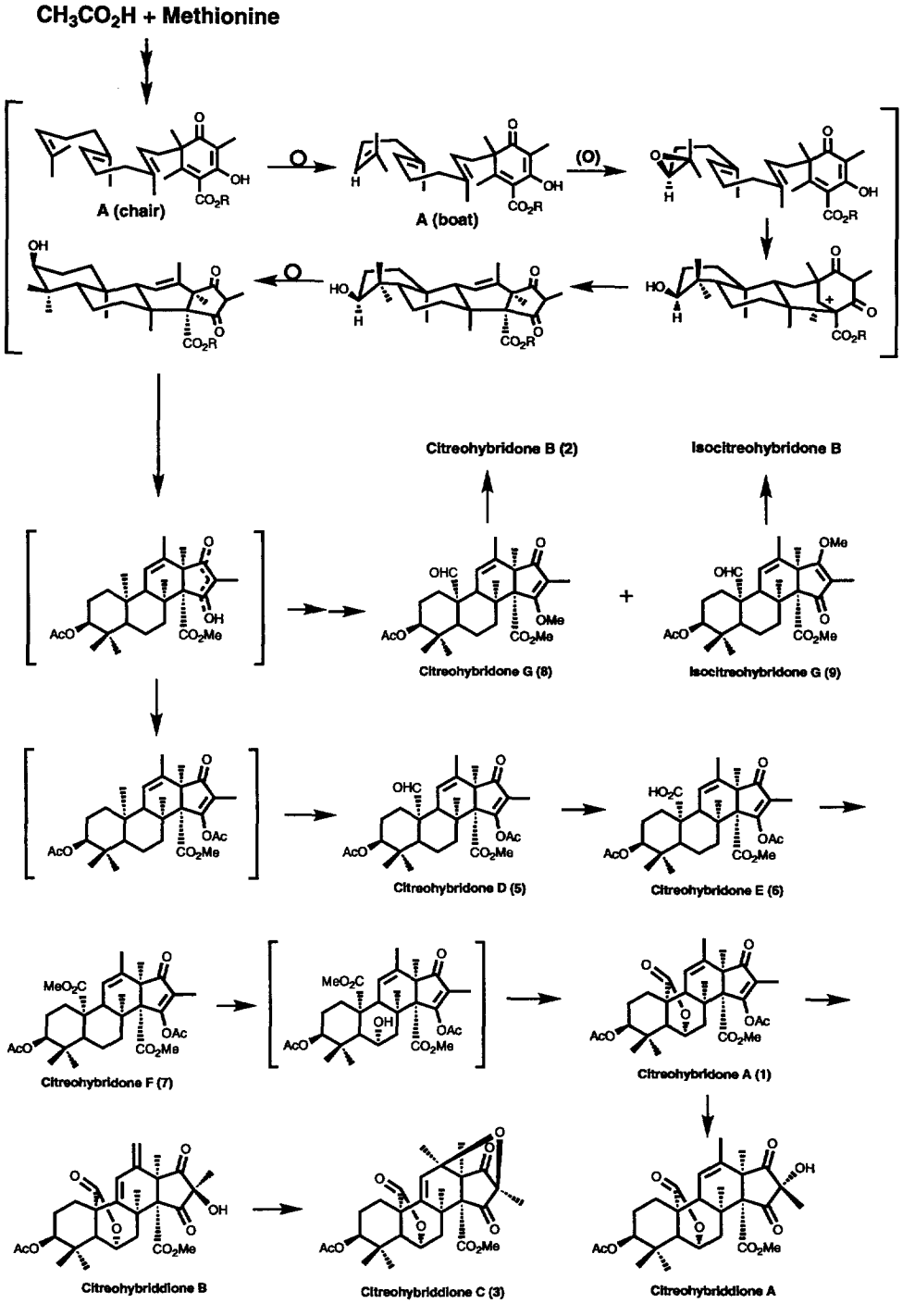


Citreohybriddione C (3)



Andrastin C (4)

Citreohybridone E (**6**)[16] and F (**7**)[17] have the molecular formula $C_{30}H_{40}O_9$ [*m/z* 544.2668 (M^+)] and $C_{31}H_{42}O_9$ [*m/z* 558.2826 (M^+)], respectively. The ^1H and ^{13}C NMR spectra were closely related to those of **5**.



Scheme 1. Proposed biosynthetic pathway for citreohybridones

Both metabolites are regarded as the further oxygenated products of the aldehyde (**3**), suggesting that **6** is a carboxylic acid [υ_{max} 3200 (br.) cm^{-1}, δC 178.4 (s), δH 10.1 (1H, s)] and **7** must be the corresponding methyl ester [υ_{max} 1745 cm^{-1}, δc 175.1 (s), 51.0 (q), δH 3.61 (3H, s)].

Citreohybridone G(**8**)[18] and isocitreohybridone G (**9**)[19] have the same molecular formula $C_{29}H_{40}O_7$ [**8**: *m/z* 500.2770 (M^+), **9**: *m/z* 500.2771 (M^+)], and their ^1H and ^{13}C NMR spectra are also closely related to those of **3** except in the following points. Citreohybridone D (**5**) has two acetoxy groups [δH 2.07 (C_3-OAc), 2.32 (C_{15}-OAc)] and a methoxy group [δH 3.61 (C_{19}-OMe)]; on the other hand, citreohybridone G (**8**) and isocitreohybridone G (**9**) have one acetoxy group [**8**: δH 2.07 (C_3-OAc), **9**: 2.09 (C_3-OAc) and two methoxyl groups [**8**: δH 4.09 (C_{15}-OMe), 3.60 (C_{19}-OMe), **9**: δH 4.10 (C_{17}-OMe), 3.60 (C_{19}-OMe)], respectively, indicating that the latter must be the methoxy derivatives of **3**. Citreohybridone D (**5**) was subjected to hydrolysis with 20% aq H_2SO_4-MeOH-$CHCl_3$ (1:3:3) followed by methylation with TMSCHN2 in MeOH-benzene to give as expected, the citreohybridone G and isocitreohybridone G in 10 and 8.3% yields, respectively.

Recently, we have reported that citreohybridones are formed *via* a mixed polyketide-terpenoid (meroterpenoid) biosynthetic pathway.[3,4] In this study, five precursors of citreohybridones were isolated from 14-days' culture medium; these metabolites have no oxygenated carbon at the C_6 position, suggesting that the oxygenation at the C_{23} position takes precedence over the oxygenation at the C_6 position in these precursors. Moreover, methylation or acetylation on the D ring of the precursor must take place before oxygenation at the C_{23} position. The proposed biosynthic pathway for citreohybridones is shown in Scheme 1. It is especially noted the formation of the hydroxy group oriented in axial direction on the A-ring of citreohybridones must be involved in the enzymic epoxidation and cyclization of intermediate A which requires boat geometry for the A-ring.

The absolute configuration of citreohybridones seems to be the same as that of andrastins, which was elucidated as an enantiomer of 5α, 14β-androstane by the X-ray analysis of 15-(p-bromobenzoyl)-andrastin A,[20] because citreohybridone D (**5**) and 15-acetyl-andrastin A[21] have the same negative optical rotation mainly contributed by an α,β-unsaturated 5-membered ring moiety.

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14. The same procedure as described in the previous papers (ref. 1-5).

15. Physical data for citreohybridone D (**5**): a colorless oil; $[\alpha]_D^{23}$ -80.2° (c = 1.0, $CHCl_3$); $C_{30}H_{40}O_8$ [*m/z* 528.2720(M^+)]; IR(film) 1780, 1740, 1715, and 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 10.1 (1H, s, H-23), 5.48 (1H, dq, J= 1.8, 1.5, H-11), 4.58 (1H, dd, J= 2.9, 2.6, H-3), 3.61 (3H, s, C_{19}-OMe), 2.36 (1H, m, H-7), 2.32 (3H, s, C_{15}-OAc), 2.30 (1H, m, Hβ-1), 2.15 (1H, dq, J= 2.4, 1.8, H-9), 2.07 (3H, s, C_3-OAc), 2.02 (1H, m, H-7), 1.65 (3H, dd, J= 2.4, 1.5, H_3-21), 1.63 (1H, m, H-2), 1.6 (1H, m, H-5), 1.55~1.6 (2H, m, H_2-6), 1.58 (3H, s, H_3-18), 1.53 (1H, m, H-2), 1.19 (3H, s, H_3-22), 1.15 (3H, s, H_3-20), 1.03 (1H, ddd, J= 13.4, 13.4, 4.2, Hα-1), 0.90 (3H, s, H_3-24), and 0.83 (3H, s, H_3-25); ^{13}C-NMR ($CDCl_3$) δ 204.7 (d, C-23), 199.9 (s, C-17), 170.3 (s, C_3-OAc), 169.7 (s, C-19), 169.3 (C-15), 165.3 (C_{15}-OAc), 132.3 (s, C-12), 131.1 (s, C-16), 123.6 (d, C-11), 77.9 (d, C-3), 67.1 (s, C-14), 59.6 (s, C-13), 53.6 (d, C-9), 52.2 (q, C_{19}-OMe), 51.7 (s, C-10), 48.5 (d, C-5), 40.7 (s, C-8), 36.7 (s, C-4), 33.1 (t, C-7), 27.3 (t, C-1), 26.9 (q, C-24), 23.0 (t, C-2), 21.4 (q, C_{15}-OAc), 21.0 (q, C_3-OAc), 20.8 (q, C-25), 19.0 (q, C-22), 18.9 (q, C-21), 16.5 (t, C-6), 15.4 (q, C-20), and 8.9 (q, C-18).
16. Physical data for citreohybridone E (**6**): a colorless oil; $[\alpha]_D^{25}$ -13.4° (c = 0.1, $CHCl_3$); $C_{30}H_{40}O_9$ [*m/z* 544.2668(M^+)]; IR(film) 3200, 1785, 1745, 1715, and 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.73 (1H, dq, J= 1.8, 1.1, H-11), 4.55 (1H, dd, J= 2.8, 2.7, H-3), 3.55 (3H, s, C_{19}-OMe), 2.41 (1H, dddd, J= 13.2, 13.2, 13.2, 3.7, Hβ-6), 2.27 (3H, s, C_{15}-OAc), 2.25 (1H, m Hβ-1), 2.23 (1H, m, Hβ-7), 2.07 (1H, dq, J= 2.6, 1.8, H-9), 2.04 (3H, s, C_3-OAc), 2.02 (1H, m, Hα-6), 1.66 (2H, m, H_2-2), 1.64 (3H, dd, J= 2.6, 1.1, H_3-21), 1.53 (3H, s, H_3-18), 1.42 (1H, m, Hα-7), 1.30 (1H, dd, J= 13.2, 2.6, H-5), 1.22 (1H, m, Hα-1), 1.20 (3H, s, H_3-22), 1.12 (3H, s, H_3-20), and 0.84 (6H, s, H_3-24, 25); ^{13}C-NMR ($CDCl_3$) δ 200.2 (s, C-17), 178.4 (s, C-23), 170.4 (s, C_3-OAc), 169.7 (s, C-19), 169.5 (C-15), 165.4 (C_{15}-OAc), 131.6 (s, C-12), 131.2 (s, C-16), 124.6 (d, C-11), 77.9 (d, C-3), 67.8 (s, C-14), 59.5 (s, C-13), 52.3 (d, C-9), 52.1 (q, C_{19}-OMe), 49.6 (d, C-5), 46.7 (s, C-10), 41.2 (s, C-8), 36.9 (s, C-4), 33.4 (t, C-7), 29.7 (t, C-1), 27.8 (q, C-24), 24.0 (t, C-2), 22.2 (q, C-25), 21.4 (q, C_{15}-OAc), 21.1 (q, C_3-OAc), 18.9 (q, C-21), 17.4 (t, C-6), 15.7 (q, C-20), 15.6 (q, C-22), and 8.9 (q, C-18).
17. Physical data for citreohybridone F (**7**): a colorless oil; $[\alpha]_D^{23}$ -36.7° (c = 0.15, $CHCl_3$); $C_{31}H_{42}O_9$ [*m/z* 558.2826(M^+)]; IR(film) 1785, 1745, 1715, and 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.73 (1H, dq, J= 1.7, 1.1, H-11), 4.58 (1H, dd, J= 2.9, 2.7, H-3), 3.61(3H, s, C_{23}-OMe), 3.59 (3H, s, C_{19}-OMe), 2.43 (1H, dddd, J= 13.2, 13.2, 13.2, 4.4, Hβ-6), 2.31 (3H, s, C_{15}-OAc), 2.3 (1H, m, Hβ-1), 2.23 (1H, m, Hβ-7), 2.08 (3H, s, C_3-OAc), 2.07 (1H, dq, J= 2.6, 1.7, H-9), 2.06 (1H, m, Hα-6), 1.68 (3H, dd, J= 2.6, 1.1, H_3-21), 1.64 (2H, m, H_2-2), 1.58 (3H, s, H_3-18), 1.50 (1H, m, Hα-7), 1.36 (1H, dd, J= 13.2, 2.5, H-5), 1.24 (1H, m, Hα-1), 1.17 (6H, s, H_3-20, 22), 0.88 (3H, s, H_3-24), and 0.82 (3H, s, H_3-25); ^{13}C-NMR ($CDCl_3$) δ 200.4 (s, C-17), 175.1 (s, C-23), 170.4 (s, C_3-OAc), 169.8 (s, C-19), 169.6 (s, C-15), 165.4 (s, C_{15}-OAc), 131.3 (s, C-12), 131.1 (s, C-16), 124.8 (d, C-11), 78.0 (d, C-3), 67.8 (s, C-14), 59.5 (s, C-13), 52.3 (d, C-9), 52.1 (q, C_{19}-OMe), 51.0 (q, C_{23}-OMe), 49.5 (d, C-5), 47.1 (s, C-10), 41.1 (s, C-8), 36.8 (s, C-4), 33.4 (t, C-7), 29.7 (t, C-1), 27.8 (q, C-24), 24.0 (t, C-2), 22.1 (q, C-25), 21.4 (q, C_{15}-OAc), 21.1 (q, C_3-OAc), 18.9 (q, C-21), 17.6 (t, C-6), 15.7 (q, C-20, 22), and 8.9 (q, C-18).
18. Physical data for citreohybridone G (**8**): a colorless oil; $[\alpha]_D^{25}$ -17.7° (c = 0.1, $CHCl_3$); $C_{29}H_{40}O_7$ [*m/z* 500.2770(M^+)]; IR(film) 1740, 1705, and 1635 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 10.1 (1H, s, H-23), 5.39 (1H, dq, J= 1.6, 1.5, H-11), 4.62 (1H, dd, J= 2.9, 2.6, H-3), 4.09 (3H, s, C_{15}-OMe), 3.60(3H, s, C_{19}-OMe), 2.54 (1H, ddd, J= 13.6, 13.2, 4.8, Hα-7), 2.34 (1H, ddd, J= 13.2, 3.3, 3.3, Hβ-1), 2.23 (1H, ddd, J= 13.6, 3.3, 3.3, Hβ-7), 2.07 (3H, s, C_3-OAc), 1.92 (3H, s, H-18), 1.9 (1H, dq, J= 2.2, 1.6, H-9), 1.9 (1H, m, H-6), 1.70 (1H, m, H-5), 1.67 (3H, dd, 2.2, 1.5, H_3-21), 1.58 (2H, m, H_2-2), 1.58 (1H, m, H-6), 1.20 (3H, s, H_3-22), 1.16 (3H, s, H_3-20), 0.96 (1H, ddd, J= 13.2, 11.7, 4.0, Hα-1), 0.92 (3H, s, H_3-24), and 0.84 (3H, s, H_3-25).
19. Physical data for isocitreohybridone G (**9**): a colorless oil; $[\alpha]_D^{25}$ -6.6° (c = 0.1, $CHCl_3$); $C_{29}H_{40}O_7$ [*m/z* 500.2771(M^+)]; IR(film) 1735, 1705, and 1630 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 10.1 (1H, s, H-23), 5.39 (1H, dq, J= 1.8, 1.4, H-11), 4.60 (1H, dd, J= 2.9, 2.6, H-3), 4.10 (3H, s, C_{17}-OMe), 3.60 (3H, s, C_{19}-OMe), 3.18 (1H, ddd, J= 13.2, 13.2, 4.2, Hα-7), 2.21 (1H, ddd, J= 13.2, 3.3, 3.3, Hβ-1), 2.09 (3H, s, C_3-OAc), 2.09 (1H, dq, J= 2.6, 1.8, H-9), 2.08 (1H, m, Hβ-7), 1.91 (3H, s, H-18), 1.9 (1H, m, H-6), 1.81 (3H, s, H_3-21), 1.80 (1H, m, H-5), 1.65 (1H, m, H-6), 1.55 (2H, m, H_2-2), 1.19 (6H, s, H_3-20, 22), 0.92 (1H, m, Hα-1), 0.91 (3H, s, H_3-24), and 0.82 (3H, s, H_3-25).
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21. 15-Acetyl-andrastin A shows a specific rotation of -108.5° (c = 0.2, $CHCl_3$).

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