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Structure and absolute configuration of citreohybridones isolated from *Penicillium* species

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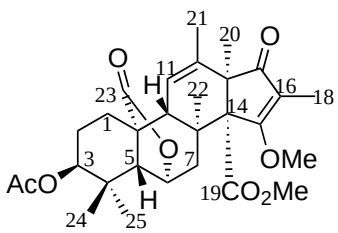
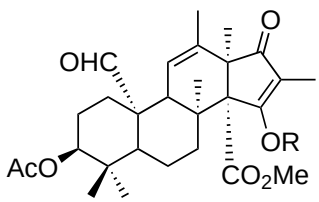
Abstract—Four new meroterpenoids have been isolated from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Their stereostructures have been elucidated on the basis of their spectral data and some chemical evidence, and their absolute configurations have also been elucidated by the modified Mosher's methods.

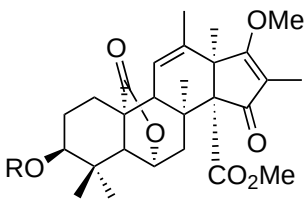
As described in previous papers,[1,2] we have succeeded in isolating several new high potent antifeeding metabolites, citreohybridone B (**1**), isocitreohybridone B (**2**) and citreohybridone D (**3**) and others, against *Plutella xylostella* from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Recently, Ōmura et al. isolated andrastins A (**4**),[3] 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.[2] In view of the biological significance of these unique meroterpenoids (mixed polyketide–terpenoid),[4,5] we further examined the metabolites in the mycelium of the hybrid strain KO 0031, incubated stationarily at room temperature for 60 days. In this communication we wish to report the structure of four new meroterpenoids and their absolute configurations.



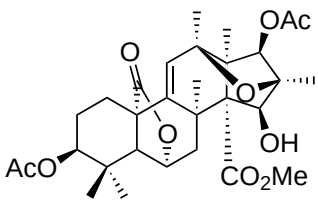
citreohybridone B (**1**)



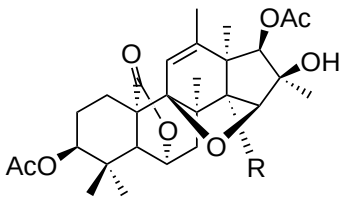
R=H; andrastin A (**4**)
R=Ac; citreohybridone D (**3**)



R=Ac; isocitreohybridone B (**2**)
R=H; isocitreohybridone C (**8**)



citreohybridone J (**5**)



R=COOMe; citreohybridone K (**6**)
R=CH_2OAc; citreohybridone L (**7**)

Keywords: *Penicillium citreo-viride* B.; meroterpenoid; citreohybridone; antifeeding; absolute configuration.
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According to essentially the same procedure as described in previous papers,[1,2] the EtOAc extract (35 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 AcOEt–hexane (1:1–2), acetone–hexane (1:1–2) and/or MeOH–$CHCl_3$ (1:10–20) to afford citreohybridone J (**5**; 0.015%), K (**6**; 0.00086%), L (**7**; 0.0011%) and isocitreohybridone C (**8**; 0.057%), respectively.

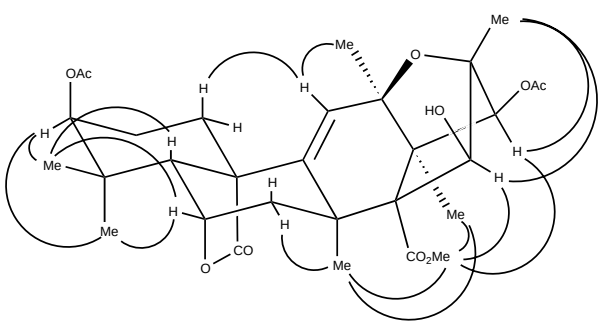
The structure of citreohybridone J (**5**)[6] was derived based on extensive spectroscopic analysis. HREIMS suggested a molecular formula of $C_{30}H_{40}O_{10}$ [m/z 560.2620 (M^+)] for **5**, thus revealing 11 degrees of unsaturation. This formula was supported by the presence of 30 carbons in its ^{13}C NMR spectrum. The carbon types, revealed by a HMQC experiment, included one methoxy, eight methyls, three methylenes, six methines and twelve non-protonated carbons, four of which were carbonyls: δ 177.6 (lactone, C-23), 174.1 (methyl ester, C-19), 169.5 (C17-OAc), and 169.2 (C3-OAc). The ^1H NMR data are similar to those of citreohybridone B (**1**) except for the absence of a proton for H-9 and the presence of two protons (δ 4.66, H-15; δ 5.03, H-17) and hydroxyl group (δ 2.62, C15-OH). Finally, the stereochemistry of citreohybridone J (**5**), especially of the configuration of the ether linkage from C12- to C16-position and D ring, was elucidated by NOE difference experiments in benzene-d_6. The NOEs between H_3-18 (δ 1.13)/H-17 (δ 5.03) and H-15 (δ 4.66); MeO-C19 (δ 3.23)/H-15 (δ 4.66), H-17 (δ 5.03), H_3-20 (δ 1.41) and H_3-22 (δ 1.61); H_3-21 (δ 1.37)/H-11 (δ 5.48) suggested the relative configuration of **5** (Fig. 1).

Citreohybridone K (**6**),[7] $C_{30}H_{40}O_{10}$ [m/z 560.2616 (M^+)] showed ^1H NMR data very similar to those of citreohybridone J (**5**). The ^1H NMR signal at δ 5.48 (1H, s, H-11) for the methine proton in **5** was replaced by a signal at 5.96 (1H, d, $J=1.1$ Hz) was observed in **6**, suggesting that **6** has an ether linkage on another position in **6**. The NOE difference experiments clarified the position of the ether linkage and the stereochemistry of citreohybridone K (**6**), as shown in Fig. 1. Especially, the NOE between Hβ-7 (δ 1.52) and H-15 (δ 4.33) suggested the ether linkage is on C9 and C15 of **6**, because of the NOE observed between these protons will be impossible by another position the ether linkage for rigid carbon skeleton.

Citreohybridone L (**7**),[8] $C_{31}H_{42}O_{10}$ [m/z 574.2804 (M^+)] showed ^1H NMR data very similar to those of citreohybridone K (**6**), except for the absence of a methoxy group and the presence of an isolated methylene group (δ 4.27 and 4.22, $J=12.1$ Hz, C-19) and an additional acetoxyl group. Citreohybridone **L** is the first compound that the carbonyl group at the C19 position of citreohybridones is reduced to alcohol.

Isocitreohybridone C (**8**),[9] $C_{27}H_{36}O_7$ [m/z 472.2459 (M^+)] showed ^1H and ^{13}C NMR data very similar to those of isocitreohybridone B (**2**). The ^1H NMR signal at δ 4.63 (1H, dd, $J=2.7$, 2.7 Hz) for the methine proton in **2** was replaced by a signal at δ 3.39 (1H, dd, $J=3.1$, 1.8 Hz) and no AcO group was observed in **8**, suggesting that **8** has an OH group instead of an AcO group on C-3. As expected, on treatment with Ac_2O–pyridine (rt, overnight), **8** was readily converted into **2**.

Recently, we reported that 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, because citreohybridone D (**3**) and 15-acetyl-andrastin A have the same negative optical rotation mainly contributed by an α,β-unsaturated five-membered ring moiety.[10] In order to confirm the absolute configuration, (*R*)- and (*S*)-MTPA esters (**9R** and **9S**)[11,12] of **8** were prepared, and the $\Delta\delta$ values (ppm) of all assignable protons were determined. It can be seen that the positive and negative $\Delta\delta$ values are irregularly dispersed on the left and right sides on the MTPA plane. Molecular models of **9R** and **9S** revealed that, in each compound, the ester group is axial and is sterically hindered by the axial 1-H and 5-H as well as



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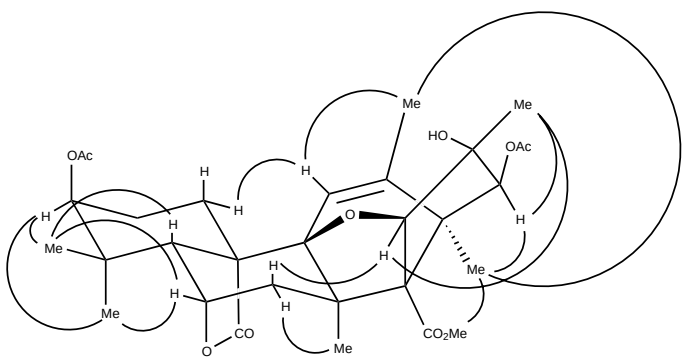

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Figure 1. NOE correlations for citreohybridones J (**5**) and K (**6**).

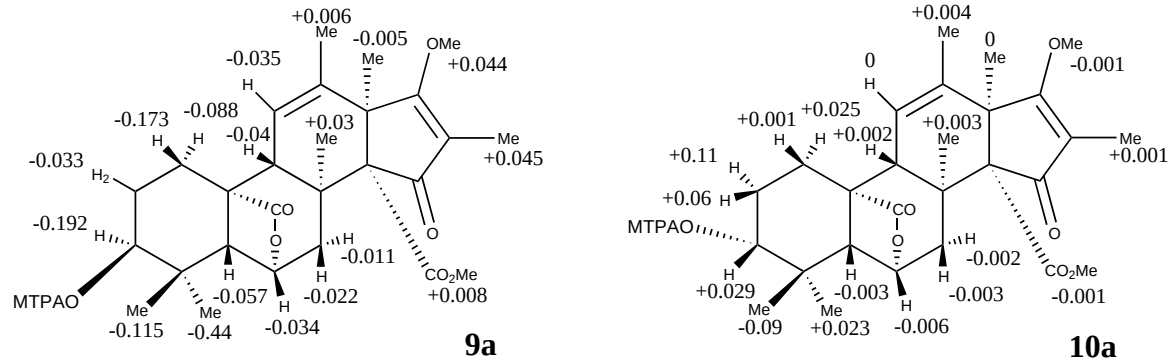

Figure 2. $\Delta\delta$ values ($\Delta\delta = \delta S - \delta R$) obtained for MTPA esters of isocitreohybridone C (**9a**) and its C3-epimar (**10a**).

by the adjacent *gem*-dimethyl groups. If this steric crowding around the ester moiety is the principal reason for the irregularity, conversion of the hydroxyl group into a less hindered one should solve the problem.[13]

C_3-Epimar of **8** was prepared by $NaBH_4$ reduction of 3-keto-compound which was obtained by treatment of **8** with pyridinium dichromate. The protons of its MTPA esters **10R** and **10S**,[14,15] in which the hydroxyl group is equatorial, have $\Delta\delta$ values that are perfectly consistent with the rule for determining absolute configurations[16] (Fig. 2). This result gave us the absolute configurations both isocitreohybridone C and its C_3-epimar, shown in the respective structures.

Acknowledgements

We are grateful to Professor Shosuke Yamamura (Keio University) for his valuable suggestion.

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6. Physical data for citreohybridone J (**5**): a colorless oil; $[\alpha]_D^{24}$ +85 (*c* 0.5, $CHCl_3$); $C_{30}H_{40}O_{10}$ [*m*/*z* 560.2620 (M^+)]; IR (film) 3520, 1765, 1740, and 1725 cm^{-1}; ^1H NMR (C_6D_6) δ 5.48 (1H, s, H-11), 5.03 (1H, s, H-17), 4.72 (1H, dd, *J*=3.7, 1.8 Hz, H-3), 4.66 (1H, d, *J*=11.1 Hz, H-15), 4.38 (1H, dd, *J*=4.0, 1.5 Hz, H-6), 3.23 (3H, s, C_{19}-OMe), 2.74 (1H, s, H-5), 2.62 (1H, d, *J*=11.1 Hz, C_{15}-OH), 2.60 (1H, dd, *J*=13.9, 1.5 Hz, Hβ-7), 1.8 (1H, dd, *J*=13.9, 4.0 Hz, Hα-7), 1.61 (3H, s, H_3-22), 1.59 (3H, s, C_{17}-OAc), 1.55 (3H, s, C_3-OAc), 1.41 (3H, s, H_3-20), 1.37 (3H, s, H_3-21), 1.13 (3H, s, H_3-18), 0.78 (3H, s, H_3-24), and 0.76 (3H, s, H_3-25), 2.0–1.8 (4H, complex, H_2-2 and H_2-1); ^{13}C NMR (C_6D_6) δ 177.6 (s, C-23), 174.1 (s, C-19), 169.5 (s, C_{17}-OAc), 169.2 (s, C_3-OAc), 148.4 (s, C-9), 123.6 (d, C-11), 81.4 (s, C-16), 80.4 (d, C-17), 80.0 (s, C-12), 79.0 (d, C-15), 77.2 (d, C-6), 75.9 (d, C-3), 62.9 (s, C-14), 53.0 (s, C-13), 51.8 (d, C-5), 51.5 (s, C_{19}-OMe), 49.1 (s, C-10), 40.5 (t, C-7), 39.6 (s, C-8), 35.2 (s, C-4), 30.2 (s, C-22), 26.7 (q, C-24), 25.7 (q, C-21), 22.9 (q, C-25), 22.6 (t, C-2), 20.4 (q, C-1), 20.3 (q, C_3-OAc), 20.1 (q, C_{17}-OAc), 14.5 (q, C-18), and 13.9 (q, C-20).
7. Physical data for citreohybridone K (**6**): a colorless oil; $[\alpha]_D^{24}$ +17 (*c* 0.03, $CHCl_3$); $C_{30}H_{40}O_{10}$ [*m*/*z* 560.2616 (M^+)]; IR (film) 3520, 1775, and 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.96 (1H, q, *J*=1.1 Hz, H-11), 4.99 (1H, s, H-17), 4.67 (1H, d, *J*=5.0 Hz, H-6), 4.61 (1H, d, *J*=2.6 Hz, H-3), 4.33 (1H, s, H-15), 3.78 (3H, s, C_{19}-OMe), 2.97 (1H, s, C_{16}-OH), 2.89 (1H, s, H-5), 2.26 (1H, dd, *J*=13.9, 5.0 Hz, Hα-7), 2.17 (3H, s, C_{17}-OAc), 2.02 (3H, s, C_3-OAc), 1.89 (3H, d, *J*=1.1 Hz, H_3-21), 1.52 (1H, d, *J*=13.9 Hz, Hβ-7), 1.42 (3H, s, H_3-22), 1.39 (3H, s, H_3-18), 1.10 (3H, s, H_3-20), 0.96 (3H, s, H_3-24), 0.89 (3H, s, H_3-25), and 2.2–1.2 (4H, complex, H_2-2 and H_2-1).
8. Physical data for citreohybridone L (**7**): a colorless oil; $[\alpha]_D^{24}$ +14 (*c* 0.04, $CHCl_3$); $C_{31}H_{42}O_{10}$ [*m*/*z* 574.2804 (M^+)]; IR (film) 3500, and 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.91 (1H, q, *J*=1.5 Hz, H-11), 5.17 (1H, s, H-17), 4.63 (1H, d, *J*=4.8 Hz, H-6), 4.56 (1H, d, *J*=3.7 Hz, H-3), 4.27 (1H, d, *J*=12.1 Hz, H-19), 4.22 (1H, d, *J*=12.1 Hz, H′-19), 3.94 (1H, s, H-15), 3.00 (1H, s, C_{16}-OH), 2.81 (1H, s, H-5), 2.11 (3H, s, OAc), 2.10 (3H, s, OAc), 2.05 (1H, Hα-7), 1.98 (3H, s, OAc), 1.84 (3H, d, *J*=1.5 Hz, H_3-21), 1.7 (1H, Hβ-7), 1.25 (3H, s, H_3-16), 1.09 (3H, s, H_3-22), 1.05 (3H, s, H_3-20), 0.93 (3H, s, H_3-24), 0.84 (3H, s, H_3-25), and 2.0–1.4 (4H, complex, H_2-2 and H_2-1).
9. Physical data for isocitreohybridone C (**8**): a colorless oil; $[\alpha]_D^{23}$ +24.6 (*c* 1.0, $CHCl_3$); $C_{27}H_{36}O_7$ [*m*/*z* 472.2459 (M^+)]; IR (film) 3510, 1765, 1740, 1690, and 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.63 (1H, dq, *J*=2.6, 1.6 Hz, H-11), 4.68 (1H, d, *J*=3.9 Hz, H-6), 4.09 (3H, s, C_{17}-OMe), 3.57 (3H, s, C_{19}-OMe), 3.39 (1H, dd, *J*=3.1, 1.8 Hz, H-3), 3.37 (1H, d, *J*=14.3 Hz, Hβ-7), 2.42 (1H, dd, *J*=14.3, 4.6 Hz, Hα-7), 2.16 (1H, dq, *J*=2.6, 2.6 Hz, H-9), 2.05 (1H, ddd, *J*=13.6, 3.8, 1.7 Hz, Hα-1), 1.91 (3H, s, H_3-18), 1.87 (1H, s, H-5), 1.82 (3H, dd, *J*=2.6, 1.6 Hz, H_3-21), 1.72 (1H, dddd, *J*=14.3, 13.6, 1.8, 1.7 Hz, Hα-2), 1.57 (1H, dddd, *J*=14.3, 5.4, 3.8, 3.1 Hz, Hβ-2), 1.42

(1H, ddd, $J=13.6$, 13.6, 5.4 Hz, Hβ-1), 1.00 (3H, s, H_3-24), and 0.77 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ 203.9 (s, C-15), 183.1 (s, C-17), 179.8 (s, C-23), 170.1 (s, C-19), 138.4 (s, C-12), 123.9 (d, C-11), 111.3 (s, C-16), 78.2 (t, C-6), 73.9 (d, C-3), 71.1 (s, C-14), 59.6 (q, C_{17}-OMe), 53.7 (d, C-5), 52.7 (s, C-13), 51.5 (q, C_{19}-OMe), 51.0 (d, C-9), 43.7 (s, C-10), 43.3 (s, C-8), 36.4 (t, C-7), 35.1 (s, C-4), 26.4 (q, C-24), 24.9 (t, C-2), 24.7 (q, C-22), 22.7 (q, C-25), 21.1 (q, C-21), 20.1 (t, C-1), 18.0 (q, C-20), and 8.7 (q, C-18).

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11. ^1H NMR data for **9S**: δ ($CDCl_3$) 1.044 (Hβ-1), 2.077 (Hα-1), 1.762 (H_2-2), 4.771 (Hα-3, dd, $J=3.1$, 1.8 Hz), 1.827 (H-5), 4.664 (H-6), 3.407 (Hβ-7), 2.426 (Hα-7), 2.145 (H-9), 5.557 (H-11), 1.921 (H_3-18), 1.271 (H_3-20), 1.857 (H_3-21), 1.263 (H_3-22), 0.796 (H_3-24), 0.857 (H_3-25), 4.135 (C_{17}-OMe), and 3.588 (C_{19}-OMe).
12. ^1H NMR data for **9R**: δ ($CDCl_3$) 1.217 (Hβ-1), 2.165 (Hα-1), 1.795 (H_2-2), 4.963 (Hα-3, dd, $J=3.1$, 1.8 Hz), 1.884 (H-5), 4.698 (H-6), 3.429 (Hβ-7), 2.437 (Hα-7), 2.185 (H-9), 5.592 (H-11), 1.876 (H_3-18), 1.277 (H_3-20), 1.851 (H_3-21), 1.266 (H_3-22), 0.911 (H_3-24), 0.901 (H_3-25), 4.091 (C_{17}-OMe), and 3.580 (C_{19}-OMe).
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14. ^1H NMR data for **10S**: δ ($CDCl_3$) 1.322 (Hβ-1), 2.415 (Hα-1), 1.790 (Hβ-2), 1.660 (Hα-2), 4.651 (Hβ-3, dd, $J=12.1$, 3.7 Hz), 1.671 (H-5), 4.704 (H-6), 3.386 (Hβ-7), 2.454 (Hα-7), 2.101 (H-9), 5.583 (H-11), 1.961 (H_3-18), 1.234 (H_3-20), 1.850 (H_3-21), 1.271 (H_3-22), 0.856 (H_3-24), 0.805 (H_3-25), 4.136 (C_{17}-OMe), and 3.590 (C_{19}-OMe).
15. ^1H NMR data for **10R**: δ ($CDCl_3$) 1.321 (Hβ-1), 2.390 (Hα-1), 1.730 (Hβ-2), 1.550 (Hα-2), 4.622 (Hβ-3, dd, $J=12.1$, 3.7 Hz), 1.674 (H-5), 4.710 (H-6), 3.389 (Hβ-7), 2.456 (Hα-7), 2.099 (H-9), 5.583 (H-11), 1.960 (H_3-18), 1.234 (H_3-20), 1.846 (H_3-21), 1.268 (H_3-22), 0.946 (H_3-24), 0.782 (H_3-25), 4.137 (C_{17}-OMe), and 3.591 (C_{19}-OMe).
16. NOE enhancement between the methoxy group of MTPA and 24-CH_3 of **10R**.