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BIOSYNTHESIS OF CITREOHYBRIDONES, THE METABOLITES OF A HYBRID STRAIN KO 0031 DERIVED FROM *PENICILLIUM CITREO-VIRIDE* B. IFO 6200 AND 4692

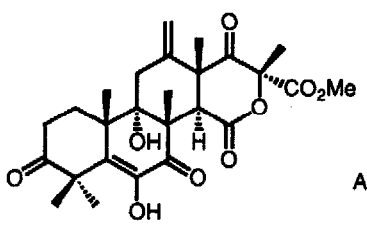
Seiji Kosemura, Hiroshi Miyata, Kimihiro Matsunaga, and Shosuke Yamamura*

Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223 Japan

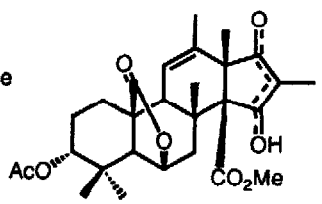
Key words: citreohybridone; biosynthesis; mixed polyketide-terpenoid(meroterpenoid) pathway

Summary: Incorporation of the ^{13}C-labelled acetate and formate into citreohybridones by cultures of a hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692 indicated that their biosynthesis proceeds *via* a mixed polyketide-terpenoid (meroterpenoid) pathway.

As described in the previous papers,[1,2] we isolated six new high potent antifeeding metabolites, citreohybridones A and B, isocitreohybridones A and B, and citreohybriddiones A and B, against *Plutella xylostella* from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. From a biogenetic point of view, these metabolites seem to be formed by successive methyl migration and skeletal rearrengement of sesterterpenoid containing five isoprene units. However, Simpson et al. has reported that terretonin(**1**),[3] which is one of mycotoxins isolated from *Aspergillus terreus* (NRRL 6273), is formed *via* a mixed polyketide-terpenoid (meroterpenoid) biosynthetic pathway. Thus, biosynthetic experiments on citreohybridones were carried out using sodium [1,2-$^{13}C_2$] acetate and sodium [^{13}C]formate, as follows.



1



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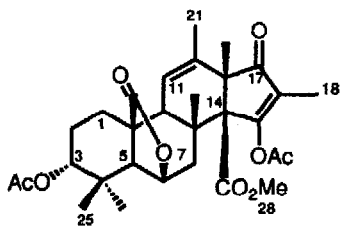


3

Table 1. ^{13}C NMR data[a] for the incorporation of [1,2-$^{13}C_2$]acetate into 3.

carbon	δ(ppm)[b]	J(Hz)	carbon	δ(ppm)	J(Hz)	carbon	δ(ppm)	J(Hz)
1	20.89	-	11	123.13	41.5	21	19.23	-
2	22.12	36.0[c]	12	134.04	-	22	22.12	36.0[c]
3	75.68	36.4	13	59.88	37.6	23	178.74	49.0
4	34.52	35.3	14	69.23	57.0	24	26.47	-
5	55.35	33.2	15	169.45	82.9	25	22.12	36.0[c]
6	76.71	33.2	16	131.96	82.9	26	170.62	59.5
7	37.70	-	17	198.96	-	27	20.89	59.5
8	41.10	36.5	18	9.52	-	28	52.32	-
9	51.64	41.5	19	169.65	57.0	29	164.91	-
10	43.70	49.0	20	17.31	37.6	30	21.55	-

a. ^{13}C NMR spectra were taken on a JNM-GX400 NMR spectrometer

b. Relative to TMS in $CDCl_3$.

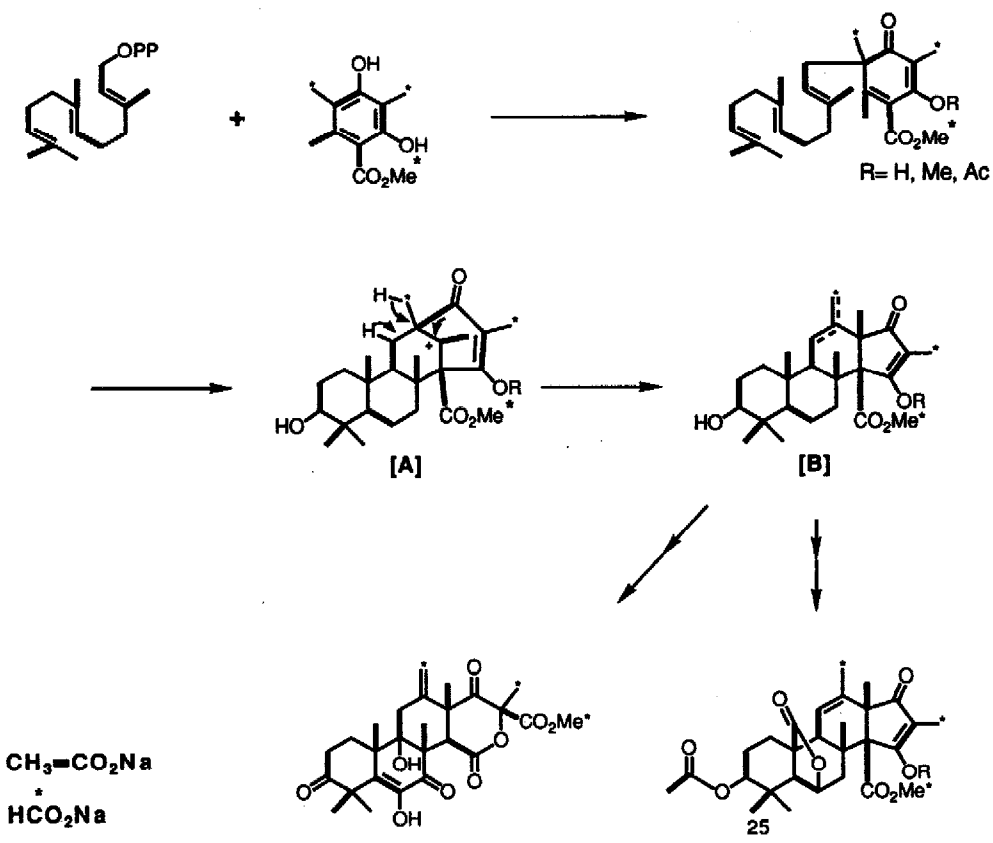
c. Overlapped with other satellites signals.

Citreohybridonol (2) exists in an equilibrium between two different tautomers on D ring in $CDCl_3$ as well as CD_3OD, resulting in some difficulties of signal assignments in the ^{13}C NMR spectrum. Therefore , 2 was treated with acetic anhydride - pyridine to afford citreohydridone A(3) and the ^{13}C NMR analysis was carried out on the basis of 2D-INADEQUATE experiments, wherein ^{13}C-^{13}C coupled signals due to doubly enriched carbon from [1,2-$^{13}C_2$]acetate were observed in the ^1H NMR complete decoupling experiments coupled with 2D-INADEQUATE experiments of labelled citreohybridone A and summarized as shown in Table 1. The average level of enrichment was estimated to be 12.7% based on the relative heights of the coupling satellites and natural abundance signals. The [^{13}C]formate-labelled sample showed signals resulting from three highly enriched carbons(300%) corresponding to C-18(δ 9.52), C-21(δ 19.23), and C-28(δ 52.32). The labelling pattern of citreohybridone A is summarised in scheme 1 and is compatible with the biosynthetic pathway proposed by Simpson.[3,7]

The present study strongly suggests that the biosynthetic intermediate [**B**] is regarded as a precursor of terretonin (**1**), as seen in scheme 1. Furthermore, it showed be noted that the acetoxyl group at C_3-position is in an α-configuration. Presumably, the α-acetoxyl group is produced by oxidation of the initially formed β-hydroxy one followed by successive reduction and acetylation.

In addition to searching for such an intermediate as [**A**], further biosynthetic study on citreohybridones is in progress using three different types of labelled 3,5-dimethylorsellinate.

According to essentially the same procedure as described in the previous papers,[1,2,4] polished rice (540g) in deionized water(1.4 l) including [1,2-$^{13}C_2$] acetate(1g)[5] was cooked using an electric rice cooker (100 °C, 20 min), and then transfered into an Erlenmyer flask(3 l x 5), which was pasteurized (121 °C, 20 min at 2.1 atm), then inoculated with a suspension of mycelium of the hybrid strain KO 0031 in a sterilized water and incubated stationarily at room temperature for 30 days and extracted with acetone and then EtOAc. The combined extracts were partitioned between EtOAc and water. The EtOAc extract (10.0 g) was directly chromatograhed on silica gel (40g, silica gel 60 K070, 70 ~230 mesh, Katayama Chemical). After elution of higher fatty acids and their esters with benzene, further elution with benzene-EtOAc(3:1) afforded a pale yellow oil (180 mg), which was further separated by repeated preparative TLC (Kieselgel PF_{254}) using acetone-$CHCl_3$(1:20~30), acetone-hexane(1:1.5~2) and then EtoAc-benzene(1:3) to give a new metabolite, named citreohybridonol (**2**) [92.2mg][6] which was formed on acid-catalyzed hydrolysis of citreohybridone A (**3**). Unexpectedly, citreohybridones have not been isolated in this experiment.



Scheme 1

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5. Incorporation of the [^{13}C]formate was carried out with the same manner of [1,2-$^{13}C_2$]acetate.
6. Citreohybridonol(3) as a colorless oil : $[\alpha]_D^{20}$ +67.3° (c 0.066, $CHCl_3$); $C_{28}H_{36}O_8$ [m/z 500.2398(M^+)]; IR(film) 3200, 1770, 1740 and 1620cm^{-1}; major tautomer(60%): δ($CDCl_3$) 5.67(1H, bs, C_{11}-H), 4.72(1H, d, J=3.9Hz, C_6-Hα), 4.65(1H, dd, J=2.5, 2.5Hz, C_3-Hβ), 3.67(3H, s, C_{28}-H_3), 3.63(1H, d, J=14.2Hz, C_7-Hα), 2.51(1H, dd, J=14.2, 4.4Hz, C_7-Hβ), 2.02(3H, s, C_3-OAc), 1.87(3H, s, C_{21}-H_3), 1.33(3H, s), 1.32(3H, s), 0.94(3H,s), and 0.89(3H, s), other signals(δ 2.25~1.25, 9H) are overlapped with one another; minor tautomer(40%): δ($CDCl_3$) 5.83(1H, bs, C_{11}-H), 4.78(1H, d, J=3.9Hz, C_6-Hα), 4.68(1H, dd, J=2.5, 2.5Hz, C_3-Hβ), 3.61(3H, s, C_{28}-H_3), 2.93(1H, d, J=14.2Hz, C_7-Hα), 2.75(1H, d, J=14.2, 4.4Hz, C_7-Hβ), 2.40(1H, dd, J=2.4, 2.4Hz, C_9-H), 2.07(3H, s, C_3-OAc), 1.87(3H, s), 1.43(3H, s), 1.25(3H, s), 0.97(3H, s), and 0.91(3H, s); other signals(δ 2.25~1.25, 8H) are overlapped with one another.
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