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CITREOHYBRIDDIONE C, A MEROTERPENOID OF A HYBRID STRAIN KO 0031 DERIVED FROM *PENICILLIUM CITREO-VIRIDE* B. IFO 6200 AND 4692

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Key Word Index—*Penicillium citreo-viride* B.; hybrid strain; meroterpenoid; citreohydbriddione C; rice.

Abstract—A new hexacyclic-meroterpenoid, citreohybriddione C, has been isolated from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Its stereostructure has been also elucidated on the basis of its spectral data. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

As described in the previous papers [1–4], we have isolated several new high potent antifeedant metabolites, citreohybridones A (**1**) and B (**2**) and citreohybriddione B (**3**), against *Plutella xylostella* from the mycelium of the hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. Recently, Shiome *et al.* isolated and determined the absolute configuration of andrastins A (**4**), B (**5**) and C (**6**) [5], new protein farnesyltransferase inhibitors, from *Penicillium* sp. FO-3929. Interestingly, it is clear that these metabolites must be precursors of citreohybridones [3, 4]. In view of the biological significance of these unique meroterpenoids [6–14], we further examined the metabolites in the same mycelium of the hybrid strain KO 0031. In this communication we wish to report the isolation and structural elucidation of a new hexacyclic-meroterpenoid (mixed polyketide-terpenoid), citreohybriddione C (**7**).

RESULTS AND DISCUSSION

Assignments of the ^{13}C and ^{1}H NMR chemical shifts derived from ^{13}C NMR, ^{1}H NMR, HMQC and HMBC experiments in benzene-d_6 are depicted in Table 1. The structure of **7** (Fig. 1) was derived based on extensive spectroscopic analysis. HR-EIMS suggested a molecular formula of $C_{28}H_{34}O_9$ ([M]$^+$, *m/z* found: 514.2201, calculated: 514.2201) for **7**, thus revealing 12 degrees of unsaturation. This formula was supported by the presence of 28 carbons in its ^{13}C NMR spectrum. The carbon types, revealed by a HMQC experiment, included one methoxy group, seven methyl groups, three methylene groups, four methine groups and 12 non-protonated carbon atoms, five of which were in carbonyl grouping: δ 204.0 (ketone, C-15), 200.8 (ketone, C-17), 177.2 (lactone, C-23), 169.4 (acethoxy, C-26) and 167.6 (methyl ester, C-19). The ^{1}H and ^{13}C NMR data are very similar to those of citreohybriddione B (**3**) [2] except for the absence of an exomethylene group on C ring and the presence of an oxygenated quaternary carbon (δ 76.2) with a tertiary methyl (δ_H 1.01, δ_C 23.6) attached. Finally the stereochemistry of citreohybriddione C (**7**), especially of the configuration of the ether linkage from C_{12}- and C_{16}-position, was elucidated by NOESY experiments in benzene-d_6. The NOEs between H-5 (δ 2.21)/H_3-24 (δ 0.83), Hβ-7 (δ 2.47)/H_3-22 (δ 1.82), H-11 (δ 5.46)/H_3-21 (δ 1.01), H_3-20 (δ 1.15)/H_3-21 (δ 1.01), H_3-20 (δ 1.15)/H_3-22 (δ 1.82), and H_3-20 (δ 1.15)/H_3-28 (δ 3.08) suggested the relative configuration of **7** (Fig. 1).

EXPERIMENTAL

Optical rotation was determined with a JASCO A-202 spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM α-400 NMR spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants are given 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. Preparative and analytical TLC were carried out on Kieselgel 60 PF_{254} (E. Merck A. G. Germany), unless otherwise stated.

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°, 7

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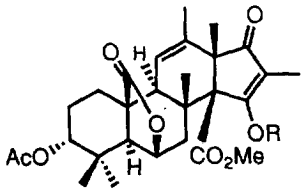
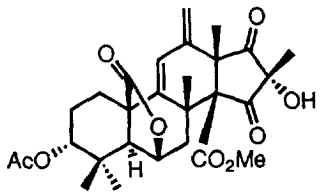
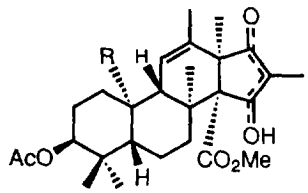


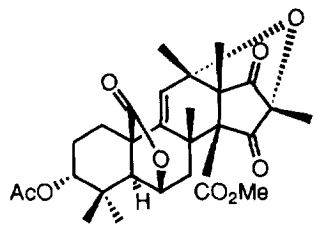
R=Ac; Citreohybridone A (1)
R=Me; Citreohybridone B (2)



Citreohybriddione B (3)



R=CHO; Andrastin A (4)
R=CH_2OH; Andrastin B (5)
R=CH_3; Andrastin C (6)



Citreohybriddione C (7)

days), using cellulase, chitinase, pectolyase and sulfatase (30°C, 60 min). 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°, 3d) to give a number of colonies, from which many new hybrid strains including *Penicillium citreoviride* KO 0031 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, Abstr., No. 4FF 1-6).

Incubation. Polished rice (*ca* 4.5 kg) in deionized water (*ca* 6 l) was cooked using an electric cooker (99°, *ca* 20 min) and transferred into 35 Erlenmeyer flasks (3 l), which were pasteurized at 121° for 20 min at 2.1 atm. After inoculation with a suspension of the mycelium of the hybrid strain KO 0031 in a sterilized water, the rice was incubated stationarily at 25° for 30 days and extracted with acetone (160 l).

Isolation and separation. The acetone extract suspended in water (800 ml) was extracted with EtOAc (1 l × 5). The EtOAc extract (dark brown syrup, 64.2 g) was chromatographed 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 using acetone–$CHCl_3$ (1:20), acetone–hexane (1:2), and then EtOAc–ben-

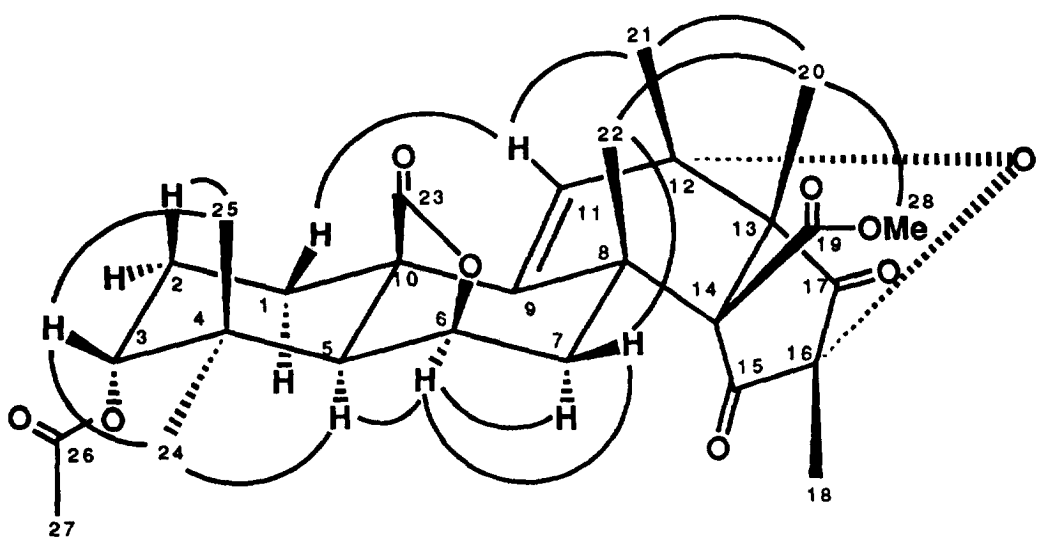


Fig. 1. NOESY experiment of citreohybriddione C (7).

Table 1. Chemical shifts assignments and ^1H–^{13}C long range connectivities for **7** (C_6D_6, 25°C)

Position	δ C* (100 MHz)	δ H (400 Hz)	Long-range coupled protons†
1	19.9 (*t*)	1.95 (*m*, 2H)	3-H
2	22.4 (*t*)	1.79 (*m*, 2H)	
3	75.7 (*d*)	4.70 (*d*, *J* = 2.7)	1-H_2, 25-H_3
4	35.0 (*s*)		25-H_3
5	50.6 (*d*)	2.21 (*s*)	24-H_3
6	76.7 (*d*)	4.41 (*dd*, *J* = 3.7, 1.5)	7-Hβ
7	38.3 (*t*)	2.81 (*dd*, *J* = 14.0, 1.5, Hα)	22-H_3
		2.47 (*dd*, *J* = 14.0, 3.7, Hβ)	
8	38.8 (*s*)		22-H_3, 11-H, 6-H
9	147.2 (*s*)		22-H_3
10	48.1 (*s*)		11-H, 2-H_2
11	123.9 (*d*)	5.46 (*s*)	21-H_3
12	76.2 (*s*)		21-H_3, 20-H_3
13	53.5 (*s*)		21-H_3, 20-H_3, 11-H
14	72.1 (*s*)		20-H_3, 22-H_3
15	204.0 (*s*)		18-H_3
16	75.7 (*s*)		18-H_3
17	200.8 (*s*)		18-H_3, 20-H_3
18	7.8 (*q*)	1.38 (*s*)	
19	167.6 (*s*)		28-H_3
20	11.0 (*q*)	1.15 (*s*)	
21	23.6 (*q*)	1.01 (*s*)	
22	31.8 (*q*)	1.82 (*s*)	
23	177.2 (*s*)		5-H, 6-H
24	26.5 (*q*)	0.83 (*s*)	
25	22.7 (*q*)	0.76 (*s*)	
26	169.4 (*s*)		
27	20.2 (*q*)	1.66 (*s*)	
28	51.6 (*q*)	3.08 (*s*)	

*Multiplicities deduced from HMQC:

†Correlations deduced from HMBC; abbreviations, *s*: singlet, *d*: doublet, *t*: triplet, *q*: quartet.

zene (1:3) to give a new meroterpenoid, named citreohybriddione C (**7**) as an oil in 0.079% yield.

Physical data for citreohybriddione C (**7**). $[\alpha]_D^{24}$ +58.4° ($CHCl_3$; *c* 1.2); $C_{28}H_{34}O_9$ (*m/z*: 514.2201 $[M]^+$); IR (film) ν_{max} 1810, 1785, 1765, 1745, and 1240 cm^{-1}: EIMS *m/z* (rel. int.): 514 $[M]^+$ (20), 472 $[M-42]^+$ (20), 454 $[M-60]^+$ (14), 440 $[M-74]^+$ (13), 378 $[M-136]^+$ (19), 341 $[M-173]^+$ (40), 323 $[M-191]^+$ (64), 311 $[M-203]^+$ (51), 253 $[M-261]^+$ (51), 207 $[M-307]^+$ (95), 183 $[M-331]^+$ (100), and 147 $[M-367]^+$ (65). HR-EIMS ($[M]^+$ obs., 514.2201, calcd. for $C_{28}H_{34}O_9$, 514.2201). ^1H and ^{13}C NMR: Table 1.

Stability of **7** *on silica gel.* **3** (2.0 mg) in $CHCl_3$–MeOH (1.0 ml, 0.1 ml) was absorbed on SiO_2 (100 mg) at room temp. for 2 days. After addition of EtOAc (15 ml), the reaction mixture was washed with water (10 ml × 2). The EtOAc solution was dried over anhydrous Na_2SO_4 and concd under red. pres. to leave an oil, whereupon TLC analysis did not show any spot of **7** except for the unchanged starting material. Thus **7** is not an artifact of **3**.

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