Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 5055–5072

TETRAHEDRON

Meroterpenoids from *Penicillium citreo-viride* B. IFO 4692 and 6200 hybrid

Seiji Kosemura*

Department of Chemistry, Hiyoshi Campus, Keio University, 4-1-1 Hiyoshi Kohoku-ku, Yokohama 223-8521, Japan

Received 27 March 2003; accepted 6 May 2003

Abstract—Twenty metabolites, citreohybridones, have been isolated from the mycelium of the hybrid strain KO 0031, prepared by cell fusion technique using *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 been also elucidated by the modified Mosher's methods. Incorporation of ^{13}C-labelled acetate, formate and ethyl 3,5-dimethylorsellinate into citreohybridones has established that their biosynthesis proceeds via a mixed polyketide-terpenoid (meroterpenoid) pathway.

1. Introduction

In connection with citreoviridin (**1**), a potent inhibitor of ATP-synthesis and ATP-hydrolysis catalyzed by mitochondrial enzyme system, a number of novel metabolites have been isolated from the mycelium of *Penicillium citreo-viride* B. Particularly, citreoviridin (**1**) and related pyrones have been mainly produced by *P. citreo-viride* B. IFO 6200.[1–4] In the case of another strain IFO 4692, however, citreovirone (**2**) and related phenols have been obtained as main products.[5] In the light of these results, more than ten hybrid strains have been produced by means of cell fusion technique using two different strains of IFO 6200 and 4692, and several interesting metabolites (**3**–**13**) have been obtained from the mycelia of the hybrid strains KO 0011[6–8] and KO 0092[9] (Fig. 1). Among them, the hybrid strain KO 0031 was used for the present study. In this communication we wish to report the isolation and structure elucidation of twenty high potent antifeeding meroterpenoids (mixed polyketide-terpenoid) against the diamondback moth (*Plutella xylostella*),[10] citreohybridones A–G (**14**–**20**), J–L (**21**–**23**), isocitreohybridones A–C (**24**–**26**), G–I (**27**–**29**), citreohybriddiones A–C (**30**–**32**), and citreohybridonol (**33**) produced by the hybrid strain KO 0031,[11–18] and their absolute configurations. Biosynthetic studies on citreohybridones (Fig. 2) will be also reported.

2. Results and discussion

2.1. Structure determination

Citreohybridone A (**14**) was obtained as a colorless prisms, mp 261.5–263°C in a sealed tube (from benzene–hexane); analyzed for $C_{30}H_{38}O_9$ by HR-EIMS [*m*/*z* 542.2491 (M^+)]. The ^{1}H NMR spectrum of **14** showed the presence of an olefinic proton (δ_H 5.68, C_{11}–H), two acetoxyl groups [δ_H 2.16 (C_3–OAc), 2.31 (C_{15}–OAc)], one methoxyl group (δ_H 3.66, C_{19}–OMe), two methyl groups with a double bond [δ_H 1.63 (H_3-18), 1.75 (H_3-21)], four methyl groups connected to quaternary carbons [δ_H 0.90 (H_3-25), 0.93 (H_3-24), 1.27 (H_3-20), 1.41 (H_3-22)]. The ^{13}C NMR spectrum showed the presence of 30 carbons including five carbonyl carbons [δ_C 164.91 (C_{15}–OAc), 169.65 (C–19), 170.62 (C_3–OAc), 178.74 (C-23), 198.96 (C-17)], four olefinic carbons [δ_C 123.13 (C-11), 131.96 (C-16), 134.04 (C-12), 169.45 (C-15)], and three oxygenated carbons {two methines [δ_C 75.68 (C-3), 76.71 (C-6)] and one carboxymethyl [δ_C 52.32 (C_{19}–OMe)]}. The gross structure of **14** was determined by detailed analyses of one and two dimentional NMR spectra.[11] The relative stereochemistry of **14** was clarified by the NOE difference spectra. Irradiation of the C_5-proton (δ_H 1.71) of **14** resulted in 2.8% NOE of the C_7-β-proton (δ_H 2.29) and 14.1% NOE of the C_9-proton (δ_H 2.51), irradiation of the C_9-proton (δ_H 2.51) resulted in 2.2% NOE of the C_7-β-proton (δ_H 2.29), irradiation of the C_{11}-proton (δ_H 5.68) resulted in 7.7% NOE of the C_1-α-proton (δ_H 2.16), irradiation of the C_{15}–OAc (δ_H 2.31) resulted in 5.7% NOE of the C_{18}-methyl group (δ_H 1.63), irradiation of the C_{20}-methyl group (δ_H 1.27) resulted in 1.2% NOE of the C_{19}-methoxyl group (δ_H 3.66) and 3.0% NOE of the C_{22}-methyl group (δ_H 1.41), irradiation of the C_{21}-methyl group (δ_H 1.75) resulted in

Keywords: *Penicillium citreo-viride* B; hybrid strain; meroterpenoid; citreohybridone; absolute configuration; biosynthesis; antifeedant.

* Tel.: +81-45-566-1379; fax: +81-45-566-1314;
e-mail: kose@hc.cc.keio.ac.jp

0040–4020/03/$ - see front matter
doi:10.1016/S0040-4020(03)00739-7

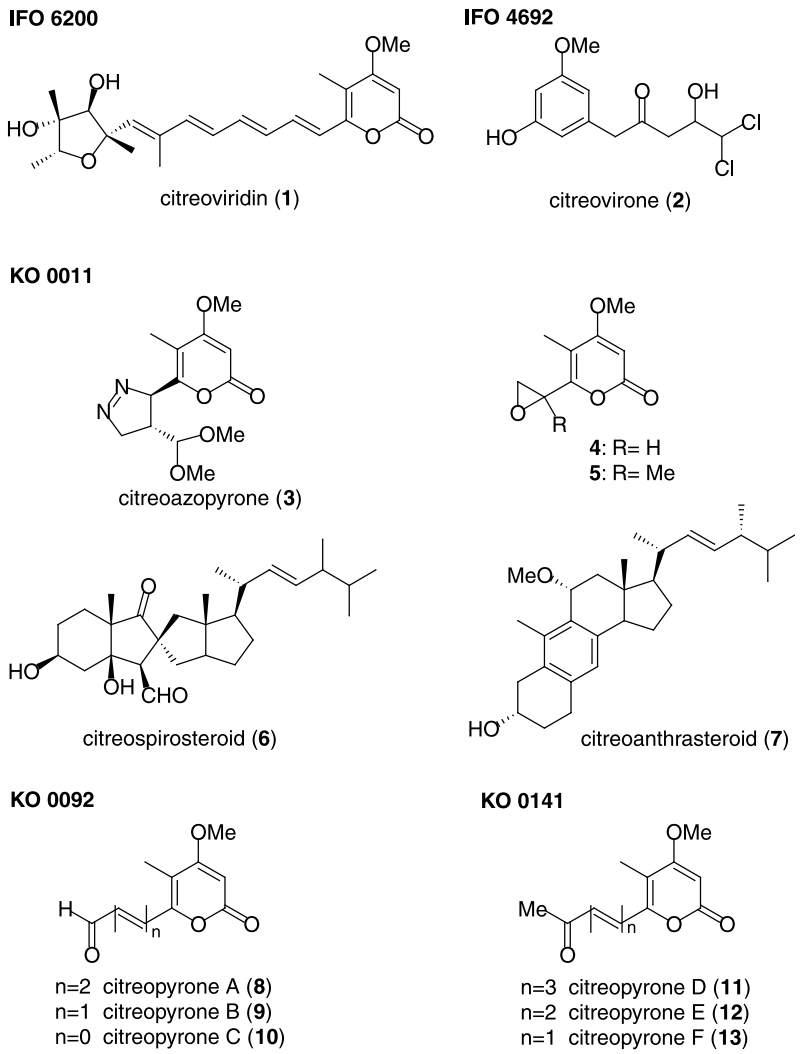


Figure 1. Metabolites of *P. citero-viride* IFO 6200, 4692 and their hybrid strains.

6.4% NOE of the C_{11}-proton (δ_H 5.68), irradiation of the C_{22}-methyl group (δ_H 1.41) resulted in 5.6% NOE of the C_7-α-proton (δ_H 2.74), and irradiation of the C_{24}-methyl group (δ_H 0.93) resulted in 8.8% NOE of the C_5-proton (δ_H 1.71) (see Figure 5, and Tables 1 and 2). As seen in the spectral data (Tables 1 and 2), both citreohybridone A (**14**) and B (**15**) are structurally quite similar to each other except for the following points. Citreohybridone A (**14**) with a molecular formula $C_{30}H_{38}O_9$ has two acetoxyl groups [δ_H 2.16 (C_3–OAc), 2.31 (C_{15}–OAc); δ_C 169.45 (C-15), 131.96 (C-16)], and one methoxyl group [δ_H 3.66 (C_{19}–OMe)], while citreohybridone B (**15**) with a molecular formula $C_{29}H_{38}O_8$ [*m/z* 514.2569 (M^+)] has two methoxyl groups [δ_H 3.64 (C_{19}–OMe), 4.15 (C_{15}–OMe); δ_C 179.12 or 178.68 (C-15), 117.03 (C-16)], and one acetoxyl group [δ_H 2.04 (C_3–OAc); δ_C 77.35 or 76.25 (C-3)], indicating that the latter has the methoxyl group instead of the acetoxyl group at C-15 position in citreohybridone A (**14**) (Fig. 3).

Citreohybridone C (**16**), $C_{27}H_{36}O_7$ [*m/z* 472.2456 (M^+)] showed ^{1}H and ^{13}C NMR data (Tables 1 and 2) very similar to those of citreohybridone B (**15**). The ^{1}H NMR signal at δ_H 4.71 (1H, dd, *J*=2.7, 2.7 Hz) for the methine proton in **15** was replaced by a signal at δ_H 3.47 (1H, dd, *J*=3.7, 1.5 Hz) and no AcO group was observed in **16**, suggesting that **16** has OH group instead of AcO group on C-3. As expected, on treatment with Ac_2O–pyridine (room temp., over night), **16** was readily converted into **15** (Fig. 3).

Citreohybridone D (**17**), $C_{30}H_{40}O_8$ [*m/z* 528.2700 (M^+)] showed ^{1}H and ^{13}C NMR data (Tables 1 and 2) similar to those of citreohybridone A (**14**). The ^{13}C NMR signal at δ_C 76.71 (d, C-6) for the methine carbon bearing an oxygen atom in **14** was replaced by a signal at δ_C 33.1 (t, methylene carbon) in **17**, suggesting that **17** does not form the lactone ring; moreover, **17** has an aldehyde group [ν_{max} 1715 cm^{-1}, δ_C 204.7 (d), δ_H 10.1 (1H, s)], suggesting that **17** is the precursor of **14**. The structure of **17** was based on its spectral data and 2D NMR experiments (COSY, HMBC, HMBC, NOESY) (Fig. 3).[17]

Citreohybridone E (**18**) and F (**19**) 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 (Fig. 3). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were closely related to those of **17**. Citreohybridone E (**18**) is a carboxylic acid [ν_{max} 3200 (br.) cm^{-1}, δ_C 178.4 (s), δ_H 10.1 (1H, s)] and citreohybridone F (**19**) must be the corresponding methyl ester [ν_{max} 1745 cm^{-1}, δ_C 175.1 (s), 51.0 (q), δ_H 3.61 (3H, s)],

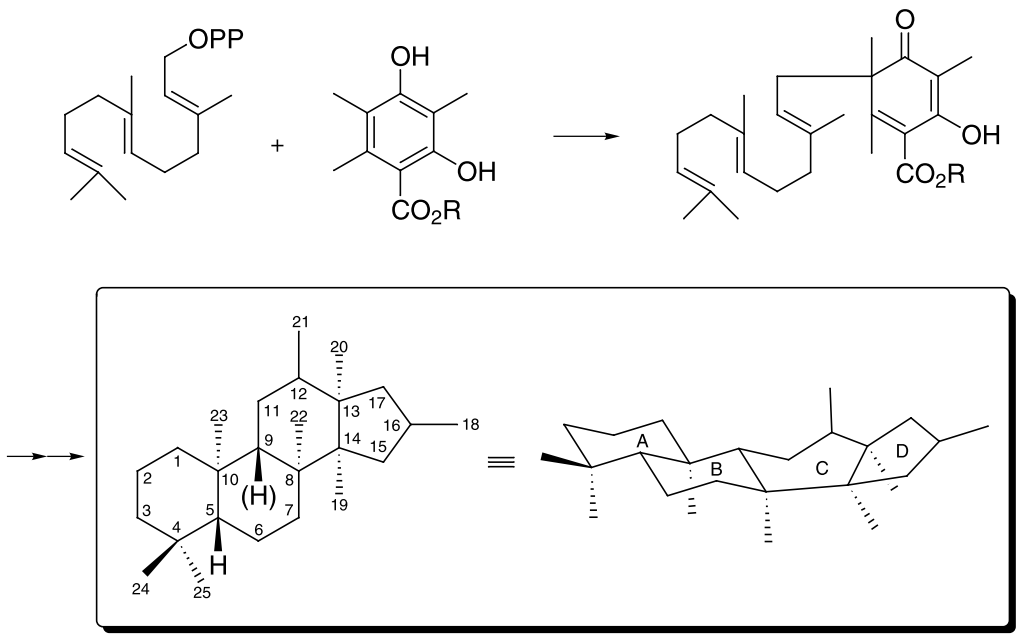


Figure 2. Carbon skeleton for citerohybridones.

Table 1. ^{13}C NMR data for citreohybridones (100 MHz, $CDCl_3$ or C_6D_6, δ in ppm)

Carbon	**14**	**15**	**16**	**17**	**18**	**19**	**21**[a]	**24**	**25**	**26**	**29**	**30**	**31**	**32**[a]
1	20.9	21.1	20.3	27.3	29.7	29.7	20.4	21.0	21.0	20.1	20.4	21.3	20.2	19.9
2	22.1	22.1	25.0	23.0	24.0	24.0	22.6	22.1	22.1	24.9	22.2	22.0	22.1	22.4
3	75.7	(76.3)	73.7	77.9	77.9	78.0	75.9	77.7	76.2	73.9	76.1	75.9	75.8	75.7
4	34.5	34.3	34.5	36.7	36.9	36.8	35.2	(34.3)	34.3	35.1	34.4	34.4	34.6	35.0
5	55.4	55.5	54.7	48.5	49.6	49.5	51.8	54.5	54.4	53.7	54.5	55.6	46.3	50.6
6	76.7	(77.4)	77.7	16.5	17.4	17.6	77.2	76.1	77.9	78.2	77.5	77.4	77.6	76.7
7	37.7	37.7	37.6	33.1	33.4	33.4	40.5	36.5	36.6	36.4	37.7	35.2	37.8	38.3
8	41.1	41.4	41.4	40.7	41.2	41.1	39.6	(43.6)	43.3	43.3	40.5	43.7	40.3	38.8
9	51.6	52.5	52.2	53.6	52.3	52.3	148.4	51.5	51.3	51.0	55.2	53.1	147.3	147.2
10	43.7	43.5	43.5	51.7	46.7	47.1	49.1	(44.0)	43.6	43.7	45.1	(39.7)	47.4	48.1
11	123.1	122.2	122.4	123.6	124.6	124.8	123.6	125.0	123.8	123.9	72.8	128.8	125.2	123.9
12	134.0	135.9	135.6	132.3	131.6	131.3	80.0	137.0	138.8	138.4	152.4	135.4	140.5	76.2
13	59.9	58.6	58.4	59.6	59.5	59.5	53.0	(52.7)	52.9	52.7	51.3	(59.4)	58.7	53.5
14	69.2	69.3	69.3	67.1	67.8	67.8	62.9	(72.4)	71.2	71.1	70.6	(74.1)	72.2	72.1
15	169.5	(179.1)	179.4	169.3	169.5	169.6	79.0	203.9	203.8	203.9	203.5	212.8	210.7	204.0
16	132.0	117.0	117.0	131.1	131.2	131.1	81.4	(125.8)	111.1	111.3	113.4	(76.2)	71.4	75.7
17	199.0	200.0	200.4	199.9	200.2	200.4	80.4	(174.2)	182.9	183.1	183.5	210.8	207.7	200.8
18	9.5	9.2	9.2	8.9	8.9	8.9	14.5	7.9	8.4	8.7	8.6	(20.1)	20.7	7.8
19	169.7	(169.7)	170.4	169.7	169.7	169.8	174.1	169.2	170.0	170.1	169.9	(167.8)	(167.3)	167.6
20	17.3	17.2	17.2	15.4	15.7	15.7	13.9	17.6	18.0	18.0	20.1	(18.9)	22.0	11.0
21	19.2	19.3	19.4	18.9	18.9	18.9	25.7	21.1	21.1	21.1	118.1	18.8	118.9	23.6
22	22.1	22.8	22.9	19.0	15.6	15.7	30.2	25.2	24.8	24.7	26.4	24.5	30.7	31.8
23	178.7	(178.7)	179.3	204.7	178.4	175.1	177.6	(179.1)	179.2	179.8	179.6	178.3	(177.6)	177.2
24	26.5	26.4	26.6	26.9	27.8	27.8	26.7	26.3	26.2	26.4	26.5	26.4	26.4	26.5
25	22.1	22.1	22.5	20.8	22.2	22.1	22.9	22.3	22.3	22.7	22.8	22.3	22.3	22.7
3-OAc	170.6	(170.2)		170.3	170.4	170.4	169.2	(170.2)	170.1		170.4	(169.9)	(170.2)	169.4
3-OAc	20.9	21.0		21.0	21.1	21.1	20.3	21.0	21.0		22.1	(20.9)	20.9	20.2
15-OAc	164.9			165.3	165.4	165.4								
15-OAc	21.6			21.4	21.4	21.4								
15-OMe		59.3	59.7											
17-OAc							169.5	(165.0)						
17-OAc							20.1	20.7						
17-OMe									59.6	59.6	59.6			
19-OMe	52.3	51.9	51.9	52.2	52.1	52.1	51.5	51.7	51.5	51.5	51.5	52.5	52.1	51.6
23-OMe						51.0								

Signals were measured in $CDCl_3$ with reference to the center peak of $CDCl_3$ (δ 77.0 ppm). ( ) Assignments were strictly empirical.
[a] Signals were measured in C_6D_6 with reference to the center peak of C_6D_6 (δ 128.0 ppm).

Table 2. ^{1}H NMR data for citreohybridones A–G and J–L (400 MHz, $CDCl_3$ and/or C_6D_6, δ in ppm)

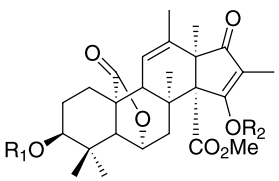
Position	14	14[a]	15	15[a]	16	17	18	19	20	21[a]	22	23
1α	2.16	2.18	2.22	2.25	2.13	1.03	1.22	1.24	0.96	(1.9)	(2.2)	(2.0)
1β	1.42	1.35	1.39	1.20	1.53	2.30	2.25	2.30	2.34	(1.8)	(1.2)	(1.4)
2	1.65–1.82	1.65	1.79	1.68	1.61, 1.78	1.53–1.63	1.66	1.64	1.58	1.8–1.9	1.2–2.2	1.4–2.0
3	4.65	4.72	4.71	4.73	3.47	4.58	4.55	4.58	4.62	4.72	4.61	4.56
5	1.71	1.57	1.89	1.75	1.92	1.60	1.30	1.36	1.70	2.74	2.89	2.81
6α						(1.55)	2.02	2.06	1.58			
6β	4.71	4.31	4.70	4.39	4.68	(1.60)	2.41	2.43	1.90	4.38	4.67	4.63
7α	2.74	2.90	2.59	2.76	2.54	(2.02)	1.42	1.50	2.54	1.80	2.26	2.05
7β	2.29	2.18	2.69	2.62	2.67	(2.36)	2.23	2.23	2.23	2.60	1.52	1.70
9	2.51	2.64	2.32	2.35	2.30	2.15	2.07	2.07	1.90			
11	5.68	5.74	5.62	5.71	5.59	5.48	5.73	5.73	5.39	5.48	5.96	5.91
15										4.66	4.33	3.94
17										5.03	4.99	5.17
18	1.63	1.64	2.00	1.79	1.95	1.58	1.53	1.58	1.92	1.13	1.39	1.25
19												4.22, 4.27
20	1.27	1.35	1.23	1.37	1.19	1.15	1.12	1.17	1.16	1.41	1.10	1.05
21	1.75	1.80	1.76	1.87	1.72	1.65	1.64	1.68	1.67	1.37	1.89	1.84
22	1.41	1.79	1.39	1.77	1.35	1.19	1.20	1.17	1.20	1.61	1.42	1.09
23						10.1			10.1			
24	0.93	0.63	0.97	0.72	1.03	0.90	0.84	0.88	0.92	0.78	0.96	0.93
25	0.90	0.75	0.92	0.76	0.81	0.83	0.84	0.82	0.84	0.76	0.89	0.84
3-OAc	2.16	1.50	2.04	1.67		2.07	2.04	2.08	2.07	1.55	2.02	(1.98)
15-OAc	2.31	2.05				2.32	2.27	2.31				
15-OMe			4.15	3.48	4.10				4.09			
17-OAc										1.59	2.17	(2.11)
19-OAc												(2.10)
19-OMe	3.66	3.19	3.64	3.23	3.60	3.61	3.55	3.59	3.60	3.23	3.78	
23-OMe								3.61				

Signals were measured in $CDCl_3$. ( ) Assignments were strictly empirical.
[a] Signals were measured in C_6D_6.

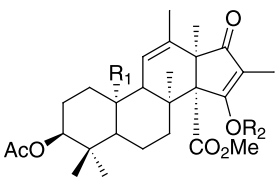
suggesting that both metabolites are regards as the further oxygenated products of the aldehyde (**17**).

Citreohybridone G (**20**) has a molecular formula $C_{29}H_{40}O_7$ [*m/z* 500.2770 (M^+)], and its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) are closely related to those of Citreohybridone D (**17**) excepted in the following points. Citreohybridone D (**17**) have two acetoxy groups [δ_H 2.07 (C_3–OAc), 2.32 (C_{15}–OAc)] and a methoxyl group [δ_H 3.61 (C_{19}–OMe)]; on the other hand, citreohybridone G (**20**) has one acetoxy group [δ_H 2.07 (C_3–OAc)], and two methoxyl groups [δ_H 4.09 (C_{15}–OMe), 3.60 (C_{19}–OMe)], indicating that **20** has the methoxyl group instead of the acetoxyl group at C-15 position in **17**. Citreohybridone D (**17**) was subjected to hydrolysis with 20% aq H_2SO_4–MeOH–$CHCl_3$ (1:3:3) followed by methylation with $TMSCHN_2$ in MeOH–benzene to give as expected, the citreohybridone G and isocitreohybridone G in 10 and 8.3% yields, respectively.

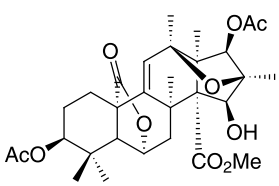
The structure of citreohybridone J (**21**) was derived based on extensive spectroscopic analysis. HR-EIMS suggested a



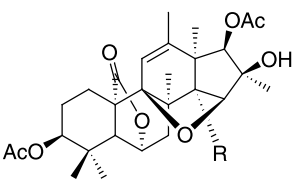
citreohybridone A (**14**): R_1= Ac, R_2= Ac
citreohybridone B (**15**): R_1= Ac, R_2= Me
citreohybridone C (**16**): R_1= H, R_2= Me



citreohybridone D (**17**): R_1= CHO, R_2= Ac
citreohybridone E (**18**): R_1= CO_2H, R_2= Ac
citreohybridone F (**19**): R_1= CO_2Me R_2= Ac
citreohybridone G (**20**): R_1= CHO, R_2= Me



citreohybridone J (**21**)



citreohybridone K (**22**): R=CO_2Me
citreohybridone L (**23**): R=CH_2OAc

Figure 3. Structures of citerohybridones A–G (**14**–**20**) and J–K (**21**-**23**).

molecular formula of $C_{30}H_{40}O_{10}$ [*m/z* 560.2620 (M^+)] for **21**, thus revealing 11 degrees of unsaturation. This formula was supported by the presence of 30 carbons in its ^{13}C NMR spectrum (Table 1). The carbon types, revealed by a HMQC experiment, included one methoxyl, eight methyls, three methylenes, six methines and twelve quaternary carbons, four of which were carbonyls: δ_C 177.6 (lactone, C-23), 174.1 (methyl ester, C-19), 169.5 (C_{17}–OAc), and 169.2 (C_3–OAc). The ^1H NMR data (Table 2) are similar to those of citreohybridone B (**15**) except for the absence of a proton for H-9 and the presence of two protons (δ_H 4.66, *J*=11.1 Hz, H-15; δ_H 5.03, H-17) and hydroxyl group (δ_H 2.62, *J*=11.1 Hz, C_{15}–O*H*). Finally, the stereochemistry of citreohybridone J (**21**), especially of the configuration of the ether linkage from C_{12}- to C_{16}-position and D ring, was elucidated by NOESY experiment in benzene-D_6. The NOEs between H_3-18 (δ_H 1.13)/H-17 (δ_H 5.03) and H-15 (δ_H 4.66); MeO–C_{19} (δ_H 3.23)/H-15 (δ_H 4.66), H-17 (δ_H 5.03), H_3-20 (δ_H 1.41) and H_3-22 (δ_H 1.61); H_3-21 (δ_H 1.37)/H-11 (δ_H 5.48) suggested the relative configuration of **21** (Figs. 3 and 5).

Citreohybridone K (**22**), $C_{30}H_{40}O_{10}$ [*m/z* 560.2616 (M^+)] showed ^1H NMR data (Table 2) very similar to those of citreohybridone J (**21**). The ^1H NMR signal at δ_H 5.48 (1H, s, H-11) for the olefinic proton in **21** was replaced by a signal at δ_H 5.96 (1H, d, *J*=1.1 Hz) was observed in **22**, suggesting that **22** has an ether linkage on another position in **22**. The NOESY experiment clarified the position of the ether linkage and the stereochemistry of citreohybridone K (**22**) as shown in Figure 5. Especially, the NOE between Hβ-7 (δ_H 1.52) and H-15 (δ_H 4.33) suggested the ether linkage is on C-9 and C-15 of **22**, because of the NOE observed between these protons will be impossible by another positions the ether linkage for rigid carbon skeleton.

Citreohybridone L (**23**) has a molecular formula $C_{31}H_{42}O_{10}$ [*m/z* 574.2804 (M^+)]. The ^1H NMR spectrum of **23** showed the presence of an olefinic proton (δ_H 5.91, C_{11}–H), three acetoxyl groups [δ_H 2.11, 2.10, 1.98, C_3–, C_{17}–, C_{19}–OAc)], one methyl groups with a double bond [δ_H 1.84 (H_3-21)], five methyl groups connected to quaternary carbons [δ_H 1.25 (H_3-18), 1.09 (H_3-22), 1.05 (H_3-20), 0.93 (H_3-24), 0.84 (H_3-25)]. Citreohybridone L (**23**), showed ^1H NMR data very similar to those of citreohybridone K (**22**), except for the absence of a methoxyl group and the presence of an isolated methylene group (δ_H 4.27 and 4.22, *J*=12.1 Hz, C-19) and an additional acetoxyl group. Citreohybridone **L** is the first compound that the carboxyl group at the C-19 position of citreohybridones is reduced to alcohol.

The spectral data (Tables 1–3) of citreohybridones A (**14**) and B (**15**) and isocitreohybridones A (**24**) and B (**25**), are quite similar to one another, particularly in their ^1H NMR spectra. In fact, citreohybridone A (**14**) was subjected to hydrolysis with 20% aq. H_2SO_4–MeOH–$CHCl_3$ (1:3:3) (60–70°C, 1 h) followed by acetylation with Ac_2O–pyridine (room temp., overnight) to give the isocitreohybridone A (**24**) and citreohybridone A (**14**) 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 (**14**) was readily converted into isocitreohybridone B (**25**) and citreohybridone B (**15**) in 47 and 40% yields, respectively. This result suggested that **24** and **25** must be the isomer of **14** and **15** on D ring, each other (see Figs. 3 and 4).

Table 3. ^1H NMR data for isocitreohybridones A–C and G–I, citreohybriddione A–C, and citreohybridonol (400 MHz, $CDCl_3$ and/or C_6D_6, δ in ppm)

Position	**24**[a]	**25**	**25**[a]	**26**	**27**	**28**	**29**	**30**[a]	**31**[a]	**32**[a]	**33** (major)[b]	**33** (minor)[c]
1α	2.26	2.16	2.29	2.05	0.92	2.12	1.8	2.12	2.07	1.95	–	–
1β	1.19	1.26	1.25	1.42	2.21	1.25	1.6	0.98	1.59	1.95	–	–
2	1.67	1.73	1.65	1.72(α)	1.55	1.69	1.97	1.67	1.78	1.79	–	–
				1.57(β)			1.70					
3	4.69	4.63	4.70	3.39	4.60	4.60	4.61	4.65	4.70	4.70	4.65	4.68
5	1.99	1.88	2.05	1.87	1.80	1.85	1.80	1.89	2.68	2.21	–	–
6	4.46	4.72	4.50	4.68	1.65–1.9	4.69	4.66	4.40	4.46	4.41	4.72	4.78
7α	2.77	2.48	2.81	2.42	3.18	2.45	2.28	2.86	2.54	2.47	2.51	2.75
7β	3.70	3.43	3.85	3.37	2.08	3.41	3.31	3.12	2.86	2.81	3.63	2.93
9	2.39	2.17	2.49	2.16	2.09	2.14	1.74	2.43			–	2.40
11	5.91	5.67	5.89	5.63	5.39	5.64	4.48	5.66	5.99	5.46	5.67	5.83
18	1.62	1.95	1.77	1.91	1.91	1.88	1.96	1.36	1.42	1.38	–	–
20	1.25	1.30	1.32	1.25	1.19	1.29	1.34	1.27	1.52	1.15	–	–
21	1.69	1.88	1.83	1.82	1.81	1.87	5.32	1.54	5.02	1.01	1.87	1.87
							5.24		5.25			
22	1.80	1.31	1.80	1.26	1.19	1.23	1.36	1.69	1.69	1.82	–	–
23					10.1							
24	0.76	0.95	0.77	1.00	0.91	0.92	0.92	0.73	0.92	0.83	–	–
25	0.73	0.88	0.74	0.77	0.82	0.85	0.87	0.70	0.78	0.76	–	–
3-OAc	1.65	2.02	1.72		2.09	1.99	2.03	1.62	1.83	1.66	2.02	2.07
17-OAc	1.58											
17-OMe		4.16	3.21	4.09	4.10		4.08					
19-OMe	3.25	3.63	3.25	3.57	3.60	3.60	3.63	2.95	2.93	3.08	3.67	3.61
17-O$CH_2$$CH_3$						4.41						
17-OC$H_2$$CH_3$						1.37						

[a] Signals were measured in C_6D_6.
[b] Four AcO signals in major tautomer of citreohybridonol (**33**) are observed at δ 1.33, 1.32, 0.94, and 0.89. Other signals (1.25–2.25) are overlapped with one another.
[c] Four AcO signals in miner tautomer of citreohybridonol (**33**) are observed at δ 1.43, 1.25, 0.97, and 0.91. Other signals (1.25–2.25) are overlapped with one another.

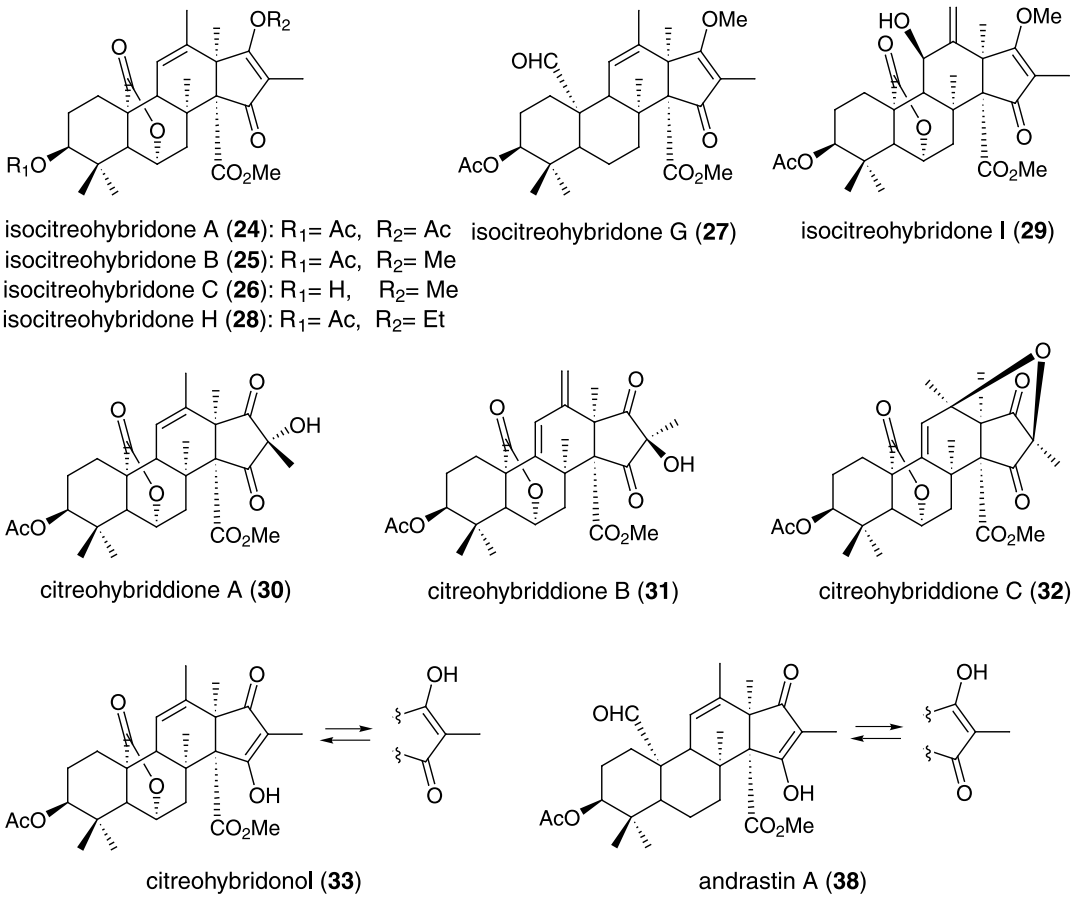


Figure 4. Structures of isocitreohybridones A–C (**24**–**26**), G–I (**27**–**29**), citreohybriddiones A–C (**30**–**32**), and citreohybridonol (**33**) and andrastin A (**38**).

Isocitreohybridone C (**26**), $C_{27}H_{36}O_7$ [*m/z* 472.2459 (M^+)] showed ^1H and ^{13}C NMR data (Tables 1 and 3) very similar to those of isocitreohybridone B (**25**). The ^1H NMR signal at δ_H 4.63 (1H, dd, *J*=2.7, 2.7 Hz) for the methine proton in **25** was replaced by a signal at δ_H 3.39 (1H, dd, *J*=3.1, 1.8 Hz) and no AcO group was observed in **26**, suggesting that **26** has OH group instead of AcO group on C-3 position. As expected, on treatment with Ac_2O–pyridine (room temp., over night), **26** was readily converted into **25** (Fig. 4).

Isocitreohybridone G (**27**) and citreohybridone G (**20**) have the same molecular formula $C_{29}H_{40}O_7$ [**20**: *m/z* 500.2770 (M^+), **27**: *m/z* 500.2771 (M^+)], and their ^1H and ^{13}C NMR spectra (Tables 1–3) are quite similar to each other excepted in the following points. Citreohybridone G (**20**) has two methoxyl groups [δ_H 4.09 (C_{15}–OMe), 3.60 (C_{19}–OMe)] on C-15 and C-19 position, on the other hand, isocitreohybridone G (**27**) has two methoxyl groups [δ_H 4.10 (C_{17}–OMe), 3.60 (C_{19}–OMe)] on C-17 and C-19 position, respectively, indicating that **27** must be the isomer of **20** on D ring (see Figs. 3 and 4).

The molecular formula of isocitreohybridone H (**28**) was determined to be $C_{30}H_{40}O_8$ (11 unsaturations) by HR-EIMS [*m/z* 528.2718 (M^+)]. The ^1H NMR spectrum (Table 3) of **28** showed the presence of one acetoxyl (δ_H 1.99), one methoxyl (δ_H 3.60), two methyl groups with a double bond (δ_H 1.88 and 1.87), four methyl groups connected to quaternary carbons (δ_H 1.29, 1.23, 0.92, and 0.85) and an ethoxyl group [δ_H 4.41 (2H, q, *J*=7.0 Hz), 1.37 (3H, q, *J*=7.0 Hz)]. The ^1H NMR data are quite similar to those of isocitreohybridone B (**25**) except for the absence of a methoxyl group and the presence of an ethoxyl group on C-17. In fact, on ethylation with ethyl bromide and K_2CO_3 in acetone (room temp., 24hr), the hydrolysis compound derived from citreohybridone A was converted into isocitreohybridone H (**28**) in 10% yield. This result suggested that isocitreohybridone H has an ethoxyl group instead of a methoxyl group on C-17 in isocitreohybridone B (**25**).

The structure of isocitreohybridone I (**29**) was derived based on extensive spectroscopic analysis. HR-EIMS suggested a molecular formula of $C_{29}H_{38}O_9$ [*m/z* 530.2515 (M^+)] for **29**, thus revealing 11 degrees of unsaturation. This formula was supported by the presence of 29 carbons in its ^{13}C NMR spectrum. The carbon types, revealed by an HMQC experiment, included one exomethylene [δ_C 118.1(t)], one acetoxy, two methoxyls, five methyls, three methylenes, five methines and 12 quaternary carbons, four of which were carbonyls: δ 203.5 (α, β unsaturated ketone, C-15), 179.6 (lactone, C-23), 169.9 (methyl ester, C-19), and 170.4 (C_3–OAc). The IR absorption at 3500 cm^{-1} suggested the presence of a hydroxyl group. The ^1H NMR data are similar to those of isocitreohybridone B (**25**) except for the absence of an olefin proton for H-11 and the presence of two exomethylene protons [δ_H 5.32 and 5.24, δ_C 118.1 (t, C_{21}) and 152.4 (s, C_{12})] and a hydroxyl group [δ_H 4.48 (dd,

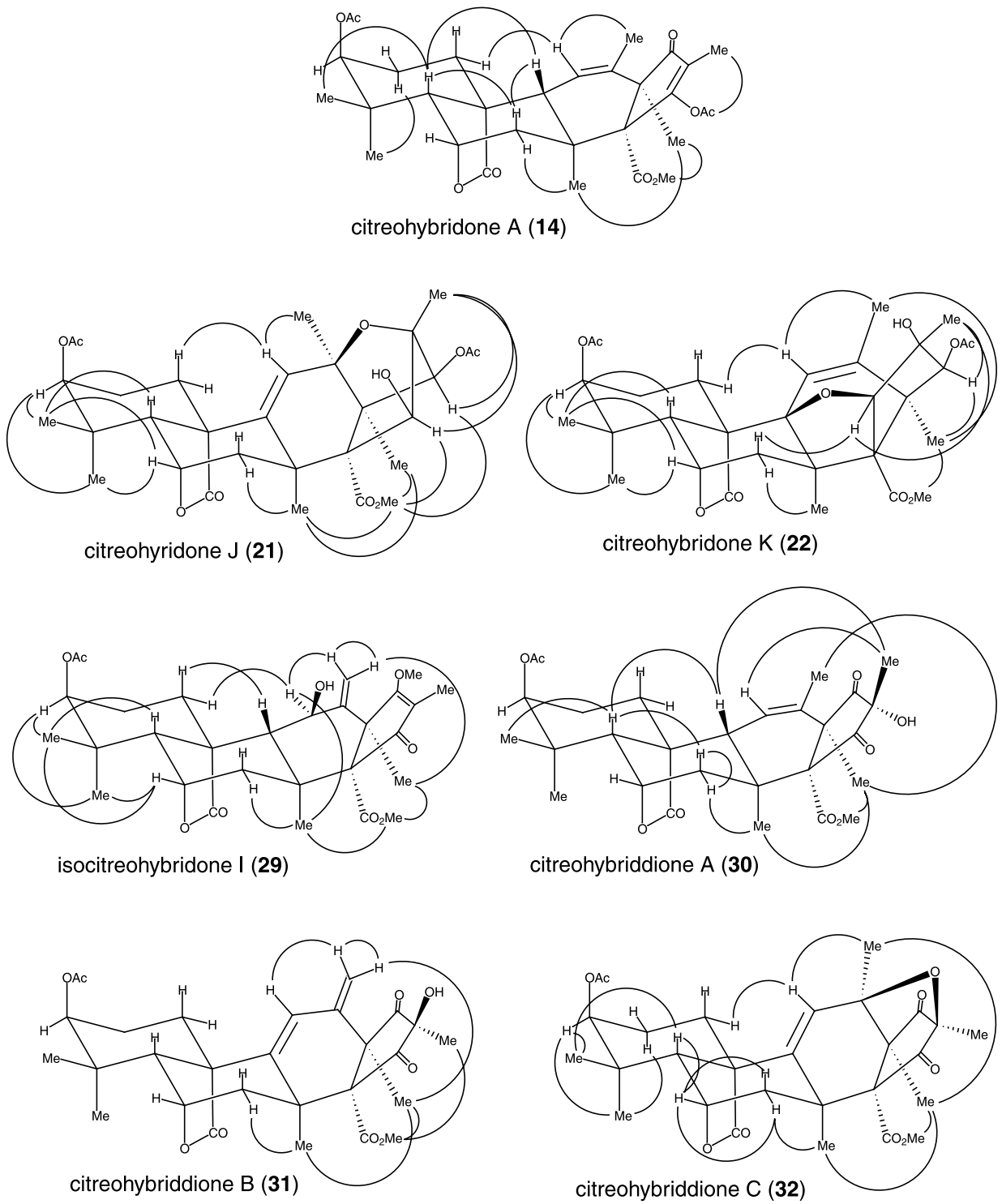


Figure 5. NOE correlations for citreohybridones A (**14**), J (**21**), K (**22**), isositreohybridone I (**29**), and citreohybriddiones A–C (**30**–**32**).

J=7.7, 1.8 Hz, C_{11}–H), 1.27 (d, *J*=1.8 Hz, C_{11}–*OH*)] on the C ring. Finally, the stereochemistry of citreohybridone I (**29**), especially of the configuration of a hydroxyl group on the C11-position, was elucidated by NOESY experiment in $CDCl_3$. The NOEs between H_3-22 (δ_H 1.36) and H-11 (δ_H 4.48) suggested the hydroxyl group on C-11 is in β configuration. Furthermore, as can be seen in Figure 5, other NOE enhancements were observed. Thus, the stereostructure of isocitreohybridone I is clearly established as depicted in the formula.

HR-EIMS suggested a molecular formula of $C_{28}H_{36}O_9$ [*m*/*z* 516.2365 (M^+)] and $C_{28}H_{34}O_9$ [*m*/*z* 514.2201 (M^+)] for citreohybriddiones A (**30**) and B (**31**), respectively. As judged from their detailed ^1H NMR decoupling experiments, probably, **30** and **31** have the same carbon skeleton. On the basis of ^1H–^{13}C COSY spectra of **30** and **31** and detailed low power selective decoupling experiments of **31** in benzene-D_6,[12] citreohybriddiones A and B have two CO groups and a tertiary OH group on D ring. Irradiation of the C_{13}-methyl group (δ_H 1.52, H_3-20) of **31** enhanced each

height of the carbon signals at C-12 position (δ_C 141.18), C-13 position (δ_C 58.70), C-14 position (δ_C 72.45), and C-17 position (δ_C 207.62), and irradiation of the C_{16}-methyl group (δ_H 1.42, H_3-18) of **31** also enhanced each height of the carbon signals at C-15 position (δ_C 210.76), C-16 position (δ_C 71.63) and C-17 position (δ_C 207.62). Citreohybriddione A (**30**) is structurally quite similar to citreohybridone A (**14**) except for D ring systems. On the other hand, citreohybriddione B (**31**) has an exomethylene group (δ_H 5.02, 5.25) on C ring and no proton at C-9 position. Finally the stereochemistry of citreohybriddiones A (**30**) and B (**31**), 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_H 1.36) of **30** resulted in 5.1% NOE of the C_9-proton (δ_H 2.43), 0.9% NOE of the C_{11}-proton (δ_H 5.66), and no enhancement of the C_{19}-methoxyl group (δ_H 2.95), irradiation of the C_5-proton (δ_H 1.89) resulted in 4.1% NOE of the C_7-β-axial proton (δ_H 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_H 1.27) and no enhancement of the C_{16}-methyl group, thereby, indicating that the C_{16}-methyl group is a β configuration. Whereas, irradiation of the C_{16}-methyl group (δ_H 1.42) of **31** resulted in 1.2% NOE of the C_{19}-methoxyl group (δ_H 2.93), and irradiation of the 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 **31** is an α configuration. Other NOE interactions are shown in Figure 5.

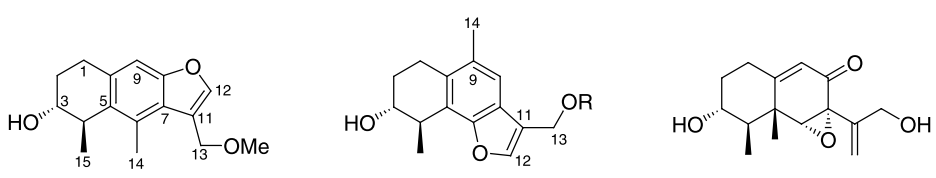
HR-EIMS suggested a molecular formula of $C_{28}H_{34}O_9$ [*m/z* 514.2201 (M^+)] for citreohybriddione C (**32**), thus revealing 12 degrees of unsaturation. This formula was supported by the presence of 28 carbons in its ^{13}C NMR spectrum. The carbon types, revealed by a HMQC experiment, included one methoxyl group, one acetoxyl group, six methyl groups, three methylene groups, three methine groups, two olefinic carbon atoms and twelve quaternary 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 (C_3–OAc), and 167.6 (methyl ester, C-19). The ^1H and ^{13}C NMR data are very similar to those of citreohybriddione B (**31**) except for the absence of an exomethylene group on C ring and the presence of an oxygenated quaternary carbon (δ_C 76.2) with a tertiary methyl (δ_H 1.01, δ_C 23.6) attached. Finally the stereochemistry of citreohybriddione C (**32**), especially of the configuration of the ether linkage from C_{12}- and C_{16}-position, was elucidated by NOESY experiment in benzene-d_6. The NOEs between H-5 (δ_H 2.21)/H_3-24 (δ_H 0.83), Hα-7 (δ_H 2.47)/H_3-22 (δ_H 1.82), H-11 (δ_H 5.46)/H_3-21 (δ_H 1.01), H_3-20 (δ_H 1.15)/H_3-21 (δ_H 1.01), H_3-20 (δ_H 1.15)/H_3-22 (δ_H 1.82), and H_3-20 (δ_H 1.15)/H_3-28 (δ_H 3.08) suggested the relative configuration of **32** (Fig. 5).

Citreohybridonol (**33**), $C_{28}H_{36}O_8$ [*m/z* 500.2398 (M^+)], exists in an equilibrium between two 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, **33** was treated with acetic anhydride–pyridine to afford citreohybridone A (**14**) and isocitreohybridone A (**24**) in 65 and 20% yields, respectively. This result suggested that citreohybridonol (**33**) will be in an equilibrium between keto–enol tautomers on a five-membered D ring.

Three sesquiterpenoid-type metabolites, citreobenzofurans A (**34**), B (**35**), and C (**36**) have been isolated from the same mycelium of the hybrid strain KO 0031.

Citreobenzofuran A (**34**) with a molecular formula $C_{16}H_{20}O_3$ [*m/z* 260.1425 (M^+)] has the IR absorption band at 3440 cm^{-1}, and its ^1H NMR spectrum has a methoxyl group (δ_H 3.40), one singlet methyl (δ_H 2.59), and a doublet methyl (δ_H 1.17). On the basis of ^1H NMR with the aid of decoupling experiments, long range coupling have been observed between C_1-methylene group (δ_H 2.90 and 3.12) and C_9-proton (δ_H 7.10, allyl type coupling) and between C_2-α-proton (δ_H 1.93) and C_4-α-proton (δ_H 3.25, W-type coupling). On the basis of NOE difference experiments, furthermore, not only gross structure but also stereostructure of **34** have been elucidated. Irradiation of the methoxyl group (δ_H 3.40) of **34** (Fig. 6) resulted in 0.85% NOE of the C_{12}-proton (δ_H 7.48), 0.66% of the C_6-methyl group (δ_H 2.59), and 1.5% NOE of C_{13}-methylene group (δ_H 4.59 and 4.60), irradiation of the methylene group (C_{13}, δ_H 4.59 and 4.60) resulted in 8.4% NOE of the C_{12}-proton, 2.6% NOE of the C_6-methyl group, and 3.7% NOE of the methoxyl group (δ_H 3.40), irradiation of the C_6-methyl group (δ_H 2.59) resulted in 13.5% NOE of the C_4-proton (δ_H 3.25), 1.5% NOE of the C_4-methyl group, 2.9% NOE of the C_{13}-methylene group, and 0.79% NOE of the methoxyl group (δ_H 3.40), and irradiation of the C_4-methyl group (δ_H 1.77) also resulted in 2.1% NOE of the C_6-methyl group, 10.6% NOE of the C_4-proton (δ_H 3.25), 7.93% NOE of the C_3-proton (δ_H 4.14), and 7.15% NOE of the C_2-β-proton (δ_H 2.08). Other NOE interactions are shown in Figure 7.

On detailed comparisons of the ^1H NMR spectra between citreobenzofurans B (**35**) and C (**36**), their structures are quite similar to each other except for the following point. The former with a molecular formula $C_{15}H_{18}O_3$ [*m/z* 246.1244 (M^+)] has two hydroxy groups (ν_{max} 3370 cm^{-1}). On the other hand, **36** with a molecular formula $C_{16}H_{20}O_3$ [*m/z* 260.1429 (M^+)] has a methoxyl



citreobenzofuran A (**34**)

citreobenzofuran B (**35**): R= H
citreobenzofuran C (**36**): R= Me

phomenone (**37**)

Figure 6. Structures of citreobenzofurans A–C (**34**–**36**) and phomenone (**37**).

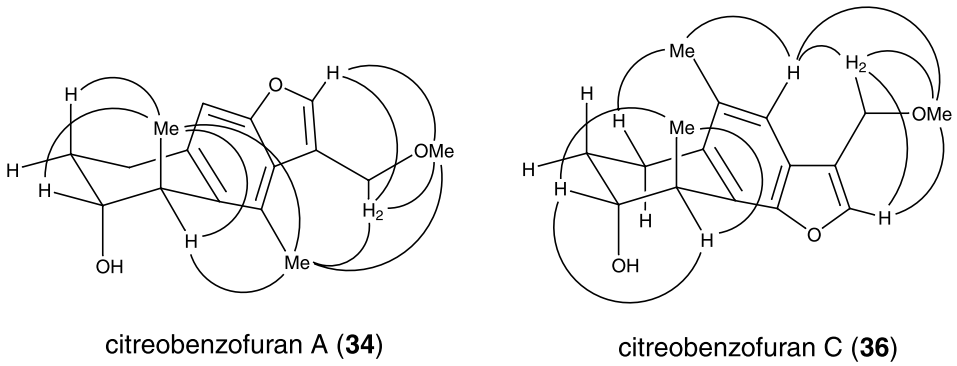


Figure 7. NOE correlations for citreobenzofuran A (**34**) and C (**36**).

group (δ_H 3.38) instead of one of the two hydroxy groups, indicating that the latter has the methoxyl group instead of the hydroxy group at C_3 or C_{13}-position in **35**. On the basis of detailed ^1H NMR decoupling experiments, **36** has a methine group (δ_H 3.37, C_4) which has a W-type long range coupling with the C_2-proton (δ_H 2.04), and an isolated methylene group (δ_H 4.57), and two isolated sp^2 protons (δ_H 7.31 and 7.53). Finally, the gross structure and the stereochemistry of **36** were elucidated by the NOE experiments. Irradiation of the C_{14}-proton (δ_H 2.32) of **36** resulted in 14.5% NOE of the C_8-proton (δ_H 7.31), and 3.5% NOE of the C_1-methylene group (δ_H 2.73 and 2.82), and irradiation of the C_{13}-methylene group resulted in 6.8% NOE of the C_{12}-proton (δ_H 7.53), 4.8% NOE of the C_8-proton (δ_H 7.31), and 2.8% NOE of the methoxyl group (δ_H 3.38). Other NOE interactions are shown in Figure 7.

R. Capasso et al. reported[19] that phomenone (**37**),[20] a known phytotoxin and mycotoxic sesquiterpenoid, afforded a new substituted benzofuran by treated with 10% H_2SO_4 in MeOH. Citreobenzofuran C (**36**) isolated by us was identical with Capasso's benzofuran. Therefore, we cannot rule out a possibility in which **36** is an artifact of phomenone (**37**). However, when **37** was treated with SiO_2 in MeOH–$CHCl_3$ (1:10) at room temperature for 5 days, any amount of **36** was not detected, suggesting that **36** is not an artifact of phomenone.

2.2. Absolute configuration

The absolute configuration of citreohybridones seems to be the same as that of andrastins (andrastin A, **38**), which was elucidated as an enantiomer of 5α, 14β-androstane by the X-ray analysis of 15-(*p*-bromobenzoyl)-andrastin A (**39**),[21] because citreohybridone D (**17**) and 15-acetyl-andrastin A have the same negative optical rotation mainly contributed by an α,β-unsaturated 5-membered ring moiety.[17] In order to confirm the absolute configuration, (*R*)- and (*S*)-MTPA esters (**40R** and **40S**) of isocitreohybridone C (**26**) was 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 **40R** and **40S** 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 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.[22]

C_3-Epimar of **26** (**41**) was prepared by $NaBH_4$ reduction of 3-keto-compound which was obtained by treatment of **26** with pyridinium dichromate. The protons of its MTPA esters **42R** and **42S**, in which the hydroxyl group is equatorial, have $\Delta\delta$ values that are perfectly consisted with the rule for determining absolute configurations (Fig. 8). This result gave us the absolute configurations both isocitreohybridone C (**26**) and its C_3-epimar (**41**), shown in the respective structures. (Fig. 9)

2.3. Biosynthesis

We isolated 20 metabolites named citreohybridones A–G, J–L, isocitreohybridones A–C, G–I, citreohybriddiones A–C, and citreohybridonol, from the mycelium of the

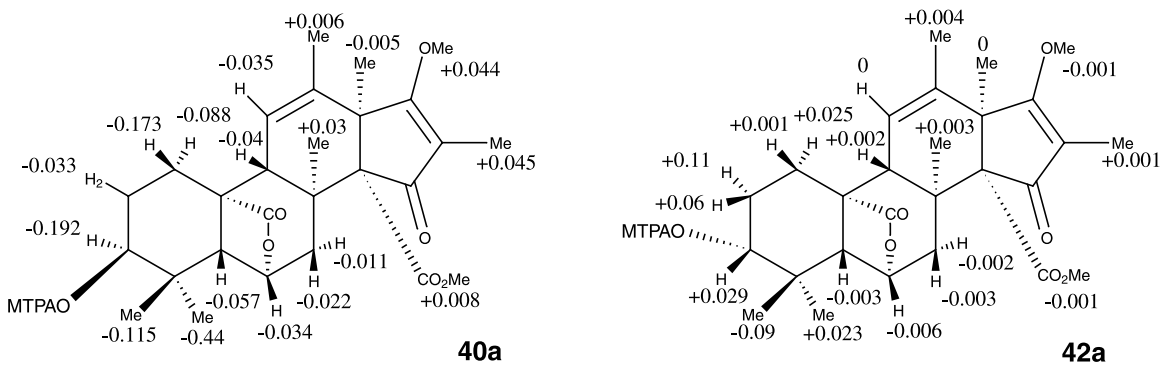


Figure 8. $\Delta\delta$ values ($\Delta\delta$=δS−δR) obtained for MTPA esters of isocitreohybridone C (**40a**) and its C_3-epimar (**42a**).

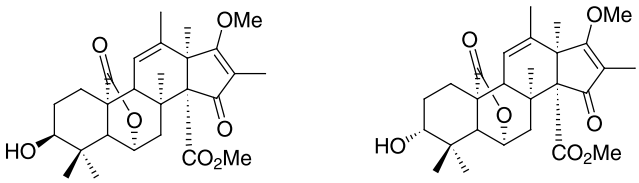
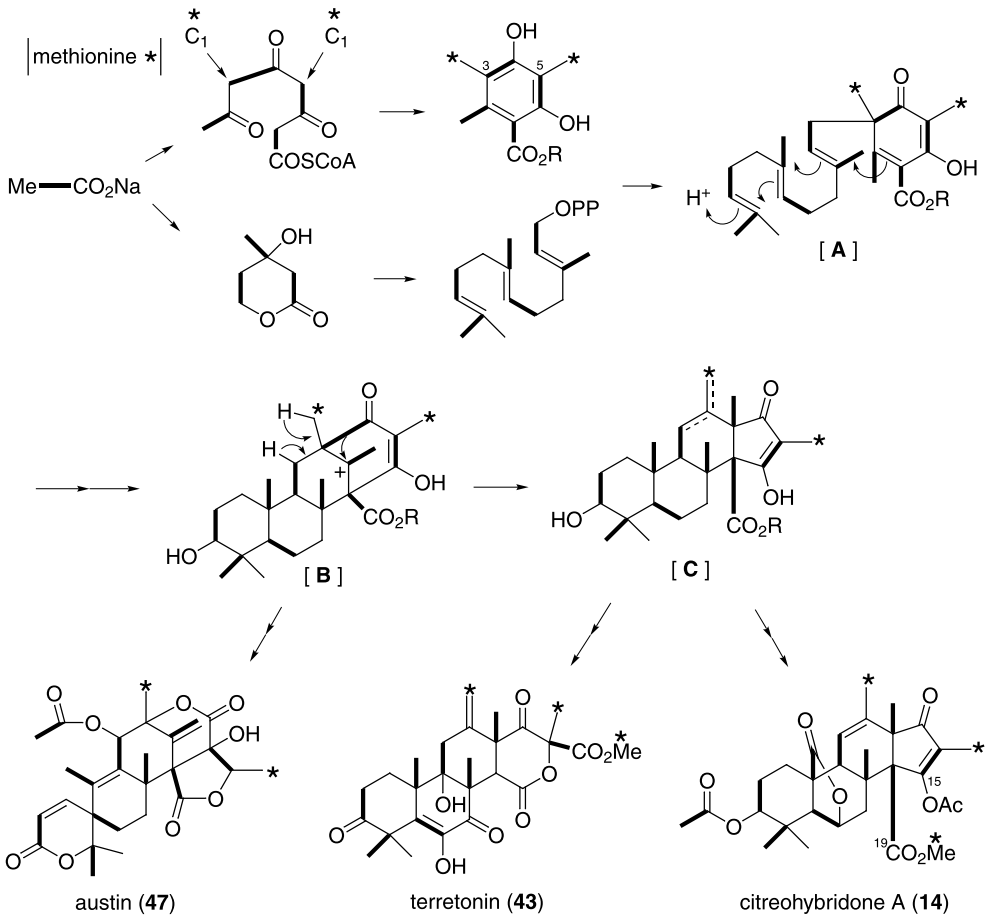


Figure 9. Absolute configuration of isocitreohybridone C (**26**) and its C_3-epimar (**41**).

hybrid strain KO 0031 derived from *P. citreo-viride* B. IFO 6200 and 4692. Interestingly, they display antifeedant and insecticidal activities against *P. xylostella*. Initial biogenetic analysis suggested that these metabolites could be formed by successive methyl migration and skeletal rearrangement of a sesterterpenoid containing five isoprene units or alternatively from a degraded triterpenoid. However, we now report biosynthetic experiments on citreohybridonol (**33**) using sodium [1,2-$^{13}C_2$] acetate, sodium [^{13}C] formate, and ethyl [*carboxy*-6-$^{13}C_2$]-3,5-dimethylorsellinate, which indicate that this metabolite also is formed *via* a mixed polyketide-terpenoid (meroterpenoid) biosynthetic pathway (see Scheme 1).

Initial biosynthetic experiments on citreohybridones were carried out using sodium [1,2-$^{13}C_2$] acetate and sodium [^{13}C] formate. According to essentially the same procedure described previously, the ethyl acetate extract of the culture medium was directly chromatographed on silica gel. Further separation and purification by repeated preparative TLC afforded citreohybridonol (**33**, 0.92%). Citreohybridonol (**33**) exists as an equilibrium between two different ring D tautomers in both $CDCl_3$ as well as CD_3OD, which causes difficulties in signal assignments in the ^{13}C NMR spectrum. Therefore, **33** was treated with acetic anhydride-pyridine to afford citreohybridone A (**14**) and ^{13}C NMR assignment (Table 4) is based on complete ^{1}H NMR decoupling experiments coupled with 2D-INADEQUATE experiments on [1,2-$^{13}C_2$] acetate-labeled citreohybridone A. The average level of enrichment was estimated to be 12.7% based on the relative heights of the coupling satellites and natural abundance signals. No significant differences in enrichment levels between the farnesyl and orsellinate derived carbons were observed. The [^{13}C] formate-labeled sample showed signals resulting from three highly enriched carbons (300%) corresponding to C-18 (δ_C 9.52), C-21 (δ_C 19.23) and C_{19}–OMe (δ_C 52.32). The resulting labeling pattern of citreohybridone A is summarized in Scheme 1, and suggests that this metabolite is formed via a mixed polyketide-terpenoid biosynthetic pathway with the same biosynthetic intermediate [**C**] proposed as a precursor of terretonin (**43**),[23,24] isolated from *Aspergillus terreus*. The pathway shown in Scheme 1 is consistent with the pathway proposed for the biosynthesis of terretonin. Ring contraction of the tetracyclic carbocation [**B**] is common to both pathways with the subsequent alternative proton losses indicated in Scheme 1 producing the exocyclic methylene



Scheme 1. Proposed biosynthetic pathway for meroterpenoids.

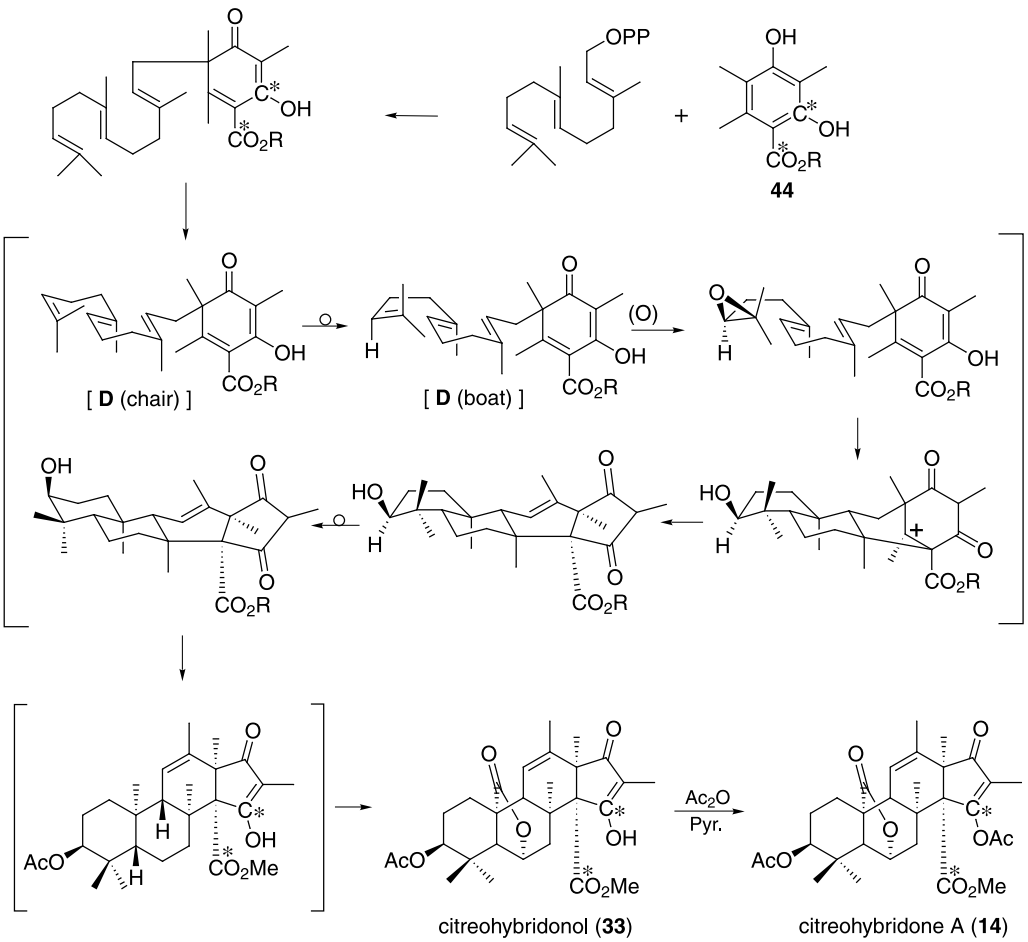
Table 4. ^{13}C NMR data for the incorporation of [1,2-$^{13}C_2$] acetate into citreohybridone A (**14**)

Carbon	δ (ppm)[a]	J (Hz)	Carbon	δ (ppm)	J (Hz)	Carbon	δ (ppm)	J (Hz)
1	20.9	–	11	123.1	41.5	21	19.2	–
2	22.1	36.0[b]	12	134.0	–	22	22.1	36.0[b]
3	75.7	36.4	13	59.9	37.6	23	178.7	49.0
4	34.5	35.3	14	69.2	57.0	24	26.5	–
5	55.4	33.2	15	169.5	82.9	25	22.1	36.0[b]
6	76.7	33.2	16	132.0	82.9	3-O*C*OCH$_3$	170.6	59.5
7	37.7	–	17	199.0	–	3-OCO*C*H$_3$	20.9	59.5
8	41.1	36.5	18	9.5	–	19-OCH$_3$	52.3	–
9	51.6	41.5	19	169.7	57.0	15-O*C*OCH$_3$	164.9	–
10	43.7	49.0	20	17.3	37.6	15-OCO*C*H$_3$	21.6	–

^{13}C NMR spectra were taken on a JEOL JNM-GX 400 NMR spectrometer.
[a] Relative to TMS in $CDCl_3$.
[b] Overlapped with other satellites' signals.

observed in terretonin or the endocyclic double bond in the citreohybridones. Further evidence for this was provided from incorporation of ^{13}C-labeled ethyl 3,5-dimethylorsellinate (**44**)[25,26] into citreohybridonol which was converted into citreohybridone A as above. The key step in their biosynthesis involves *C*-alkylation of the tetraketide-dirived intermediate, 3,5-dimethylorsellinic acid, with farnesyl pyrophosphate, giving [**A**] as shown in Scheme 1. This intermediate is then cyclized and undergoes oxidation and other modifications to produce the above metabolites. It is especially noted the formation of the hydroxyl group oriented in axial direction on the A-ring of citreohybridones must be involved in the enzymatic epoxidation and cyclization of intermediate [**D**] which requires boat geometry for the A-ring. The [*carboxy*-6-$^{13}C_2$]-3,5-dimethylorsellinate-labeled citreohybridone A showed signals resulting from two highly enriched carbons (1100%) corresponding to C-15 (δ_C 169.45, $^2J_{CC}$ 3.9 Hz) and C-19 (δ_C 169.65, $^2J_{CC}$ 3.9 Hz). The labeling pattern of citreohybridone A is summarized in Scheme 2 and confirms the intact incorporation of 3,5-dimethylorsellinate (**44**).

As mentioned above, we have clarified that the citreohybridones are formed via a mixed polyketide-terpenoid



Scheme 2. Intact incorporation of ethyl [carboxy-6-$^{13}C_2$]-3,5-dimethylorsellinate and proposed mechanism for the formation of the axial hydroxyl group on A-ring of citreohybridones.

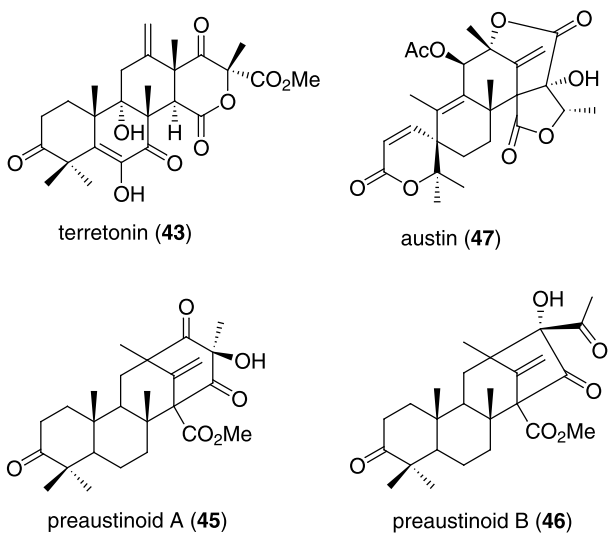


Figure 10. Structures of meroterpenoids from another origins.

(meroterpenoid) biosynthetic pathway. Furthermore, five precursors of citreohybridones, citreohybridones D–G (**17**–**20**) and isocitreohybridone G (**27**) 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.

Recently, preaustinoid A (**45**) and B (**46**)[27] related to austin (**47**)[28] have been discovered in the rice culture of *Penicillium* sp isolated from the root bark of *Melia azedarach* (Fig. 10). Surprisingly, preaustinoid A (**45**) was quite similar to the biosynthetic intermediate [**B**] of citreohybridones in Scheme 1. The meroterpenoid pathway can now be seen to be relatively widespread in fungi.

3. Experimental

3.1. General

Melting points were determined on a Mitamura Riken apparatus and uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a JASCO A-202 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer in $CDCl_3$, C_6D_6 or CD_3OD with tetramethylsilane as an internal standard. Coupling constants are give 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. Thin layer chromatography was performed using preparative (20×20 cm) glass plates coated with a 0.5 mm layer of silica gel (Merck Art. 5744 Kieselgel 60 PF_{254}). UV light of wavelength 254 nm was used to visualize chromatograms. Dimethyl [1,3-$^{13}C_2$] malonate was obtained from Amersham International plc UK, ^{13}C-labeled formate and acetate from ISOTEC Inc., USA.

3.2. Cell fusion technique

Each protoplast corresponding to *P. 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 d), using cellulose, 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 d) to give a number of colonies, from which many new hybrid strains including *P. citreo-viride* KO 0031 were obtained.

3.3. Incubation

Polished rice (ca. 4.5 kg) in deionized water (ca. 6 l) was cooked using an electric cooker (99°C, ca. 20 min) and transfered into thirty-five Erlenmyer flasks (3 l), which were pasteurized at 121°C for 20 min at 2.1 atm. After inoculated with a suspension of the mycelium of hybrid strain KO 0031 in a sterilized water, the rice was incubated stationarily at 25°C for 14, 30, or 60 days and extracted with acetone (160 l).

3.4. Isolation and separation

3.4.1. From 14-days' rice culture. The acetone extract suspended in water was extracted with EtOAc. The EtOAc extract (dark brown syrup, 10.2 g) was chromatographed on silica gel (300 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 (10:1–2) afforded a pale yellow oil (3.5 g), which was further separated by repeated preparative TLC using EtOAc–benzene (1:4), MeOH–$CHCl_3$ (1:10), acetone–hexane (1:3), and then acetone–$CHCl_3$ (1:10) and/or AcOEt–hexane (1:1–2) to afford citreohybridones D (**17**; 0.11%), E (**18**; 0.022%), F (**19**; 0.015%), and G (**20**; 0.0069%), and isocitreohybridone G (**27**; 0.0069%), respectively.

3.4.2. From 30-days' rice culture. The acetone extract suspended in water was extracted with EtOAc. 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–benzene (1:1.5–3) and/or AcOEt–hexane (1:1–2) to afford citreohybridones A (**14**; 0.17%) and B (**15**; 0.057%), isocitreohybridones A (**24**; 0.038%) and B (**25**; 0.053%), citreohybriddiones A (**30**; 0.016%), B (**31**; 0.031%), and C (**32**; 0.079%), and citreobenzofurans A (**34**; 0.0014%), B (**35**; 0.0.0011%), and C (**36**; 0.0038%), together with phomenone (**37**; 0.050%), respectively.

3.4.3. From 60-days' rice culture. The acetone extract was partitions between H_2O and EtOAc. 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 repeated preparative TLC using AcOEt–hexane (1:1–2), acetone–hexane (1:1–2) and/or MeOH–$CHCl_3$ (1:10–20) to afford citreohybridones C (**16**; 0.029%), J (**21**; 0.015%), K (**22**; 0.00086%), and L (**23**; 0.0011%), isocitreohybridones C (**26**; 0.057%), H (**28**; 0.00057%), and I (**29**; 0.0091%), respectively.

3.5. Physical data for isolated metabolites

3.5.1. Physical data for citreohybridone A (14). A colorless prisms: mp 261.5–263°C in a sealed tube (from benzene–hexane); $[\alpha]_D^{19}=-25.4°$ (*c* 1.00, $CHCl_3$); $C_{30}H_{38}O_9$ [*m/z* 542.2491 (M^+)]; IR (film) 1775, 1740, 1715, 1660, and 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.68 (1H, s, H-11), 4.71 (1H, d, *J*=4.3 Hz, H-6), 4.65 (1H, dd, *J*=3.4, 1.8 Hz, H-3), 3.66 (3H, s, C_{19}–OMe), 2.74 (1H, dd, *J*=14.7, 4.3 Hz, Hα-7), 2.51 (1H, dq, *J*=2.4, 2.4 Hz, H-9), 2.31 (3H, s, C_{15}–OAc), 2.29 (1H, d, *J*=14.7 Hz, Hβ-7), 2.16 (3H, s, C_3–OAc), 2.16 (1H, m, Hα-1), 1.75 (3H, dd, *J*=2.4, 1.5 Hz, H_3-21), 1.82–1.65 (2H, m, H_2-2), 1.71 (1H, s, H-5), 1.63 (3H, s, H_3-18), 1.42 (1H, ddd, *J*=13.4, 13.4, 6.2 Hz, Hβ-1), 1.41 (3H, s, H_3-22), 1.27 (3H, s, H_3-20), 0.93 (3H, s, H_3-24), 0.90 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 198.96 (s, C-17), 178.74 (s, C-23), 170.62 (s, C_3–OAc), 169.65 (s, C-19), 169.45 (s, C-15), 164.91 (s, C_{15}–OAc), 134.04 (s, C-12), 131.96 (s, C-16), 123.13 (d, C-11), 76.71 (d, C-6), 75.68 (d, C-3), 69.23 (s, C-14), 59.88 (s, C-13), 55.35 (d, C-5), 52.32 (q, C_{19}–OMe), 51.64 (d, C-9), 43.70 (s, C-10), 41.10 (s, C-8), 37.70 (t, C-7), 34.52 (q, C-4), 26.47 (q, C-24), 22.12 (t, q, and q, C-2, 22, and −25), 21.55 (C_{15}–OAc), 20.89 (t, and q, C-1 and C_3–OAc), 19.23 (q, C-21), 17.31 (q, C-20), and 9.52 (q, C-18).

3.5.2. Physical data for citreohybridone B (15). A colorless oil: $[\alpha]_D^{19}=+4.7°$ (*c* 1.00, $CHCl_3$); $C_{29}H_{38}O_8$ [*m/z* 514.2569 (M^+)]; IR (film) 1770, 1740, 1700, 1630, and 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.62 (1H, s, H-11), 4.71 (2H, H-3 and −6), 4.15 (3H, s, C_{15}–OMe), 3.64 (3H, s, C_{19}–OMe), 2.69 (1H, d, *J*=14.4 Hz, Hβ-7), 2.59 (1H, dd, *J*=14.2, 4.2 Hz, Hα-7), 2.32 (1H, dq, *J*=2.4, 2.4 Hz, H-9), 2.22 (1H, ddd, *J*=13.0, 4.9, 2.4 Hz, Hα-1), 2.04 (3H, s, C_3–OAc), 2.00 (3H, s, H_3-18), 1.89 (1H, s, H-5), 1.79 (2H, m, H_2-2), 1.76 (3H, dd, *J*=2.4, 1.5 Hz, H_3-21), 1.39 (1H, ddd, *J*=13.0, 11.0, 7.5 Hz, Hβ-1), 1.39 (3H, s, H_3-22), 1.23 (3H, s, H_3-20), 0.97 (3H, s, H_3-24), and 0.92 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 200.04 (s, C-17), 179.12 (s, C-15 or-23), 178.68 (s, C-15 or −23), 170.20 (s, C-19 or C_3–OAc), 169.67 (s, C-19 or C_3–OAc), 135.94 (s, C-12), 122.24 (d, C-11), 117.03 (s, C-16), 77.35 (d, C-3 or −6), 76.25 (d, C-3 or −6), 69.25 (s, C-14), 59.32 (q, C_{15}–OMe), 58.57 (s, C-13), 55.49 (d, C-5), 52.49 (d, C-9), 51.93 (q, C_{19}–OMe), 43.48 (s, C-10), 41.39 (s, C-8), 37.66 (t, C-7), 34.26 (s, C-4), 26.43 (q, C-24), 22.83 (q, C-22), 22.08 (t and q, C-2 and −25), 21.08 (t, C-1), 20.95 (q, C_3–OAc), 19.30 (q, C-21), 17.16 (q, C-20), and 9.21 (q, C-18).

3.5.3. Physical data for citreohybridone C (16). A colorless oil; $[\alpha]_D^{23}=-8.3°$ (*c* 1.0, $CHCl_3$); $C_{27}H_{36}O_7$ [*m/z* 472.2456 (M^+)]; IR (film) 3510, 1765, 1740, 1695, and 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.59 (1H, dq, *J*=2.6, 1.5 Hz, H-11), 4.68 (1H, d, *J*=4.0 Hz, H-6), 4.10 (3H, s, C_{15}–OMe), 3.60 (3H, s, C_{19}–OMe), 3.47 (1H, dd, *J*=3.7, 1.5 Hz, H-3), 2.67 (1H, d, *J*=14.3 Hz, Hβ-7), 2.54 (1H, dd, *J*=14.3, 4.0 Hz, Hα-7), 2.30 (1H, dq, *J*=2.6, 2.6 Hz, H-9), 2.13 (1H, ddd, *J*=13.4, 4.2 Hz, 1.8, Hα-1), 1.95 (3H, s, H_3-18), 1.92 (1H, s, H-5), 1.78 (1H, dddd, *J*=14.2, 13.8, 3.7 Hz, 1.8, Hα-2), 1.72 (3H, dd, *J*=2.6, 1.5 Hz, H_3-21), 1.61 (1H, dddd, *J*=14.2, 5.5, 4.2, 1.5 Hz, Hβ-2), 1.53 (1H, ddd, *J*=13.8, 13.4, 5.5 Hz, Hβ-1), 1.35 (3H, s, H_3-22), 1.19 (3H, s, H_3-20), 1.03 (3H, s, H_3-24), and 0.81 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ 200.4 (s, C-17), 179.4 (s, C-15), 179.3 (s, C-23), 170.4 (s, C-19), 135.6 (s, C-12), 122.4 (d, C-11), 117.0 (s, C-16), 77.7 (t, C-6), 73.7 (d, C-3), 69.3 (s, C-14), 59.7 (q, C_{15}–OMe), 58.4 (s, C-13), 54.7 (d, C-5), 52.2 (d, C-9), 51.9 (q, C_{19}–OMe), 43.5 (s, C-10), 41.4 (s, C-8), 37.6 (t, C-7), 34.5 (s, C-4), 26.6 (q, C-24), 25.0 (t, C-2), 22.9 (q, C-22), 22.5 (q, C-25), 20.3 (t, C-1), 19.4 (q, C-21), 17.2 (q, C-20), and 9.2 (q, C-18).

3.5.4. Physical data for citreohybridone D (17). 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}; ^1H NMR ($CDCl_3$) δ_H 10.1 (1H, s, H-23), 5.48 (1H, dq, *J*=1.8, 1.5 Hz, H-11), 4.58 (1H, dd, *J*=2.9, 2.6 Hz, 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 Hz, H-9), 2.07 (3H, s, C_3–OAc), 2.02 (1H, m, H-7), 1.65 (3H, dd, *J*=2.4, 1.5 Hz, 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 (3H, 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 Hz, Hα-1), 0.90 (3H, s, H_3-24), and 0.83 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 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).

3.5.5. Physical data for citreohybridone E (18). A colorless oil; $[\alpha]_D^{25}=-13.4°$ (*c* 1.0, $CHCl_3$); $C_{30}H_{40}O_9$ [*m/z* 544.2668 (M^+)]; IR (film) 3200, 1785, 1745, 1715, and 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.73 (1H, dq, *J*=1.8, 1.1 Hz, H-11), 4.55 (1H, dd, *J*=2.8, 2.7 Hz, H-3), 3.55 (3H, s, C_{19}–OMe), 2.41 (1H, dddd, *J*=13.2, 13.2, 13.2, 3.7 Hz, 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 Hz, 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 Hz, 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 Hz, 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_C 200.2 (s, C-17), 178.4 (s, C-23), 170.4 (s, C_3–OAc), 169.7 (s, C-19), 169.5 (s, C-15), 165.4 (s, 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).

3.5.6. Physical data for citreohybridone F (19). 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}; ^1H NMR ($CDCl_3$) δ_H 5.73 (1H, dq, J=1.7, 1.1 Hz, H-11), 4.58 (1H, dd, J=2.9, 2.7 Hz, 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 Hz, 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 Hz, H-9), 2.06 (1H, m, Hα-6), 1.68 (3H, dd, J=2.6, 1.1 Hz, 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 Hz, 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_C 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).

3.5.7. Physical data for citreohybridone G (20). 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}; ^1H NMR ($CDCl_3$) δ_H 10.1 (1H, s, H-23), 5.39 (1H, dq, J=1.6, 1.5 Hz, H-11), 4.62 (1H, dd, J=2.9, 2.6 Hz, 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 Hz, Hα-7), 2.34 (1H, ddd, J=13.2, 3.3, 3.3 Hz, Hβ-1), 2.23 (1H, ddd, J=13.6, 3.3, 3.3 Hz, Hβ-7), 2.07 (3H, s, C_3–OAc), 1.92 (3H, s, H-18), 1.9 (1H, dq, J=2.2, 1.6 Hz, H-9), 1.9 (1H, m, H-6), 1.70 (1H, m, H-5), 1.67 (3H, dd, J=2.2, 1.5 Hz, 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 Hz, Hα-1), 0.92 (3H, s, H_3-24), and 0.84 (3H, s, H_3-25).

3.5.8. Physical data for citreohybridone J (21). 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_H 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}–O*H*), 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_C 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).

3.5.9. Physical data for citreohybridone K (22). 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_H 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}–O*H*), 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).

3.5.10. Physical data for citreohybridone L (23). 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_H 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}–O*H*), 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_3-18), 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 (4 H, complex, H_2-2 and H_2-1).

3.5.11. Physical data for isocitreohybridone A (24). A colorless oil: $[\alpha]_D^{19.6}$=+22.6° (c 1.00, $CHCl_3$); 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}; ^1H NMR (C_6D_6) δ_H 5.91 (1H, s, H-11), 4.69 (1H, dd, J=3.3, 1.8 Hz, H-3), 4.46 (1H, d, J=4.4 Hz, H-6), 3.70 (1H, d, J=14.4 Hz, Hβ-7), 3.25 (3H, s, C_{19}–OMe), 2.77 (1H, dd, J=4.4 Hz, Hα-7), 2.39 (1H, dq, J=2.4, 2.4 Hz, H-9), 2.26 (1H, ddd, J=14.3, 4.8, 2.0 Hz, Hα-1), 1.99 (1H, s, H-5), 1.80 (3H, s, H_3-22), 1.69 (3H, dd, J=2.4, 1.6 Hz, H_3-21), 1.67 (2H, m, H_2-2), 1.65 (3H, s, C_3–OAc), 1.62 (3H, s, H_3-18), 1.58 (3H, s, C_{17}–OAc), 1.25 (3H, s, H_3-20), 1.19 (1H, ddd, J=13.4, 13.4, 6.2 Hz, Hβ-1), 0.76 (3H, s, H_3-24), and 0.73 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 203.90 (s), 179.05 (s), 174.18 (s), 170.17 (s), 169.19 (s), 164.96 (s), 137.00 (s), 125.82 (s), 124.97 (d), 77.67 (d), 76.05 (d), 72.39 (s), 54.49 (d), 52.74 (s), 51.72 (q), 51.54 (d), 43.96 (s), 43.61 (s), 36.51 (t), 34.30 (s), 26.27 (q), 25.23 (q), 22.33 (q), 22.07 (t), 21.13 (q), 21.04 (q), 20.98 (t), 20.67 (q), 17.61 (q), and 7.91 (q).

3.5.12. Physical data for isocitreohybridone B (25). A colorless prisms: mp 263–264.5°C in a sealed tube (from benzene–hexane); $[\alpha]_D^{19.6}$=+51.1° (c 1.00, $CHCl_3$); $C_{29}H_{38}O_8$ [m/z 514.2548 (M^+)]; IR (film) 1765, 1740, 1690, 1620, and 1240 cm^{-1}; ^1H NMR (C_6D_6) δ_H 5.89 (1H, s, H-11), 4.70 (1H, dd, J=2.0, 1.0 Hz, H-3), 4.50 (1H, d, J=4.5 Hz, H-6), 3.85 (1H, d, J=14.3 Hz, Hβ-7), 3.25 (3H, s, C_{19}–OMe), 3.21 (3H, s, C_{17}–OMe), 2.81 (1H, dd, J=14.3, 4.5 Hz, Hα-7), 2.49 (1H, dq, J=2.6, 2.6 Hz, H-9), 2.29 (1H, ddd, J=13.6, 3.3, 3.3 Hz, Hα-1), 2.05 (1H, s, H-5), 1.83 (3H, dd, J=2.0, 1.5 Hz, H_3-21), 1.80 (3H, s, H_3-22), 1.77 (3H, s, H_3-18), 1.72 (3H, s, C_3–OAc), 1.65 (2H, m, H_2-2), 1.32 (3H, s, H_3-20), 1.25 (1H, ddd, J=13.6, 13.6, 5.7 Hz, Hβ-1), 0.77 (3H, s, H_3-24), and 0.74 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 203.80 (s), 182.89 (s), 179.21 (s), 170.10 (s), 169.96 (s), 138.75 (s), 123.78 (d), 111.11 (s), 77.92 (d), 76.15 (d), 71.19 (s), 59.58 (q), 54.39 (d), 52.87 (s), 51.50 (q), 51.33 (d), 43.60 (s), 43.34 (s), 36.57 (t), 34.28 (s), 26.24 (q), 24.79 (q), 22.34 (q), 22.09 (t), 21.06 (q), 21.01 (q), 20.95 (t), 17.98 (q), and 8.36 (q).

3.5.13. Physical data for isocitreohybridone C (26). 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_H 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), 1.26 (3H, s, H_3-22), 1.25 (3H, s, H_3-20), 1.00 (3H, s, H_3-24), and 0.77 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 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).

3.5.14. Physical data for isocitreohybridone G (27). 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}; ^1H NMR ($CDCl_3$) δ_H 10.1 (1H, s, H-23), 5.39 (1H, dq, J=1.8, 1.4 Hz, H-11), 4.60 (1H, dd, J=2.9, 2.6 Hz, 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 Hz, Hα-7), 2.21 (1H, ddd, J=13.2, 3.3, 3.3 Hz, Hβ-1), 2.09 (3H, s, C_3–OAc), 2.09 (1H, dq, J=2.6, 1.8 Hz, 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).

3.5.15. Physical data for isocitreohybridone H (28). A colorless oil; $[\alpha]_D^{24}$=+24° (c 0.02, $CHCl_3$); $C_{30}H_{40}O_8$ [m/z 528.2718 (M^+)]; IR (film) 1765, 1735, and 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.64 (1H, dq, J=2.9, 1.8 Hz, H-11), 4.69 (1H, d, J=4.4 Hz, H-6), 4.60 (1H, dd, J=2.9, 2.6 Hz, H-3), 4.41 (2H, q, J=7.0 Hz, C_{17}–OCH_2-CH_3), 3.60 (3H, s, C_{19}–OMe), 3.41 (1H, d, J=14.3 Hz, Hβ-7), 2.45 (1H, dd, J=14.3, 4.4 Hz, Hα-7), 2.14 (1H, dd, J=2.9, 2.6 Hz, H-9), 2.12 (1H, complex, Hα-1), 1.99 (3H, s, C_3–OAc), 1.88 (3H, s, H_3-18), 1.87 (3H, dd, J=2.6, 1.8 Hz, H_3-21), 1.85 (1H, s, H-5), 1.69 (2H, complex, H_2-2), 1.37 (3H, t, J=7.0 Hz, C_{17}–OCH_2-CH_3), 1.29 (3H, s, H_3-20), 1.25 (1H, complex, Hβ-1), 1.23 (3H, s, H_3-22), 0.92 (3H, s, H_3-24), and 0.85 (3H, s, H_3-25).

3.5.16. Physical data for isocitreohybridone I (29). A colorless oil; $[\alpha]_D^{24}$=+29° (c 0.3, $CHCl_3$); $C_{29}H_{38}O_9$ [m/z 530.2515 (M^+)]; IR (film) 3500, 1760, 1735, and 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.32 (1H, s, H-21), 5.24 (1H, s, H′-21), 4.66 (1H, d, J=4.8 Hz, H-6), 4.61 (1H, s, H-3), 4.48 (1H, dd, J=7.7 Hz, 1.8, H-11), 4.08 (3H, s, C_{17}–OMe), 3.63 (3H, s, C_{19}–OMe), 3.31 (1H, d, J=14.5 Hz, Hβ-7), 2.28 (1H, dd, J=14.5, 4.8 Hz, Hα-7), 2.03 (3H, s, C_3–OAc), 1.96 (3H, s, H_3-18), 1.80 (1H, s, H-5), 1.97 (1H, complex, H-2), 1.74 (1H, d, J=7.7 Hz, H-9), 1.70 (1H, complex, H-2), 1.8–1.6 (2H, complex, H_2-1), 1.36 (3H, s, H_3-22), 1.34 (3H, s, H_3-20), 1.27 (1H, d, J=1.8 Hz, C_{11}–OH), 0.92 (3H, s, H_3-24), and 0.87 (3H, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 203.5 (s, C-15), 183.5 (s, C-17), 179.6 (s, C-23), 170.4 (s, C_3–OAc), 169.9 (s, C-19), 152.4 (s, C-12), 118.1 (t, C-21), 113.4 (s, C-16), 77.5 (d, C-6), 76.1 (d, C-3), 72.8 (d, C-11), 70.6 (s, C-14), 59.6 (q, C_{17}–OMe), 55.2 (d, C-9), 54.5 (d, C-5), 51.5 (q, C_{19}–OMe), 51.3 (s, C-13), 45.1 (s, C-10), 40.5 (s, C-8), 37.7 (t, C-7), 34.4 (s, C-4), 26.5 (q, C-24), 26.4 (q, C-22), 22.8 (q, C-25), 22.2 (t, C-2), 22.1 (q, C_3–OAc), 20.4 (t, C-1), 20.1 (q, C-20), and 8.6 (q, C-18).

3.5.17. Physical data for citreohybridone A (30). 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}; ^1H NMR (C_6D_6) δ_H 5.66 (1H, s, H-11), 4.65 (1H, dd, J=2.8, 2.8 Hz, H-3), 4.40 (1H, d, J=4.3 Hz, H-6), 3.12 (1H, d, J=14.4 Hz, Hβ-7), 2.95 (3H, s, C_{19}–OMe), 2.86 (1H, dd, J=14.4, 4.3 Hz, Hα-7), 2.69 (1H, brs, C_{16}–OH), 2.43 (1H, dd, J=2.7, 2.7 Hz, H-9), 2.12 (1H, ddd, J=13.5, 4.9, 2.3 Hz, Hα-1), 1.89 (1H, s, H-5), 1.69 (3H, s, H_3-22), 1.67 (2H, m, H_2-2), 1.62 (3H, s, C_3–OAc), 1.54 (3H, dd, J=2.7, 1.3 Hz, H_3-21), 1.36 (3H, s, H_3-18), 1.27 (3H, s, H_3-20), 0.98 (1H, ddd, J=13.5, 11.5, 7.2 Hz, Hβ-1), 0.73 (3H, s, H_3-24), 0.70 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 212.79 (s), 210.77 (s), 178.25 (s), 169.93 (s), 167.78 (s), 135.37 (s), 128.83 (d), 77.41 (d), 76.24 (s), 75.88 (d), 74.12 (s), 59.40 (s), 55.59 (d), 53.05 (d), 52.53 (q), 43.67 (s), 39.74 (s), 35.16 (t), 34.40 (s), 26.36 (q), 24.49 (q), 22.31 (q), 22.01 (t), 21.25 (t), 20.85 (q), 20.06 (q), 18.93 (q), and 18.75 (q).

3.5.18. Physical data for citreohybridone B (31). 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}; ^1H NMR (C_6D_6) δ_H 5.99 (1H, s, H-11), 5.25 (1H, s, H-21), 5.02 (1H, s, H-21), 4.70 (1H, dd, J=1.5, 1.5 Hz, H-3), 4.46 (1H, dd, J=3.4, 1.5 Hz, H-6), 2.93 (3H, s, C_{19}–OMe), 2.86 (1H, d, J=14.7 Hz, Hβ-7), 2.68 (1H, s, H-5), 2.54 (1H, dd, J=14.7, 3.4 Hz, Hα-7), 2.17 (1H, s, C_{16}–OH), 2.07 (1H, ddd, J=12.7, 2.0, 2.0 Hz, Hα-1), 1.83 (3H, s, C_3–OAc), 1.78 (2H, m, H_2-2), 1.69 (3H, s, H_3-22), 1.59 (1H, ddd, J=12.7, 9.8, 2.4 Hz, Hβ-1), 1.52 (3H, s, H_3-20), 1.42 (3H, s, H_3-18), 0.92 (3H, s, H_3-24), and 0.78 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$) δ_C 210.66 (s), 207.74 (s), 177.59 (s), 170.22 (s), 167.31 (s), 147.28 (s), 140.50 (s), 125.19 (d), 118.92 (t), 77.58 (d), 75.83 (d), 72.20 (s), 71.43 (s), 58.65 (s), 52.10 (q), 47.40 (s), 46.32 (d), 40.31 (s), 37.84 (t), 34.57 (s), 30.68 (q), 26.38 (q), 22.28 (q), 22.11 (t), 21.95 (q), 20.88 (q), 20.68 (q), and 20.20 (t); ^{13}C NMR (C_6D_6) δ_C 210.76 (s), 207.62 (s), 176.81 (s), 169.45 (s), 167.64 (s), 148.40 (s), 141.18 (s), 125.09 (d), 118.31 (t), 77.06 (d), 75.90 (d), 72.45 (s), 71.63 (s), 58.70 (s), 51.23 (q), 47.62 (s), 46.53 (d), 40.71 (s), 38.40 (t), 34.68 (s), 30.96 (q), 26.24 (q), 22.29 (t), 22.16 (q), 21.98 (q), 20.67 (q), 20.58 (t), and 20.49 (q).

3.5.19. Physical data for citreohybridone C (32). A colorless oil; $[\alpha]_D^{24}$=+58.4° (c 0.1, $CHCl_3$); $C_{28}H_{34}O_9$ [m/z 514.2201 (M^+)]; IR (film) 1810, 1785, 1765, 1745, and 1240 cm^{-1}; ^1H NMR (C_6D_6) δ_H 5.46 (1H, s, H-11), 4.70 (1H, d, J=2.7 Hz, H-3), 4.41 (1H, dd, J=3.7, 1.5 Hz, H-6), 3.08 (3H, s, C_{19}–OMe), 2.81 (1H, dd, J=14.0, 1.5 Hz, Hβ-7), 2.47 (1H, dd, J=14.0, 3.7 Hz, Hα-7), 2.21 (1H, s, H-5), 1.95 (2H, m, H_2-1), 1.82 (3H, s, H_3-22), 1.79 (2H, m, H_2-2), 1.66 (3H, s, C_3–OAc), 1.38 (3H, s, H_3-18), 1.15 (3H, s, H_3-20), 1.01 (3H, s, H_3-21), 0.83 (3H, s, H_3-24), 0.76 (3H, s,

H_3-25); ^{13}C NMR (C_6D_6) δ_C 204.0 (s, C-15), 200.8 (s, C-17), 177.2 (s, C-23), 169.4 (s, C_3–OAc), 167.6 (s, C-19), 147.2 (s, C-9), 123.9 (d, C-11), 76.7 (d, C-6), 76.2 (s, C-12), 75.7 (d, C-3), 75.7 (s, C-16), 72.1 (s, C-14), 53.5 (s, C-13), 51.6 (q, C_{19}–OMe), 50.6 (d, C-5), 48.1 (s, C-10), 38.8 (s, C-8), 38.3 (t, C-7), 35.0 (s, C-4), 31.8 (q, C-22), 26.5 (q, C-24), 23.6 (q, C-21), 22.7 (q, C-25), 22.4 (t, C-2), 20.2 (q, C_3–OAc), 19.9 (t, C-1), 11.0 (q, C-20), 7.8 (q, C-18).

3.5.20. Physical data for citreohybridonol (33). 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 1620 cm^{-1}; major tautomer (60%): ^1H NMR ($CDCl_3$) δ_H 5.67 (1H, brs, H-11), 4.72 (1H, d, *J*=3.9 Hz, H-6), 4.65 (1H, dd, *J*=2.5, 2.5 Hz, H-3), 3.67 (3H, s, C_{19}–OMe), 3.63 (1H, d, *J*=14.2 Hz, Hβ-7), 2.51 (1H, dd, *J*=14.2, 4.4 Hz, Hα-7), 2.02 (3H, s, C_3–OAc), 1.87 (3H, s, H_3-21), 1.33 (3H, s), 1.32 (3H, s), 0.94 (3H, s), and 0.89 (3H, s), other signals (δ_H 2.25–1.25, 9H) are overlapped with one another; minor tautomer (40%): ^1H NMR ($CDCl_3$) δ_H 5.83 (1H, brs, H-11), 4.78 (1H, d, *J*=3.9 Hz, H-6), 4.68 (1H, dd, *J*=2.5, 2.5 Hz, H-3), 3.61 (3H, s, C_{19}–OMe), 2.93 (1H, d, *J*=14.2 Hz, Hβ-7), 2.75 (1H, dd, *J*=14.2, 4.4 Hz, Hα-7), 2.40 (1H, dd, *J*=2.4, 2.4 Hz, H-9), 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_H 2.25–1.25, 8H) are overlapped with one another.

3.5.21. Physical data for citreobenzofuran A (34). A colorless oil; $[\alpha]_D^{28}=+0.67°$ (*c* 0.045, $CHCl_3$); $C_{16}H_{20}O_3$ [*m/z* 260.1425 (M^+)]; IR (film) 3440 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 7.48 (1H, s), 7.10 (1H, s), 4.60 (1H, d, *J*=11.8 Hz), 4.59 (1H, d, *J*=11.8 Hz), 4.14 (1H, ddd, *J*=4.7, 2.5, 2.5 Hz), 3.40 (3H, s), 3.25 (1H, ddd, *J*=2.1, 1.0, 7.1 Hz), 3.12 (1H, ddd, *J*=17.9, 12.2, 7.2 Hz), 2.90 (1H, ddd, *J*=17.9, 6.8, 2.1 Hz), 2.59 (3H, s), 2.08 (1H, m), 1.93 (1H, m), and 1.17 (3H, d, *J*=7.1 Hz).

3.5.22. Physical data for citreobenzofuran B (35). A colorless oil; $[\alpha]_D^{28}=+19.4°$ (*c* 0.035, $CHCl_3$); $C_{15}H_{18}O_3$ [*m/z* 246.1254 (M^+)]; IR (film) 3370 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 7.55 (1H, s), 7.33 (1H, s), 4.80 (2H, s), 4.07 (1H, ddd, *J*=4.7, 2.6, 2.5 Hz), 3.36 (1H, dq, *J*=2.6, 7.2 Hz), 2.77 (2H, m), 2.32 (3H, s), 2.05 (2H, m), and 1.38 (3H, d, *J*=7.2 Hz).

3.5.23. Physical data for citreobenzofuran C (36). A colorless oil; $[\alpha]_D^{28}=+9.0°$ (*c* 0.125, $CHCl_3$); $C_{15}H_{18}O_3$ [*m/z* 246.1254 (M^+)]; IR (film) 3440 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 7.53 (1H, s), 7.31 (1H, s), 4.57 (2H, s), 4.06 (1H, ddd, *J*=5.3, 2.8, 2.8 Hz), 3.38 (3H, s), 3.37 (1H, m), 2.82 (1H, ddd, *J*=17.6, 10.1, 6.8 Hz), 2.73 (1H, ddd, *J*=17.6, 6.2, 2.7 Hz), 2.32 (3H, s), 2.04 (2H, m), 1.38 (3H, d, *J*=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ_C 153.7 (d), 142.0 (d), 131.1 (s), 130.2 (s), 124.4 (s), 122.8 (s), 118.2 (d), 117.3 (s), 70.6 (d), 65.0 (t), 57.8 (q), 36.4 (d), 25.3 (t), 22.0 (t), 20.0 (q), and 19.9 (q).

3.5.24. ^1H NMR data for 40S. δ_H ($CDCl_3$) 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).

3.5.25. ^1H NMR data for 40R. δ_H ($CDCl_3$) 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).

3.5.26. ^1H NMR data for 42S. δ_H ($CDCl_3$) 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).

3.5.27. ^1H NMR data for 42R. δ_H ($CDCl_3$) 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).

3.6. Hydrolysis of citreohybridone A (14) followed by acetylation

A solution of citreohybridone A (**14**, 3.5 mg) in 20% aq. H_2SO_4–MeOH–$CHCl_3$ (1:3:3) was stirred at 60–70°C for 1 h and then dil. with aq. $NaHCO_3$ and extracted with EtOAc. The EtOAc solution was dried (Na_2SO_4) and then concentration under reduced pressure to give an oil, which was purified by preparative TLC using EtOAc–benzene (2:3) to afford a colorless oil of the corresponding keto–enol tautomers. To a solution of this keto–enol tautomers in pyridine (0.3 ml) were added acetic anhydride (0.1 ml) with stirring. The reaction mixture was stirred at room temp for 12 h after which work-up gave an oil which was subjected to preparative TLC using benzene–EtOAc (3:1) to afford citreohybridone A (**14**, 76%) and isocitreohybridone A (**24**, 15%), respectively.

3.7. Hydrolysis of citreohybridone A (14) followed by methylation

A solution of citreohybridone A (**14**, 3.9 mg) in 20% aq. H_2SO_4–MeOH–$CHCl_3$ (1:3:3) was stirred at 60–70°C for 1 h and then dil. with aq. $NaHCO_3$ and extracted with EtOAc. The EtOAc solution was dried (Na_2SO_4) and then concentration under reduced pressure to give an oil, which was purified by preparative TLC using EtOAc–benzene (2:3) to afford a colorless oil of the corresponding keto–enol tautomers. To a solution of this keto–enol tautomers in MeOH–benzene (0.4 ml, 4:1) were added $TMSCHN_2$ (0.15 ml) with stirring. The reaction mixture was stirred at room temp for 20 min after which work-up gave an oil which was subjected to preparative TLC using benzene–EtOAc (3:1) to afford citreohybridone B (**15**, 40%) and isocitreohybridone B (**25**, 47%), respectively.

3.8. Acetylation of isocitreohybridone C (26)

A solution of isocitreohybridone C (**26**, 5.0 mg) in acetic anhydride (0.1 ml)–pyridine (0.2 ml) was stirred at room temp for 12 h after which work-up gave an oil which was

subjected to preparative TLC using hexane–EtOAc (2:1) to afford isocitreohybridone B (**25**, 85%).

3.9. Hydrolysis of citreohybridone A (14) followed by ethylation

A solution of citreohybridone A (**14**, 28 mg) in 20% aq. H_2SO_4–MeOH–$CHCl_3$ (1:3:3) was stirred at 60–70°C for 2 h and then dil. with aq. $NaHCO_3$ and extracted with EtOAc. The EtOAc solution was dried (Na_2SO_4) and then concentration under reduced pressure to give an oil, which was purified by preparative TLC using EtOAc–benzene (2:3) to afford a colorless oil of the hydrolysis compound. To a solution of this compound in acetone (10 ml) was added EtBr (0.15 ml) and K_2CO_3 (50 mg). The reaction mixture was stirred at room temp for 24 h, and then poured into H_2O and extracted with EtOAc. The EtOAc solution was washed with satd aq NaCl and dried (Na_2SO_4). Removal of solvent afforded an oil, was subjected to preparative TLC using benzene–EtOAc (3:1) to afford citreohybridone H (**28**, 10%).

3.10. Stability of citreohybriddione C (32) on silica gel

Citreohybriddione B (**31**) (2.0 mg) in $CHCl_3$–MeOH (1.0 ml, 1.0 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 concentration under reduced pressure to leave an oil, whereupon TLC analysis did not show any spot of **32** except for the unchanged starting material. Thus **32** is not an artifact of **31**.

3.11. Acetylation of citreohybridonol (33)

A solution of citreohybridonol (**33**, 74.5 mg) in acetic anhydride (1.0 ml)–pyridine (2.0 ml) was stirred at 0°C for 16 h after which work-up gave an oil which was subjected to preparative TLC using benzene–EtOAc (5:2) to afford citreohybridone A (**14**, 65%) and isocitreohybridone A (**24**, 20%), respectively.

3.12. Conversion of phomenone (37) into citreobenzofuran C (36)

(1) Phomenone (14.5 mg) was added to a 5% solution of concd H_2SO_4 (0.025 ml) in MeOH–H_2O (0.375 ml, 0.1 ml). The reaction was stirred at 70–80°C for 1 h and at room temp for 13 h. TLC analysis indicated the presence of the starting material (R_f 0.16), new UV-active component with an R_f of 0.58 [EtOAc–benzene 1:1.5 (v/v)] and too many components. The reaction mixture was neutralized with sat. aq $NaHCO_3$, diluted with H_2O (5 ml) and extracted with EtOAc (6 ml×3). The combined organic layers were dried on anhydrous Na_2SO_4 and evaporated under reduced pressure giving a crude residue (14.0 mg) which was purified by preparative TLC [EtOAc–benzene 1:1.5 (v/v)] to afford **36** (0.62 mg, 4.3% yield, 8.2% conversion yield) as a main product in addition to the recovered phomenone (6.95 mg, 48% yield).

(2) Phomenone (3.0 mg) in MeOH–$CHCl_3$ (0.1 ml, 1.0 ml) was absorbed on SiO_2 (100 mg) at room temp for 5 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 concentrated under reduced pressure to leave an oil, whereupon TLC analysis did not show any spot of **36** except for the unchanged starting material.

3.13. Incorporation of sodium [1,2-$^{13}C_2$] acetate and sodium [^{13}C] formate into citreohybridonol

Polished rice (540 g) in deionized water (1.4 dm^3) including sodium [1,2-$^{13}C_2$] acetate (1 g) or sodium [^{13}C] formate (1 g) was cooked using an electric rice cooker (100°C, 20 min), and then transferred into an Erlenmeyer flask (3 dm^3×5). This was sterilized (121°C, 20 min at 2.1 atm) and then inoculated with a suspension of mycelium of the hybrid strain KO 0031 in sterilized water and incubated at room temperature for 30 days. The culture was extracted with acetone and then ethyl acetate. The combined extracts were partitioned between ethyl acetate and water. The ethyl acetate extract (10.0 g) was directly chromatographed on silica gel (40 g, silica gel 60 K070, 70–230 mesh, Katayama Chemical). After elution of higher fatty acids and their esters with benzene, further elution with benzene–ethyl acetate (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 ethyl acetate–benzene (1:3) to give citreohybridonol (**33**) as a colourless oil (92.2 mg, 0.92%).

3.14. Incorporation of ethyl 3,5-dimethylorsellinate into citreohybridonol (33)

Ethyl [carboxy-6-$^{13}C_2$]-3,5-dimethylorsellinate (96.0 atom% ^{13}C; 90.8 mg) was dissolved in hot distilled water (80 cm^3) containing 'Tween 80' detergent (8 cm^3). This sterilized solution was distributed evenly, by injection through the mycelial mat, into 4-day old stationary cultures of the hybrid strain KO 0031 (360 g of cooked rice in each of four 3 dm^3 Erlenmeyer flasks). The cultures were then incubated for a further 31 days at 25°C.

The acetone extract was concentrated under reduced pressure to an acetone-free aqueous solution (1 dm^3) and then extracted with ethyl acetate (8 dm^3). The combined extracts (dark brown syrup, 4.9 g) were partitioned between ethyl acetate and water. The ethyl acetate extract (4.9 g) was directly chromatographed on silica gel (60 g, silica gel 60 K070, 70–230 mesh Katayama Chemical). After elution of higher fatty acids and their esters with benzene, further elution with benzene–ethyl acetate (3:1) afforded a pale yellow (560 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 ethyl acetate–benzene (1:3) to give citreohybridonol (**33**) in 1.5% yield.

Acknowledgements

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

References

1. Sakabe, N.; Goto, T.; Hirata, Y. *Tetrahedron* **1977**, *33*, 3077.
2. Nishiyama, S.; Shizuri, Y.; Yamamura, S. *Tetrahedron Lett.* **1985**, *26*, 231.
3. Nishiyama, S.; Shizuri, Y.; Imai, D.; Yamamura, S.; Terada, Y.; Niwa, M.; Kawai, K.; Furukawa, H. *Tetrahedron Lett.* **1985**, *26*, 3243.
4. Nishiyama, S.; Shizuri, Y.; Yamamura, S.; Terada, Y.; Kawai, K.; Furukawa, H. *Tetrahedron Lett.* **1985**, *26*, 6239.
5. Shizuri, Y.; Nagahama, M.; Yamamura, S.; Kawai, K.; Kawai, N.; Furukawa, H. *Chem. Lett.* **1986**, 1129.
6. Kosemura, S.; Uotsu, S.; Yamamura, S. *Tetrahedron Lett.* **1995**, *36*, 7481–7482.
7. Kosemura, S.; Yamamura, S. *Tetrahedron Lett.* **1997**, *38*, 3025–3026.
8. Kosemura, S.; Uotsu, S.; Yamamura, S. *Tetrahedron Lett.* **1997**, *38*, 7373–7374.
9. Kosemura, S.; Kojima, S.; Yamamura, S. *Chem. Lett.* **1997**, 33–34.
10. Hermawan, W.; Tsukada, R.; Nakajima, S.; Fujisaki, K.; Nakasuji, F. *Appl. Entomol. Zool.* **1998**, *33*, 239–241.
11. Kosemura, S.; Matsunaga, K.; Yamamura, S.; Kubota, M.; Ohba, S. *Tetrahedron Lett.* **1991**, *32*, 3543–3546.
12. Kosemura, S.; Matsunaga, K.; Yamamura, S. *Chem. Lett.* **1991**, 1811–1814.
13. Kosemura, S.; Nagamatsu, H.; Yamamura, S. *Bull. Chem. Soc. Jpn* **1992**, *65*, 926–928.
14. Kosemura, S.; Miyata, H.; Matsunaga, K.; Yamamura, S. *Tetrahedron Lett.* **1992**, *33*, 3883–3886.
15. Kosemura, S.; Miyata, H.; Yamamura, S.; Albone, K.; Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 135–139.
16. Kosemura, S.; Matsuo, S.; Yamamura, S. *Phytochemistry* **1996**, *43*, 1231–1234.
17. Kosemura, S.; Yamamura, S. *Tetrahedron Lett.* **1997**, *38*, 6221–6224.
18. Kosemura, S. *Tetrahedron Lett.* **2002**, *43*, 1253–1256.
19. Capasso, R.; Palumbo, G.; Randazzo, G.; Bavoso, A.; Blasio, B. D.; Pavone, U. *Tetrahedron* **1986**, *42*, 4493.
20. Riche, C.; Pascard-Billy, C.; Devts, M.; Gaudemer, A.; Barbier, M.; Fousquet, J. F. *Tetrahedron Lett.* **1974**, *15*, 2765.
21. Uchida, R.; Shiomi, K.; Inokoshi, J.; Sunazuka, T.; Tanaka, H.; Iwai, Y.; Takayanagi, H.; Ōmura, S. *J. Antibiot.* **1996**, *49*, 418–424.
22. Otani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
23. Springer, J. P.; Dorner, J. W.; Cole, R. J.; Cox, R. H. *J. Org. Chem.* **1976**, *98*, 6748.
24. McIntyre, C. R.; Scott, F. E.; Simpson, T. J.; Trimble, L. A.; Vederas, J. C. *Tetrahedron* **1989**, *45*, 2307.
25. Holker, J. S. E.; Simpson, T. J. *J. Chem. Soc., Chem. Commun.* **1978**, 626.
26. McIntyre, C. R.; Scott, F. E.; Simpson, T. J.; Trimble, L. A.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1986**, 502.
27. Geris dos Santos, R. M.; Rodrigues-Fo, E. *Phytochemistry* **2002**, *61*, 907–912.
28. Chexal, K. K.; Springer, J. P.; Clardy, J.; Cole, R. J.; Kirksey, J. W.; Dorner, J. W.; Cutler, H. G.; Strawter, W. J. J. *J. Am. Chem. Soc.* **1976**, *98*, 6748–6750.