BIOSYNTHESIS OF SOME CURVULARIN-TYPE METABOLITES BY A HYBRID STRAIN ME 0005 DERIVED FROM *PENICILLIUM CITREO-VIRIDE* B. IFO 6200 AND 4692

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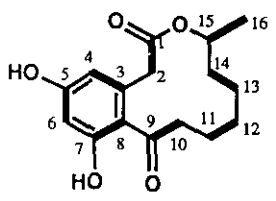
Abstract - ^{13}C Nmr analyses were carried out to deduce the labeling patterns of the curvularin-type metabolites of the hybrid strain ME 0005 derived from [1,2-$^{13}C_2$] acetate, indicating that eight acetate molecules are incorporated into these metabolites. And citreofuran, 12-oxocurvularin, and 11β-hydroxy-12-oxocurvularin are presumably formed from curvularin by enzymatic oxygenation. In addition, the structure of 11β-hydroxy-12-oxocurvularin was also supported by the present experiments.

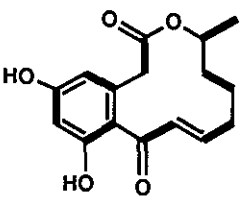
From view points of physiological activity and biogenesis, both curvularin (**1**) and dehydrocurvularin (**2**) are quite attractive because of remarkable activities against sea urchin embryo cells: These macrolides induce barrel-like spindles resulting in inhibition of cell proliferation, as reported by Kobayashi *et al.* [1] Recently, furthermore, biosynthetic study on dehydrocurvularin (**2**) was carried out by Vederas *et al.* [2] in detail, indicating that **2** is a typical polyketide with a head-to-tail arrangement of eight acetate units. Their further experiments have supported the hypothesis that both **3** and **4** are enzyme-bound intermediates during the assembly of dehydrocurvularin (**2**).[3] However, biogenetic relationship between curvularin (**1**) and dehydrocurvularin (**2**) is still uncertain, because a possibility that the former is derived from dehydrocurvularin (**2**) by enzymatic reduction seems not to be always ruled out. We describe herein the biosynthesis of citreofuran (**5**), 12-oxocurvularin (**6**), and 11β-hydroxy-12-oxocurvularin (**7**) using [1,2-$^{13}C_2$] acetate.

According to essentially the same procedure as described in the previous papers,[4] the EtOAc extract was chromatographed on silica gel using a gradient solvent of MeOH-$CHCl_3$. Further separation and purification by recrystallization or repeated preparative tlc led to the isolation of labeled citreofuran (**5**, 0.16%), 12-oxocurvularin (**6**, 0.27%), and 11β-hydroxy-12-oxocurvularin (**7**, 0.18%) in addition to labeled curvularin (**1**, 3.3%),[5,6] dehydrocurvularin (**2**, 16%),[7] *cis*-dehydrocurvularin (0.12%),[4] and 11-hydroxycurvularins (2.3%).[4]

In the ^{13}C nmr spectra of citreofuran (**5**), 12-oxocurvularin (**6**), and 11β-hydroxy-12-oxocurvularin (**7**), ^{13}C-^{13}C coupled signals due to doubly enriched carbons from [1,2-$^{13}C_2$] acetate were observed and summarized as shown in Table 1.



1



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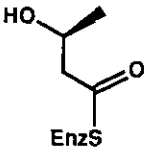

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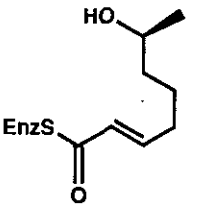

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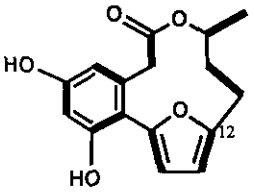

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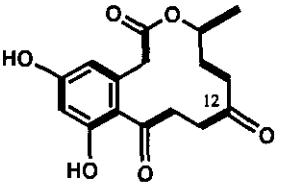

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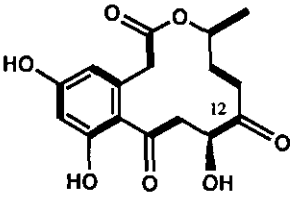

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The results show that citreofuran (**5**), 12-oxocurvularin (**6**), and 11β-hydroxy-12-oxocurvularin (**7**) must be octaketides with a head-to-tail linkage of the eight acetate units, in accordance with the labeling patterns of curvularin[6] and dehydrocurvularin.[2,3] In these metabolites, furthermore, the carbon atom (C_{12}) bearing the oxygen function is originally derived from the methyl group of acetate molecule, clearly indicating that they are not formed in such a β-oxidation manner as seen in dehydrocurvularin (**2**)[2,3] but directly formed by enzymatic oxygenation from such a less oxidized precursor as curvularin (**1**) which has been proved to be biosynthesized from **4**.[3]

11β-Hydroxy-12-oxocurvularin (**7**) was obtained this time as colorless needles (mp 67-69 °C) by recrystallization from $CHCl_3$. The ^{13}C nmr data [δ 197.8 (J= 40.1 Hz), 43.2 (J= 40.1 Hz); and δ 82.7 (J= 40.8 Hz), 207.3 (J= 40.8 Hz)] indicate that the secondary hydroxyl group is adjacent to the carbonyl group at C_{12}-position, and also support the structure (**7**) which was previously proposed based on the ^1H nmr spectral data, particularly J values, coupled with molecular mechanics calculations.[4]

EXPERIMENTAL

Melting points were determined on a Mitamura Riken apparatus and uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter. Infrared spectra were recorded on a JASCO A-202 spectrophotometer. ^1H Nmr and ^{13}C nmr spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer in methanol-d_4.

Incubation Polished rice (300 g) in deionized water (800 ml) was cooked using an electric cooker (99 °C, 20 min), then transfered into an Erlenmyer flask (5 l) and pasteurized at 120 °C at 2 atm. The pasteurized rice was, after addition of sodium [1,2-$^{13}C_2$] acetate (1 g), successively inoculated with a suspension of mycelium of the hybrid strain ME 0005 in sterilized water, incubated stationarily at 25 °C for 23 days, and then extracted with acetone.

Table 1 ^{13}C Nmr spectral data* of the [1,2-$^{13}C_2$] enriched curvularin-type compounds

	1		2**		5		6		7	
Carbon	δ	J	δ	J	δ	J	δ	J	δ	J
1	170.8	57.0	172.9	58.6	173.3	59.4	172.9	57.3	175.8	60.1
2	38.5	57.0	42.6	58.6	41.6	59.4	40.2	57.3	38.8	60.1
3	135.2	61.9	137.0	62.2	138.9	62.2	137.6	61.9	136.2	64.0
4	110.2	61.9	112.4	62.2	111.3	62.2	111.9	61.9	113.7	64.0
5	159.1	67.5	162.2	67.1	158.7	68.2	161.7	67.5	166.3	67.1
6	100.7	67.5	102.9	67.1	101.6	68.2	102.9	67.5	97.8	67.1
7	157.4	68.9	162.4	65.3	156.5	70.3	159.1	68.6	169.2	57.7
8	118.8	68.9	117.4	65.3	111.0	70.3	121.2	68.6	113.1	57.7
9	207.8	40.4	199.5	54.9	155.2	71.0	208.2	40.8	197.8	40.1
10	42.6	40.4	133.0	54.9	106.7	71.0	40.9	40.8	43.2	40.1
11	25.7	34.5	153.6	41.2	110.1	73.1	39.3	39.4	82.7	40.8
12	21.8	34.5	34.0	41.2	147.8	73.1	213.0	39.4	207.3	40.8
13	22.8	34.8	25.2	35.4	36.3	34.8	38.9	35.2	42.5	37.6
14	30.9	34.8	35.0	35.4	25.7	34.8	31.0	35.2	26.3	37.6
15	71.8	39.4	74.1	39.7	73.0	39.7	72.3	39.4	73.2	38.0
16	18.5	39.4	20.3	39.7	20.3	39.7	20.2	39.4	20.3	38.0

* ^{13}C Nmr spectra were measured in CD_3OD at 100.4 MHz on a JEOL JNM-GX 400 NMR spectrometer. Chemical shifts (δ) are given in ppm, and coupling constants (J), in Hz.

**The ^{13}C nmr spectral data are compatible with those cited in the reference 2.

Isolation and Purification As reported in the previous papers,[4] the acetone extract was concentrated *in vacuo* to an acetone-free aqueous solution (170 ml) and then extracted with EtOAc (150 ml x 7). The EtOAc extract (dark brown syrup, 4.424 g) was subjected to a column chromatography on silica gel (300 g, Katayama Chemicals, Type 60) using $CHCl_3$ (1500 ml) and then a gradient solvent of MeOH-$CHCl_3$ (1-2 % MeOH-$CHCl_3$, 1000 ml for each; 3%, 5%, 10%, 15%, and 20% MeOH-$CHCl_3$, 500 ml for each).Further separation and purification were performed by recrystallization or repeated preparative tlc (Kieselgel 60 PF_{254} and F_{254}) with conceivable solvent systems as follows. Elution with 5% MeOH-$CHCl_3$ afforded a dark brown oil. The oil was separated by repeated preparative tlc using hexane-EtOAc (1 : 2), $CHCl_3$-MeOH [(15 : 1) x 2] followed by recrystallization, yielding the labeled citreofuran (**5**; 7.0 mg, 0.16 %) as brownish needles (from $CHCl_3$-MeOH) in addition to labeled dehydrocurvularin (**2**; 710 mg, 16%; yellowish needles from C_6H_6-MeOH) and *cis*-dehydrocurvularin [4] (yellowish powder; 5.2 mg, 0.12%). Concentration of the 10% MeOH-$CHCl_3$ eluant

gave a yellow residue, which was separated by repeated preparative tlc with hexane-EtOAc (1: 2), hexane-acetone (2 : 1; 1 : 1) and purified by recrystallization, affording labeled 11β-hydroxy-12-oxocurvularin (**7**; 8.02 mg, 0.18%) as colorless needles (from $CHCl_3$) and curvularin (**1**; 146 mg, 3.3%) as colorless plates (from C_6H_6-MeOH). The brown syrup (410 mg) from 15% and 20% MeOH-$CHCl_3$ elutes was separated by repeated preparative tlc using $CHCl_3$-acetone (2 : 1), hexane-acetone (2 : 1), $CHCl_3$-MeOH (10 : 1) and $CHCl_3$-MeOH [(15 : 1) x 2] to give rise to labeled 12-oxocurvularin (**6**; yellowish powder, 12 mg, 0.27%) and 11-hydroxycurvularins[4] (colorless needles,101.1 mg, 2.3%).

The physical constants (mp, $[\alpha]_D$) and spectral data (ir, ^1H nmr) of these 1,2-^{13}C enriched macrolides are completely identical with those of the authentic non-enriched compounds,[4-6] respectively.

^{13}C Nmr spectral data of citreofuran (**5**), 12-oxocurvularin (**6**), and 11β-hydroxy-12-oxocurvularin (**7**), as well as curvularin (**1**) and dehydrocurvularin (**2**) were recorded as shown in Table 1.

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