Biosynthesis of Dykellic acid: Origin of the Carbon Skeleton

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A novel apoptosis inhibitor, dykellic acid, was recently isolated from the fermentation broth of *Westerdykella multispora* F50733 and its structure was determined by NMR spectroscopy and X-ray crystallographic analysis (Fig. 1)[1]. Dykellic acid inhibited the etoposide-induced apoptotic cell death of human monocytic leukemia U937 cells dose dependently. The biosynthesis of dykellic acid was of interest to better understand which precursors, if added to the broth, might increase the fermentation yield. The biosynthetic origin of the carbon atoms of dykellic acid was unambiguously determined by feeding experiments using ^{13}C-labeled precursors followed by ^{13}C-NMR analysis of the isolated product. These studies show that dykellic acid is derived from six acetate units and two methyl units of methionine.

Materials and Methods

Labeled Compounds

^{13}C-labeled compounds were 99% ^{13}C atom purity. Sodium [1-^{13}C]acetate, sodium [2-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate, sodium [1-^{13}C]propionate and L-[*methyl*-^{13}C]methionine were purchased from Aldrich, U.S.A.

Incorporation of Stable Isotope-labeled Precursors into Dykellic acid

A loopful of surface growth of *W. multispora* F50733 grown on potato dextrose agar slant culture was inoculated into a 250-ml Erlenmeyer flask containing 50 ml of medium (glucose 1.0%, tryptone 0.5%, yeast extract 0.3%, malt extract 0.3%, pH 6.5). The inoculated flask was shaken on a rotary shaker at 180 rpm for 3 days at 25°C. This seed culture (50 ml) was transferred to a 5-liter fermentor containing 3 liters of the same medium. Ten milliliters of each ^{13}C-labeled precursor solution at a concentration of 20 mg/ml in sterile water was added into the culture twice equally, at the 84th and 96th hour of cultivation. The fermentation was continued 48 hours after the feed of ^{13}C-labeled precursor.

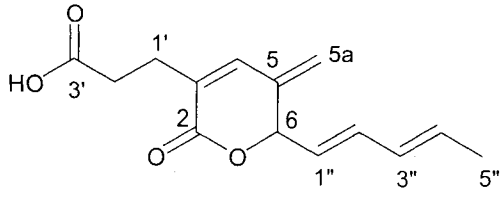
Isolation of Dykellic acid

Each harvested fermentation broth containing dykellic acid, which was fed with ^{13}C-labeled precursors was filtered with the aid of filter paper. The filtrate was applied to a column of Diaion HP-20. After washing with water and 50% MeOH, dykellic acid was eluted with 80% MeOH. The active fractions were evaporated, dissolved in water and extracted twice with equal volume of EtOAc. After washing with saturated NaCl solution, the extract was concentrated to dryness *in vacuo*. The extract was chromatographed on a silica gel (Merck, type 60) and eluted with the mixture of $CHCl_3$ and MeOH (10 : 1). The fractions containing dykellic acid were collected and concentrated *in vacuo*. The residue was further purified by Sephadex LH-20 column chromatography with MeOH. The fractions containing dykellic acid were collected and concentrated *in vacuo* to give white powder.

NMR

^{13}C-NMR spectra were recorded on a Bruker DRX 300 spectrometer at room temperature. Each ^{13}C-enriched dykellic acid was dissolved in CD_3OD at the concentration of about 10 mg in a NMR tube. The increment of signal intensities caused by ^{13}C-enrichment was determined from each signal intensity of ^{13}C-enriched dykellic acids by comparison with that of natural dykellic acid.

Fig. 1. The structure of dykellic acid.



Results and Discussion

Biosynthetic origin of each carbon of dykellic acid was investigated by ^{13}C-NMR analyses of ^{13}C-enriched dykellic acids which were obtained from the culture broth of *W. multispora* F50733 by feeding experiments of ^{13}C-labeled precursors. Firstly, as the carbon skeleton of dykellic acid is similar to that of other fungal polyketides[2], feeding experiments using ^{13}C-labeled acetates were performed. The ^{13}C-NMR spectra of dykellic acid derived from [1-^{13}C]acetate, [2-^{13}C]acetate and natural abundance media are shown in Fig. 2. The arrowheads depicted in the signals indicate the enriched carbon signals. Enrichment ratios of carbon signals in [1-^{13}C]acetate and [2-^{13}C]acetate-labeled dykellic acid, and ^{13}C-^{13}C coupling constants in [1,2-$^{13}C_2$]acetate-labeled dykellic acid are listed in Table 1. Enrichment ratios of [1-^{13}C]acetate- and [2-^{13}C]acetate-labeled dykellic acid were expressed relative to the C-5a signals as 1.0. As shown in Table 1, high levels of enrichments were observed for C-4, C-6, C-1′, C-3′, C-2″ and C-4″ in [1-^{13}C]acetate-labeled dykellic acid. These enrichments correspond to high level of enrichment for C-5, C-1″, C-3, C-2′, C-3″ and C-5″ in [2-^{13}C]acetate-labeled dykellic acid respectively. The ^{13}C-^{13}C coupling constants of intact [1,2-$^{13}C_2$]acetate units were observed at these positions. These results indicate the incorporation of six acetates into dykellic acid.

The two carbons at C-2 and C-5a were not enriched with ^{13}C-labeled acetates. In the biosynthesis of fungal polyketide metabolites the extra C_1 units are derived from the methyl group of L-methionine activated as *S*-adenosyl-L-methionine or from C-3 of propionate[3]. In order to establish two extra C_1 units feeding experiments using L-[*methyl*-^{13}C]methionine and sodium [1-^{13}C]propionate were performed. In the ^{13}C-NMR spectrum of dykellic acid obtained by feeding L-[*methyl*-^{13}C]methionine, high levels of enrichments were observed for C-2 and C-5a as shown in Fig. 2. The enrichment ratios of L-[*methyl*-^{13}C]methionine-labeled dykellic acid were calculated from the relative intensity of C-5″ signal as 1.0. Enrichment ratios were up to 17.5 and 17.9 for C-2 and C-5a, respectively (Table 1). Sodium [1-^{13}C]propionate was not incorporated into dykellic acid.

The results obtained from the above feeding experiments with ^{13}C-labeled precursors demonstrate that dykellic acid is derived from a hexaketide intermediate obtained by head-to-tail condensation of six acetate units. The δ-lactone carbony carbon (C-2) and exo-methylene carbon (C-5a) of dykellic acid are derived from the methyl group of methionine. Hence, the origin of the all carbon atoms of

Fig. 2. ^{13}C-NMR spectra of dykellic acids derived from ^{13}C-labeled precursors in CD_3OD.

(A) sodium [1-^{13}C]acetate, (B) sodium [2-^{13}C]acetate, (C) L-[*methyl*-^{13}C]methionine, (D) natural abundance.

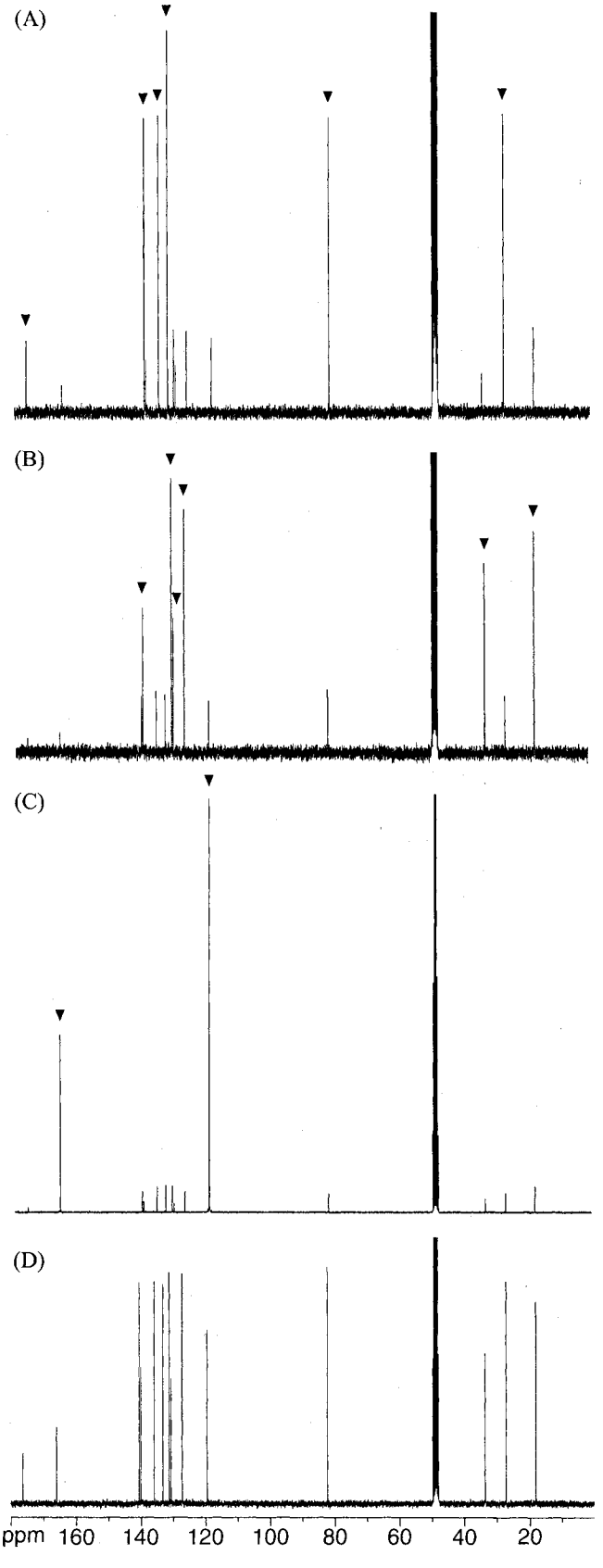
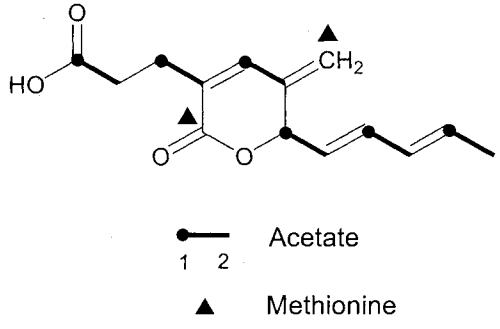


Table 1. ^{13}C-NMR chemical shifts, enrichment ratios of dykellic acids derived from ^{13}C-labeled precursors, and the $J_{C\text{-}C}$ of sodium [1,2-$^{13}C_2$]acetate-labeled dykellic acid.

Carbon No.	δ (ppm)	Natural response factor	Enrichment ratios* of dykellic acid derived from			[1,2-$^{13}C_2$]AcONa $J_{C\text{-}C}$ (Hz)
			[1-^{13}C]AcONa	[2-^{13}C]AcONa	L-[*Me*-^{13}C]Met	
2	166.2	0.4	0.8	0.9	**17.5**	-
3	130.9	0.7	0.9	**3.6**	0.5	46.7
4	140.6	1.3	**3.1**	0.9	0.7	51.1
5	140.2	0.8	0.9	**3.6**	0.6	51.1
5a	119.7	1.0	1.0	1.0	**17.9**	-
6	82.4	1.4	**2.9**	0.9	0.6	49.8
1′	27.3	1.3	**3.1**	0.8	0.7	46.7
2′	33.7	0.9	0.6	**4.2**	0.7	55.0
3′	176.4	0.3	**3.4**	0.9	0.7	55.0
1″	127.4	1.3	0.8	**3.6**	0.7	49.8
2″	136.1	1.3	**3.1**	0.9	0.9	55.6
3″	131.4	1.3	0.8	**4.0**	0.9	55.6
4″	133.4	1.3	**4.1**	0.9	0.9	43.3
5″	18.2	1.2	1.0	**3.7**	1.0	43.3

* Enrichment ratios were relative to the intensities of C-5a signals on [1-^{13}C]AcONa and [2-^{13}C]AcONa as 1.0, and to the intensity of C-5″ signal on L-[*Me*-^{13}C]Met as 1.0, respectively.

Fig. 3. The origin of the carbon atoms of dykellic acid.



dykellic acid has been established and can be summarized as shown in Fig. 3.

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