STRUCTURE AND BIOSYNTHESIS OF A NEW ANTIFUNGAL ANTIBIOTIC, PHTHORAMYCIN

Sir:

We have demonstrated that the feeding experiments using ^{13}C enriched precursors are useful means for structure elucidation of polyketide antibiotics.[1,2] In the course of screening for antifungal substances, a new macrolide antibiotic, phthoramycin (**1**: MP 116~119°C, $C_{40}H_{68}O_{12}$) was found in the cultured broth of *Streptomyces* sp. WK-1875.[3] We now report structural elucidation and biosynthesis[4,5] of **1** by means of feeding experiments using ^{13}C-labeled precursors and 400 MHz ^1H NMR spectroscopy.

The ^{13}C NMR spectral data of **1** and its pentaacetate (**2**: $C_{50}H_{78}O_{17}$, obtained by acetylation with Ac_2O - pyridine) suggested that the antibiotic possesses a polyketide skeleton derived biosynthetically from malonate, methylmalonate and so on. In the ^{13}C NMR spectrum of **2**, the appearance of a new signal at δ 207.9 (ketone) and disappearance of a hemiacetal carbon at δ 98.1 which was observed in **1** demonstrated that the cleavage of a hemiacetal ring occurred in the presence of pyridine base during acetylation of **1**. Extensive ^1H-^1H and ^{13}C-^1H correlation spectroscopy (COSY) NMR studies of **2** led to a proposed structure for **1** consisting of a 22-membered macrolide containing a hemiacetal and hemiketal ring with the precise connectivity between C-9 – C-10, C-11 – C-12, C-23 – C-24 and C-25 – C-26 undefined. The ^1H and ^{13}C chemical shift assignments for **2** were finally established by means of feeding experiment of ^{13}C-labeled compounds, as shown in Table 1. In order to elucidate the complete structure of **1**, biosynthetic studies were performed with ^{13}C-labeled precursors (99% enriched sodium [1-^{13}C]- and [1,2-$^{13}C_2$]acetate, sodium [1-^{13}C]-propionate, sodium [1-^{13}C]isobutyrate and sodium [1-^{13}C]isocaproate). The ^{13}C precursors (0.2%, w/v) were added to a 13~26-hour fermentation broth (medium; glycerol 1.8%, soybean meal 2.0%, NaCl 0.3%, pH 7.0) and the cultivation continued at 27°C for 72~96 hours. ^{13}C-Labeled phthoramycins (1~5 mg) were isolated from the cultured broth (1~2 liters) by the isolation procedure described in the previous paper[3] and were acetylated to afford the pentaacetates. ^{13}C NMR spectra (100 MHz, in $CDCl_3$) of the acetates were examined.

The feeding experiment using [1-^{13}C]acetate showed an enrichment for nine carbons (C-1, C-7, C-11, C-13, C-15, C-17, C-21, C-23 and C-27). In the feeding experiment with [1-^{13}C]-propionate, six carbons (C-3, C-5, C-9, C-19, C-25 and C-29) were highly enriched. Therefore, six methyls on the lactone ring of **2** should be located on the neighboring carbon to the above carbons enriched with ^{13}C arising from the carboxyl carbon of a propionate unit. The ^{13}C spectrum of pentaacetate (**2**) labeled with [1,2-$^{13}C_2$]acetate exhibited additional satellite peaks arising from intramolecular ^{13}C-^{13}C coupling patterns of acetate units. The 2D-INADEQUATE spectrum revealed direct evidence for C-C connectivities of nine acetate units (C-1 – C-2, C-7 – C-8, C-11 – C-12, C-13 – C-14, C-15 – C-16, C-17 – C-18, C-21 – C-22, C-23 – C-24 and C-27 – C-28) in the phthoramycin molecule.

The incorporation pattern of ^{13}C-precursors into phthoramycin molecule, in addition to extensive NMR studies allowed deduction a complete structure for **1** possessing additional C_4- and C_{11}-alkyl chains at C-16 and C-21, respectively, in a 22-membered macrolide skeleton (Fig. 1). Phthoramycin structurally corresponds to decymarosyl-8-deoxy derivative of cytovaricin which has been reported by Isono *et al.*[6,7] The feeding experiment with [1-^{13}C]isobutyrate was carried out for the determination of biosynthetic origin of the C_4 unit (C-32, C-33, C-34 and C-35) which had not been enriched with either ^{13}C-labeled acetate and propionate. A high incorporation of [1-^{13}C]isobutyrate into C-32 was observed, as in the case of cytovaricin,[8] together with the enrichment of ^{13}C into the carbons which correspond to carboxyl carbons of acetate and propionate unit derived from ^{13}C enriched isobutyrate.

In addition, to our surprise, an extremely high incorporation of the ^{13}C into C-15 (δ 128.1 in **2**) was observed in the feeding experiment with sodium [1-^{13}C]isocaproate without β-oxidation of the precursor into an acetate and isobutyrate during the fermentation (Fig. 1). Although there are some reports[9~11] that partially elaborate polyketides (C_6 and C_8 fatty acids) were incorporated into polyketide molecule, it seems

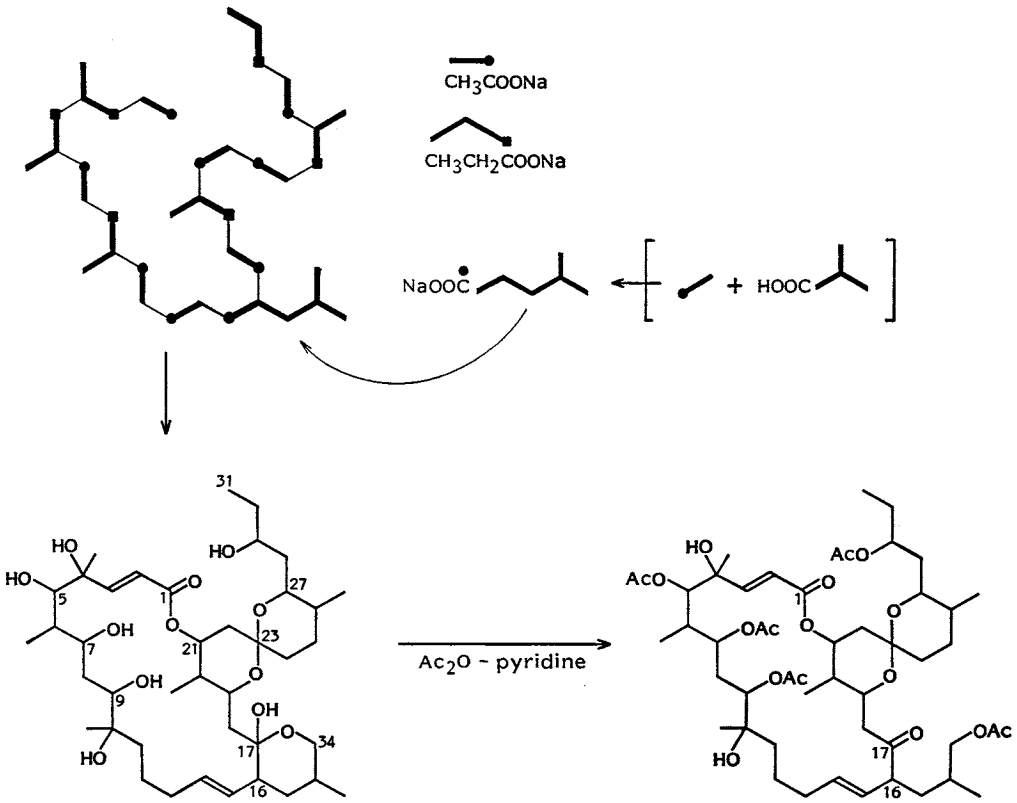
Table 1. ^{1}H and ^{13}C chemical shift assignments and ^{13}C enrichments of pentaacetylphthoramycin (2).

Carbon No.	δ_H	δ_C	^{13}C enrichment factor[a]			
			[1-^{13}C]-Acetate	[1-^{13}C]-Propionate	[1-^{13}C]-Isobutyrate	[1-^{13}C]-Isocaproate
1		165.0	2.4	0.8	6.3	0.8
2	6.12	121.3	1.4	1.0	1.0	1.0
3	6.92	148.4	1.4	9.3	6.6	0.8
4		75.7	1.5	1.0	0.73	1.0
5	4.86	78.0	1.5	8.1	11.3	0.9
6	2.19	36.0	1.2	0.7	1.1	0.7
7	4.93	74.7	2.5	0.8	7.6	0.7
8	1.83	31.8	1.5	1.0	0.8	1.0
9	4.60	75.2	1.4	8.4	7.5	0.7
10		74.0	0.9	1.1	0.7	1.0
11	1.32	39.0	2.6	1.0	7.9	1.0
12	1.30, 1.41	22.8	1.0	0.9	0.7	0.9
13	1.90, 2.13	32.7	2.5	1.0	8.4	1.0
14	5.60	134.6	1.8	0.9	0.8	0.4
15	5.33	128.1	3.2	0.7	4.8	73.0
16	3.18	55.2	1.0	1.0	1.0	1.0
17		207.9	2.4	0.9	4.8	0.5
18	2.56, 2.69	41.9	1.0	0.9	1.1	0.9
19	4.25	66.4	1.3	9.4	8.6	0.6
20	2.06	33.6	1.0	0.9	1.0	0.9
21	5.29	70.3	4.0	1.4	10.6	1.0
22	1.64, 1.73	35.5	1.0	1.0	0.9	0.4
23		97.1	2.5	1.0	5.4	1.0
24	1.45, 1.64	35.4	1.2	1.2	1.0	1.2
25	1.47	27.6	1.6	9.8	7.2	0.9
26	1.31	34.2	1.0	0.9	0.8	0.9
27	3.33	72.8	2.7	1.1	7.4	1.0
28	1.59, 1.8	37.7	1.0	0.9	0.9	0.9
29	5.01	73.1	1.9	9.7	8.0	0.7
30	1.63	28.0	1.3	0.8	1.4	0.8
31	0.90	9.5	1.4	1.4	0.7	1.3
32	1.32, 1.82	34.8	1.0	0.8	50.7	0.8
33	1.73	30.3	1.0	0.9	1.0	0.9
34	3.87	68.6	1.0	1.1	1.7	1.2
4-CH_3	1.20	26.6	1.0	1.0	1.4	1.0
6-CH_3	0.97	8.1	1.5	0.7	0.7	0.5
10-CH_3	1.11	22.3	0.9	0.7	0.9	0.7
20-CH_3	0.85	5.2	0.7	0.8	0.9	0.8
26-CH_3	0.81	17.8	1.1	0.9	0.8	0.8
33-CH_3	0.93	17.1	1.0	1.0	1.0	0.7
$COCH_3$	1.96[b]	169.9[c]				
		20.7				
$COCH_3$	2.01[b]	170.4[c]				
		20.8				
$COCH_3$	2.03[b]	170.6[c]				
		20.9				
$COCH_3$	2.08[b]	170.7[c]				
		21.1				
$COCH_3$	2.10[b]	171.0[c]				
		21.3				

[a] Peak height enriched sample/natural abundance from spectra under essentially identical instrumental conditions.

[b,c] Assignments may be interchanged.

Fig. 1. Structure and biosynthetic building units of phthoramycin.



Phthoramycin (**1**) Pentaacetylphthoramycin (**2**)

to be the first finding in the biosynthesis of microbial metabolites that isocaproate was incorporated as an intact precursor into the middle of position of the polyketide chain. Further investigation on the sequence of precursor assembly and the chain-elongation process are in progress.

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