NOTE

BIOSYNTHESIS OF TETROCARCIN. INCORPORATION OF ^{14}C- AND ^{13}C-LABELED COMPOUNDS INTO TETROCARCIN

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(Received for publication December 4, 1982)

Micromonospora chalcea KY11091 produces tetrocarcin, showing antibacterial activity against Gram-positive bacteria and antitumor activity against experimental murine tumors.[1~4]

Tetrocarcin A, a major component, consists of a thirteen-membered macrocyclic ring containing tetronic acid (tetronolide),[5] a nitro sugar (tetronitrose, the same as kijanose[6]) and deoxy sugars. The antibiotics known to have a similar aglycone to tetronolide are chlorothricin[7] and kijanimicin[5]. But they are different from tetrocarcin in several points. The unusual structural features of tetrocarcin prompted us to initiate a study of its biosynthesis. In this report, the incorporation of ^{14}C- and ^{13}C-labeled compounds into tetrocarcin is described.

Compounds labeled with ^{14}C were added at a concentration of 0.25 μCi/ml to a producing culture of *M. chalcea* KY11091 in a chemically defined medium[9] two days after inoculation. Labeled tetrocarcin A was extracted with ethyl acetate at pH 2 from the culture filtrate and purified by preparative TLC (Silica gel 60 F_{254}, Merck, $CHCl_3$ - MeOH, 9: 1 v/v). Compounds labeled with ^{13}C were added at a concentration of 0.05% to a producing medium consisting of 2% soluble starch, 0.1% yeast extract, 0.05% soy bean meal, 1% peptone A, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.1% $CaCO_3$ two days after inoculation. The culture filtrate was extracted with ethyl acetate at pH 2, followed by hydrolysis with 0.2 N HCl - acetone at 80°C for 2 hours to give tetronitrosyl tetronolide. Tetronitrosyl tetronolide labeled with ^{13}C was purified by preparative TLC (Silica gel 60 F_{254}, Merck, $CHCl_3$ - MeOH, 9: 1 v/v) and subjected to the measurement of ^{13}C NMR spectra.

Table 1. Incorporation of ^{14}C-labeled compounds into tetrocarcin A.

Labeled compounds	*Incorporation into tetrocarcin A (%)
[1-^{14}C]Acetate	0.4
[2-^{14}C]Acetate	1.1
[1-^{14}C]Propionate	1.4
D-[*U*-^{14}C]Glucosamine	0.2
D-[*U*-^{14}C]Glucose	0.3
L-[*U*-^{14}C]Leucine	0.3
L-[*U*-^{14}C]Tyrosine	0.5
L-[*U*-^{14}C]Lysine	<0.1
L-[*Methyl*-^{14}C]methionine	0.2

* The incorporation ratio represents: (Total dpm of [^{14}C]tetrocarcin A/Total dpm of added ^{14}C-compound) × 100.

As shown in Table 1, [1-^{14}C]propionate and [2-^{14}C]acetate were efficiently incorporated into tetrocarcin A (1.4% and 1.1%, respectively). Furthermore, [1-^{14}C]propionate, [2-^{14}C]acetate and [1-^{14}C]acetate were efficiently incorporated into tetronitrosyl tetronolide obtained by hydrolysis of the tetrocarcin complex from the culture filtrate (2.4%, 2.0% and 1.2%, respectively), while they were not efficiently incorporated into the deoxy sugars moiety (lower than 0.1%) (data not shown). The reason that the incorporation was higher into tetronitrosyl tetronolide than into tetrocarcin A is that the tetronitrosyl tetronolide was derived from all the components of the tetrocarcin complex, while tetrocarcin A was just one of these components. Therefore, it is presumed that the main carbon skeleton of tetronitrosyl tetronolide is synthesized *via* the polyketide route from acetate and propionate. The incorporation of L-[*U*-^{14}C]leucine, L-[*U*-^{14}C]-tyrosin and L-[*methyl*-^{14}C]methionine into tetrocarcin A was lower in efficiency than that of [2-^{14}C]acetate and [1-^{14}C]propionate, although these compounds promoted the production of tetrocarcin A in the chemically defined medium.[9] In contrast, WAITZ *et al.* reported that L-[*methyl*-^{14}C]methionine was efficiently incorporated along with acetate and propionate into kijani-

Table 2. Chemical shift assignments and enrichments of carbons of tetronitrosyl tetronolide labeled with ^{13}C-labeled compounds.

Carbon atom	Chemical shift δ (ppm)	Relative enrichment*			
		[1-^{13}C]Acetate	[2-^{13}C]Acetate	[1-^{13}C]Propionate	[3-^{13}C]Propionate
Tl[a]- 1	166.6	4.6	1.0	0.7	0.8
2	100.8	0.7	2.9	1.3	0.9
3	206.4	1.0	1.1	5.3	1.0
4	51.2	0.7	0.8	0.7	0.8
5	42.9	1.0	1.0	5.2	1.0
6	31.0	1.0	1.7	0.8	0.9
7	41.7	0.9	1.0	5.3	0.8
8	39.2	0.8	1.8	1.0	1.1
9	75.9	3.4	1.0	1.0	1.2
10	34.8	1.0	1.8	0.7	0.7
11	126.2	2.8	1.0	1.0	0.8
12	125.7	1.0	2.4	1.0	0.8
13	54.3	1.0	0.9	5.2	1.0
14	136.2	0.8	1.1	0.7	0.7
15	123.0	3.6	0.7	0.9	0.8
16	31.0	0.7	2.1	0.7	0.8
17	77.8	0.8	0.8	2.9	1.0
18	141.0	0.6	1.3	0.7	1.1
19	118.4	3.9	0.8	1.0	1.2
20	44.8	0.7	3.2	0.9	0.9
21	69.4	3.3	0.8	0.9	0.8
22	149.7	0.7	2.9	0.9	1.1
23	136.2	3.8	0.8	0.9	0.9
24	29.7	0.8	0.9	1.0	1.0
25	83.9	0.8	0.8	0.8	0.8
26	201.4	0.7	0.7	1.0	0.7
27	15.1	1.0	1.3	1.0	4.8
28	22.1	1.0	1.1	0.7	4.9
29	13.0	0.9	1.2	0.7	4.7
30	14.3	0.8	1.1	0.8	5.2
31	16.1	0.9	1.2	0.9	5.3
32	192.5	0.7	2.9	1.0	1.1
Tn[b]- 1	96.4	0.7	0.7	0.9	0.9
2	35.9	0.8	0.8	0.9	0.8
3	91.6	0.8	0.7	1.0	0.9
4	53.7	0.8	0.7	0.8	0.8
5	69.4	1.0	0.8	0.9	1.0
6	16.9	0.8	0.8	0.9	1.0
3-CH_3	25.3	0.8	0.8	0.8	0.8
4-$NHCO_2CH_3$	157.5	1.5	1.3	1.5	1.1
4-$NHCO_2CH_3$	52.7	0.8	0.8	0.7	0.9

[a] tetronolide, [b] tetronitrose

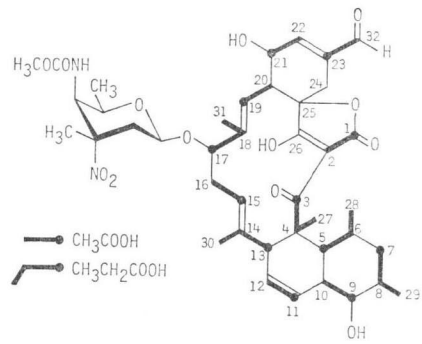
* Relative enrichment is estimated as follows: Intensity of carbon atom of enriched tetronitrosyl tetronolide/intensity of carbon atom of unenriched tetronitrosyl tetronolide.

micin.[10] The incorporation of L-[*U*-^{14}C]tyrosine into tetrocarcin A was a little higher than that of [1-^{14}C]acetate. This suggests that tyrosine might also contribute to the biosynthesis of the aglycone of tetrocarcin. However, this result might be similar to the case of chlorothricin, where tyrosine was only incorporated into chlorothricin in an indirect fashion.[11,12] Furthermore, the incorporation of L-[*U*-^{14}C]tyrosine into tetronitrosyl tetronolide was substantially lower than [1-^{14}C]acetate (1.2%), [2-^{14}C]acetate (2.0%) and [1-^{14}C]propionate (2.4%). Therefore, whether leucine, tyrosine and methionine are the possible precursors of tetrocarcin A could not be clarified in this study.

Table 2 shows the chemical shifts in the ^{13}C NMR spectrum of tetronitrosyl tetronolide and the relative enrichment of each carbon atom of tetronitrosyl tetronolide with ^{13}C-labeled precursors. The signal assignments of tetronitrosyl tetronolide were reported.[13,14] The ^{13}C NMR spectrum of tetronitrosyl tetronolide labeled with [1-^{13}C]acetate showed enrichment for C-1, C-9, C-11, C-15, C-19, C-21 and C-23, while [2-^{13}C]-acetate enriched C-2, C-10, C-12, C-16, C-20, C-22 and C-32. Enrichment at C-8 by [2-^{13}C]acetate can be interpreted as the consequence of an indirect incorporation of the precursor through propionate as shown in the case of macbecin,[15] and macrolides.[16,17] One molecule of acetate was incorporated into C-23 and C-32, the methyl group having been oxidized to aldehyde group. Five carbon atoms, C-3, C-5, C-7, C-13 and C-17 were enriched by [1-^{13}C]propionate, while [3-^{13}C]-propionate enriched five carbon atoms, C-27, C-28, C-29, C-30 and C-31. Therefore, it was concluded that the five methyl carbon atoms at C-4, C-6, C-8, C-14 and C-18 were derived from the methyl group of propionate. These precursors were not incorporated into tetronitrose. For the most part, the formation of tetronolide follows a similar pattern to the biosynthesis of chlorothricolide, the aglycone of chlorothricin.[12] The origin of three carbon atoms, C-24, C-25 and C-26 in tetronic acid moiety of tetronolide remained unresolved as in the case of chlorothricolide. Furthermore, the biosynthesis of sugar moieties (tetronitrose and deoxy sugars) in tetrocarcin A was not clarified.

Based on the results presented above, it was concluded that the aglycone of tetrocarcin A, tetronolide, was synthesized *via* the polyketide route from seven acetate and five propionate units, leaving three carbon atoms of tetronic acid moiety unresolved, as shown in Fig. 1.

Fig. 1. Labeling pattern of tetronitrosyl tetronolide.



Acknowledgment

The authors express their thanks to Mrs. MAYUMI YOSHIDA for the analysis of ^{13}C NMR spectra. They are also grateful to Miss RITSUKO YAMASHIRO for her technical assistance.

References

1) TOMITA, F.; T. TAMAOKI, K. SHIRAHATA, M. KASAI, M. MORIMOTO, S. OHKUBO, K. MINEURA & S. ISHII: Novel antitumor antibiotics, tetrocarcins. J. Antibiotics 33: 668~670, 1980
2) TOMITA, F. & T. TAMAOKI: Tetrocarcins, novel antitumor antibiotics. J. Antibiotics 33: 940~945, 1980
3) TAMAOKI, T.; M. KASAI, K. SHIRAHATA, S. OHKUBO, M. MORIMOTO, K. MINEURA, S. ISHII & F. TOMITA: Tetrocarcins, novel antitumor antibiotics. J. Antibiotics 33: 946~950, 1980
4) TAMAOKI, T.; M. KASAI, K. SHIRAHATA & F. TOMITA: Tetrocarcins E_1, E_2, F and F-1, new antibiotics. J. Antibiotics 35: 979~984, 1982
5) HIRAYAMA, N.; M. KASAI, K. SHIRAHATA, Y. OHASHI & Y. SASADA: The structure of tetronolide, the aglycone of antitumor antibiotic, tetrocarcin. Tetrahedron Lett. 21: 2559~2560, 1980
6) MALLAMS, A. K.; M. S. PUAR & R. R. ROSSMAN: Kijanimicin. 1. Structures of individual sugar components. J. Am. Chem. Soc. 103: 3938~3940, 1981
7) KELLER-SCHIERLEIN, W.; R. MUNTWYLER & H. ZAHNER: Stoffwechselprodukte von Mikroorganismen. Chlorothricin und Des-chlorothricin. Helv. Chim. Acta 52: 127~142, 1969
8) MALLAMS, A. K.; M. S. PUAR & R. R. ROSSMAN:

Kijanimicin. 2. Structure and absolute stereochemistry of kijanimicin. J. Am. Chem. Soc. 103: 3940~3943, 1981

9) TAMAOKI, T. & F. TOMITA: Biosynthesis and production of tetrocarcin A, a new antitumor antibiotic in chemically defined medium. Agric. Biol. Chem. 46: 1021~1026, 1982

10) WAITZ, J. A.; A. C. HORAN, M. KALYANPUR, B. K. LEE, D. LOEBEVBERG, J. A. MARQUEZ, G. MILLER & M. G. PATEL: Kijanimicin, a novel antibiotic produced by *Actinomadura kijaniata* SCC 1256. J. Antibiotics 34: 1101~1106, 1981

11) HOLZBACH, R.; H. PAPE, D. HOOK, E. F. KREUTZER, C. CHANG & H. G. FLOSS: Biosynthesis of macrolide antibiotic chlorothricin. Biochemistry 17: 556~560, 1978

12) MASCARETTI, O. A.; C. CHANG, D. HOOK, H. OTSUKA, E. F. KREUTZER & H. G. FLOSS: Biosynthesis of macrolide antibiotic chlorothricin. Biochemistry 20: 919~924, 1981

13) KASAI, M.; M. YOSHIDA, N. HIRAYAMA, F. TOMITA, T. TAMAOKI, Y. SASADA, Y. OHASHI & K. SHIRAHATA: Structure of new antitumor antibiotics, tetrocarcins. The 23rd Symposium Papers, The Chemistry of Natural Products. pp. 584~591, Nagoya, 1980

14) KASAI, M.; M. YOSHIDA, N. HIRAYAMA, F. TOMITA, T. TAMAOKI, Y. SASADA, Y. OHASHI & K. SHIRAHATA: Structure of new antitumor antibiotics, tetrocarcins. in preparation

15) HATANO, K.; M. MUROI, E. HIGASHIDE & M. YONEHARA: Biosynthesis of macbecin. Agric. Biol. Chem. 46: 1699~1702, 1982

16) ŌMURA, S.; A. NAKAGAWA, H. TAKESHIMA, K. ATSUMI, J. MIYAZAWA, F. PIRIOU & G. LUKACS: Biosynthesis studies using ^{13}C enriched precursors on the 16-membered macrolide antibiotic leucomycin A_3. J. Am. Chem. Soc. 97: 6600~6602, 1975

17) WHITE, R. J.; E. MARTINELLI, G. G. GALLO, G. LANCINI & P. BEYNON: Rifamycin biosynthesis studied with ^{13}C enriched precursors and carbon magnetic resonance. Nature 243: 273~277, 1973