BIOSYNTHESIS OF MANUMYCIN: ORIGIN OF THE POLYENE CHAINS

RALF THIERICKE and AXEL ZEECK*

Institut für Organische Chemie,
Universität Göttingen,
Tammannstr. 2, D-3400 Göttingen, FRG

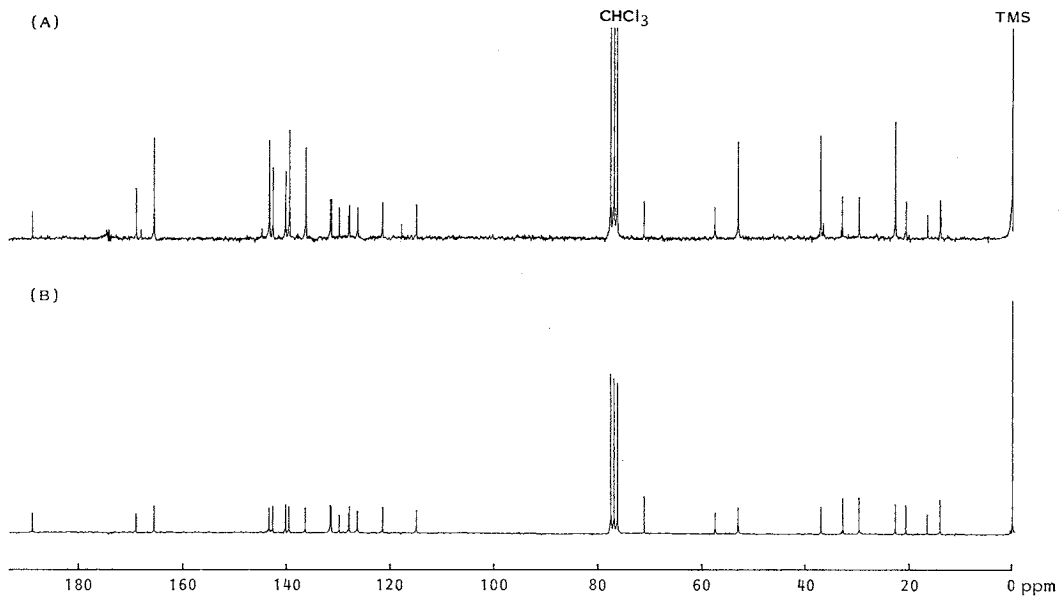
(Received for publication September 7, 1987)

Manumycin[1~3] and the closely related manumycin-group antibiotics[4~6] consist of structural units obviously derived from different biosynthetic pathways. For example, the 2-amino-3-hydroxycyclopent-2-enone moiety in manumycin (**1**) may well be biosynthesized from succinate and glycine *via* an intramolecular cyclization of 5-aminolevulinic acid as FLOSS *et al.*[7] found for the antibiotic asukamycin. In this communication we describe the incorporation of [^{13}C]-acetate into manumycin (**1**). This allowed the biosynthetic source of the methyl-branched C_{13}-side chain and the triene chain to be determined. The remaining multifunctional, *meta*-substituted, six-membered ring carries a nitrogen and an additional carbon atom; the incorporation pattern of acetate into this *m*-C_7N unit is also reported.

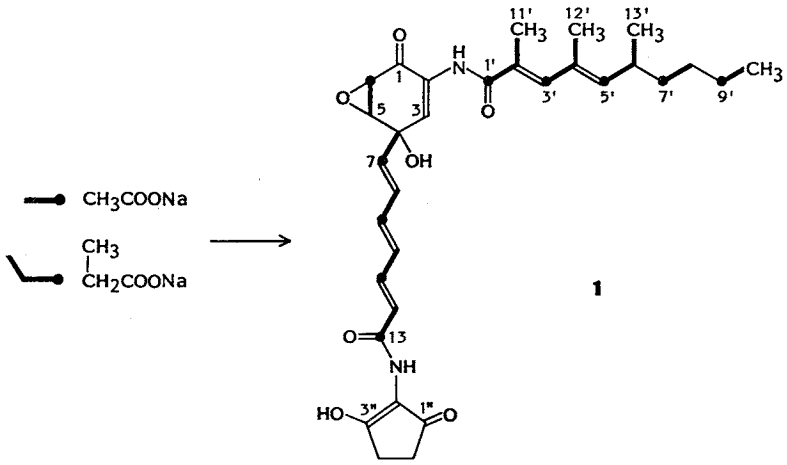
Experiments were carried out using cultures of the manumycin producer, *Streptomyces parvullus* (strain Tü 64), grown at 28°C in 1,000-ml Erlenmeyer flasks filled with 100 ml of medium (degreased soybean mean 2%, mannitol 2%, pH adjusted to 7.0). Precursors, which were dissolved in small amounts of deionized water and adjusted to pH 7.0 with 1 M NaOH, were added into the stationary growing phase[2] about 40 hours after inoculation. Both the [1-^{13}C]acetate and [2-^{13}C]acetate precursor experiments were carried out using 4×100 ml of culture medium and an amount of 10.8 mmol/liter of labeled acetate. The culture broth was adjusted to pH 4.5, 34 hours later and centrifuged (3,000 rpm, 30 minutes). The mycelium was extracted twice with acetone. After evaporation the remaining water residue was extracted with $CHCl_3$. Evaporation of the organic layer resulted in a dark red crude product which was chromatographed twice on a Sephadex LH-20 column (80×2.5 cm, $CHCl_3$). The purified pale yellow manumycin (**1**) (yield: 25.3 mg/liter of [1-^{13}C]acetate labeled **1**; 48.0 mg/liter of [2-^{13}C]acetate labeled **1**) corresponded completely with an authentic sample[2].

Based on the NMR signal assignments of manumycin (**1**)[2], the ^{13}C NMR spectra of [1-^{13}C]acetate (Fig. 1) and [2-^{13}C]acetate enriched

Fig. 1. Proton decoupled ^{13}C NMR spectra of [1-^{13}C]acetate-enriched manumycin (**1**) (A) and reference sample (B) recorded at 50.3 MHz in $CDCl_3$.



Scheme 1.



1

1 displayed the depicted labeling pattern (enhancements of the signals see Table 1). The C_{18}-side chain was labeled by incorporation of [1-^{13}C]acetate at positions C-1′, C-3′, C-5′, C-7′ and C-9′ indicating its polyketide origin. Feeding with [2-^{13}C]acetate allowed us to clarify whether the methyl groups derive from acetate *via* propionyl-coenzyme A or from methionine. The significant enhancements of C-11′, C-12′ and C-13′ as well as the noticeable distribution of the label over the whole chain with the exception of C-7′ and C-9′ indicated the methyl group's polyketide origin. A feeding experiment with [1-^{13}C]propionate (99% enriched) with signal enhancements at C-1′ (988%), C-3′ (925%) and C-5′ (831%) proved this result. The not expected overall dilution of the [2-^{13}C]-acetate label obviously is an indication of a highly active TCA-cycle. This suggested that the C_{18}-side chain is assembled by a starter acetyl-CoA (C-9′ and C-10′) and extended by one malonyl-CoA and three propionyl-CoA molecules. We believe further support of this pathway is provided by feeding experiments with unlabeled methionine (10 mmol/liter and 0.1 mmol/liter) in which drastic inhibition of the secondary metabolism and lack of manumycin production is observed.

Furthermore, the ^{13}C signal enhancements of the all-*trans* triene chain of **1** at C-9, C-11 and C-13 for [1-^{13}C]acetate incorporation and at C-8, C-10 and C-12 for [2-^{13}C]acetate incorporation, are only explainable by polyketide metabolism.

Table 1. Chemical shifts and enhancements (standardized to the C-2″ signal intensity) of the proton noise decoupled ^{13}C NMR resonances of manumycin (**1**) after feeding with [1-^{13}C]acetate and [2-^{13}C]acetate.

Spectra recorded at 50.4 MHz.

C-Atom	δ (ppm)	Enhancement (%)	
		[1-^{13}C]Acetate	[2-^{13}C]Acetate
1	188.9	+6.5	+26.8
2	128.0	−19.7	+12.0
3	126.3	−0.6	+15.4
4	71.3	−32.3	+71.0
5	57.5	+5.1	+108.3
6	52.9	+138.4	+76.1
7	136.5	+134.7	+77.5
8	131.5	+4.0	+98.6
9	139.6	+166.1	+18.9
10	131.7	−5.9	+85.1
11	143.4	+150.7	+23.9
12	121.6	+0.1	+80.3
13	165.5	+119.8	−5.3
1′	168.8	+65.1	+56.8
2′	128.4	−22.8	+88.7
3′	140.2	+56.1	+64.8
4′	129.9	+0.4	+111.7
5′	142.7	+64.2	+81.5
6′	32.9	−27.0	+88.0
7′	37.1	+140.4	+39.9
8′	29.8	−21.7	+52.9
9′	22.8	+130.6	+44.5
10′	14.1	−20.3	+87.0
11′	14.0	−20.0	+89.7
12′	16.5	−20.0	+135.0
13′	20.7	−10.9	+102.8
2″	115.0	0	0

In addition, [1-^{13}C]acetate was incorporated into the *m*-C_7N unit of **1** at C-6 and C-7, [2-^{13}C]-acetate at C-4, C-5, C-6 and C-7, a fact of high distribution of the [2-^{13}C]acetate label *via* the TCA-cycle, whereas the remaining three carbon atoms of this multifunctional ring showed no enhancements. The labeling pattern with the back to back association of two acetate molecules suggested succinate to be a likely biosynthetic precursor of the *m*-C_7N unit and a preliminary feeding experiment with [1,4-$^{14}C_2$]succinate showed an incorporation rate of 2.5%, but this might also be due to incorporation into the C_5N-moiety[7]. The origin of the remaining three carbon atoms of the *m*-C_7N unit is still under study[8]. The present results, however, lead to the prediction of a novel biosynthetic pathway for the *m*-C_7N unit found in the manumycin group antibiotics. This is in agreement with results recently reported in the case of asukamycin[9].

Acknowledgment

We thank Prof. H. ZÄHNER, Institut für Biologie II, der Universität Tübingen (FRG), for making available to us the strain *Streptomyces parvullus* (Tü 64).

References

1) SCHRÖDER, K. & A. ZEECK: Manumycin. Tetrahedron Lett. 1973: 4995~4998, 1973
2) ZEECK, A.; K. SCHRÖDER, K. FROBEL, R. GROTE & R. THIERICKE: The structure of manumycin. I. Characterization, structure elucidation and biological activity. J. Antibiotics 40: 1530~1540, 1987
3) THIERICKE, R.; M. STELLWAAG, A. ZEECK & G. SNATZKE: The structure of manumycin. III. Absolute configuration and conformational studies. J. Antibiotics 40: 1549~1554, 1987
4) KAKINUMA, K.; N. IKEKAWA, A. NAKAGAWA & S. ŌMURA: The structure of asukamycin, a possible shunt metabolite from 3-dehydroquinic acid in the shikimate pathway. J. Am. Chem. Soc. 101: 3402~3404, 1979
5) SLECHTA, L.; J. I. CIALDELLA, S. A. MIZSAK & H. HOEKSEMA: Isolation and characterization of a new antibiotic U-62162. J. Antibiotics 35: 556~560, 1982
6) BRODASKY, T. F.; D. W. STROMAN, A. DIETZ & S. MIZSAK: U-56,407, a new antibiotic related to asukamycin: Isolation and characterization. J. Antibiotics 36: 950~956, 1983
7) NAKAGAWA, A.; T. SHIANG WU, P. J. KELLER, J. P. LEE, S. ŌMURA & H. G. FLOSS: Biosynthesis of asukamycin. Formation of the 2-amino-3-hydroxycyclopenten-2-one moiety. J. Chem. Soc. Chem. Commun. 1985: 519~521, 1985
8) BEALE, J. M.; R. E. HERROLD, H. G. FLOSS, R. THIERICKE, A. ZEECK, A. NAKAGAWA & S. ŌMURA: Studies on the biosynthesis of the *meta*-C_7N unit in the antibiotics manumycin and asukamycin. J. Am. Chem. Soc. 110: 1987, in press
9) FLOSS, H. G.; P. J. KELLER & J. M. BEALE: Studies on the biosynthesis of antibiotic. J. Nat. Prod. 49: 957~970, 1986