

NOTE

BIOSYNTHESIS OF ANTIBIOTIC A23187
INCORPORATION OF PRECURSORS
INTO A23187

MILTON J. ZMIJEWSKI, Jr.

Department of Medicinal Chemistry, College of
Pharmacy, University of Utah, Salt Lake City,
Utah 84112, U.S.A.

(Received for publication February 2, 1980)

Streptomyces chartreusis NRRL 3881 produces the divalent cation ionophore antibiotic A23187¹⁾. This antibiotic is primarily active against Gram-positive bacteria and fungi and has been used extensively to investigate the role of divalent cations in regulating cellular physiological processes^{2,3,4)}. The chemical structure of A23187^{5,6)} indicates that it is a member of a new class of ionophorous antibiotics, the pyrrole polyethers.⁷⁾ The only other antibiotic in this class is the recently discovered X-14547A⁸⁾. In this report, the incorporation and labeling pattern of ¹⁴C- and ¹³C-labeled precursors into A23187 is described.

Antibiotic A23187 (1a) is a mono-carboxylic acid consisting of three structural units; an α -ketopyrrole, a substituted benzoxazole and a spiroketal ring⁹⁾. The α -ketopyrrole moiety could arise from proline or acetate while the

spiroketal ring system could be formed by propionate and acetate⁹⁾ or by condensation of acetate units with C-methyl groups arising through transmethylation from a C₁-donor¹⁰⁾. The aromatic ring of the benzoxazole moiety could be derived from 3-hydroxyanthranilic acid *via* tryptophan¹¹⁾ or from glucose metabolism *via* a shikimic type intermediate¹²⁾. These possibilities were tested by determining the incorporation of ¹⁴C-labeled precursors into the antibiotic (Table 1).

Radioisotope labeled substrate ($\sim 10 \mu\text{Ci}$ to $50 \mu\text{Ci}/50 \text{ ml}$ culture broth) were added to individual cultures of *S. chartreusis* three days after inoculation. Cultures were harvested twenty-four hours later and A23187 was isolated, converted to its acid form, methylated with diazomethane, and purified by chromatography as described elsewhere¹⁾.

The results (Table 1) indicated that methionine, propionate, acetate, glycerol, glucose, proline and ornithine were all efficiently incorporated into A23187 methylester (1b). Information on the labeling pattern of these precursors was obtained by chemical degradation of the specifically radioisotope labeled antibiotic (Table 2).

KUHN-ROTH oxidation¹³⁾ of the antibiotic made possible the isolation of carbons 19' and 19, 17' and 17, 15' and 15, and 11' and 11 in the form of sodium acetate. SCHMIDT degradation¹³⁾ of the sodium acetate derived from the K-R oxidation yields monomethyl amine and CO₂ from C-2 and C-1 of the acetate, respectively. The results (Table 2) indicate that the C-3 and C-2 of propionate are the source of the methyl and adjacent methylenes of the spiroketal ring of A23187. The specific activity of the sodium acetate derived from A23187 labeled from propionate [^{3-¹⁴C}] and [^{2-¹⁴C}] is one quarter that of the labeled antibiotic.

Table 1. Incorporation of ¹⁴C-labeled substrates into A23187 methylester.

Substrate (sodium salt)	% Incorporation
Propionate-3- ¹⁴ C	28.4
Propionate-2- ¹⁴ C	18.0
Propionate-1- ¹⁴ C	8.1
Acetate-2- ¹⁴ C	1.6
Acetate-1- ¹⁴ C	1.8
L-Proline-U- ¹⁴ C	24.2
D,L-Ornithine-1- ¹⁴ C	0.55
D-Glucose-U- ¹⁴ C	1.3
Glycerol-U- ¹⁴ C	0.5
D,L-Tryptophan-7a- ¹⁴ C	0
L-Methionine-5- ¹⁴ CH ₃	22.0

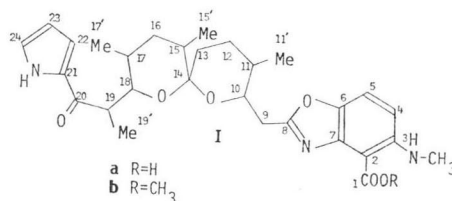


Table 2. Degradation of specifically labeled A23187 methylesters.

Precursor	Spec. act. ($\times 10^3$) of A23187 ME ($\mu\text{Ci}/\mu\text{mole}$)	Spec. act. of sodium acetate from KRO $\times 10^3$ ($\mu\text{Ci}/\mu\text{mole}$)	SCHMIDT degradation % of recovered radioactivity in		% of Total precursor label found in sodium acetate
			CH_3NH_2	CO_2	
Propionate-3- ^{14}C	3.0	0.78	95.3	4.7	100
Propionate-2- ^{14}C	2.4	0.54	5.8	94.2	100
Propionate-1- ^{14}C	1.6	0	N.D.	N.D.	—
Acetate-2- ^{14}C	1.9	0.064	56	44	30
Acetate-1- ^{14}C	1.2	0.060	22	88	10
Methionine ($\text{S-}^{14}\text{CH}_3$)	1.1	0	N.D.	N.D.	—
Glucose ($\text{U-}^{14}\text{C}$)	0.61	0	N.D.	N.D.	10

KRO; K-R oxidation. N.D.; Not determined.

This is consistent with the idea that all four of the methyl group and adjacent methylenes of the spiroketal ring are derived from C-3 and C-2 of propionate, respectively. The results of the SCHMIDT degradation of the [$3\text{-}^{14}\text{C}$] and [$2\text{-}^{14}\text{C}$] propionate labeled acetates are also consistent with this proposal. The finding that 100% of the label that [$3\text{-}^{14}\text{C}$] and [$2\text{-}^{14}\text{C}$] propionate contribute to the antibiotic molecule is found at these carbons indicates that propionate is a direct precursor and not metabolically transformed prior to incorporation. The sodium acetate derived from the K-R oxidation of [$2\text{-}^{14}\text{C}$] and [$1\text{-}^{14}\text{C}$] acetate labeled A23187 methylester was also found to be labeled but the results are consistent with the metabolic conversion of acetate to propionate prior to its incorporation. Other attempts to degrade the antibiotic proved fruitless and so in order to examine the role of proline and methionine in the biosynthesis of A23187, ^{13}C -labeled precursors in conjunction with ^{13}C -NMR was used (Table 3).

Comparison of the FT-proton noise decoupled ^{13}C -NMR natural abundance spectrum with that of the D,L-proline-1- ^{13}C enriched antibiotic (Table 3) demonstrated a 7-fold enrichment of the peak at 194.3 ppm which had been assigned previously¹⁴⁾ to C-20 (the α -keto carbon of the α -ketopyrrole moiety). The spectrum of methionine ($\text{S-}^{13}\text{CH}_3$) enriched A23187 methylester also showed only one enriched signal (30.3 ppm) assigned to the N-methyl group of the benzoxazole moiety.

The origin of the substituted aromatic ring of the benzoxazole moiety remains to be determined. Tryptophan-7a- ^{14}C was not incorporated into the antibiotic indicating that its degradation pro-

duct, 3-hydroxyanthranilic acid, is not a precursor for this part of the molecule. The finding that glucose- $\text{U-}^{14}\text{C}$ labels the antibiotic but poorly labeled the carbons derived from KUHN-ROTH oxidation (Table 2), suggests that this part of the antibiotic may be derived from a shikimate type intermediate as has been suggested for the aromatic functionalities found in the streptovaricins¹⁵⁾, mitomycins¹⁶⁾, geldanamycin¹⁶⁾, rifamycin¹²⁾ and pactamycin¹⁷⁾.

The results allow the following conclusions to be drawn: (1) the spiroketal ring of A23187 is derived from the condensation of propionate and possibly acetate as has been reported for the streptovaricins and rifamycins¹⁴⁾, (2) proline, probably *via* ornithine, is the precursor for the α -ketopyrrole moiety and (3) methionine contributes only one carbon to the antibiotic at the C-3' position (N-methyl). Additional studies with ^{13}C -labeled precursors are underway to determine more conclusively the role of acetate and propionate in forming the spiroketal ring, to establish the precursors for the benzoxazole moiety, and to demonstrate the role of ornithine in the biosynthesis of the α -ketopyrrole moiety.

Acknowledgments

This work was supported by a grant from the University of Utah, College of Pharmacy, *Ad Hoc* Committee. Special thanks to Dr. R. HAMILL of Eli Lilly for reference samples of A23187 and to Dr. L. H. HURLEY of the University of Kentucky for the C-13 labeled methionine. The technical assistance of JOHN W. PIZ and DAVID WINGATE are also gratefully acknowledged.

Table 3. Incorporation of D,L-proline-1-¹³C and D,L-methionine-CH₃-¹³C into A23187 methylester as determined by ¹³C-NMR.^a

Chemical shift ppm (TMS)	D,L-Proline-1- ¹³ C enrichment × natural abundance	D,L-Methionine-CH ₃ - ¹³ C enrichment × natural abundance	Assignment	Functional group
194.3	7.0	0.6	C-20	>C=O
168.6	0.9	0.6	C-1	-COOH
165.9	0.7	0.6	C-8	-C(=O)-N-
150.5	1.0	0.6	$\left. \begin{array}{l} \text{C-3, C-6, C-7,} \\ \text{C-21, C-22,} \\ \text{C-23, C-24,} \\ \text{C-4, C-5} \end{array} \right\}$	=C-
142.2	0.9	0.5		
133.6	0.6	0.4		
123.6	0.9	0.5		
116.1	1.0	0.4		
109.8	0.9	0.7		
107.7	1.0	0.6		
100.4	0.7	0.4	C-2	=C-COOH
98.5	0.8	0.5	C-14	>C(O)-O-
73.8	0.9	1.0	{C-10} {C-18}	H-C-O
68.3	0.8	1.2		
51.8	1.0	1.0	C-1	-COOCH ₃
42.7	0.9	1.0	N.A.	
35.3	0.9	1.2	N.A.	
32.5	1.4	1.1	N.A.	
30.3	1.0	16.4	C-3'	N-CH ₃
29.0	0.8	1.0	N.A.	
28.4	0.9	1.1	N.A.	
25.5	1.1	1.2	N.A.	
16.1	1.0	1.4	$\left. \begin{array}{l} \text{C-11', C-15'} \\ \text{C-1', C-19'} \end{array} \right\}$	-C-CH ₃
12.4	0.8	1.4		
11.1	1.0	1.4		
10.6	0.9	1.2		

^a Proton decoupled ¹³C-NMR FT spectrum were recorded on a Varian XL-100 spectrometer at 25.2 MHz. N.A. Not Assigned.

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