ISOLATION AND CHARACTERIZATION OF 10-DEOXYMETHYNOLIDE PRODUCED BY *Streptomyces venezuelae*

Sir:

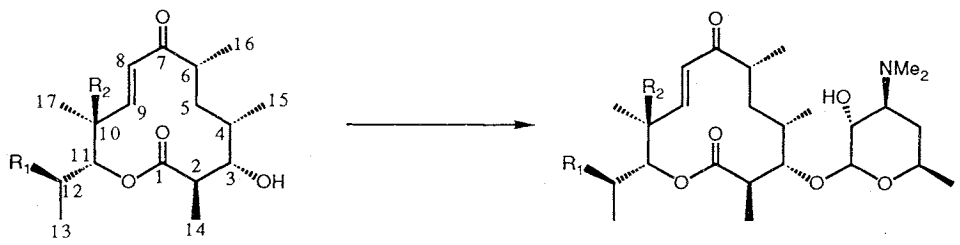
Methymycin (**V**) and neomethymycin (**VI**) are macrolide antibiotics co-produced by fermenting cultures of *Streptomyces venezuelae* ATCC 15439. Methynolide (**II**), neomethynolide (**III**)[1] and YC-17 (**IV**)[2] have all been reported as biosynthetic precursors of **V** and **VI**. In previous work we demonstrated intact incorporation of isotopically labeled advanced precursors into **V** and **VI** thus providing further confirmation for the processive chain elongation mechanism proposed for macrolide biosynthesis.[3] In this paper, we report the isolation and characterization of 10-deoxymethynolide (**I**), the parent aglycone of this series of metabolites **II**~**VI**.

I is accumulated in liquid cultures of *S. venezuelae* grown in the presence of xanthotoxin (8-methoxypsoralen) a reported inhibitor of monooxygenase activity.[4] A concentrated spore suspension (0.1 ml) of *S. venezuelae* was used to inoculate 100 ml SCM medium consisting of soluble starch 15 g, Soytone 20 g, $CaCl_2$ 0.1 g, yeast extract 1.5 g and MOPS 10.5 g per 1 liter nanopure H_2O, pH 7.2. The culture was grown at 27°C on a rotary shaker at 250 rpm for 48 hours. Two 2.8-liter baffled flasks containing 2 × 500 ml SCM media each had 108 mg (0.5 mmol) xanthotoxin in 1 ml DMSO added after autoclaving and were inoculated with 2 × 15 ml 48-hour seed culture. These vegetative cultures were then grown under the same conditions for 46 hours. The cultures were harvested by centrifugation at 10,000 × *g* for 10 minutes and the supernatant pH adjusted to 9.5 with KOH. The broth was extracted with 5 × 100 ml $CHCl_3$. Emulsions were clarified with Celite. Drying ($MgSO_4$) and concentration *in vacuo* provided 223 mg oil. The mycelia were extracted with 2 × 100 ml acetone, the acetone removed by rotovapor, and the yellow concentrate extracted with 3 × 75 ml $CHCl_3$. Drying ($MgSO_4$) followed by concentration *in vacuo* provided 75 mg of recovered xanthotoxin.

The crude supernatant extract was purified by flash chromatography on silica gel. Elution with a gradient of 0~20% (v/v) MeOH in $CHCl_3$ provided 8 mg each of **I**, **V** and **VI**. Only minor amounts of other unidentified metabolites had accumulated in the broth. A total of 150 mg xanthotoxin was recovered from both the mycelia and broth. This experiment was repeated with a 10-liter culture of *S. venezuelae* grown in the presence of 2 mM xanthotoxin in a 14-liter New Brunswick fermentor to obtain 250 mg **I**. Cultures of *S. venezuelae* grown under the same conditions in the absence of xanthotoxin typically produced 50~75 mg/liter of both **V** and **VI** with no significant accumulation of other macrolides.

Characterization of **I**, $[\alpha]_D^{25}$ +67.75° (*c* 8.0 mg/ml, $CHCl_3$), UV λ_{max}^{MeOH} nm (ε) 226 (9,100), IR ν_{max} (neat) cm^{-1} 3464, 2970, 2937, 2879, 1726, 1687, 1628, 1459, 1383, 1325, CI-MS (NH_4^+) Calcd for $C_{17}H_{28}O_4$ 297.2066 $(M+H)^+$, Found 297.2071 $(M+H)^+$, ^1H NMR and ^{13}C NMR (Table 1), confirmed the structure to be that of 10-deoxymethynolide (**I**). All NMR assignments were confirmed by $^1H,^1H$-COSY, $^1H,^{13}C$-HETCOR, INEPT, and *J* Resolved experiments (data not shown). Assignments of relative stereochemistry are based on comparison of chemical shifts and coupling constants with ^1H NMR spectra of **V** and **VI**.

Scheme 1. Methymycin and related metabolites of *Streptomyces venezuelae* ATCC 15439.



		R_1	R_2
I	10-Deoxymethynolide	–H	–H
II	Methynolide	–H	–OH
III	Neomethynolide	–OH	–H

		R_1	R_2
IV	YC-17	–H	–H
V	Methymycin	–H	–OH
VI	Neomethymycin	–OH	–H

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data of 10-deoxymethynolide (**I**).

^{13}C	δ (ppm)	m	^{1}H	δ (ppm)	m	J	Hz
C-1	174.7	CO_2				$J_{2,3}$	10.38
C-2	43.4	CH	2-H	2.6	dq	$J_{2,14}$	6.90
C-3	78.2	CH	3-H	3.55	d	$J_{3,4}$	0
C-4	33.22	CH	4-H	1.27[a]	m	$J_{4,5a}$	*
C-5	33.24	CH_2	5-Ha	1.65	m	$J_{4,5b}$	*
			5-Hb	1.32[a]	m	$J_{4,15}$	6.24
C-6	45.1	CH	6-H	2.53	ddq	$J_{5a,5b}$	*
C-7	204.9	CO				$J_{5a,6}$	12.75[b]
C-8	125.7	CH	8-H	6.42	dd	$J_{5b,6}$	3.70[b]
C-9	147.1	CH	9-H	6.74	dd	$J_{6,16}$	6.98
C-10	38.0	CH	10-H	2.64	m	$J_{8,9}$	15.75
C-11	73.7	CH	11-H	5.00	ddd	$J_{8,10}$	1.20
C-12	25.1	CH_2	12-Ha	1.71	ddq	$J_{9,10}$	5.45
			12-Hb	1.55	ddq	$J_{10,11}$	2.41
C-13	10.3	CH_3	13-H	0.91	t	$J_{10,17}$	6.85
C-14	16.4	CH_3	14-H	1.30	d	$J_{11,12a}$	8.70
C-15	17.4	CH_3	15-H	1.00	d	$J_{11,12b}$	5.45
C-16	17.7	CH_3	16-H	1.22	d	$J_{12a,12b}$	13.90
C-17	9.6	CH_3	17-H	1.12	d	$J_{12ab,13}$	7.43

All shifts relative to TMS.
Asterisks indicate that the coupling constant could not be determined due to non-first order effects.
[a,b] These values may be interchanged.

I proved difficult to crystallize and was therefore derivatized as the 3,5-dinitrobenzoate ester (**I**-DNB) by stirring 23 mg **I** and an approximate five-fold excess of 3,5-dinitrobenzoyl chloride (freshly recrystallized from pentane) in 1 ml 50% (v/v) pyridine (dried over KOH) in CH_2Cl_2 (distilled from P_2O_5) with a catalytic amount of DMAP for 48 hours at room temperature. The reaction mixture was then diluted with 1 ml CH_2Cl_2 and washed with 1 × 1 ml 5% (v/v) HCl followed by 1 × 1 ml saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$ and concentrated to 32 mg crude orange solid. Preparative TLC purification (20 × 20 cm × 1 mm silica, 4% (v/v) MeOH in $CHCl_3$) provided 25 mg (66%) white amorphous solid, mp 225~228°C; EI-MS Calcd for $C_{24}H_{30}N_2O_9$ 490.1951, Found 490.1998. The ^{1}H NMR spectrum of **I**-DNB ($CDCl_3$, 400 MHz) was consistent with the expected structure of the derivative.

Thus growing *S. venezuelae* in the presence of xanthotoxin provides 10-deoxymethynolide. This material may serve as a valuable standard in enzymatic studies of the methymycin polyketide synthase currently in progress in our laboratory.

Acknowledgment

We thank Dr. JAMES VAN EPP for the exact mass analyses and assistance with the 2D NMR spectroscopy. This work was supported by a grant from the National Institutes of Health, GM22172.

RALPH H. LAMBALOT
DAVID E. CANE*

Department of Chemistry
Brown University
Providence, Rhode Island 02912, U.S.A.

(Received June 1, 1992)

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