COMMUNICATIONS TO THE EDITOR

Production of a New Methylated 6,8a-Seco-6,8a-deoxy Derivative of the Avermectins by a Transformant Strain of *Streptomyces avermitilis*

Sir:

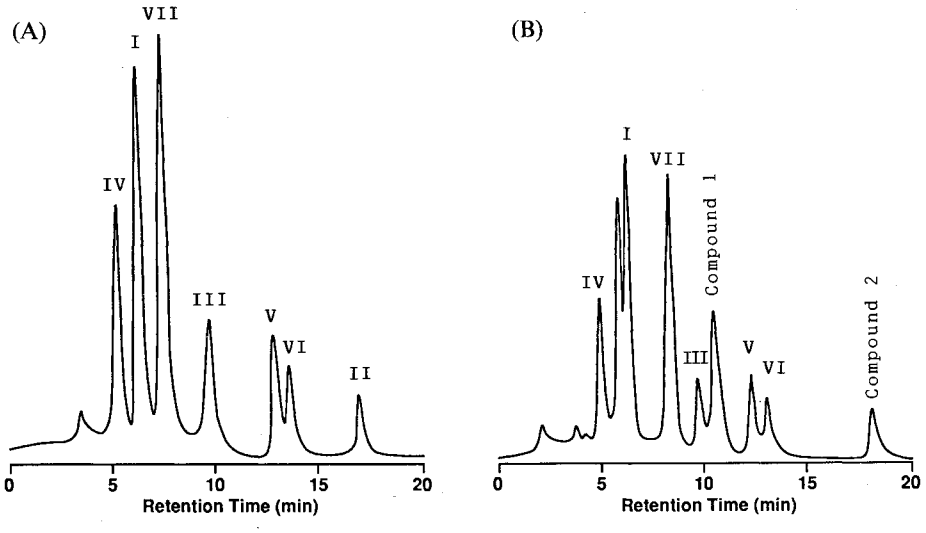
Recently, we have reported several new 6,8a-seco-6,8a-deoxy derivatives of avermectins produced by a mutant strain of *Streptomyces avermitilis* K2057[1] derived by mutation from strain K2038 producing avermectins B1a and B2a selectively[2]. The parent strain K2038 carries two types of mutations, C-5 *O*-methylation (*aveD*) and the selective incorporation of branched-chain fatty acid into the avermectin molecule (*X*). The mutant strain K2057 has an additional mutation (*aveE*) affecting furan ring formation at C-6 and C-8a positions besides both mutations *aveD* and *X*. We could not detect methylated 6,8a-seco-6,8a-deoxy derivatives of avermectins from mutant K2057 because the mutant lacks functions required for methylation at position C-5. Although some of methylated 6,8a-seco-6,8a-deoxy derivatives of avermectins have been reported by CHEN and INAMINE[3], 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin A1a has not been described. Since the mutant K2057 produced a relatively large amount of 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin B1a, if C-5 *O*-methylation (*aveD*) could be restored, the revertant would likely produce the new compound, 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin A1a.

In this communication, we describe the production of the new 6,8a-seco-6,8a-deoxy derivative of avermectins by introducing the gene for the C-5 *O*-methylation step from the wild type strain of *S. avermitilis* into mutant K2057.

Transformants were obtained from the mutant strain K2057 (*aveD aveE X*) by introduction of the recombinant plasmid pKU109 :: *aveD139*, which consisted of *Streptomyces* vector pKU109[4] and the 3.4 kbp *Bam*HI fragment encoding the C-5 *O*-methylation step of avermectin biosynthesis from wild type strain K139. Conditions for the production were as described previously[5,6] except that 5 μg of thiostrepton per milliliter was added in the medium to prevent plasmid loss.

Mutant K2057 produces seven compounds lacking a furan ring at C-6 and C-8a positions[1]. Transformants of K2057 carrying a gene for C-5 *O*-methylation step produced at least nine avermectin-related compounds. Seven of them were identical to the compounds produced by K2057. It was anticipated that the other two components would be methylated derivatives. Mycelia were harvested from 600-ml culture by centrifugation and the avermectin derivatives were extracted with acetone. The acetone extract was evaporated and the concentrate was extracted with methylene chloride to provide about 730 mg of oily material, which was applied to preparative silica gel thin layer chromatography and developed with *n*-hexane - 2-propanol (85 : 15). The fractions corresponding to new components were collected and eluted with methylene chloride - methanol (3 : 1). The eluate was concentrated to dryness and

Fig. 1. Analytical HPLC of mycelial extracts from mutant K2057 (A) and the pKU109::*aveD139* transformant (B).



Cultivation and preparation of the mycelial extract were described previously[1]. Conditions for HPLC were as described in references 1 and 4. Identified peaks were as follows; I: 6,8a-seco-6,8a-deoxyavermectin B2a, II: 6,8a-seco-6,8a-deoxyavermectin B1a, III: 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin B1a, IV: 4′-deoleandrosyl-6,8a-seco-6,8a-deoxy-5-oxoavermectin B2a, V: 6,8a-seco-6,8a-deoxy-2,5-didehydroavermectin B2a, VI: 4′-deoleandrosyl-6,8a-seco-6,8a-deoxy-5-oxoavermectin B1a and VII: 6,8a-seco-6,8a-deoxy-5-oxoavermectin B1a aglycone.

Table 1. Physico-chemical properties of compounds **1** and **2**.

	Compound **1**	Compound **2**
IR ν_{max}^{KBr} (cm^{-1})	3440, 1700	3440, 1700
UV λ_{max}^{MeOH} (nm)	242.5	242
FAB-MS (*m/z*)		
$(M+Na)^+$	607	751
$(M+DEA)^+$*	690	834
Elementary analysis		
Found:	H 8.90	H 8.88
	C 72.12	C 68.92
Calcd:	H 8.96	H 8.85
	C 71.89	C 69.20
Formula	$C_{35}H_{52}O_7$	$C_{42}H_{64}O_{10}$
MW	584.8	729.0

* DEA; diethylamine.

66.2 mg of oily material was obtained. Subsequently, each component was purified by preparative high performance liquid chromatography to give white powders of compound **1** (2.31 mg) and of compound **2** (2.03 mg), as shown in Fig. 1.

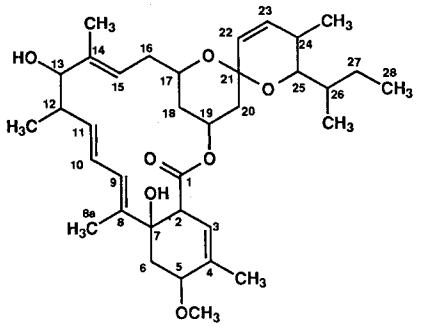
The physico-chemical properties of these two compounds are summarized in Table 1. Structures were determined by comparison of their spectral and other physico-chemical properties with those of the natural avermectins and 6,8a-seco-6,8a-deoxy derivatives. Based on their molecular weight and formula, compounds **1** and **2** were similar to avermectin A1a aglycone and 4'-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin B1a, respectively. ^1H NMR spectra of compound **1** and avermectin A1a aglycone were extremely similar but two protons of the H-6 (4.04 ppm, 1H, d, $J=5.5$ Hz) and H-8a (4.65 ppm, dd, $J=14.0$, 2.0 Hz; 4.70 ppm, dd, $J=$

Table 2. 400-MHz ^1H-NMR spectrum data for compounds **1**, **2** and 4'-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin B1a (**3**).

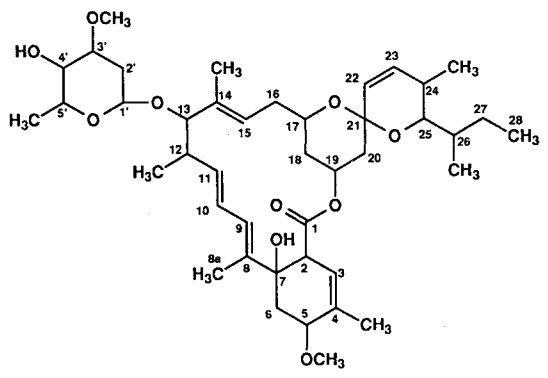
	1	**2**	**3**
C2-H	2.19 (dd, $J=13.0$, 6.0)	2.20 (dd, $J=13.0$, 2.5)	2.13 (dd, $J=13.0$, 6.5)
C3-H	5.29 (d, $J=1.5$)	5.30 (d, $J=2.5$)	5.29 (d, $J=1.5$)
C4-H	1.80 (s)	1.81 (s)	1.85 (s)
C5-H	3.87 (m)	3.90 (m)	3.89 (m)
C5-OCH_3	3.36 (s)	3.37 (s)	—
C6-H	3.45 (m)	3.46 (m)	3.46 (m)
C8-CH_3	1.72 (s)	1.72 (s)	1.71 (s)
C9-H	6.26 (d, $J=11.0$)	6.27 (d, $J=11.0$)	6.26 (d, $J=11.0$)
C10-H	6.06 (dd, $J=15.0$, 11.0)	6.00 (dd, $J=15.0$, 11.0)	5.99 (dd, $J=15.0$, 11.0)
C11-H	5.57 (dd, $J=14.0$, 10.0)	5.57 (dd, $J=14.0$, 10.0)	5.57 (dd, $J=14.0$, 10.0)
C12-H	2.52 (m)	2.44 (m)	2.44 (m)
C12-CH_3	1.21 (d, $J=7.0$)	1.17 (d, $J=7.0$)	1.17 (d, $J=7.0$)
C13-H	3.98 (br, s)	3.95 (br, s)	3.95 (br, s)
C14-H	1.59 (s)	1.54 (s)	1.53 (s)
C15-H	5.15 (br, m)	4.83 (br, m)	4.83 (br, dd, $J=10.0$, 3.0)
C16-Ha	Unresolved in spectra	Unresolved in spectra	Unresolved in spectra
C16-He	Unresolved in spectra	Unresolved in spectra	2.27 (m)
C17-H	4.04 (br, dd, $J=7.0$)	4.04 (dd, $J=7.0$)	4.47 (dd, $J=7.0$)
C18-Ha	1.75 (br, dd, $J=2.2$)	1.74 (br, dd, $J=2.2$)	1.77 (br, t)
C18-He	Unresolved in spectra	Unresolved in spectra	Unresolved in spectra
C19-H	5.34 (tt)	5.38 (tt)	5.37 (tt)
C20-H	1.54 (m)	1.57 (m)	1.48 (m)
C20-H	1.91 (ddd, $J=12.0$, 4.5, 1.5)	1.92 (ddd, $J=12.0$, 4.5, 1.5)	1.91 (ddd, $J=12.0$, 4.5, 1.5)
C22-H	5.75 (dd, $J=10.0$, 2.0)	5.76 (dd, $J=10.0$, 1.8)	5.75 (dd, $J=9.8$, 1.5)
C23-H	5.55 (dd, $J=10.0$, 2.5)	5.55 (dd, $J=10.0$, 2.5)	5.55 (dd, $J=10.0$, 2.5)
C24-H	2.33 (m)	2.27 (m)	2.27 (m)
C24-CH_3	0.88 or 0.91 (d, $J=7.0$)	0.88 or 0.90 (d, $J=7.0$)	0.87 or 0.91 (d, $J=6.5$)
C25-H	3.45 (m)	3.46 (m)	3.47 (m)
C26-H	1.58 (m)	1.55 (m)	1.54 (m)
C26-CH_3	0.91 or 0.88 (d, $J=7.0$)	0.90 or 0.88 (d, $J=7.0$)	0.90 or 0.87 (d, $J=6.5$)
C27-H_2	1.45 (dddd, $J=7.0$)	1.55 (dddd, $J=7.0$)	1.44 (dddd, $J=7.0$)
C28-H_3	0.92 (t, $J=7.0$)	0.89 (t, $J=7.0$)	0.89 (t, $J=7.0$)
C1'-H	—	4.78 (d, $J=3.2$)	4.78 (d, $J=3.0$)
C2'-H	—	2.27 (m)	2.27 (m)
C3'-H	—	3.60 (ddd, $J=11.0$, 9.0, 5.0)	3.59 (ddd, $J=11.0$, 9.0, 5.0)
C3'-OCH_3	—	3.53 (s)	3.52 (s)
C4'-H	—	3.17 (t, $J=9.0$)	3.16 (t, $J=9.0$)
C5'-H	—	3.90 (m)	3.88 (m)
C5'-CH_3	—	1.28 (d, $J=6$)	1.27 (d, $J=6$)

Spectra were recorded in $CDCl_3$ solution; chemical shifts are given in ppm and coupling constants are given in Hz.

Fig. 2. Structures of 6,8a-seco-6,8a-deoxyavermectin A1a aglycone (**1**) and 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin A1a (**2**).



1



2

14.0, 2.0 Hz) in avermectin A1a aglycone were not found in compound **1** and two different signals, a methylene proton of H-6 (3.45 ppm, 2H, m) and a methyl proton of H-8a (1.72 ppm, 3H, s) were observed. These results indicate that compound **1** is identical to 6,8a-seco-6,8a-deoxyavermectin A1a aglycone[3,7] which is considered to be derived from 6,8a-seco-6,8a-deoxyavermectin B1a aglycone by methylation at 5-OH with the gene product of the *aveD* gene. Interestingly, this aglycone, 6,8a-seco-6,8a-deoxyavermectin B1a aglycone, was not detected among the fermentation products of the parent K2057. The ^{1}H NMR spectrum of compound **2** was extremely similar to that of 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin B1a although a new proton signal was observed with compound **2** (Table 2). The signal was identified to the methoxy proton (3.37 ppm, 3H, s) at C-5 position. The mass spectral pattern of compound **2** indicates the addition of one carbon and two hydrogen atoms and it was concluded that compound **2** is a new avermectin derivative, 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin A1a (Fig. 2).

The recombinant strain, K2057 carrying pKU109::*aveD139*, and the mutant isolated by CHEN and INAMINE[3] are isogenic except for the *X* phenotype, but the recombinant strain produced a new compound, 4′-deoleandrosyl-6,8a-seco-6,8a-deoxyavermectin A1a. The recombinant strain contains about 50 copies of the methylation gene (*aveD*) per chromosome (data not shown) although chromosomal *aveD* gene is not functional in the recombinant strain because the parent strain K2057 was derived from *aveD* mutant. The *aveD* gene in the mutant isolated by CHEN and INAMINE[3] would be a single copy in the chromosome. Since the methylation activities of the recombinant strain was much higher than that of wild type strain K139 (data not shown), the recombinant strain could accumulate a new methylated compound. The *aveE* mutant might also be expected to produce this new methylated compound if a high copy number plasmid carrying the C-5 *O*-methylation gene, *aveD*, was introduced.

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