ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1996, p. 595–601 Vol. 40, No. 3
0066-4804/96/$04.00+0

Stereospecific Reduction of Virginiamycin M_1 as the Virginiamycin Resistance Pathway in *Streptomyces virginiae*

CHANG-KWON LEE, MASASHI MINAMI, SHOHEI SAKUDA, TAKUYA NIHIRA, AND YASUHIRO YAMADA*

Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565, Japan

Received 31 July 1995/Returned for modification 6 September 1995/Accepted 28 December 1995

In a cell extract of *Streptomyces virginiae*, virginiamycin M_1 was inactivated in the presence of NADPH, while virginiamycin S remained intact. The inactivated product of virginiamycin M_1 was isolated, and structure analysis revealed that the inactivation involves reduction of a C-16 carbonyl group leading to the formation of 16-dihydrovirginiamycin M_1. Acetonide and benzylidene acetal derivatives were synthesized from the two hydroxyl groups on C-14 and C-16, and the C-16 stereochemistry was determined by ^{13}C nuclear magnetic resonance spectroscopy. Two methyl groups of the acetonide derivative gave ^{13}C signals of 20.1 and 30.1 ppm, indicating that the relative stereochemistry of the C-14 and C-16 hydroxy groups is *syn*. Furthermore, irradiation of the benzylidene methine proton gave clear nuclear Overhauser effect enhancement of the C-14 or C-16 methine protons, indicating that H-14 and H-16 were in an *axial* configuration. From the (14S) absolute configuration of natural virginiamycin M_1 and the *syn* relative configuration for the C-14 and C-16 hydroxyl groups of the inactivated product, the C-16 absolute configuration of the inactivated product was thus identified as *R*.

Studies of the inactivation of antibiotics by the microorganisms that produce them are important for understanding the self-defense mechanism against antibiotics and providing clues for locating the gene clusters necessary for antibiotic biosynthesis. The antibiotic virginiamycin M_1 (VM_1) is a member of the virginiamycin family (alternatively designated streptogramins, synergimycins, or pristinamycins). The virginiamycin family, which has been known for more than 30 years (3), has been used successfully as a performance promoter in animal husbandry (1). The family constitutes a unique class of antibiotics consisting of two groups of compounds: type A, exemplified by VM_1 (Fig. 1), and type B, exemplified by virginiamycin S. Although the two types have quite different structures, they act synergistically to provide greatly enhanced levels of antibacterial activity (2). However, very few studies concerning the inactivation of antibiotics in the virginiamycin family have been reported. Mikamycin B, a type B virginiamycin compound, is hydrolyzed to mikamycin B seco acid by its producer strain, *Streptomyces mitakaensis* (6, 7). VM_1 is inactivated by acetylation of the C-14 hydroxyl group by a resistant strain of *Staphylococcus aureus* (4, 11). However, despite the long history of virginiamycin production by *Streptomyces virginiae*, no study of the inactivation of VM_1 by *S. virginiae* has been reported. Here, we report the results of recent investigations into VM_1 inactivation, which have revealed that *S. virginiae* stereospecifically reduces the C-16 keto group of VM_1, converting it into an inactive derivative.

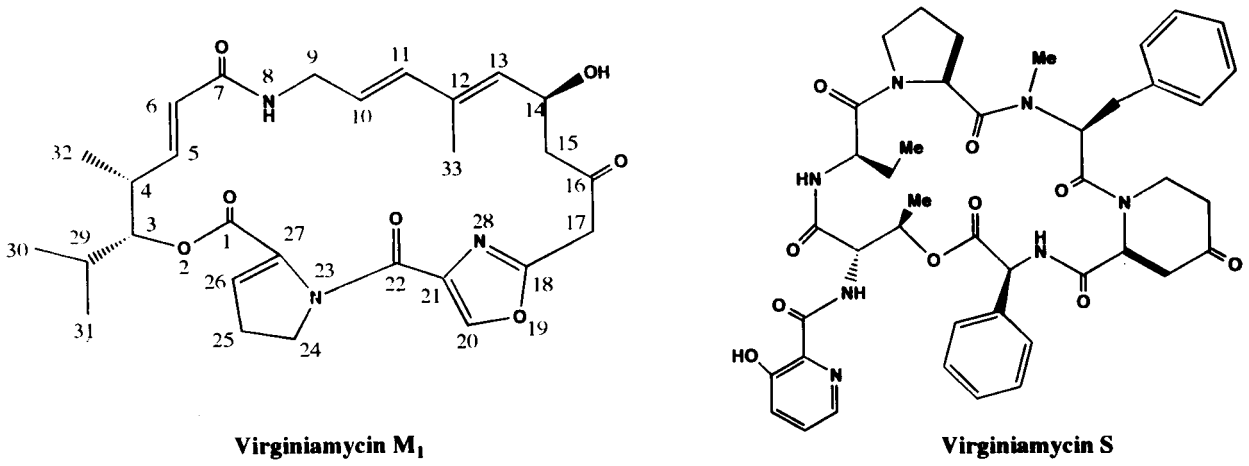

FIG. 1. Structures of VM_1 and virginiamycin S. Me, methyl.

* Corresponding author. Mailing address: Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565, Japan. Phone: 06-879-7431. Fax: 06-879-7448.

TABLE 1. Assignments of ^{1}H and ^{13}C NMR spectra of natural epimer 1, epimer 1 acetonide, epimer 2, and epimer 2 acetonide[a]

Position and structure	Spectrum for:							
	Natural epimer 1		Epimer 1 acetonide		Epimer 2		Epimer 2 acetonide	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1 C═O		167.3		166.9		167.4		166.5
3 CH	4.91 (dd, 2, 10)	81.2	4.90 (dd, 3, 10)	81.4	4.91 (dd, 3, 10)	81.1	4.92 (dd, 2, 10)	80.6
4 CH	2.70 (m)	37.5	2.75 (m)	34.2	2.75 (m)	37.3	2.80 (m)	37.3
5 CH═	6.57 (dd, 6, 16)	143.8	6.67 (dd, 3, 16)	149.9	6.51 (dd, 5, 16)	143.4	6.56 (dd, 5, 16)	140.0
6 CH═	5.93 (dd, 2, 16)	123.9	5.99 (d, 16)	123.9	5.88 (dd, 2, 16)	123.2	5.84 (dd, 2, 16)	123.9
7 C═O		160.6		160.2		159.8		160.3
8 NH═	6.79 (ddt, 1, 3, 6)		6.35 (m)		6.40 (m)		5.62 (m)	
9 CH_2	4.07 (m)	44.1	3.90 (m)	40.6	4.49 (m)	42.4	4.41 (m)	41.0
	3.95 (m)		3.61 (m)		4.47 (m)		4.39 (ddt, 4, 6, 10)	
10 CH═	5.62 (ddd, 4, 7, 16)	133.0	5.59 (ddd, 4, 6, 16)	132.6	5.56 (ddd, 4, 10, 16)	133.1	5.66 (m)	133.6
11 CH═	5.97 (d, 16)	124.8	6.06 (d, 16)	124.3	6.08 (d, 16)	124.9	6.21 (d, 16)	124.5
12 C═		134.3		136.0		133.7		134.2
13 CH═	5.07 (dd, 1, 9)	134.6	5.37 (d, 10)	134.9	5.60 (d, 9)	136.0	5.98 (d, 8)	136.1
14 HO—CH	4.67 (dt, 4, 9)	67.0	4.67 (dt, 4, 9)	65.1	4.93 (m)	66.8	4.91 (m)	66.9
15 CH_2	1.90 (dt, 4, 9)	40.6	1.96 (m)	37.9	1.99 (m)	41.6	2.30 (m)	33.6
	1.95 (dt, 4, 9)		1.97 (m)		1.96 (m)		1.96 (d, 15)	
16 HO—CH	3.98 (m)	67.6	4.27 (m)	65.3	4.46 (m)	67.6	4.38 (m)	64.4
17 CH_2	3.00 (dd, 5, 15)	36.6	3.08 (dd, 6, 15)	33.2	3.1 (dd, 4, 16)	35.6	3.12 (dd, 3, 16)	36.8
	2.91 (dd, 8, 15)		2.97 (dd, 5, 15)		2.87 (dd, 10, 16)		2.80 (dd, 11, 16)	
18 C═		160.9		160.3		161.2		160.6
20 CH═	7.94 (s)	143.9	8.16 (s)	145.5	8.07 (s)	143.8	8.12 (s)	144.1
21 C═		136.2		136.5		136.2		136.7
22 C═O		161.5		160.7		161.6		160.7
24 CH_2	4.26 (ddd, 7, 10, 12)	50.8	4.42 (q, 10)	51.0	4.23 (ddd, 8, 10, 12)	50.4	4.21 (ddt, 2, 3, 10)	51.4
	4.17 (ddd, 7, 10, 12)		4.30 (m)		4.15 (ddd, 6, 10, 12)		4.08 (dd, 10, 20)	
25 CH_2	2.74 (m)	29.8	2.84 (m)	29.6	2.72 (ddt, 2, 5, 3, 8)	29.8	2.74 (m)	29.3
	2.74 (m)		2.69 (m)		2.66 (m)		2.52 (m)	
26 CH═	6.14 (t, 3)	125.0	6.22 (t, 3)	124.7	6.06 (d, 3)	125.7	6.14 (d, 3)	125.2
27 C═		137.4		137.3		137.4		137.4
29 CH	1.98 (m)	30.0	1.96 (m)	30.0	2.05 (m)	30.0	2.01 (m)	30.0
30 CH_3	0.96 (d, 3)	19.5	0.99 (d, 6)	19.6	0.99 (d, 7)	19.6	1.02 (d, 6)	19.4
31 CH_3	0.95 (d, 2)	18.9	0.97 (d, 6)	18.9	0.97 (d, 7)	18.8	0.99 (d, 6)	18.9
32 CH_3	1.11 (d, 7)	13.4	1.14 (d, 7)	13.2	1.11 (d, 7)	13.0	1.15 (d, 6)	13.0
33 CH_3	1.74 (d, 1)	12.1	1.70 (s)	12.8	1.67 (d, 1)	11.3	1.73 (s)	11.1
34 C				98.6				100.0
35 CH_3			1.50 (s)	30.1			1.49 (s)	28.7
36 CH_3			1.37 (s)	20.1			1.47 (s)	27.2

[a] Chemical shifts are expressed as parts per million, and values in parentheses are the coupling constants measured in hertz. Splitting patterns are expressed as multiplets (m), quartets (q), triplets (t), doublets (d), and singlets (s). Carbon atoms 34, 35, and 36 correspond to a quaternary C and two methyl carbons of acetonide derivatives.

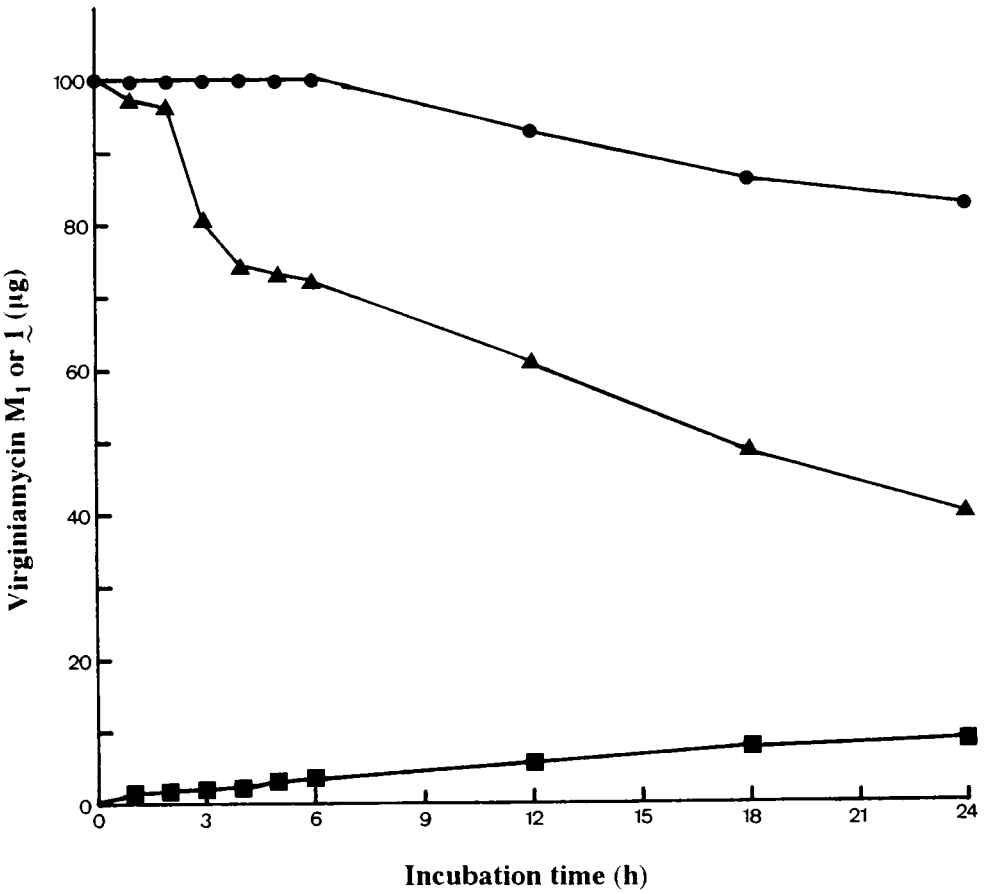

FIG. 2. Loss of VM_1 during incubation with cell extract of *S. virginiae*. The experimental conditions are described in Materials and Methods. The loss of VM_1 incubated with 0.05 M MOPS-KOH buffer (pH 7.45) alone (●) or with cell extract of *S. virginiae* (▲) and the amount of natural epimer 1 produced in the presence of cell extract (■) are shown. At the indicated times, a sample (50 μl) was withdrawn, mixed with a fourfold volume of ethanol, and centrifuged (11,000 × *g*, 5 min, room temperature). Fifty microliters of the supernatant was analyzed by HPLC.

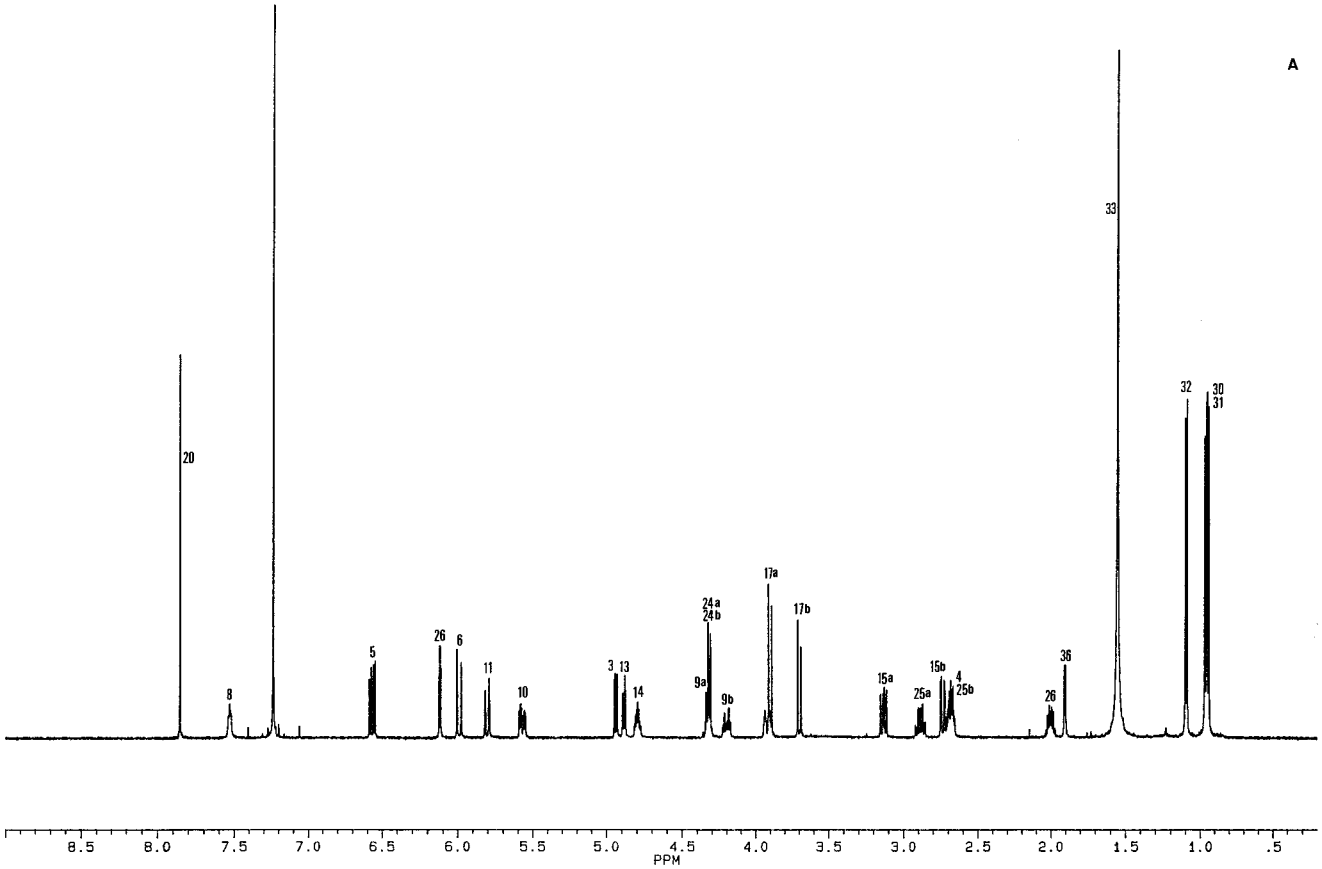

FIG. 3. ^{1}H NMR (600 MHz) spectra of VM_1 (A) and natural epimer 1 (B).

MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. virginiae* MAFF 10-06014 (National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan) was used throughout this study. It was grown at 28°C for 32 h as previously described (8).

Preparation of cell extract and reaction with virginiamycin. Cells (1 g [wet weight]) were suspended in 5 ml of 0.05 M 3-(*N*-morpholino)propanesulfonic acid (MOPS)-KOH buffer (pH 7.45) and disrupted by sonication (three times, 1 min each) at 4°C. After centrifugation (24,000 × *g*, 15 min, 4°C), the supernatant was dialyzed twice at 4°C with a 100-fold volume of the buffer, divided into aliquots, and either stored at −80°C or used immediately.

The inactivation reaction was initiated by the addition of 4 μl of purified VM_1 or virginiamycin S (10 mg/ml in ethanol) to the cell extract (400 μl) containing 1.1 mM NADPH, and incubation was carried out at 28°C with shaking at 40 strokes per min. At appropriate times, the remaining virginiamycins were measured either by bioassay against *Bacillus subtilis* PCI219 (16) or by C_{18} reverse-phase high-performance liquid chromatography (HPLC) (column: Cosmosil 5C18, 4.6 by 100 mm [Nacalai Tesque Co., Ltd., Kyoto, Japan]; detection at 210 nm; flow rate, 0.75 ml/min; gradient elution with CH_3CN from 0 to 80% in water for 20 min) using VM_1 (Sigma Chemical Co., St. Louis, Mo.) and purified virginiamycin S as standards.

Chemicals. Authentic VM_1 was purchased from Sigma Chemical Co. VM_1 and virginiamycin S were extracted from STAFAC 500 (Smith Kline-RIT, Rixensart, Belgium) with CH_3CN by incubation for 16 h at 120 rpm and 30°C, evaporation of the extract to dryness, and separation of the antibiotics by C_{18} reverse-phase HPLC (Cosmosil 5C18, 10 by 250 mm; Nacalai Tesque Co.) with 50% CH_3CN in water as the mobile phase at 40°C at a flow rate of 3 ml/min with detection at 305 nm. VM_1 and virginiamycin S were eluted at 7.6 and 13.1 min, respectively. Their identities were confirmed by mass spectrometry (MS) and 600-MHz ^{1}H nuclear magnetic resonance (NMR) spectrometry (9). VM_1 (75 mg/10 ml of ethanol) was chemically reduced with intermittent addition of $NaBH_4$ (1.2 eq) for 45 min at 4°C. The reaction mixture was acidified with 1 N acetic acid, evaporated, and redissolved in ethanol. Insoluble material was removed by filtration, and the two epimers of 16-dihydrovirginiamycin M_1 (epimers 1 and 2) in the filtrate were purified by C_{18} reverse-phase HPLC (Cosmosil 5C18, 10 by 250 mm [Nacalai Tesque Co.]) using 35% CH_3CN in water as the mobile phase at a flow rate of 3 ml/min, yielding epimers 1 (23.8 mg) and 2 (13.9 mg). The retention times of epimers 1 and 2 were 15.6 and 17.6 min, respectively.

Acetonide and benzylidene acetal derivatives of epimers 1 and 2 were synthesized with dimethoxypropane (10) and benzaldehyde dimethylacetal (15), respectively. Acetonide derivatives were purified by silica gel HPLC (Nucleosile 100-10, 10.7 by 300 mm [Wako Pure Chemicals Co., Ltd., Osaka, Japan]) using ethyl acetate as the mobile phase at a flow rate of 3 ml/min. Epimer 1 acetonide and epimer 2 acetonide were eluted at 12.3 and 16.4 min, respectively. Benzylidene derivatives were purified by C_{18} reverse-phase HPLC (Cosmosil 5C18, 10 by 250 mm [Nacalai Tesque Co.]) using 60% CH_3CN in water as the mobile phase at a flow rate of 3 ml/min. Benzylidene derivative of epimer 1 was eluted at 14.0 min.

^{1}H and ^{13}C NMR assignments are given in Table 1. Physicochemical properties: VM_1, fast atom bombardment-MS (FAB-MS) *m/z* 548 $(M + Na)^+$, 526 $(M + H)^+$, 508 $(M - H_2O + H)^+$; Fourier-transform-infrared (FT-IR) (film) (cm^{-1}) 3,317, 2,970, 1,728, 1,720, 1,667, 1,612, 1,580, 1,550; UV absorption (CH_3CN) λ_{max} (nm) 228 (ε, 3.23×10^4); natural epimer 1, FAB-MS *m/z* 550 $(M + Na)^+$, 528 $(M + H)^+$, 510 $(M\text{-}H_2O + H)^+$; FT-IR (film) (cm^{-1}) 3,357, 2,930, 1,725, 1,669, 1,620, 1,580, 1,542; UV absorption (CH_3CN) λ_{max} (nm) 235 (ε, 3.07×10^4); synthetic epimer 1, identical with natural epimer 1; epimer 2, FAB-MS *m/z* 550 $(M + Na)^+$, 528 $(M + H)^+$, 510 $(M\text{-}H_2O + H)^+$; FT-IR (film) (cm^{-1}) 3,352, 2,920, 1,732, 1,666, 1,622, 1,587, 1,548; UV absorption (CH_3CN) λ_{max} (nm) 235 (ε, 2.73×10^4); epimer 1 acetonide, FAB-MS *m/z* 568 $(M + H)^+$; FT-IR (film) (cm^{-1}) 2,934, 1,726, 1,677, 1,641, 1,583, 1,550, 1,529, 1,514; UV absorption (CH_3CN) λ_{max} (nm) 236 (ε, 3.67×10^4); epimer 2 acetonide, FAB-MS *m/z* 568 $(M + H)^+$; FT-IR (film) (cm^{-1}) 3,379, 3,318, 2,963, 1,725, 1,666, 1,621, 1,586, 1,550; UV absorption (CH_3CN) λ_{max} (nm) 236 (ε, 3.03×10^4); epimer 1 benzylidene, FAB-MS *m/z* 616 $(M + Na)^+$; FT-IR (film) (cm^{-1}) 3,309, 2,963, 1,729, 1,665, 1,632, 1,580, 1,549.

Analytical methods. IR spectra were determined as films on a JEOL JIR-AQS20M spectrometer. NMR spectra were obtained at 600 and 150 MHz for ^{1}H and ^{13}C, respectively, on a Bruker AM-600 spectrometer in $CDCl_3$ using $CHCl_3$ δH 7.27 for ^{1}H and $CHCl_3$ δC 77.2 for ^{13}C as the internal standard. FAB-MS were obtained with a JEOL JMS-DX 303HF spectrometer using *m*-nitrobenzoic acid as matrix. UV spectra were recorded with a Hitachi U 3000 spectrophotometer.

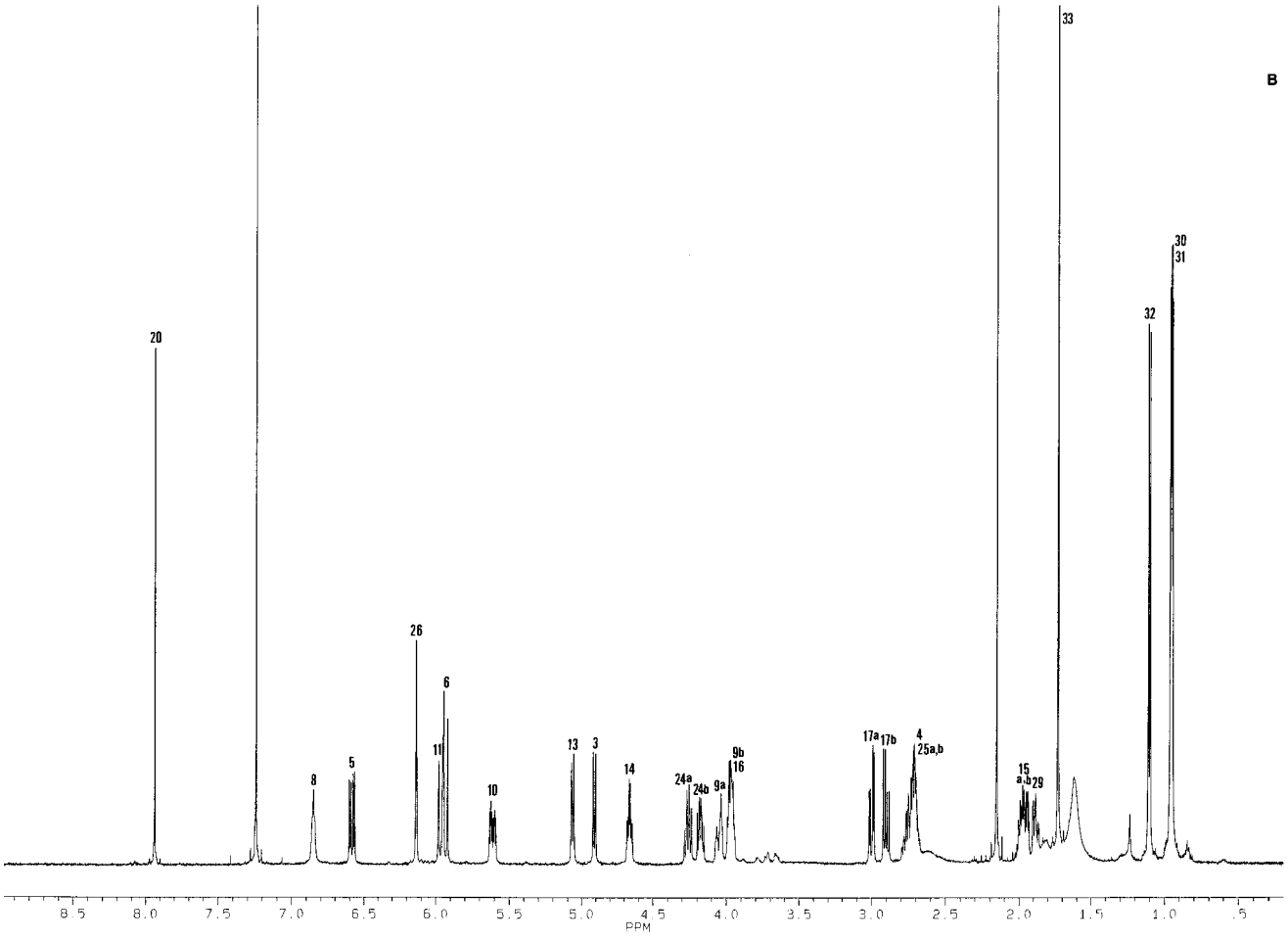

FIG. 3—*Continued.*

RESULTS AND DISCUSSION

To determine whether *S. virginiae* has the ability to modify virginiamycins as a potential self-resistance pathway, we incubated VM_1 or virginiamycin S with a cell extract of *S. virginiae* for 24 h at 28°C and analyzed the remaining antibiotic activity by bioassay using *B. subtilis* as a test organism. While no loss of antibiotic activity was observed for virginiamycin S, VM_1 was inactivated (data not shown). HPLC analyses confirmed that all of the virginiamycin S remained intact after the incubation (data not shown), while the amount of VM_1 decreased gradually (Fig. 2); the optimum pH was 7.5 (data not shown). Heat (20 min at 90°C) or pronase treatment (0.1 mg/ml, 30 min, 28°C) of the cell extract removed its inactivating capacity, suggesting an enzyme-catalyzed inactivation process. Furthermore, dialysis of the extract prevented the inactivation of VM_1, while the addition of NADPH, but not NADH, restored the inactivation ability. Concomitant with the inactivation was the appearance of a new compound eluting earlier than VM_1. This finding and the fact that addition of ATP, acetyl coenzyme A, or *S*-adenosylmethionine (each 2 mM) had no effect suggest the participation of some kind of NADPH-dependent reductase in the VM_1 inactivation. Intact mycelia of *S. virginiae* showed no reducing activity of VM_1 in the absence and presence of NADPH or NADH, suggesting the intracellular localization of the reducing enzyme(s).

To further clarify the inactivation pathway, the inactivated product was purified on a preparative scale. From 20 mg of VM_1, 4.9 mg of the compound was purified by reverse-phase C_{18} HPLC (see Materials and Methods). A comparison of its physicochemical properties with those of VM_1 indicated that the inactivation product was 2 mass units larger (see Materials and Methods), suggesting the reduction of a double bond or a carbonyl group. The ^1H NMR spectra of VM_1 and the inactivation product were also compared (Fig. 3), revealing that the product possessed the same olefinic protons on C-5, -6, -10, -11, -13, -20, and -26 but differed significantly with respect to signals arising from the methylene protons at C-15 and C-17, thus indicating that a carbonyl group on C-16 was possibly reduced to give a hydroxyl group. To confirm this, the homonuclear J-correlated (H-H COSY) spectrum of the inactivation product was measured (Fig. 4). Starting from a signal (4.67 ppm) for the C-14 methine proton, clear coupling connections were observed for the C-15 methylene protons (1.90 and 1.95 ppm), the C-16 methine proton (3.98 ppm), and the C-17 methylene protons (2.91 and 3.0 ppm). Thus, a new carbinol methine signal (3.98 ppm) appeared in the inactivation product. On the basis of these results, the structure of the compound was assigned as 16-dihydrovirginiamycin M_1 (epimer 1) (Fig. 5).

The MIC of natural epimer 1 for *S. virginiae* was determined to be >1 mg/ml, which is 20-fold higher than that for VM_1 (MIC = 0.05 mg/ml), indicating that the reduction of a C-16

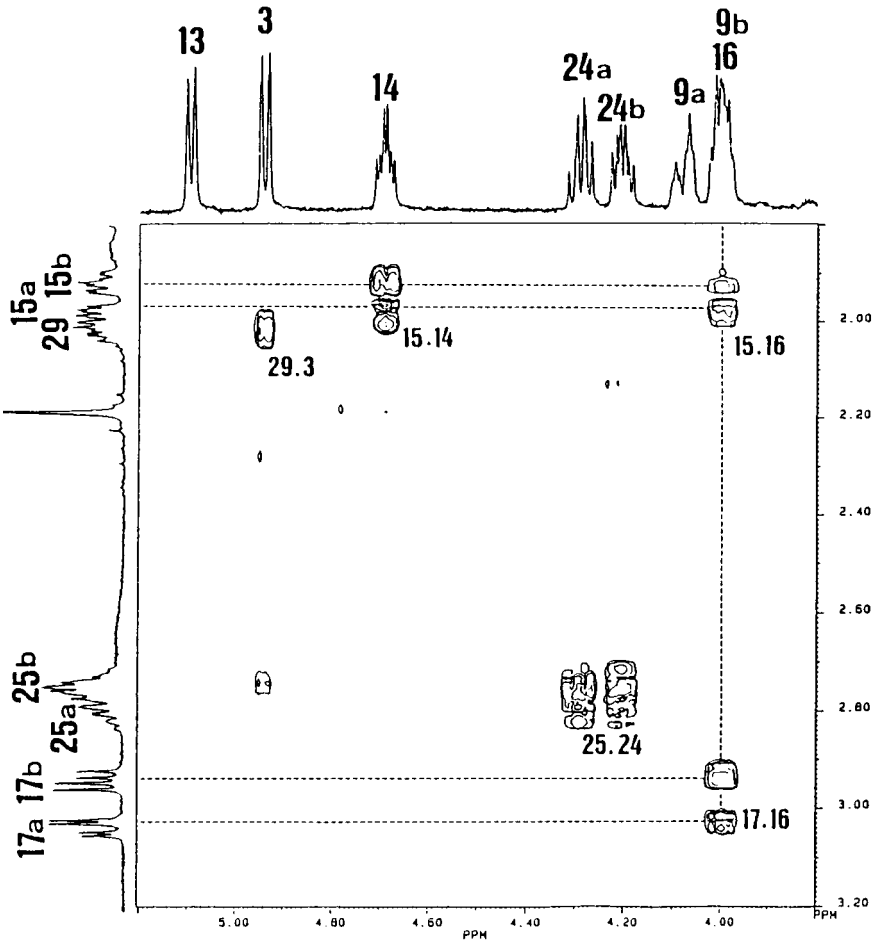

FIG. 4. H-H COSY spectrum of natural epimer 1.

carbonyl group resulted in the loss of antibiotic activity. Using synthetic epimer 1 (see below) as a substrate, we observed no reoxidation into VM_1 by cell extract or intact mycelia in the presence and absence of 1 mM NAD or NADP. Furthermore, epimer 1 is unstable in aqueous solution: it is rapidly degraded nonenzymatically into more-fragmented compounds. Therefore, the reduction process can be concluded to be an irreversible process of inactivation of VM_1.

Since the amount of natural epimer 1 was too small to determine the stereochemistry at C-16, we chemically reduced VM_1 with $NaBH_4$, which led, as expected, to the formation of two compounds (synthetic epimers 1 and 2) for the two possible epimers (Fig. 6). Synthetic epimer 1 showed the same retention on C_{18} reverse-phase HPLC as natural epimer 1 as well as the same ^1H NMR signals (data not shown). Therefore, epimer 1 was concluded to be identical with natural epimer 1, and epimer 2 was concluded to be the C-16 epimer of epimer 1. To determine the relative stereochemistry of the two hydroxyl groups on C-14 and C-16, we prepared acetonide derivatives and determined the ^{13}C chemical shifts of the acetonide methyl groups. The acetonide from a *syn*-1,3-diol adopts a chair conformation, so the *equatorial* methyl group appears at 30 ppm and the *axial* one appears at 20 ppm (5, 13, 14). In contrast, the acetonide from an *anti*-1,3-diol adopts a twist-boat conformation, so the two acetonide methyl groups are in a steric environment midway between *axial* and *equatorial*, leading to the appearance of similar ^{13}C signals of the two acetonide methyl groups at between 20 and 30 ppm. As shown in Table 1, the acetonide methyl groups of epimer 1 acetonide appeared at 20.1 and 30.1 ppm, while those of epimer 2 appeared at 27.1 and 28.7 ppm, which strongly suggests that the C-14 and C-16 alcohols in epimer 1 are *syn* and that those in epimer 2 are *anti*. Irradiation of H-13 enabled the coupling constants between H-14 and H-15 ($J_{14,15}$ = 12 and 3 Hz) to be measured, and the result indicated that the acetonide ring of epimer 1 acetonide existed in the chair form shown in Fig. 7.

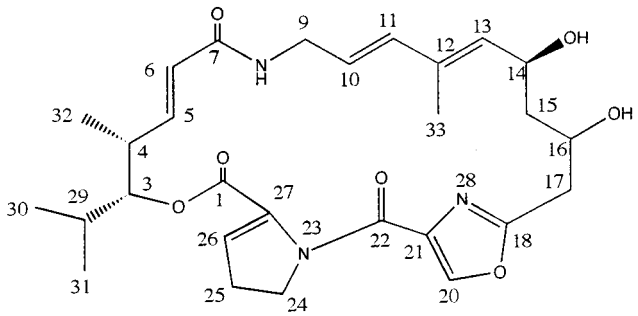

FIG. 5. Structure of 16-dihydrovirginiamycin M_1 (epimer 1).

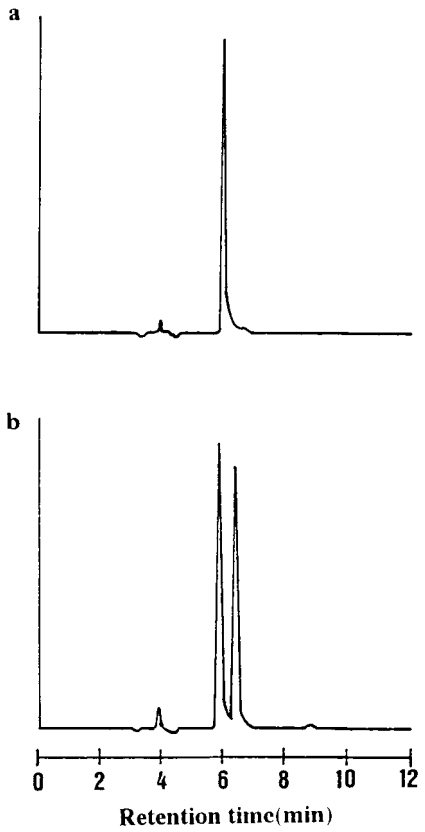

FIG. 6. HPLC profiles of natural epimer 1 (a) and its chemically prepared counterparts (synthetic epimers 1 and 2) (b) on a C_{18} reverse-phase column. CH_3CN (50%) in water was used as the mobile phase. The retention time of VM_1 was 9.6 min.

To confirm this structure, we prepared a benzylidene acetal derivative of epimer 1 and measured the NOE between the benzylidene methine proton and H-14 or H-16. NOE enhancement (8.9 and 8.7%, respectively) for H-14 and H-16 by irradiation of the benzylidene methine proton clearly supported the *syn*-1,3-diol structure in epimer 1. Because natural VM_1 has been determined to have a (14*S*) absolute configuration (12), epimer 1 is considered to have a (14*S*,16*R*) absolute configuration.

It can be concluded that *S. virginiae* has a stereospecific reduction pathway for VM_1 leading to the formation of (14*S*,16*R*)-16-dihydrovirginiamycin M_1. The product lost its antibiotic activity against *S. virginiae*, and the reduction may constitute part of the self-defense pathway.

REFERENCES

1. **Biot, A. M.** 1984. Virginiamycin: properties, biosynthesis, and fermentation, p. 695–720. *In* E. J. Vandamme (ed.), Biotechnology of industrial antibiotics. Marcel Dekker, Inc., New York.
2. **Cocito, C.** 1979. Antibiotics of the virginiamycin family, inhibitors which contain synergistic compounds. Microbiol. Rev. **43:**145–198.
3. **Delpierre, G. R., F. W. Eastwood, G. E. Graeam, D. G. I. Kingston, P. S. Sarin, L. Todd, and D. H. Williams.** 1966. Antibiotics of the ostreogrycin complex. Part II. Structure of ostreogrycin A^2. J. Chem. Soc. **1966:**1653–1669.
4. **De Meester, C., and J. Rondelet.** 1976. Microbial acetylation of M factor of virginiamycin. J. Antibiot. **29:**1297–1305.
5. **Evans, D. A., D. L. Rieger, and J. R. Gage.** 1990. ^{13}C NMR chemical shift correlations in 1,3-diol acetonides. Implications for the stereochemical assignment of propionate-derived polyols. Tetrahedron Lett. **131:**7099–7100.
6. **Kim, C. H., T. Endo, and H. Yonehara.** 1976. Purification and some properties of mikamycin B lactonase. Agric. Biol. Chem. **40:**1559–1564.
7. **Kim, C. H., N. Otake, and H. Yonehara.** 1974. Studies on mikamycin B lactonase. I. Degradation of mikamycin B by *Streptomyces mitakaensis*. J. Antibiot. **27:**903–908.
8. **Kim, H.-S., T. Nihira, H. Tada, M. Yanagimoto, and Y. Yamada.** 1989. Identification of binding protein of virginiae butanolide C, an autoregulator in virginiamycin production, from *Streptomyces virginiae*. J. Antibiot. **42:**769–778.
9. **Kingston, D. G. I., L. Todd, and D. H. Williams.** 1966. Antibiotics of the ostreogrycin complex. Part III. The structure of ostreogrycin A. Evidence based on nuclear magnetic double resonance experiments and high-resolution mass spectrometry. J. Chem. Soc. **1966:**1669–1676.
10. **Kitamura, M., M. Isobe, Y. Ichikawa, and T. Goto.** 1984. Stereocontrolled total synthesis of (−)-maytansinol. J. Am. Chem. Soc. **106:**3252–3257.
11. **Le Goffic, F., M. L. Capmau, K. L. Bonnet, C. Cerceau, C. J. Soussy, A. Dublanchet, and J. Duval.** 1977. Plasmid-mediated pristinamycin resistance: PACIIA, a new enzyme which modifies pristinamycin IIA. J. Antibiot. **30:** 665–669.
12. **Paris, J. M., J. C. Barriere, C. Smith, and P. E. Bost.** 1990. The chemistry of pristinamycins, p. 183–248. *In* G. Lukacs and M. Ohno (ed.), Recent progress in the chemical synthesis of antibiotics. Springer-Verlag, Berlin.
13. **Rychnovsky, S. D., and D. J. Skalitzky.** 1990. Stereochemistry of alternating polyol chains: ^{13}C NMR analysis of 1,3-diol acetonides. Tetrahedron Lett. **131:**945–948.
14. **Rychnovsky, S. D., D. J. Skalitzky, C. Pathirana, P. R. Jensen, and W. Fenical.** 1992. Stereochemistry of the macrolactins. J. Am. Chem. Soc. **114:** 671–677.
15. **Sakuda, S., and Y. Yamada.** 1991. Stereochemistry of butyrolactone autoregulators from *Streptomyces*. Tetrahedron Lett. **132:**1817–1820.
16. **Yamada, Y., K. Sugamura, K. Kondo, M. Yanagimoto, and H. Okada.** 1987. The structure of inducing factors for virginiamycin production in *Streptomyces virginiae*. J. Antibiot. **40:**496–504.

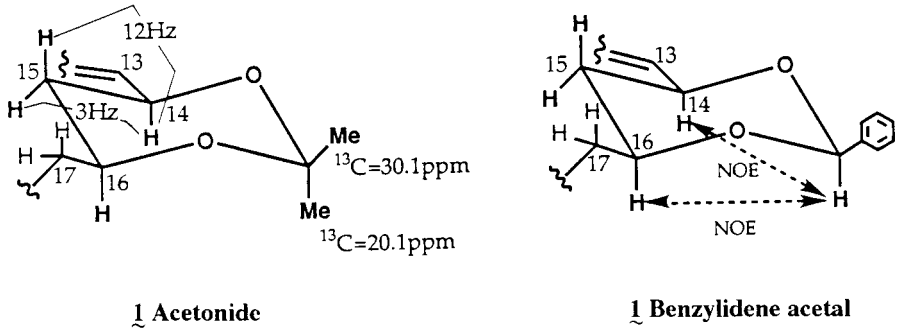

FIG. 7. Relative stereochemistry of acetonide and benzylidene acetal derivatives of epimer 1.