NEW CYCLITOLS, DEGRADATION OF VALIDAMYCIN A BY *FLAVOBACTERIUM SACCHAROPHILUM*

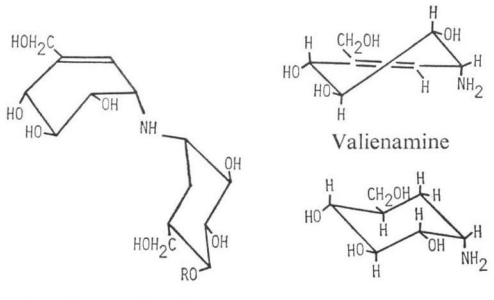
Sir:

Validamycin A, a main component of the validamycin complex produced by *Streptomyces hygroscopicus* var. *limoneus*, is extensively used as a curative fungicide for sheath blight of rice plants, soil-borne and other diseases caused by *Basidiomycetes*. In the viewpoint of microbial clearance of fungicides in fields, we have attempted to elucidate degradation processes of validamycins by soil bacteria for a past few years. Previous papers have described transformations of validamycins C, E and F to validamycin A, validamycin A and D to validoxylamine A on selective hydrolysis by various strains of microorganisms, and the further decomposition of validoxylamine A by *Pseudomonas denitrificans* proceeds *via* validamine[1,2] and valienamine[2] as intermediates.

A new species, named *Flavobacterium saccharophilum*[3] being capable of potently decomposing validamycin A was isolated from the rice field of Kanazawa City, Japan, and similar results as in the degradation processes by *P. denitrificans* were obtained. We now report two new cyclitols, intermediates of microbial degradation of validamycin A by *F. saccharophilum*, in addition to validamine and valienamine reported previously.

Flavobacterium saccharophilum was cultured with shaking at 27°C in a medium consisting of validamycin A 1%, $(NH_4)_2SO_4$ 1%, K_2HPO_4 0.7%, KH_2PO_4 0.3% and $MgSO_4 \cdot 7H_2O$ 0.01% (pH 7.1). The 7-day culture broth (4 liters) was passed through columns of Amberlite IR-120 (H form, 800 ml) and IR-45 (OH form, 800 ml). The concentrate of the neutral fraction was chromatographed on a column of Dowex 1-X2 (OH form, 100 ml) and eluted with water to give two components. Further chromatography on a column of charcoal with 5% ethanol yielded the homogeneous compounds, X-I (120 mg) and X-II (25 mg).

Fig. 1.



Compound X-I: colorless, amorphous ($C_7H_{14}O_4$; m.p. 131~133°C, $[\alpha]_D^{25}$ +48.1° (*c* 1, H_2O)). The ^{13}C-nmr spectrum (in D_2O) of compound X-I showed the presence of three methylenes and four methines.

Compound X-I forms a tetraacetate, colorless needles (m.p. 90~91°C). The ^{1}H-nmr spectrum (in C_6D_6) of compound X-I tetraacetate, **Ib**, shows a pair of quartets centered at δ 3.69 and δ 4.01 of two side-chain methylene protons ($-CH_2O-Ac$, H-7), which are coupled with a proton (δ 1.43, H-1) on the tertiary ring carbon atom. The splitting patterns of H-2 and H-3 protons ($J_{1,2}$=9.6 Hz, $J_{2,3}$=9.1 Hz, $J_{3,4}$=9.1 Hz) suggest all *trans*-axial protons H-1, H-2, H-3 and H-4.

These data established the structure of compound X-I as (1*R*)-(2,4/1,3)-1-hydroxymethyl-2,3,4-cyclohexanetriol, namely, 1-epivalidatol.[2]

Compound X-II: colorless, amorphous ($C_7H_{14}O_4$; m.p. 74~75°C, $[\alpha]_D^{25}$ +37.1° (*c* 1, H_2O)). The ^{13}C-nmr spectrum (in D_2O) of compound X-II also showed the presence of three methylenes and four methines.

Compound X-II forms a tetraacetate, a colorless oil. The ^{1}H-nmr spectrum (in C_6D_6) of compound X-II tetraacetate, **IIb**, revealed a pair of quartets (δ 3.90, δ 4.06) of the side-chain methylene protons coupled with a proton (δ 2.04, H-1) on the tertiary ring carbon atom. The splitting pattern of the H-2 proton ($J_{1,2}$=11.4 Hz, $J_{2,3}$=2.6 Hz) is typical of an axial proton with vicinal axial and equatorial protons, clearly suggesting the axial conformation of the H-1 proton. The splittings of the H-4 proton ($J_{3,4}$= 2.6 Hz, $J_{4,5eq.}$=6.4 Hz, $J_{4,5ax.}$=9.5 Hz) indicates the axial conformation of the H-4 proton. The fairly large coupling constant of $J_{4,5eq.}$ (6.4 Hz) can be explained by the effects of the configuration of electronegative substituents contributing to vicinal proton-proton coupling constants. WILLIAMS and BHACCA reported[4] that, when a

Table 1. ^{1}H-nmr spectral data (100 MHz in C_6D_6).

	Compound X-I-tetraacetate (**Ib**)		Compound X-II-tetraacetate (**IIb**)	
	δ (ppm)*	J (Hz)	δ (ppm)*	J (Hz)
H-1	1.56 (1 H, m)	$J_{1,2}=9.6$	2.00 (1 H, m)	$J_{1,2}=11.4$
H-2	5.00 (1 H, t)	$J_{2,3}=9.1$	4.86 (1 H, q)	$J_{2,3}=2.6$
H-3	5.24 (1 H, t)	$J_{3,4}=9.1$	5.88 (1 H, t)	$J_{3,4}=2.6$
H-4	4.90 (1 H, m)		4.78 (1 H, m)	$J_{4,5eq.}=6.4$ $J_{4,5ax.}=9.5$
H-5, 6	0.74~1.80 (4 H, m)		0.70~1.80 (4 H, m)	
H-7	3.69 (1 H, q)	$J=11.1$, 3.4	3.90 (1 H, q)	$J=11.1$, 3.4
	4.01 (1 H, q)	$J=11.1$, 5.1	4.06 (1 H, q)	$J=11.1$, 5.1
—C—CH_3 (O)	1.67 (3 H, s)		1.65 (3 H, s)	
	1.70 (3 H, s)		1.70 (6 H, s)	
	1.71 (3 H, s)		1.77 (3 H, s)	
	1.74 (3 H, s)			

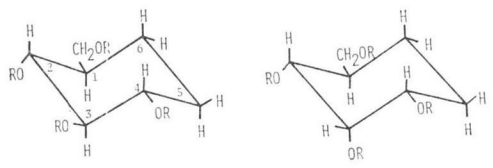
* with TMS as internal standard.

hydroxyl or acetate function is equatorial in a cyclohexane chair system, the observed J_{ae}* value is about 5.5±1.0 Hz, greater than the J_{ea}* coupling of approximately 2.5~3.2 Hz occurring when the substituent is axial.

The above data established the structure of compound X-II as (1*R*)-(2,3,4/1)-1-hydroxymethyl-2,3,4-cyclohexanetriol, namely, 1,3- epivalidatol.

From the structure relationships of degradation intermediates obtained so far, it can be assumed that compound X-I is formed by enzymatic deamination of validamine, furthermore, compound X-II formed by epimerization at the 3-position of compound X-I. We are interested in the enzymes involved in the individual steps of the degradation. The isolation of the enzymes is being attempted.

Fig. 2.



Ia R=H; Compound X-I
Ib R=$COCH_3$
IIa R=H; Compound X-II
IIb R=$COCH_3$

* The notation J_{ae} is used to denote the coupling of an axial proton on the carbon atom bearing the electronegative substituent to an equatorial proton, whereas J_{ea} is employed for the analogous coupling in which the proton on the electronegatively substituted carbon atom is equatorial.

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(Received July 19, 1980)

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