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

Sir:

Validamycin A possesses unique structural features (Fig. 1) and interesting biological activities. It is effective against sheath blight of rice plants and some soil-borne diseases, in spite of having no antibiotic activity *in vitro*. We are interested in the degradation of validamycin A by soil bacteria from rice fields. In the preceding paper[1], we reported on cyclitols, 1-*epi*- and 1,3-*epi*-validatol, and aminocyclitols, validamine and valienamine, as intermediates of the microbial degradation of validamycin A in growing cultures of *Flavobacterium saccharophilum*. In order to study the degradation processes in more detail, we carried out the bioconversion with resting cells of *F. saccharophilum*.

In this communication, we report the isolation and structure elucidation of three additional intermediates and discuss the relationship between the intermediates and the degradation processes.

F. saccharophilum was cultured in nutrient broth with shaking at 27°C for 24 hours. The cells were harvested by centrifugation at 20,000 × *g*. The washed cells (50 g, wet weight) were suspended in 1,000 ml of 0.05 M phosphate buffer, pH 7.0, containing 10 g of validamycin A, and the suspension was incubated at 27°C for 48 hours under shaking conditions. The cells were centrifuged and the supernatant passed through columns of Amberlite IR-120 (H^+ form, 200 ml) and IR-45 (OH^- form, 300 ml). The concentrate of the neutral fraction was chromatographed on a silica gel column with the solvent system $CHCl_3$ - MeOH (10: 1) and separated into fractions A and B. Further chromatography of fraction B with columns of Dowex 1-×2 (OH^- form) and active carbon gave the homogeneous compound **III** (15 mg), in addition to 1-*epi*- and 1,3-*epi*-validatol reported previously. Fraction A was chromatographed on a column of silica gel with the solvent system EtOAc - $CHCl_3$ (10: 1) and rechromatographed on a column of Dowex 1-×2 (CH_3COO^- form) to give homogeneous compounds **IV** (130 mg) and **V** (48 mg).

Compound **III**: colorless oil, $[\alpha]_D^{25}$ +20.6° (*c* 1, H_2O). The ^{13}C NMR spectrum (in D_2O) of compound **III** showed the presence of three methylenes and four methines.

Compound **III** forms a tetraacetate, colorless

Fig. 1. Microbial degradation of validamycin A by *Flavobacterium saccharophilum*.

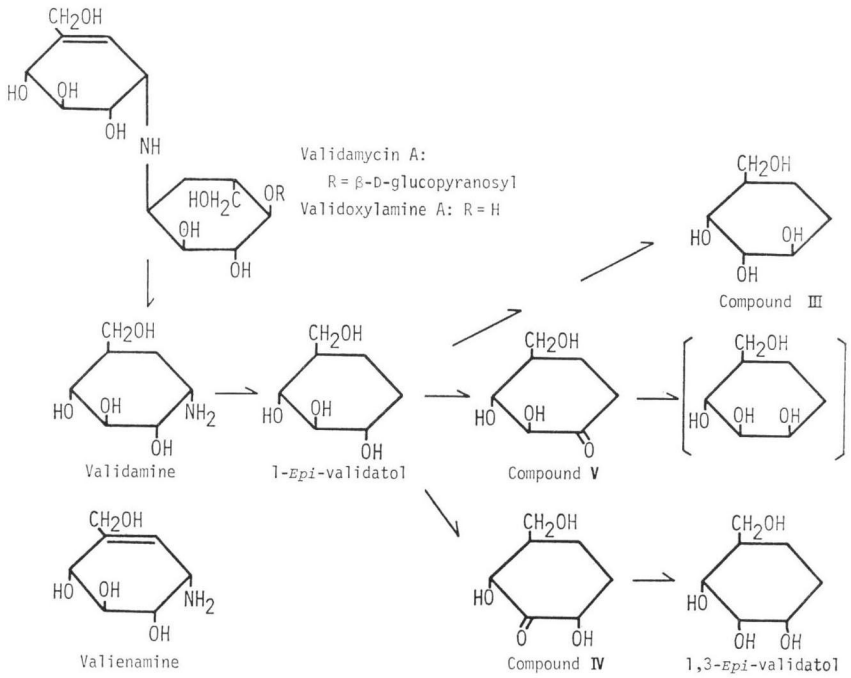


Fig. 2. ^{1}H NMR spectrum of compound **IV** (100 MHz in D_2O).

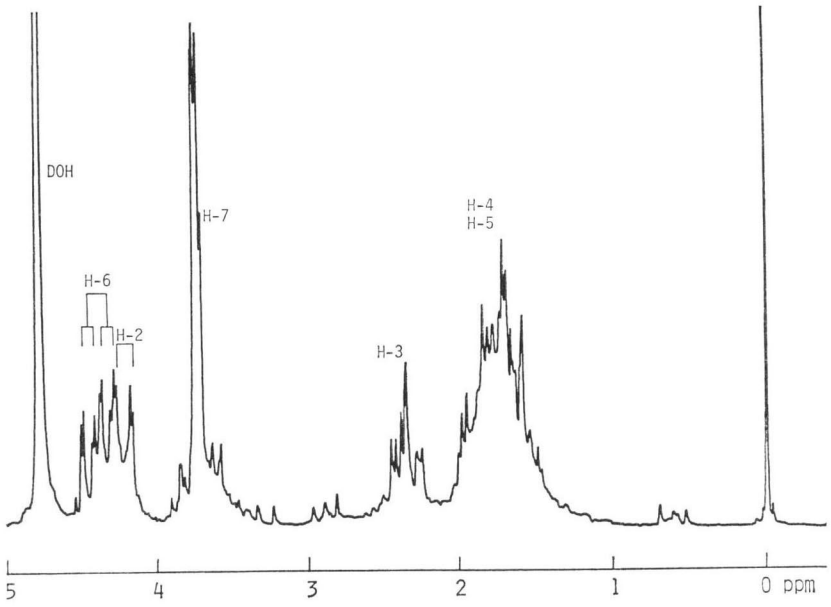
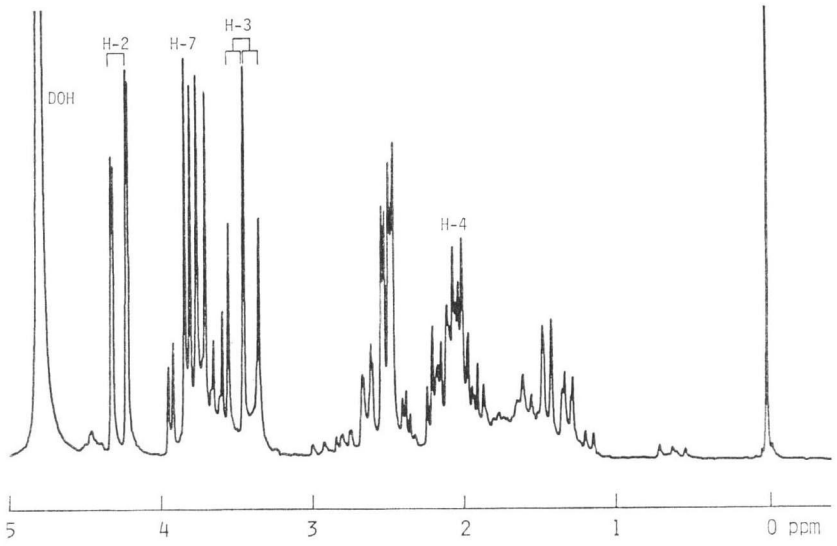


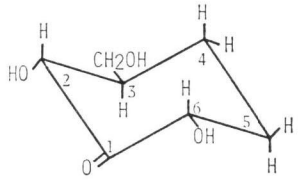
Fig. 3. ^{1}H NMR spectrum of compound **V** (100 MHz in D_2O).



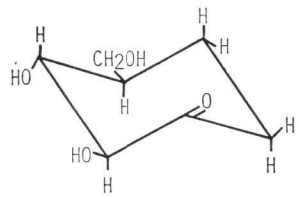
oil, $C_{15}H_{22}O_8$ (mass spec.: M^+ *m/z* 330). The ^{1}H NMR spectrum (in C_6D_6) of compound **III** tetraacetate showed a pair of quartets centered at δ 3.94 and δ 4.04 of two side-chain methylene protons (-CH_2O-Ac, H-7, J=11.1 Hz), which are coupled with the H-4 proton (δ 2.04, $J_{4,7a}$=3.4Hz, $J_{4,7b}$=5.1 Hz) on the tertiary ring carbon atom. The splitting pattern of the H-3 proton (δ 5.30, $J_{2,3}$=3.1 Hz, $J_{3,4}$=10.3 Hz) is typical of an axial proton with vicinal axial and equatorial protons, clearly suggesting the axial conformation of the H-4 proton. The splittings of the H-1 proton (δ 5.10, $J_{1,2}$=4.3 Hz, $J_{1,6a}$=3.4 Hz, $J_{1,6b}$=3.4 Hz) indicate the equatorial conformation for the H-1 proton.

These data established the structure of compound **III** as (1L)-1,4/2,3-4-hydroxymethylcyclohexanetriol, namely, 1,3,4-*epi*-validatol.

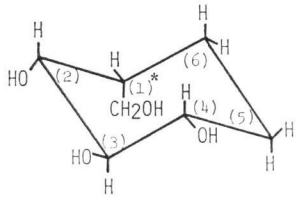
Fig. 4.



Compound **IV**:
(2D)-2,6/3-3-hydroxymethyl-2,6-dihydroxycyclohexanone; 3-keto-l-*epi*-validatol



Compound **V**:
(2L)-2,4/3-4-hydroxymethyl-2,3-dihydroxycyclohexanone; 4-keto-l-*epi*-validatol



Validatol

* The numbering system for validatol

Compound **IV**: colorless amorphous, m.p. 112~116°C, $[\alpha]_D^{25}$+25.7° (*c* 1, H_2O), calcd. for $C_7H_{12}O_4$: C 52.49, H 7.55, found: C 53.21, H 7.64. The infrared spectrum taken in KBr disk showed the presence of a carbonyl function (1720 cm^{-1}). The ^{13}C NMR spectrum (in D_2O) of compound **IV** showed the presence of three methylenes, three methines and a singlet at δ 214.7 (ppm from DSS) suggestive of a carbonyl group. The ^1H NMR spectrum (in D_2O) is shown in Fig. 2. In the splitting patterns of H-2 and H-6, the effect of long-range coupling was observed. The splitting pattern of the H-6 proton ($J_{5eq,6}$=6.6 Hz, $J_{5ax,6}$=11.4 Hz) indicates axial conformation for the H-6 proton. The H-2 proton appears as a doublet peak with a large coupling constant ($J_{2,3}$=10.5 Hz) and suggests *trans*-axial protons H-2 and H-3.

These data established the structure of compound **IV** as (2D)-2,6/3-3-hydroxymethyl-2,6-dihydroxycyclohexanone, namely, 3-keto-1-*epi*-validatol.

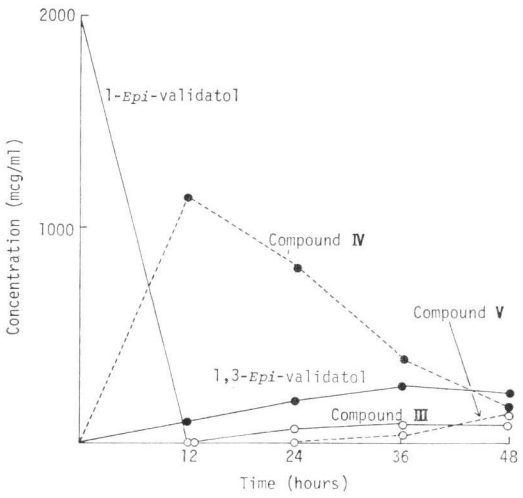
Compound **V**: colorless amorphous, m.p. 85~88°C, $[\alpha]_D^{25}$+6.5° (*c* 1, H_2O), calcd. for $C_7H_{12}O_4$: C 52.49, H 7.55, found: C 52.40, H 7.53. The infrared spectrum (in KBr disk) showed the presence of a carbonyl function (1720 cm^{-1}). The ^{13}C NMR spectrum (in D_2O) showed the presence of three methylenes, three methines and a carbonyl group at δ 214.2. The splitting patterns of H-2 and H-3 protons ($J_{2,3}$=9.7 Hz, $J_{3,4}$=9.7 Hz) in the ^1H NMR spectrum (Fig. 3) suggest all *trans*-axial protons H-2, H-3 and H-4.

These data established the structure of compound **V** as (2L)-2,4/3-4-hydroxymethyl-2,3-dihydroxycyclohexanone, namely, 4-keto-1-*epi*-validatol.

In experiments on the microbial conversion using resting cells of *F. saccharophilum*, we found that each of the five neutral intermediates obtained so far was derived from validamine. In the case of the conversion of 1-*epi*-validatol as a substrate, as shown in Fig. 5, it was found that two keto-cyclitols and other cyclitols were formed from 1-*epi*-validatol.

Epimerization at C-4 position of hexoses is well-known. Since it had been found that the reaction at C-4 required the presence of catalytic amounts of NAD^+, it was assumed that the epimerization proceeded through a 4-ketose intermediate[2,3]. However, the formation of such an intermediate has not been demonstrated conclusively. Evidence has been accumulating that *syllo*-inositol is formed from *myo*-inositol by an epimerization reaction thought to proceed through a keto compound, *myo*-inosose-2[4~6].

Fig. 5. Bioconversion of 1-*epi*-validatol by resting cells of *Flavobacterium saccharophilum*.



We could also demonstrate that 3-keto-1-*epi*-validatol was reduced stereospecifically by resting cells of *F. saccharophilum* to give 1,3-*epi*-validatol. Though the role of 4-keto-1-*epi*-validatol is not clear, it may be the intermediate of the epimer with the mannose-type configuration, which has not been isolated so far.

Further investigations are undertaken to elucidate the process of formation of 1,3,4-*epi*-validatol, and to isolate the enzymes catalyzing the individual steps of the degradation.

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