Communications to the editor

STUDIES ON VALIDAMYCINS, NEW ANTIBIOTICS. V
DEGRADATION STUDIES

Sir:

In a screening study of antibiotics active against sheath blight disease of rice plants, the validamycins[1] were obtained as water-soluble, polyhydroxy, weakly basic compounds from a culture filtrate of *Streptomyces hygroscopicus* var. *limoneus*.

Hydrogenolysis of validamycin A[1], a main component of the validamycin complex, over platinum oxide results in absorption of approximately 2.3 moles of hydrogen to yield a mixture of several degradation products, including compound AH-1 (about 55 % yield) and two new neutral cyclitols, designated validatol (about 40 % yield) and deoxyvalidatol (about 15 % yield). Compound AH-1 [pKa′ 8.4; one primary amino group (VAN SLYKE), nmr* (D_2O) δ 4.77 (doublet, J=7.5 Hz, anomeric proton)], one of the anthrone-positive, basic degradation products, yields D-glucose and a new aminocyclitol designated validamine, on acid hydrolysis.

The structural studies of validamine, (1*S*)-(1,2,4/3,5)-1-amino-5-hydroxymethyl-2,3,4-cyclohexanetriol and validatol, (1,2,4/3)-1-hydroxymethyl-2,3,4-cyclohexanetriol, will be reported in detail in an accompanying communication[2].

A solution of validamycin A in 1 N sulfuric acid heated on the steam bath gives rise to two products. One of these was identified as D-glucose. The second product was a weakly basic compound designated validoxylamine A (m.p. 101~105°C; $[\alpha]_D$ +170° (H_2O); pKa′ 6.2; negative to anthrone test) and having the tentative molecular formula $C_{14}H_{23\sim27}NO_{8\sim9}$.** Validamine, validatol, and deoxyvalidatol were formed from validoxylamine A by hydrogenolysis.

The nmr spectrum (D_2O) of validoxylamine A indicates the signals of two ring-methylene protons and one tertiary ring proton in addition to the signals of a vinyl proton appearing as a doublet at δ 6.2. The tertiary ring proton (δ 2.48, multiplet), the axial methylene proton (δ 1.44), which shows splitting (large, large, small) due to coupling with three protons (geminal, axial, and equatorial), and the equatorial methylene proton (δ 2.18), which similarly shows coupling (large, small, small) with geminal and neighboring protons, were well resolved in pyridine-d_5 solution.

An axial conformation of the tertiary ring proton was assigned with the aid of double resonance experiments, while validatol has four ring-methylene protons and one tertiary ring proton whose conformation is equatorial. These results establish that the ring-methylene protons and the tertiary ring proton of validamine are originally presented in both validoxylamine A and validamycin A, and the ring-methylene protons and the tertiary ring proton of validatol*** arise by catalytic hydrogenation.

The presence of the unsaturated cyclitol moiety, which has the partial structure $HOCH_2$-$\overset{1}{C}$=CH- in validoxylamine A, was confirmed by nmr and spin-decoupling studies of acetyl validoxylamine A.

Since validoxylamine A has no primary amino group (VAN SLYKE), the amino group of validamine must be attached to the unsaturated cyclitol moiety at the α position of the allylic system.

These data also indicate that validamycin A consists of D-glucose and validoxylamine A, and validoxylamine A is cleaved into validatol, which must arise from the unsaturated cyclitol moiety, and validamine by hydrogenolysis.

A β-glycosidic linkage between the D-glucose and validamine moieties of validoxylamine A was assigned by the nmr spin coupling constant (doublet, J=7.5 Hz) of the anomeric proton (δ 4.75) of validamycin A and was further confirmed by HUDSON's rule.

Hydrogenolysis of validamycin B[1], the

* Nmr spectra were taken at 100 MHz with TMS standard.

** Compounds characterized by melting point gave satisfactory elemental analyses.

*** It is thought that reduction occurs predominantly by equatorial attack at C-5 in this case.

biologically less active component, afforded several degradation products, including validatol, deoxyvalidatol, and compound BH-1 by the same procedure as employed with validamycin A.

Compound BH-1, one of the anthrone test-positive, basic degradation products, gave D-glucose and a new aminocyclitol designated hydroxyvalidamine by acid hydrolysis. The structural study of hydroxyvalidamine will be reported in the accompanying communication[2].

Validamycin B on acid hydrolysis yields D-glucose and a weakly basic compound with pKa′ 5.0, which was named validoxylamine B. Hydroxyvalidamine, validatol, and deoxyvalidatol were detected in the reaction mixture from hydrogenolyzed validoxylamine B.

In the nmr spectra, validamycin B, validoxylamine B, compound BH-1 and hydroxyvalidamine differ from the corresponding compounds for validamycin A by the absence of signals for ring-methylene protons. This evidence also indicates that the methylene protons of validatol arise by hydrogenation.

The β-anomeric linkage between D-glucose and the hydroxyvalidamine moiety of validoxylamine B was shown by its nmr spectrum (δ 4.75, doublet, J=7.5 Hz).

Acknowledgement

The authors are grateful to Dr. R. TAKEDA and Dr. A. MIYAKE for their interest throughout this work. Thanks are also due to Mr. E. MIZUTA for the nmr spectral data and Mr. K. KAWAHARA for his technical assistance.

SATOSHI HORII
TAKASHI IWASA
YUKIHIKO KAMEDA

Microbiological Research Laboratories,
Research and Development Division,
Takeda Chemical Industries, Ltd.,
Higashiyodogawa-ku, Osaka, Japan

(Received September 24, 1970)

References

1) IWASA, T.; Y. KAMEDA, M. ASAI, S. HORII & K. MIZUNO: Studies on validamycins, new antibiotics. IV. Isolation and characterization of validamycin A and B. J. Antibiotics (in press)
2) HORII, S.; T. IWASA, E. MIZUTA & Y. KAMEDA: Studies on validamycins, new antibiotics. VI. Validamine, hydroxyvalidamine and validatol, new cyclitols. J. Antibiotics (in press)