Communications to the editor

β-HYDROXY-L-VALINE, A CONSTITUTIONAL AMINO ACID OF ANTIBIOTICS YA-56 X AND Y*

Sir:

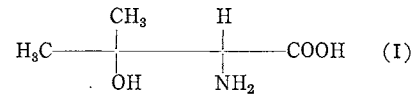
In the previous communication[1] dealing with the isolation and characterization of the phleomycin - bleomycin - zorbamycin[2] group antibiotic YA-56 X and Y, we have reported that at least ten ninhydrin-positive spots were observed on a thin-layer chromatogram of the acid hydrolyzates of these antibiotics. By the direct comparison of these spots with those of phleomycin and bleomycin hydrolysates on TLC, the existence of β-amino-β-(4-amino-6-carboxy-5-methylpyrimidine-2-yl)-propionic acid, L-β-aminoalanine and β-hydroxyhistidine, which are common to this group of antibiotics, was suggested in YA-56 X and Y.

Now, we wish to report on an unique constitutional amino acid of YA-56 X and Y which is designated H-1 and determined to be β-hydroxy-L-valine.

Antibiotic YA-56 was hydrolyzed with 6 N HCl in a sealed tube for 20 hours at 105°C. The hydrolysate was evaporated to dryness to remove excess acid. The amino acids in the hydrolysate were then roughly fractionated on Dowex 50 W×8 ion-exchange column (H^+, 100~200 mesh) with dil. HCl. The evaporated fractions containing H-1 were rechromatographed on Dowex 50 W×4 (H^+, 200~400 mesh) column.

Hydrochloric acid was used for elution in stepwise increasing concentration and H-1 was eluted separately from the other amino acids with 0.2 N HCl. The eluates showing only a single ninhydrin-positive spot on TLC (Rf : 0.41, violet ; cellulose plate Merck ; *n*-BuOH - pyridine - AcOH - H_2O (15 : 10 : 3 : 12)) were combined and concentrated *in vacuo* to give a crude hydrochloride of H-1. The free base of H-1 was obtained by treating the hydrochloride with silver oxide in water. Removing excess silver oxide with H_2S gas, followed by evaporation of the solution gave a white powder which was recrystallized from H_2O-acetone to afford colorless prisms of H-1: M.p. 196~198°C (decomp.), $[\alpha]_D^{22}$ +10.0° (*c* 2.0, 5 N HCl), (Lit.[4] m.p. 196~197°C, $[\alpha]_D^{24}$ +13.5° (*c* 2.0, 5 N HCl)). Anal. Calcd. for $C_5H_{11}NO_3$: C 45.11, H 8.26, N 10.52, M.W. 133. Found: C 45.05, H 8.44, N 10.52%, M. W. 138 (titration). pKa′ values (2.0 and 8.9) suggested that H-1 is an α-amino acid[5]. The N.M.R. spectrum of H-1 (60 MHz in D_2O, DSS internal reference) showed the presence of one *gem*-dimethyl group (δ 1.23 (3H, singlet) and 1.43 (3H, singlet)) and one α-methine proton of an α-amino acid (3.58 (1H, singlet)). Infrared absorption band at 1159 cm^{-1} (in KBr) indicates the presence of *t*-OH in H-1 as shown in Fig. 1[4,5,6].

The above results allowed us to conclude that H-1 is shown by the structure I.



(I)

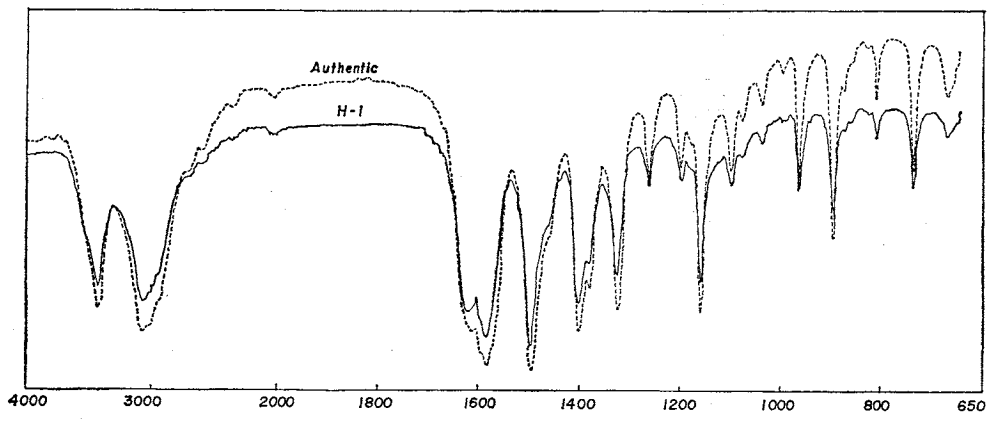
The fact that H-1 has a similar retention time with threonine on an amino acid analyzer accords with the structure I. Since it is known[8] that β-hydroxy-α-amino acid is cleaved with $Ba(OH)_2$ and yields glycine as a degradation product, H-1 was heated with saturated aq. $Ba(OH)_2$ for 12 hours at 120°C in a sealed tube. TLC of the reaction mixture showed the presence of glycine. Thus, the structure of H-1 was chemically supported. The amino acid H-1 was finally identified as β-hydroxy-L-valine[4] on their I.R. (Fig. 1), N.M.R., T.L.C. and $[\alpha]_D$.

Hitherto, several authors have reported on the finding of β-hydroxyvaline in nature. However, later investigations showed that the natural occurrence of the amino acid was questionable**. Consequently, our finding of β-hydroxy-L-valine in nature is thought to be the first unambigous one.

* Presented at the 182 nd meeting of Japan Antibiotics Research Association (Jan. 28, 1972).

** Private communication of Dr. G. W. EDWARDS. Also confer references cited in J. OH-HASHI & K. HARADA : Bull. Chem. Soc. Japan 39 : 2287~2289, 1966.

Fig. 1. IR Spectra of amino acid H-1 and β-hydroxy-L-valine (KBr)



It is interesting that YA-56 X and Y are constituted of β-hydroxyvaline instead of threonine which is a common constituent of phleomycins and bleomycins.

Acknowledgement

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