Communications to the editor

A NEW PEPTIDE ANTIBIOTIC COMPLEX S-520. III

ISOLATION OF L-*THREO*-β-HYDROXY-GLUTAMIC ACID FROM THE HYDROLYSATE

Sir:

We previously reported the isolation of a new peptide antibiotic complex S-520 from a streptomyces strain identified with *Streptomyces diastaticus*[1]. Isolation and structure determination of a new amino acid, a constituent of the antibiotic, will be reported here.

An acid hydrolysate of the antibiotic complex (6 N HCl, 105°C, 16 hours) was distributed between water and *n*-butanol. The aqueous fraction, which contained glycine, valine, isoleucine, ornithine, lysine and an unknown amino acid tentatively named a-I, was subjected to preparative paper chromatography carried out on Toyo Roshi No. 525 with *n*-butanol - acetic acid - water (4 : 1 : 2). By this procedure, two mixtures (ornithine and lysine; and a-I and glycine), valine, and isoleucine were isolated. The mixture of a-I and glycine was passed through an IR-4B (OH) column. Glycine appeared in the effluent, and a-I adsorbed on the column was eluted with 1 N HCl. The preparation of a-I thus separated, was further purified by adsorption on a Dowex 50 (H) column followed by elution with 1 N NH_4OH. Concentration of the eluate and crystallization from water-ethanol gave colorless crystals of a-I mono ammonium salt, dec. above 190°C in a sealed tube.

Anal. Found:
C 33.06, H 6.62, N 15.27.
Calcd. for $C_5H_8NO_5 \cdot NH_4$:
C 33.33, H 6.71, N 15.55 %.

The amino acid a-I, was positive to the reaction of periodate followed by NESSLER's reagent for α-amino-β-hydroxy acid, also to ninhydrin reaction. It behaved as an acidic amino acid on paper electrophoresis and an amino acid analyzer. The IR spectrum (Fig. 1) showed characteristic absorptions for a primary amino acid. These informations and analytical data suggested the amino acid to be β-hydroxyglutamic acid. The NMR spectrum in D_2O (Fig. 3) gave four protons, whose chemical shifts and splittings were in agreement with the above postulation (assignments are shown in the figure). To further confirm this structure, the amino acid was treated with hydrogen chloride-saturated methanol and then acetylated with acetic anhydride in pyridine. O-Acetyl-β-hydroxypyrroglutamic acid methyl ester, whose structure was supported by IR (Fig. 2) and NMR spectra (Fig. 4), was produced.

The O.R.D. of the amino acid was $[\phi]_{250}0$, $[\phi]_{215}+801$ (peak), $[\phi]_{203}-1308$ (*c*, 0.0764, H_2O); $[\phi]_{250}+707$, $[\phi]_{225}+2874$ (peak), $[\phi]_{205}-3770$ (*c*, 0.0764, 0.5 N HCl). From this, an L-configuration was deduced[2] for the α-carbon. In order to determine the configuration of the β-carbon, the amino acid was converted to an anhydride (Fig. 5) by

Fig. 1. IR spectrum of ammonium L-*threo*-β-hydroxyglutamate (KBr).

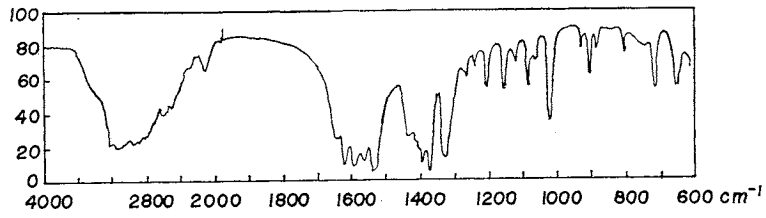
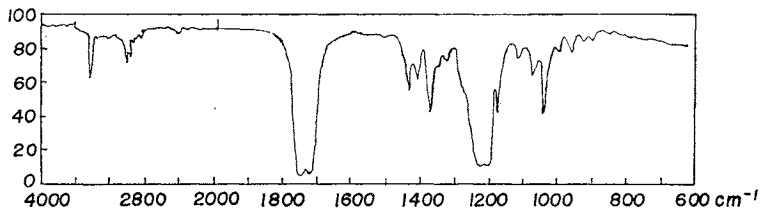


Fig. 2. IR spectrum of O-acetyl-β-hydroxypyrroglutamic acid methyl ester (in chloroform).



the following procedure: the α-amino group was protected by DNP by the usual method, then dehydration with dicyclohexylcarbodiimide in acetonitrile afforded the anhydride. In the NMR spectrum measured on deutrated acetonitrile solution, $J_{\alpha\beta}=10.0$ cps, $J_{\beta\gamma(a)}=10.0$ cps and $J_{\beta\gamma(e)}=6.0$ cps were observed. From these coupling constants, the configuration between α and β-hydrogens was deduced to be *trans*. Thus, the total configuration of the amino acid was determined as L-*threo*-β-hydroxyglutamic acid.

Quantitative analysis by an amino acid analyzer gave 0.46 moles of this amino acid per mole of S-520 hydrolysate (6 NHCl, 105°C, 24 hours). However, as the recovery rate of the amino acid under the same condition of hydrolysis was estimated as 53.6 %, the content of β-hydroxyglutamic acid in the antibiotic S-520 was indicated to be one mole.

Fig. 3. NMR spectrum of ammonium L-*threo*-β-hydroxyglutamate (D_2O).

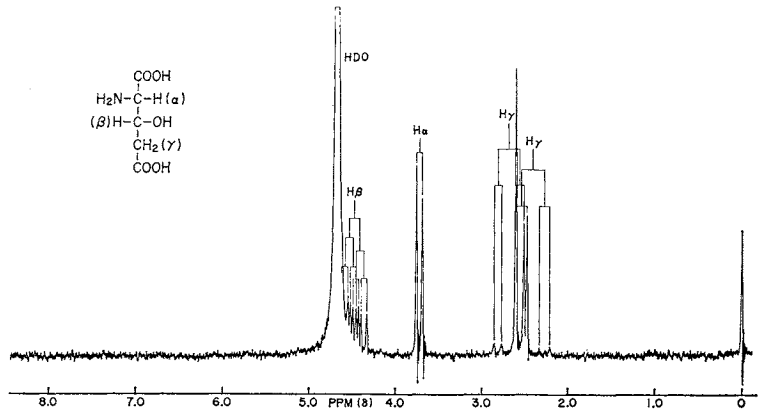


Fig. 4. NMR spectrum of O-acetyl-β-hydroxypyrroglutamic acid methyl ester ($CDCl_3$).

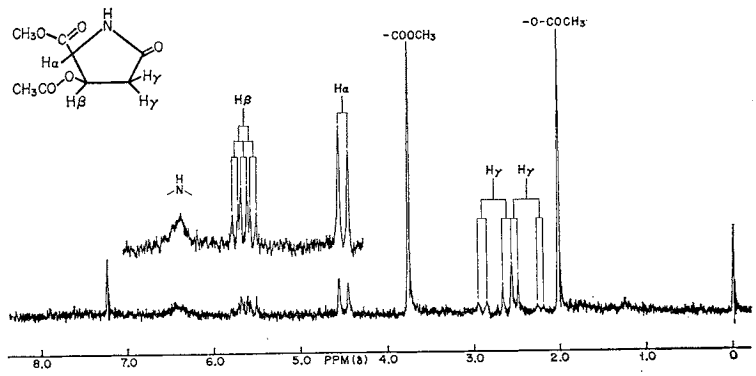
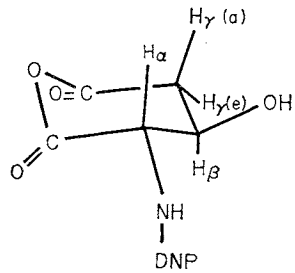


Fig. 5.



DAKIN (1918) has reported the isolation of β-hydroxyglutamic acid from casein hydrolysate. However, this work has been doubted by many workers, and DENT and his coworker (1954)[3] have proved the absence of the amino acid in the DAKIN's specimens. Therefore, the work reported here presents the first example of the isolation of β-hydroxyglutamic acid as a natural product.

JUN'ICHI SHOJI
RYUZI SAKAZAKI

Shionogi Research Laboratory,
Shionogi & Co., Ltd.
Fukushima-ku, Osaka, Japan

(Received June 8, 1970)

References

1) SHOJI, J.; S. KOZUKI, M. MAYAMA & N. SHIMAOKA: A new peptide antibiotic complex S-520. I. Isolation and characterization. J. Antibiotics (in press).
2) JENNINGS, J. P.; W. KLYNE & PATRICIA M. SCOPES: Optical rotatory dispersion. X. Amino-acids. J. Chem. Soc. 1965 : 294~296, 1965
3) DENT, C. E. & DOROTHY I. FOWLER: Paper-chromatographic analysis of DAKIN's samples of β-hydroxyglutamic acid. Biochem. J. 56 : 54~55, 1954