A NEW PEPTIDE ANTIBIOTIC COMPLEX S-520. IV

ISOLATION OF THREE NEW AMINO ACIDS FROM THE HYDROLYSATE

Sir:

In previous papers[1,2], the isolation of a new peptide antibiotic complex S-520 from a streptomyces and the occurrence of four unknown amino acids, named a-I, n-I, n-II and n-III, has been reported. In this communication, isolation and structure elucidation of n-I, n-II and n-III are reported.

An acid hydrolysate of the antibiotic complex (6 N HCl, 105°C, 24 hours) was distributed between water and *n*-butanol. The *n*-butanol fraction, which contained small amounts of valine and isoleucine, and the unknown amino acids, n-I, n-II and n-III, was subjected to ion-exchange resin chromatography carried out on a Dowex 50×4 (200~400 mesh) column (2.2×80 cm) with 0.4 M pyridine-acetic acid buffer, pH 4.50. The amino acid n-I was eluted in a fraction of 320~350 ml, n-II in a fraction of 410~470 ml, and n-III in a fraction of 570~650 ml. The fraction containing n-I was adsorbed on a small column of Dowex 50×8 (NH_4^+ form), and eluted with 75 % aqueous methanol containing 1 N NH_4OH. The eluate was concentrated to dryness. The resultant residue was dissolved in hot methanol, and upon concentration, the methanol solution afforded colorless crystals of n-I. The same procedure with the fractions containing n-II and n-III gave respective crystalline preparations.

n-I: colorless plates, mp 274~278°C (dec. in a sealed tube).

Fig. 1. Infrared absorption spectrum of n-I(L-α-aminoisoheptanoic acid) on KBr tablet.

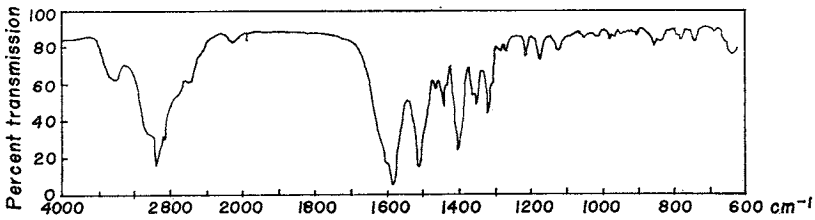


Fig. 2. Infrared absorption spectrum of n-II(L-α-aminoisooctanoic acid) on KBr tablet.

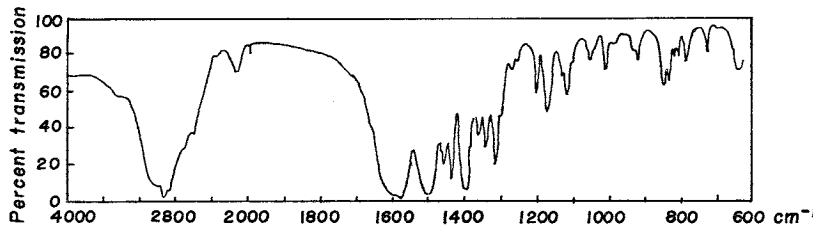


Fig. 3. Infrared absorption spectrum of n-III(L-α-aminoisononanoic acid) on KBr tablet.

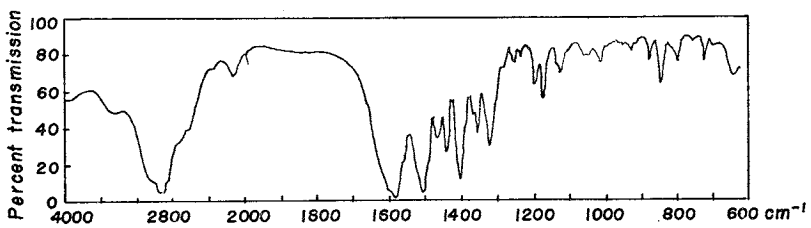


Fig. 4. NMR spectrum of *n*-I(L-α-aminoisoheptanoic acid) in DCl.

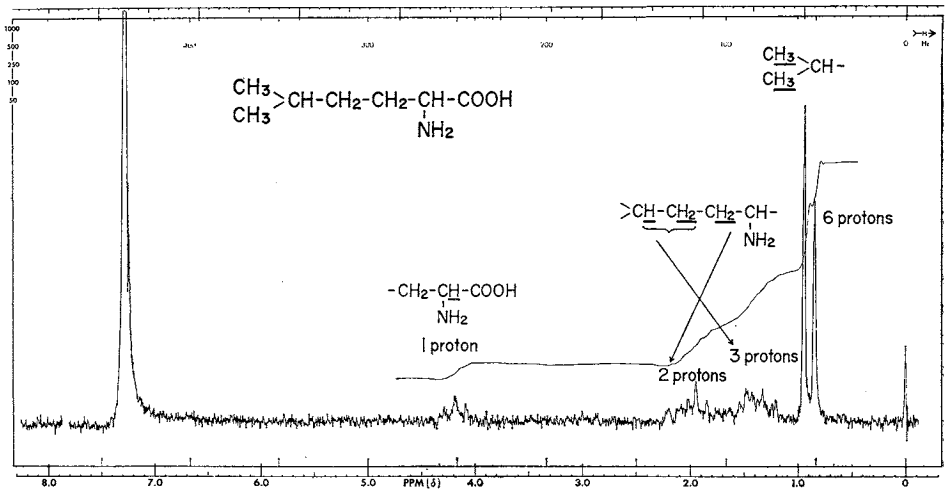
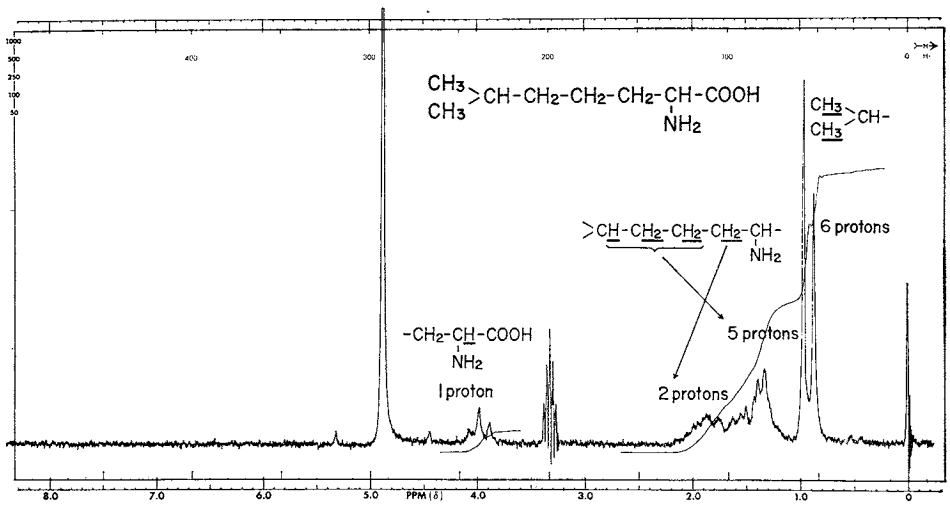


Fig. 5. NMR spectrum of *n*-II(L-α-aminoisooctanoic acid) hydrochloride in CD_3OD.



Anal. Found:

C 57.53, H 10.75, N 9.57, O 21.03.

Calcd. for $C_7H_{15}NO_2$:

C 57.90, H 10.41, N 9.67, O 22.04%.

n-II: colorless plates, mp 240~245°C (dec. in a sealed tube).

Anal. Found:

C 60.43, H 11.05, N 9.00, O 19.41.

Calcd. for $C_8H_{17}NO_2$:

C 60.34, H 10.76, N 8.80, O 20.10%.

n-III: colorless plates, mp 254~260°C (dec. in a sealed tube).

Anal. Found:

C 62.24, H 11.29, N 8.32, O 18.18.

Calcd. for $C_9H_{19}NO_2$:

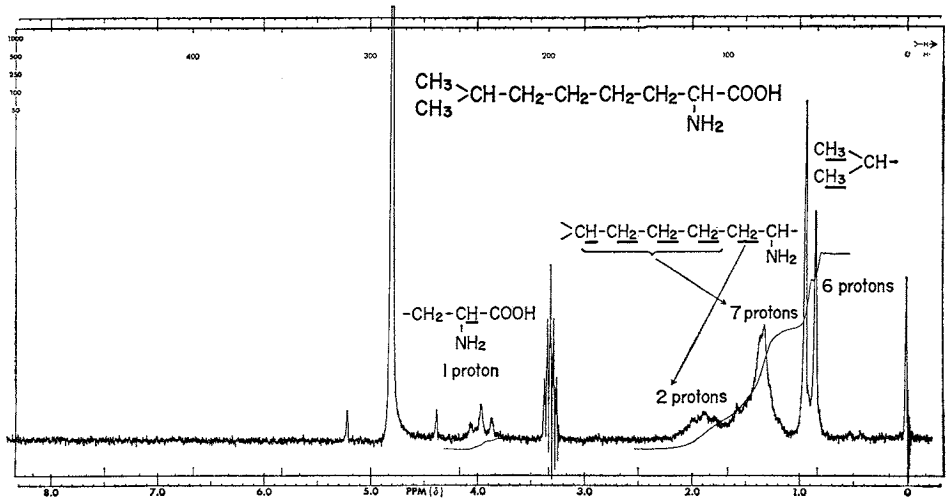
C 62.39, H 11.05, N 8.09, O 18.47%.

The IR spectra of n-I, n-II and n-III (Figs. 1, 2 and 3) suggested that they are primary amino acids.

The NMR spectrum of n-I in DCl (Fig. 4) and the spectra of the hydrochlorides of n-II and n-III in CD_3OD (Figs. 5 and 6) clarified their structures as α-aminoisoheptanoic acid for n-I, α-aminoisooctanoic acid for n-II, and α-aminoisononanoic acid for n-III. Methylation with hydrogen chloride-saturated methanol followed by acetylation with acetic anhydride in pyridine, gave N-acetylated methyl esters of these amino acids (NMR ($CDCl_3$): $-COCH_3$, 2.00 ppm, 3H; $-OCH_3$, 3.73 ppm, 3H).

The ORD of these amino acids were as

Fig. 6. NMR spectrum of *n*-III(L-α-aminoisononanoic acid) hydrochloride in CD_3OD.



follows: n-I, $[\phi]_{300}$ +578, $[\phi]_{225}$ +4357 (peak), $[\phi]_{215}$ +2816 (*c* 0.1960, 0.5 N HCl); n-II, $[\phi]_{300}$ +740, $[\phi]_{225}$ +4954 (peak), $[\phi]_{211}$ 0 (*c* 0.1543, 0.5 N HCl); n-III, $[\phi]_{300}$ 0, $[\phi]_{225}$ +4130 (peak), $[\phi]_{212}$ 0 (*c* 0.1636, 0.5 N HCl). The positive COTTON effects at 225 mμ indicated that these amino acids belong to the L-series[3].

The solubility of n-I is somewhat similar to that of leucine; considerably soluble in water and sparingly soluble in methanol and ethanol, but n-II and n-III are rather soluble in methanol and ethanol and sparingly soluble in water. This fatty nature of n-II and n-III was reflected in their behaviors on an amino acid analyzer. As already reported[2], the amino acids n-II and n-III were strongly retarded on a sulphonated polystyrene resin column. This great extent of sporption to the resin, as with basic amino acids, is thought to be due largely to non-ionic interaction[4] between the longer aliphatic side chains of these amino acids and the hydrophobic polystyrene matrix.

All three of these amino acids, *i.e.* L-α-aminoisoheptanoic acid, L-α-aminoisooctanoic acid and L-α-aminoisononanoic acid, have been hitherto unknown as natural products. Their contents in the antibiotic complex S-520 have been reported in the previous paper[2]. It is noteworthy that they have a considerably more fatty nature than the usual amino acids.

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