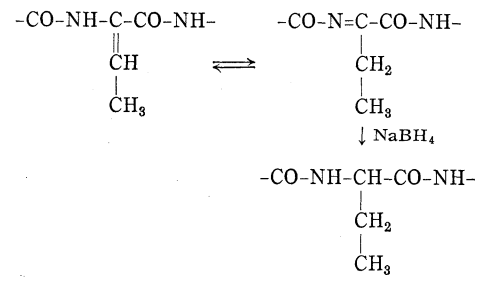
ISOLATION OF α-AMINOBUTYRIC ACID FROM REDUCED THIOSTREPTON

Sir:

The amino acid constituents of the antibiotic thiostrepton[1] were described earlier[2]. The formation of pyruvic acid on treatment of the compound with trifluoroacetic acid, pointing to the presence of dehydroalanyl or α-substituted alanyl derivatives in the molecule, was also reported[3]. In recent investigations on the structure of the antifungal antibiotic stendomycin[4,5], reduction of a dehydro-α-aminobutyryl residue to an α-aminobutyryl moiety could be accomplished not only through catalytic hydrogenation but also with sodium borohydride. The latter reagent was applied on thiostrepton for the study of the source of pyruvic acid, because this antibiotic is rich in sulfur, and therefore its catalytic hydrogenation did not seem too promising*. In addition to the expected newly formed alanyl residues, the presence of a significant amount of α-aminobutyric acid was observed by quantitative amino acid analysis[6] of a hydrolysate. About 0.8 mole of this amino acid was liberated from one mole of the parent molecule. Since not even a small quantity of α-aminobutyric acid was detected in acid hydrolysates of (unreduced) thiostrepton**, reduction of a double bond leading to butyrine has to be assumed. Sodium borohydride does not react with carbon-carbon double bonds but can reduce carbon-nitrogen double bonds. In the present case a tautomeric equilibrium between two forms of dehydrobutyryl residue might serve as an explanation for the reduction:



To substantiate the observations made by amino acid analysis, a large sample (10 g) of the antibiotic was dissolved in a mixture of tetrahydrofuran (400 ml) and methanol (60 ml) and was treated with sodium borohydride (5 g) at room temperature for five hours. After acidification with hydrochloric acid and removal of the solvents by evaporation, the residue was hydrolyzed with constant boiling hydrochloric acid (500 ml) under reflux in an atmosphere of nitrogen for 25 hours. A preliminary fractionation of the hydrolyzed material was carried out by successive extractions with ether, ethyl acetate and then by countercurrent distribution[7] in a system of *n*-butanol – 0.01 N hydrochloric acid. A 30-tube CRAIG apparatus was used and the single withdrawal technique was applied through 110 transfers. From here on no more upper layer was added and the distribution was continued for an additional 30 transfers (complete withdrawal). The fractions rich in butyrine (tubes no. 12~19 of the distribution train) were pooled and chromatographed on a cation exchange column (Dowex 50×12; 2.5 cm×40 cm in H^+ cycle). First 0.2 N HCl (1,400 ml) was used for elution followed by 0.3 N HCl; 100 ml fractions were collected. Fractions no. 31~36 were pooled and evaporated to dryness. The residue was dissolved in ethanol and treated with pyridine. A white crystalline precipitate (0.11 g) was collected, washed with alcohol and dried. It was indistinguishable from an authentic sample of DL-butyrine on thin-layer

* Catalytic hydrogenation was nevertheless attempted and hydrolysis of the reduction product afforded α-aminobutyric acid albeit in low yield. Additional results of the experiments involving catalytic hydrogenation will be reported separately.

** However, the presence of α-ketobutyric acid could be detected.

*** It should be noted that a thiazoline from dehydrobutyryl-cysteine was postulated by CROSS, KENNER, SHEPPARD and STEHR[9] as a partial structure formed from a threonyl-cysteine thiazoline during alkaline degradation of thiostrepton.

chromatograms and by infrared and nmr spectra. $[\alpha]_D^{25}$ $-0.3°$ (c 2, 5 N HCl). Lit.[8] for L-butyrine $[\alpha]_D^{25}$ $+20.6°$ (5 N HCl). The isolation of racemic material adds support to the evidence that no butyrine occurs in thiostrepton, but a derivative of this amino acid at a higher state of oxidation is present in the antibiotic***.

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Ichiro Muramatsu
Miklos Bodanszky

Department of Chemistry
Case Western Reserve University
Cleveland, Ohio 44106 U. S. A.

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