ISOLATION OF D(-)-2,3-DIAMINOPROPIONIC ACID FROM DIGESTIVE FLUID OF SILKWORM LARVAE, BOMBYX MORI.

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During the studies of the amino acid patterns of digestive fluid of silkworm larvae, Bombyx mori, which were grown on fresh mulberry leaves as the sole diet, an unknown ninhydrin-reactive compound that behaved like a basic amino acid was found in a fresh fluid.

The present paper deals with the isolation of this unknown compound and its identification as being D(-)-2,3-diamino-propionic acid.

Main amino acids in digestive fluid of fifth instar larvae that had been fasted for 20 hours.

Amino acid	Micromoles/ml*
Ammonia	0.6736
Diaminopropionic acid	3.6457
Aspartic acid	0.2350
Serine	1.5695
Glutamic acid	0.2676
Glycine	7.449

* Values measured by Hitachi automatic amino acid analyzer (Model KLA-2).

The digestive fluid of fifth instar larvae that had been fasted for 20 hours, was collected either by the electric shock method, which involves a short time contact (ca. 60 second) with

40 volt A.C.-current at the body of silkworm larvae, or by the treatment of larvae with chloroform vapour in a suitable cage. The fluid, 300 ml. collected from 1,000 larvae, was immediately treated with 1,500 ml. of cold acetone, and precipitates were removed by filtration. The acetone was evaporated under reduced pressure and the remaining aqueous solution was extracted with n-butanol (900 ml.) and the organic layer was discarded. The aqueous solution, which contained the unknown compound as well as amino acids, peptides and other water soluble substances, was passed through a column packed with Amberlite IR-120 (H, 3 x 30 cm.). A mixture of amino acids was eluted from the column with 6.0% ammonium hydroxide and the solvent evaporated under reduced pressure. The amino acid mixture was again adsorbed on Amberlite IRC-50 (H, 3 x 30 cm) and the column was washed with water to remove the accompanying non-basic amino acids. The basic amino acid was finally eluted with 6.0% ammonium hydroxide solution. The excess of ammonium hydroxide in the eluate was removed under reduced pressure and the solution was brought to pH 4.0 with hydrochloric acid. After treatment with activated charcoal the solution was evaporated under reduced pressure to a small volume and allowed to stand in an ice-box, whereupon needle crystals appeared. Recrystallization from water gave colorless crystals, which were dried in a vacuum desiccator over phosphorus pentoxide. In one experiment, a quantity of 170 mg of the crystals was obtained from one liter of fresh digestive fluid which corresponds to 3,000 larvae; m.p., 243-5° (decomp.); Analysis: Calcd. for $C_3H_9O_2N_2Cl$: C 25.63 H 6.45 N 19.93 Cl 25.22; Found: C 25.74 H 6.50 N 19.74 Cl 25.20.

On the automatic amino acid analyzer (Hitachi) the peak due to this unknown compound appeared between those of lysine and

ammonia. The nuclear magnetic resonance spectrum (Fig. 1) of

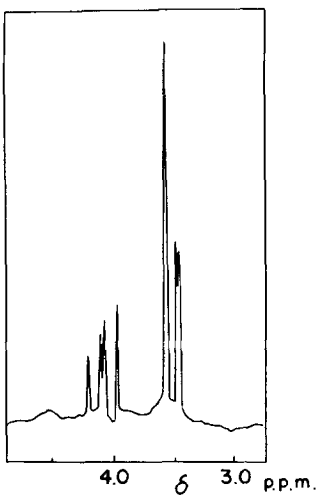

Fig. 1. NMR spectrum of D(-)-2,3-diaminopropionic acid monohydrochloride (Natural) in D_2O, at 60 Mc, p.p.m. from TMS.

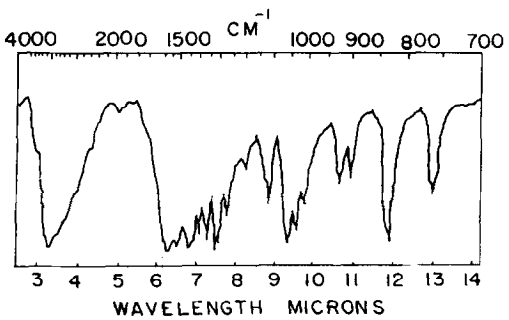

Fig. 2. Infrared spectrum of natural D(-)-2,3-diaminopropionic acid monohydrochloride (KBr).
(The spectrum of synthetic D-specimen was superimposable on that of natural compound)

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the amino acid showed a signal of ABX type, which led us to the assignment of the structure of 2,3-diaminopropionic acid. This assumption was substantiated by the comparison of the infrared

spectrum (Fig. 2) with that of L(+)-2,3-diaminopropionic acid, $[\alpha]_D^{25}$ +25.2° (c=2, n-HCl)* (Greenstein J.P. et al., 1961; Koegel R.J. et al., 1955).

The Rf value of the compound on paper chromatography (phenol/water 80/20, ammonia atmosphere) was 0.56 and the specific rotation $[\alpha]_D^{25}$ -24.7° (c=1, n-HCl), the latter being in complete accord with the reported value ($[\alpha]_D^{25}$ -25.2° c=2, n-HCl) for D(-)-2,3-diaminopropionic acid by Birnbaum S.M. et al. (1952).

Furthermore, the synthetic L(+)-2,3-diaminopropionic acid and the natural compound were inseparable by paper chromatography and by ion-exchange chromatography. For direct comparison the authors have synthesized D(-)-2,3-diaminopropionic acid by the reaction of D-aspartic acid with sodium azide in acidic medium. Physical properties (NMR, IR and m.p.) of the compound thus obtained were identical with those of the natural specimen, which unambiguously demonstrated that the newly isolated acid was D(-)-2,3-diaminopropionic acid.

It has been reported that L(+)-2,3-diaminopropionic acid occurred as a constituting amino acid of an antibiotic Viomycin (Haskell T.H. et al. 1952), and also in the free form in seeds of Mimosa palmeri and Mimosa hemiendyta (Gmelin, R. et al. 1959).

It was somewhat surprising to note that the digestive fluid from the larvae on normal diet contained no appreciable quantity of this unusual amino acid. It seems relevant therefore to assume that the amino acid has not arisen from the diet. The occurrence of D(-)-2,3-diaminopropionic acid in digestive fluid of silkworm larvae is the first instance of the discovery of

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the D-isomer of this amino acid in nature and may have a direct bearing on the presence of D-serine in the silkworm blood (Srinivasan N.G. et al. 1962).

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