The Natural Occurrence of Allomucic (Allaric) Acid: a Contribution to the Assignment of Structure of an Insecticidal Exotoxin from *Bacillus thuringiensis* Berliner

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Summary Allomucic acid has been identified as a constituent of an insecticidal exotoxin from *Bacillus thuringiensis* Berliner.

THE ability of the centrifuged culture media from various strains of *B. thuringiensis* and closely related organisms to kill a variety of insects[1] is now well authenticated.[2] Purification or partial purification of the responsible compound or compounds has now been reported from four laboratories.[2a,3] It is not yet established whether the purified materials so obtained are identical, but even if they are not, they represent a closely related class of high-molecular-weight (800—900) adenine nucleotides containing one phosphate group per adenine.[3b,c,4]

The importance of this compound or class of compound has been demonstrated by Šebesta and Horská, who have shown that their exotoxin inhibits the DNA-dependent RNA-polymerase from *Escherichia coli* competitively with ATP.[5]

The compound isolated in our laboratory contains, in addition to adenine and a phosphate group, allomucic acid, a compound not previously known to occur naturally.

When the purified exotoxin is hydrolysed with wheat germ or potato acid phosphatase, calf intestinal alkaline phosphatase, or, chemically, with acetate buffer at pH 4 and 100°,[3b] there results a dephosphorylated material which can be purified by ion-exchange chromatography on Whatman DE52 cellulose with 0·025M-ammonium carbonate buffer as eluant. The dephosphorylated material migrates towards the anode on high-voltage electrophoresis in buffer at pH 7. It has $E^{1\%}_{1\text{cm}}$ 200 at 260 nm. in water which indicates a molecular weight[3b] of 770 assuming ϵ 15,400. The molecular weight of the purified exotoxin has been similarly estimated to be 850.[3b]

Treatment of the dephosphorylated material with 1N-H_2SO_4 for 2 hr. at 100° results in, *inter alia*, the release of adenine.[3b] The hydrolysis mixture was fractionated on a column of Deacidite G (100—200 mesh, free-base). With water, the adenine and a neutral sugar fraction which reduced Fehling's solution were eluted without retention. The column was then eluted with 1N-H_2SO_4 and the eluate concentrated by freeze drying. From the concentrated solution, crystals were obtained as prisms melting at 188—192° with decomposition and evolution of gas. The i.r.

spectrum was identical with that of authentic allomucic acid (prepared by nitric acid oxidation of di-isopropylideneallose[6] and by similar oxidation of D-allonolactone obtained by cyanohydrin synthesis from D-ribose[7]). Heating the crystals under reflux in methanolic HCl afforded a methyl ester lactone whose mass spectrum was identical with that of material similarly prepared from authentic allomucic acid.

The "allomucic acid" of literature prior to 1934 is DL-altromucic acid. This point is made in the first edition of Rodd[8] but is omitted from the second edition.[9]

Dr. K. Šebesta has kindly informed me that his group have also isolated allomucic acid from their exotoxin.[10]

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