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THE STRUCTURE OF THE ALLARIC PORTION OF EXOTOXIN FROM BACILLUS THURINGIENSIS

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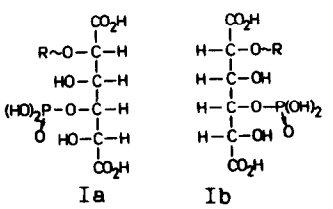
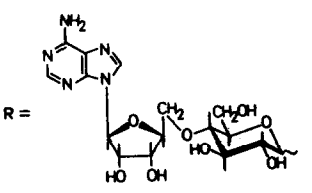
The insecticidal exotoxin which is produced by Bacillus thuringiensis, has been isolated by several European teams almost simultaneously. The mechanism of action of this toxin has been elucidated in our Institute[1]; in accordance with other authors, exotoxin has been identified as a nucleotide containing adenine bound to an anomalous sugar moiety. On the basis of degradations and some other evidence, the structure Ia or alternatively Ib was proposed for exotoxin[2]. Shortly thereafter, the structure of a characteristic derivative of the fundamental fragment II of exotoxin was confirmed by synthesis[3].

The position of the bond between the glucopyranosyl residue (of the adenine-ribofuranose-glucopyranose sequence) and allaric acid has been deduced by Farkaš and coworkers[2] from the formation of two five-membered lactones of the dephosphorylated exotoxin. It was concluded that the glucoside bond is situated in one of the α-positions

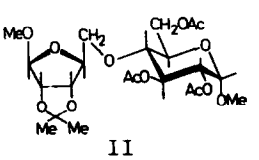


Ia

Ib



R =

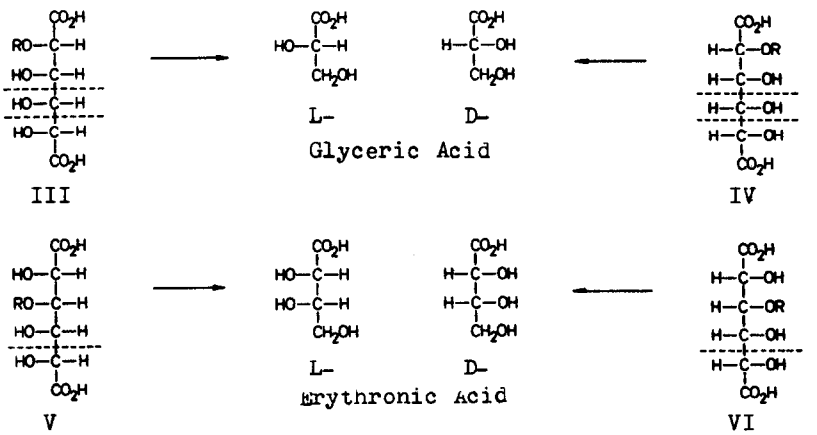


II

in respect to the carboxylic function of allaric acid (the absolute configuration of the allaric acid residue has not been determined). Owing to the well known low stability of aldaric acid lactones and the danger of isomerisation during isolation procedure, some additional chemical evidence was felt desirable to confirm or to reject the original idea on structural relations in the allaric acid portion of the exotoxin molecule.

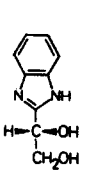
In connection with the synthesis of the dephosphorylated exotoxin it appeared of interest to reinvestigate the attachment of the glucopyranosyl residue to allaric acid and to determine the absolute configuration of the allaric acid residue. The α-configuration of the glucosidic bond has been determined by Bond and coworkers[4] by analysis of the NMR spectrum of the dephosphorylated exotoxin. We have assumed that the dephosphorylated exotoxin possesses the structure of one of the four optically active allaric acid O-glycosyl derivatives III-VI. Oxidative fission of the C-C bonds of allaric acid at the indicated positions (see Scheme 1), analogous fission of the glycosyl residue R (of the adenine-ribofuranose-glucopyranose sequence), reduction of the thus-obtained aldehydes, and hydrolytic removal of the modified glycosyl residue should lead to L- or D-glyceric acid, or, to L- or D-erythronic acid.

Scheme 1

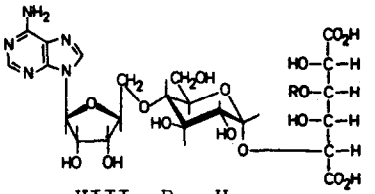


The dephosphorylated exotoxin was successively subjected to oxidation with an alkali periodate, sodium borohydride reduction, and a mild acidic hydrolysis to afford a mixture of hydroxy acids which was converted to a mixture of the corresponding benzimidazoles by reaction with o-phenylene diamine in acidic media. From this mixture, there was isolated the benzimidazole VII (overall yield, 39%), the CD and mass

spectra were identical with those of the benzimidazole derived[5] from D-glyceric acid. Consequently, the dephosphorylated exotoxin may be ascribed the structure VIII, i.e., the structure of (2R)-2-O-α-glycosylallaric acid. As indicated by the different course of the alkali periodate oxidation of exotoxin on one hand and the dephosphorylated exotoxin on the other[2], exotoxin possesses the structure IX.



VII



VIII, R = H

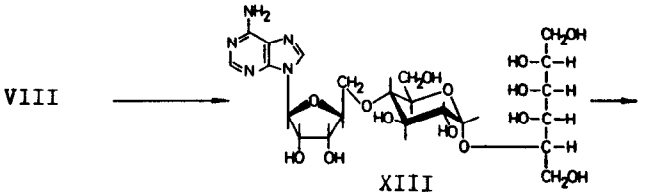
IX, R = $PO(OH)_2$

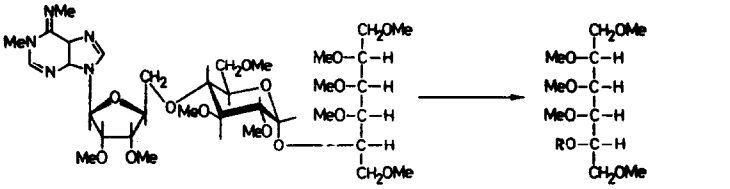
The structure of the allaric portion of exotoxin was also confirmed by an independent process consisting in permethylation of the alkali stable analogue of the dephosphorylated exotoxin. The direct permethylation of the dephosphorylated exotoxin itself with methyl iodide and silver oxide failed or was accompanied by eliminations[6] when performed under more drastic conditions with a mixture of methyl iodide and sodium hydride in dimethylformamide. The dephosphorylated exotoxin possessing one of the four structures III-VI (see above) should be reduced to an alkali stable O-glycosylallitol which could be then permethylated. Hydrolysis of such a permethyl derivative should lead to a penta-O-methylallitol with a free hydroxylic function at the position of the original glycosyl residue-allaric acid bond. In connection with this idea, (5R)-1,2,3,4,6-penta-O-methylallitol (X), its 5-O-acetyl derivative XI, and (3S)-1,2,4,5,6-penta-O-methylallitol (XII) were prepared by unambiguous procedures.

The dephosphorylated exotoxin was treated with diazomethane and the thus-obtained dimethyl ester was reduced by using more than a hundredfold excess of sodium borohydride in ethanol to afford the glycosyl derivative XIII (see Scheme 2). Permethylation of the latter derivative with a mixture of methyl iodide and sodium hydride in dimethylformamide afforded the permethyl derivative XIV (mass spectrum: M^+ = 761 and characteristic maxima at m/e 57, 71, 45, 101, 163 (B+1), and 164 (B+2)) which was subjected to methanolysis (0.01M-HCl in methanol, 2 hours at 100°C). The mass spectrum of the fragment XV (M^+ + 1=253 and characteristic maxima at m/e 45, 101, 89, 71, 59, 87, 145, and 175) was identical with that of the allitol derivative X. The comparison of the CD

spectrum (water) of the acetylated fragment XVI with that of the acetate XI [$\lambda([\Theta])$: 240 (0), 204 (+870), and 195 nm (+735)] proved the acetylated fragment XVI to be the enantiomer of the acetate XI. This finding leads to the same conclusion on the structure of the allaric portion of the dephosphorylated exotoxin as the preceding periodate oxidation. The earlier proposed[2] structure of exotoxin, particularly that of the allaric portion of the molecule, is thus closely related to the structure which was rigorously proved in the present paper.

Scheme 2





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