NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLX.*

DETERMINATION OF THE STRUCTURE OF THE ALLARIC PORTION OF EXOTOXIN FROM *Bacillus thuringiensis* BY MEANS OF PERIODATE OXIDATION

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Oxidation of the dephosphorylated exotoxin with alkali periodate and the subsequent sodium borohydride reduction afforded D-glyceric acid which was characterised by conversion to the benzimidazole derivative *V*. Consequently, the dephosphorylated exotoxin possesses the structure of a (2*R*)-2-O-glycosylallaric acid *II* and exotoxin is a 4-phosphate of the nucleoside *II*.

Exotoxin which shows a remarkable insecticidal activity, has been isolated almost simultaneously by several European teams[1-5] from *Bacillus thuringiensis*. The mechanism of exotoxin action has been elucidated in our Institute[6,7]. In contrast to the present structure *I*, the original proposal[8] did not include the configuration of the allaric acid residue and configuration of the glucosidic bond. Soon thereafter, the structure of the fundamental sugar fragment[9] and of the adenine-ribofuranose-glucopyranose sequence[10] has been confirmed by synthesis.

The structure of the allaric portion of the dephosphorylated exotoxin has been proposed by Farkaš and coworkers[8] on the basis of the formation of two five-membered lactones. It has been concluded that the glucosidic bond is situated in one of the α-positions in respect to the carboxylic function of allaric acid. In view of the well known low stability of aldaric acid lactones and the danger of isomerisation during the isolation process, it was felt desirable to present some additional evidence.

In connection with preparations for the total synthesis of exotoxin it was necessary to prove rigorously the structure of the allaric portion, namely, to determine the absolute configuration of the allaric acid residue and the position of the glucosidic bond. It was not possible to use the method of the X-ray analysis since exotoxin and its salts have not been so far obtained in crystalline form. Of the chemical methods, the oxidation with alkali periodate appeared most promising for our purpose. We have assumed that the dephosphorylated exotoxin possesses the structure of one of the four possible O-glycosyl derivatives of allaric acid *IIIa*, *IIIb* and *IVa*, *IVb*, the oxidation of which should be accompanied by cleavage of the C—C bonds

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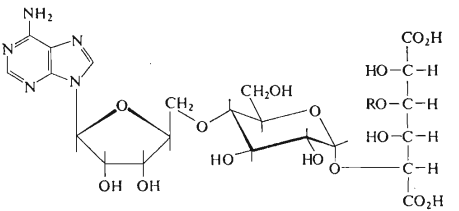
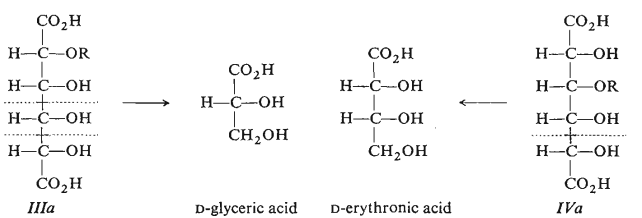
at indicated positions of the allaric acid residue and cleavage of the glycosyl residue (for the isomers *IIIa* and *IVa* see Scheme 1; R is the residue of the adenine-ribofuranose-glucopyranose sequence; the enantiomers *IIIb* and *IVb* should behave analogously). Reduction of the resulting aldehyde with sodium borohydride and the subsequent hydrolysis of the modified glycosyl residue should then lead to the corresponding D- or L-glyceric acid, or, D- or L-erythronic acid.

When the dephosphorylated exotoxin was successively subjected to oxidation with alkali periodate, reduction with sodium borohydride, and mild hydrolysis, a mixture of hydroxy acids was obtained. Since the isolation of micro amounts of hydroxy acids is difficult, the formation of the corresponding benzimidazole derivatives was made use of. Thus, the mixture of hydroxy acids was converted by reaction with *o*-phenylenediamine in acidic media to a mixture of benzimidazole derivatives, from which the compound *V* was isolated. Since the CD and mass spectra of compound *V* are identical with those of the benzimidazole derived from D-glyceric acid, the dephosphorylated exotoxin may be unequivocally assigned the structure of (2*R*)-2-O-glycosylallaric acid *II*. The α-configuration of the glucosidic bond in the dephos-

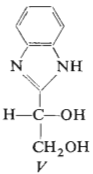


I, R = $PO(OH)_2$
II, R = H



SCHEME 1

phorylated exotoxin has been determined earlier by Bond[4] from the NMR spectral analysis. Owing to the different course of the periodate oxidation[8] of exotoxin on the



V

one hand and the dephosphorylated exotoxin on the other, the phosphate group of exotoxin is situated at position 4 of the allaric residue. Conclusively, exotoxin possesses the structure *I*.

EXPERIMENTAL

Mass spectra were measured on a MS-902 apparatus. CD spectra were taken on a Roussel-Jouan Dichrograph II, Model CD-185. The ion exchange resins Dowex 1X8 and Dowex 50 WX 8 were used (100—200 mesh).

Oxidative Cleavage of the Dephosphorylated Exotoxin *II* with Sodium Periodate

To a solution of the dephosphorylated exotoxin (*II*; 12·4 mg; 0·02 mmol) in 0·2M phosphate buffer[11] (2 ml; pH 6·8), there was added 0·1M aqueous sodium periodate (0·8 ml), the mixture kept at room temperature for 45 min, and then rapidly frozen. A solution of sodium borohydride (50 mg) in water (5 ml) was added and the mixture was allowed to melt at room temperature. After 2 h, the mixture was acidified with acetic acid to pH 5 and applied to a column (1·5 × × 10·0 cm) of Dowex 1 ($CH_3CO_2^-$) ion exchange resin. The column was washed with water and then eluted with 10% aqueous formic acid (150 ml). The acidic eluate was kept at room temperature overnight, evaporated under diminished pressure, and the residue coevaporated with two 30 ml portions of water. The final residue was applied to a column (10 ml) of Dowex 1 ($CH_3CO_2^-$) ion exchange resin, the column washed with water (100 ml) and eluted with 10% aqueous formic acid (100 ml). The acidic eluate was evaporated under diminished pressure, the residue coevaporated with two 50 ml portions of water, the final residue mixed with 0·1M aqueous *o*-phenylenediamine dihydrochloride (1 ml), the mixture evaporated, and heated at 135—140°C for 2 h. The mixture of benzimidazoles was dissolved in water (2 ml) and the aqueous solution applied to a 2·2 × 26 cm column of Whatman CM-70 cellulose which had been previously washed with 1 : 1 2M-HCO_2H–2M-HCO_2NH_4 (500 ml) and then with water until neutral. The column was eluted with an exponential gradient (500 ml) of water (1 000 ml) and 1 : 1 0·4M-HCO_2H–0·4M-HCO_2NH_4 (500 ml) to give fractions 1—50. The ultraviolet absorbing fractions 25—30 were combined and evaporated. The residue was purified by electrophoresis on paper Whatman 3 MM in 1 : 1 0·4M-HCO_2H–0·4M-HCO_2NH_4 (8 V/cm). The electrophoreogram showed in ultraviolet light two fluorescent bands. The less mobile band (positive to the *cis*-diol test) was eluted with water and the eluate applied to a 5 ml column of Dowex 50 (H^+) ion exchange resin. The column was washed with water (50 ml) and then eluted with 5% aqueous ammonia. Evaporation of the ammonia eluate afforded 1·4 mg (39%) of the benzimidazole

derivative *V*. CD spectrum (1M-HCl), $\lambda([\Theta])$: 263 (+1240), 266 (+960), 269 (+1180), 272 (+800), and 275 (+1120). Mass spectrum: ion at *m/e* 178 (M), ion at *m/e* 148 (M-30), and ions at *m/e* 118 and 119 (B + 1 and B + 2). These spectra were in accordance with those of the authentic specimen[12].

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