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SYNTHESIS OF A PART OF THE MOLECULE OF B. THURINGIENSIS EXOTOXIN

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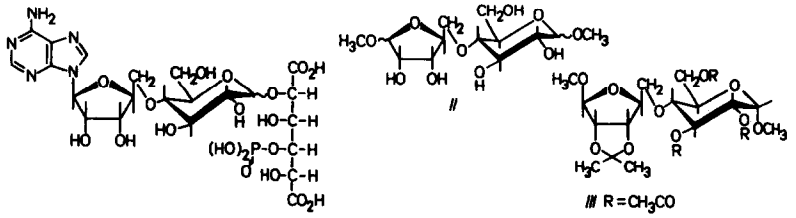
Last year, the structure of the insecticidal exotoxin produced by B. thuringiensis was derived in this Institute from structural studies on individual fragments of the toxin[1]. The exotoxin (I) is an anomalous adenosine derivative containing a disaccharide of a type so far not found in nature, in which the ribose and the glucose residue are bound together by an ethereal bond, and allaric acid which has not been observed among natural products. Allaric acid is bound to the disaccharide by a glycosidic bond and bears one phosphate group. An essential role in the determination of the exotoxin structure was played by the dimethylglycoside ether, which arises from acid catalyzed methanolysis of enzymatically dephosphorylated exotoxin, and to which the structure II has been ascribed. The anomeric mixture II has been transformed into the ether III, the cis-diol arrangement of which is protected by isopropylidene group and the hydroxyl groups by acetyl groups[2]. The product III gave a well resolved NMR spectrum[2].



/// R=CH_3CO

To prove the structure of the nucleoside moiety of the molecule of the exotoxin (I), we synthetized the most complicated fragment represented by the derivative of structure III. We have been able to form the anomalous ethereal bond by diaxial cleavage of the epoxide ring of 2-O-benzyl-1,6:3,4-dianhydro--β-D-galactopyranose (V) by methyl-2,3-O-isopropylidene-β-D-ribofuranoside

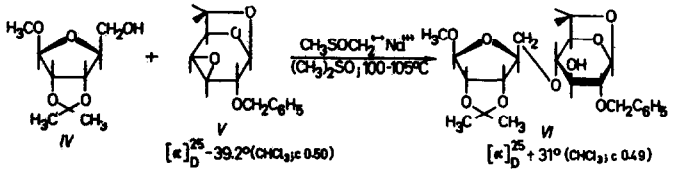
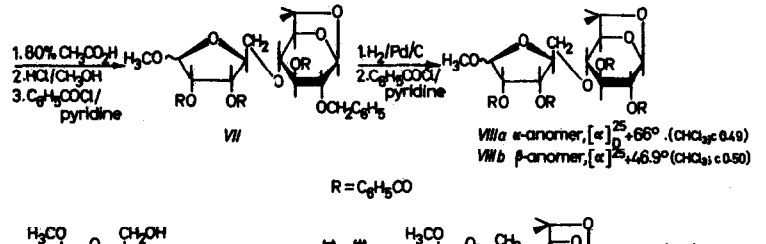


Chart 1



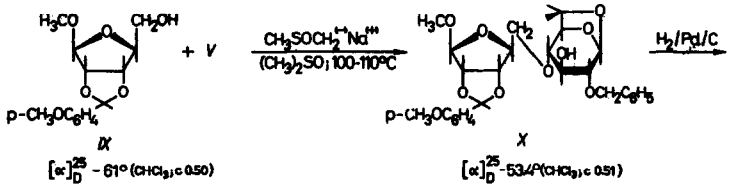
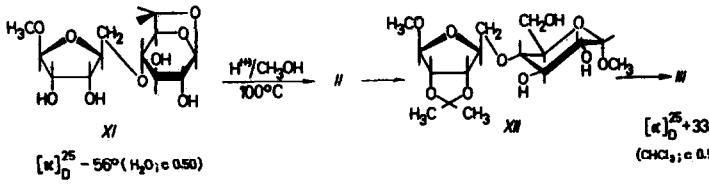


Chart 2



(IV) under alkaline conditions. The product VI (59%) was converted in several steps into the amorphous tribenzoate VII, and the latter by hydrogenolysis and benzoylation into anomeric mixture of tetrabenzoates VIIIa and VIIIb that was quantitatively resolved by chromatography (Chart 1).

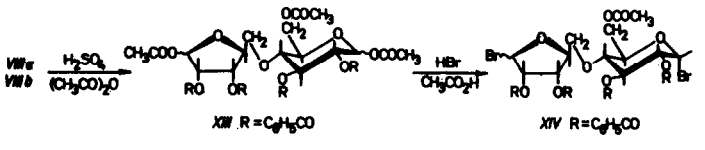
We shortened the procedure leading to the anomeric mixture VIIIa and VIIIb by replacing IV by methyl 2,3-O-p-anisylidene-β-D-ribofuranoside (IX). The benzylepoxide V was treated with IX to give 2-O-benzyl-4-O-(methyl 2,3-O-p-anisylidene-5-deoxy-β-D-ribofuranoside-5-yl)-1,6-anhydro-β-D-glucopyranose (X), m.p. 126-133°C. Hydrogenolysis of X (over Pd/C in acetic acid) afforded the ether XI, m.p. 132-133°C, which is identical with the product of alkaline methanolysis of tetrabenzoate VIIIb (Chart 2).

The free ether XI was subjected to acid catalyzed methanolysis at 100°C to afford a mixture similar in composition to the diglycoside fragment of exotoxin

II. The anomeric mixture was then converted into the isopropylidene derivative XII, the acetylation of which afforded the compound III (Chart 2). III was shown to be identical with the sample obtained by transformation of the exotoxin fragment II. NMR spectrum of III (C_6D_6): δ 1.09 and 1.33 (s, CH_3 of isopropylidene group), 1.56, 1.61, and 1.72 (s, 3x CH_3 of acetyl groups), 2.85 (s, CH_3 in C_1'-OCH_3), 2.97 (s, CH_3 in C_1-OCH_3), 3.28 (q, 4′-H, $J_{4',3'}$ 9.2 and $J_{4',5'}$ 9.5), 3.60-4.40 (m, 2x 5-H), 3.61 (sextet, 5′-H, $J_{5',6'} = J_{5',6''}$ 3.5), 4.20 (d, 2x 6′-H), 4.36 (q, 4-H, $J_{4,3} < 1$), 4.51 (s, 2-H, 3-H, $J_{2,1} = J_{3,2} = 0$ and $J_{3,4} < 1$), 4.70 (d, 1′-H, $J_{1',2'}$ 3.6), 4.86 (q, 2′-H, $J_{2',3'}$ 9.8), 4.89 (s, 1-H, $J_{1,2} = 0$) and 5.69 p.p.m. (q, 3′-H, $J_{3',4'}$ 8.8 c.p.s.). The glucose residue of the product III is present in C 1-configuration. By this approach we were able to prove the correctness of the proposed structure of the nucleoside moiety of the exotoxin molecule.

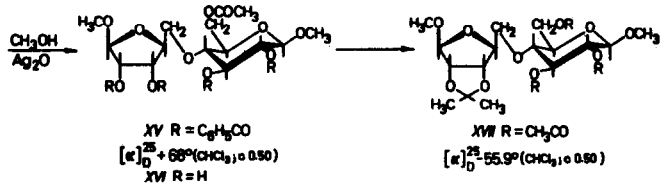
The anomeric tetrabenzoates VIIIa and VIIIb were subjected under controlled conditions to acetolysis to afford a high yield of the triacetate XIII. The latter was then used as a versatile intermediate for the preparation of the dibromide XIV, which afforded the blocked dimethyl diglycoside ether XVI with β-configuration in positions 1 and 1′. XVI was converted analogously into XVII, m.p. 118°C, which differed in β-configuration in position 1′ from the derivative of the exotoxin fragment III (Chart 3).

Since dihalogenose XIV contains two reactive centers, the decisive role in the preparation of the exotoxin model played selective glycosylation of blocked adenine by the ribose moiety of the molecule carried out in a way leaving the reactive glucose part intact. The exact conditions of the glycosylation



XIII R = C_6H_5CO XIV R = C_6H_5CO

Chart 3

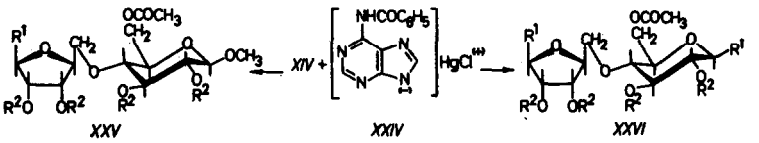


XIV R = C_6H_5CO
$[\alpha]_D^{25}$ + 68° (CHCl₃; c 0.50)
XVI R = H

XVII R = CH_3CO
$[\alpha]_D^{25}$ -55.9° (CHCl₃; c 0.50)

XVIII R^1=CH_3COO ; *XIX* R^1=Br *XXI* R^1=CH_3COO ; *XXII* R^1=Br
XX R^1=N^6-benzoyladenine *XXIII* R^1=N^6-benzoyladenine
R =C_6H_5CO

were explored in experiments in which as a model of dihalogenose XIV was chosen a mixture of halogenoses XIX and XXII prepared from equimolar amounts of the corresponding acetates XVIII and XXI. The mixture of XIX and XXII was allowed to react with the chloromercuric salt of N^6-benzoyladenine (cf.ref.[3]) at a molar ratio of 1:1:1. Under these conditions a mixture of 53% of the ribosyl derivative XX and of 18% of the glucosyl derivative XXIII, m.p. 165°C, was formed. When the molar ratio of components XVIII, XXI, and XXIV was altered to 1:1:0.5, 50% XX was formed in addition to less than 1% XXIII. Selective glycosylation (Chart 4) was affected by 4-min boiling of 0.4 equivalent of



XXV *XIV* + *XXIV* *XXVI*

R^1=N^6-benzoyladenine ; R^2=C_6H_5CO

Chart 4

XXIV with dihalogenose XIV in acetonitrile. The reaction mixture was decomposed immediately afterwards by mixture of methanol and silver oxide. The blocked model of exotoxin XXV was isolated by tlc on silica gel. XXV showed the frequency of NH-group at 3 402 cm^{-1}. The reaction of XIV with an excess of XXIV afforded a so far unknown type of nucleoside XXVI with two basic residues bound to the sugar residue by nucleosidic bond.

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