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SYNTHESIS OF EXOTOXIN PRODUCED BY Bacillus thuringiensis

L. Kalvoda , M. Prystaš, and F. Šorm

Institute of Organic Chemistry and Biochemistry,

Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia

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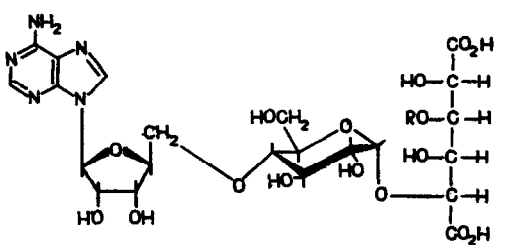
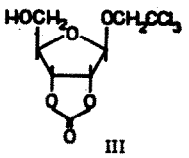
The insecticidal exotoxin produced by Bacillus thuringiensis was almost simultaneously isolated by several Europian teams. The structure of exotoxin (without the configuration of the glucosidic bond and the allaric portion) was proposed in this Institute on the basis of degradations[1]. The correctness of this proposal was proved by synthesis of the fundamental sugar fragment[2]. The structure of the allaric portion of exotoxin was rigorously proved by two independent chemical routes[3]. The complex structure of exotoxin (I), especially the α-configuration of the glucosidic bond[4] and position of the phosphate group on the allaric acid moiety[1] has been now established by synthesis.

The total synthesis of exotoxin required realisation of several fundamental steps, namely, formation of an ethereal bond between the ribofuranose and glucopyranose component (1), selective nucleosidation (2), formation of α-glucosidic bond connecting the allaric portion of correct configuration (3), and its unequivocal phosphorylation (4). It has been then determined on the basis of numerous experiments that the order (1), (3), (2), and (4) is advantageous for the realisation of the total synthesis. In step (1) we have modified the method of preparing diglycoside ethers[2] by the trans-diaxial opening of the epoxide ring of 1,6:3,4-dianhydrogalactopyranoses with alcohols[5-7]. The reaction of 2-O-benzyl-1,6:3,4-dianhydro- β -D-galactopyranose (II) and 2,2,2-trichloroethyl 2,3-O-cyclocarbonyl- β -D-ribofuranoside (III) in benzene in the presence of 1 mol.% of $BF_3.Et_2O$



I, R=$PO(OH)_2$


II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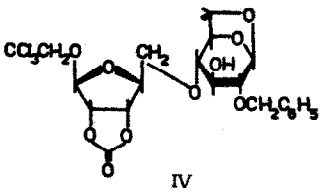
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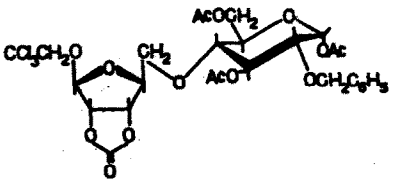
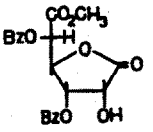
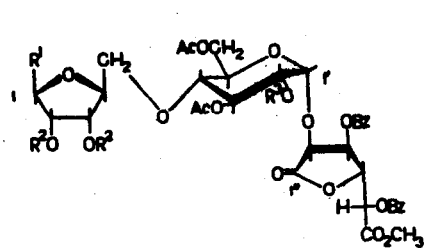
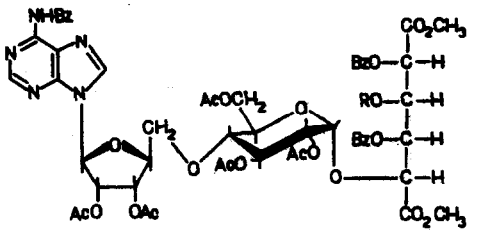

V


VI

(O°C, 24 hours) afforded the diglycoside ether IV, m.p. 117-118°C, $[\alpha]_D^{25}$ -78.2° ($CHCl_3$, c1). The anomeric center of the ribose component of the ether IV is deactivated by the presence of the carbonate group to such an extent that further reactions may be performed selectively on the glucose portion; this observation was made use of in realisation of step (3). Acetolysis of the 1,6-anhydro ring of compound IV (4% $BF_3.Et_2O$ in acetic anhydride) afforded the triacetate V, m.p. 149-151°C, $[\alpha]_D^{25}$+13.1° ($CHCl_3$, c 1), which was condensed (by the action of 10% $BF_3.Et_2O$ in chloroform) with the lactone ester of allaric acid VI to give the glycosyllactone VII. The latter compound was without any isolation hydrogenolyzed (over 10% Pd/C in acetic acid) under the formation of the α-glycoside VIII, $[\alpha]_D^{25}$+10.4° ($CHCl_3$, c 1); NMR spectrum ($CDCl_3$): δ 2.02 and 2.14 (s, 2x OAc), 3.90 (s, 6''-CO_2Me), 4.04 and 4.17 (d, OCH_2CCl_3, J_{gem} 11.0), 5.31 (d, 1'-H, $J_{1',2'}$ 3.5 c.p.s.) and 5.42 p.p.m. (s, 1-H). Acetylation of compound VIII (0.2M-CF_3CO_2H in Ac_2O) afforded the triacetate IX, m.p. 64°C (m-xylene; solvate with 2 molecules of the solvent).

In subsequent step (2), it was necessary to activate the ribose moiety; the cyclocarbonyl group was therefore replaced by acetyl groups. The hydrolysis of compound IX (pyridine-water, 100°C, 30 min) was accompanied by a simultaneous opening of the lactone ring which was reclosed by the action of N,N'-dicyclohexylcarbodiimide to give the dihydroxy derivative X (ν CO of the lactone, 1 804 cm^{-1}; ν CO of esters, 1 735 cm^{-1}) which was acetylated under the formation of the pentaacetate XI. The trichloroethyl group of the pentaacetate XI was removed by reduction (Zn/HCl in acetone) and the thus-obtained hydroxy derivative XII was converted into an anomeric mixture of the peracetyl derivatives XIII. By the action of hydrogen bromide in a toluene-chloroform mixture, the a-





VII, R^1=CCl_3CH_2O; R^2= -OCOO- ; R^3=$PhCH_2$

VIII, R^1=CCl_3CH_2O; R^2= -OCOO- ; R^3=H

IX, R^1= CCl_3CH_2O; R^2= -OCOO- ; R^3=Ac

X, R^1=CCl_3CH_2O; R^2=OH; R^3=Ac

XI, R^1=CCl_3CH_2O; R^2=R^3=Ac

XII, R^1=OH; R^2=R^3=Ac

XIII, R^1=OAc; R^2=R^3=Ac

XIV, R^1=Br; R^2=R^3=Ac

XV, R^1=N^6-benzoyladenine; R^2=R^3=Ac

XVI, R=H

XVII, R=$POCl_2$

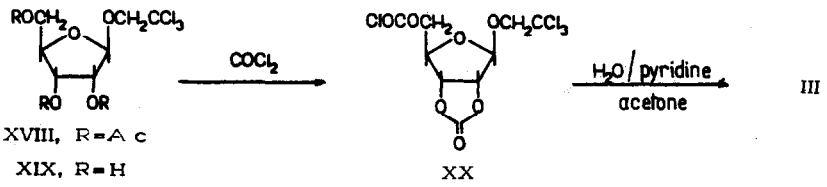
nomeric mixture XIII was transformed into the halogenose XIV which was treated with chloromercuri salt of N^6-benzoyladenine in acetonitrile to afford the nucleoside XV, λ_{max}^{MeOH} 232 and 278 nm, NMR spectrum ($CDCl_3$): δ 1.97, 2.05 and 2.13 (s, 5x OAc), 3.80 (s, 6''-CO_2Me), 5.55 (d, 1'-H, $J_{1',2'}$ ~ 3.5), 5.83 (d, 2''-H, $J_{2'',3''}$ ~ 6 c.p.s.), 8.42 and 8.82 p.p.m. (s, 8-H and 2-H of adenine). The phosphorylation of the nucleoside XV (step 4) was started with opening of the lactone ring by the action of 1M-C_5H_5N in methanol (reflux, 40 min) to afford the dimethylester XVI which was then phosphorylated with phosphorus oxychloride in benzene in the presence of pyridine. The thus-obtained chlorophosphate XVII was hydrolyzed with sodium hydroxide in aqueous pyridine to give exotoxin (I) which was in all respects identical[8] with naturally occurring toxin.

The above route together with another procedure which will be published later may be used in investigation in the field of exotoxin analogues.

The starting ribofuranoside III was prepared (Chart 1) by reaction of anomeric tetra-O-acetyl-D-ribofuranoses[9] with 2,2,2-trichloroethanol in the presence of $BF_3.Et_2O$, the alkaline methanolysis of the product XVIII, and treatment of the free glycoside XIX, m.p. 114-115°C, $[\alpha]_D^{25}$ -60.9° (EtOH, c 1), with excess phosgene in the presence of N,N-dimethylaniline; the chloride XX was converted by a mild hydrolysis (in the presence of pyridine in acetone) into the ribofuranoside III, $[\alpha]_D^{25}$ -85.9° (EtOH, c 1).

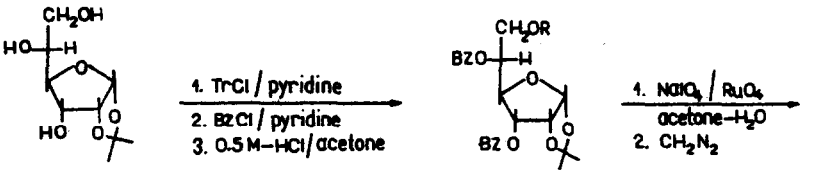
In the synthesis of the allaric acid lactone ester VI (Chart 2), the starting 1,2-O-isopropylidene-α-D-allofuranose[10] was protected by trityl group at position 6 and by benzoyl groups at positions 3 and 5 to afford compound XXI. Removal of the trityl group (HCl/acetone) afforded the alcohol XXII, m.p. 106-108°C, which was oxidized[11] with so-

Chart 1

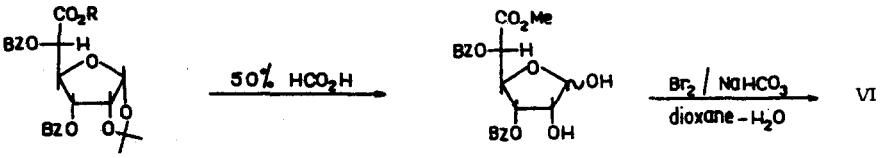


XVIII, R=Ac
XIX, R=H
XX

Chart 2



XXI, R=Ph_3C
XXII, R=H



XXIII, R=H
XXIV, R=CH_3
XXV

dium periodate in the presence of ruthenium tetraoxide. The thus-obtained acid XXIII was treated with diazomethane to give the methyl ester XXIV. Removal of the isopropylidene group led to the dihydroxy derivative XXV, m.p. 124-130°C, which was oxidized with bromine to afford the lactone VI, m.p. 191-193°C, $[\alpha]_D^{25}$ -34° ($CHCl_3$, c 1).

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