NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CXXXIII.*

SYNTHESIS OF THE SUGAR FRAGMENT OF EXOTOXIN FROM *Bacillus thuringiensis*

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trans-Diaxial opening of the epoxide ring of the tosyl derivative *XVII* by the action of methyl 2,3-O-cyclocarbonyl-(*X*), methyl 2,3-di-O-benzoyl- (*XIV*) and methyl 2,3-di-O-*p*-toluenesulfonyl-β-D-ribofuranoside (*XV*) in the presence of acids afforded the diglycoside ethers *XXIII*, *XXVII*, and *XXVIII*. The multistep transformation of compound *XXIII* led to a very poor yield of the acetylated ether *XXVI* containing the methyl riboside and laevoglucosan residues. Treatment of esters *XXIII* and *XXVII* with alkoxides afforded exclusively the epoxide *XXIX* which was isolated in the form of the dibenzoate *XXX*. *trans*-Diaxial opening of the epoxide ring of the benzyl derivative *XIX* with methyl isopropylideneribofuranoside (*XVI*) under alkaline conditions was more successful. The resulting diglycoside ether *XXXI* was readily transformed into the versatile triacetate *XXXVII* containing the ribose and glucose components. Treatment of the acetate *XXXVII* with hydrogen bromide in acetic acid afforded the dihalogenose *XXXVIII*, which was converted by reaction with methanol in the presence of silver oxide into dimethyl diglycoside ether *XXXIX* possessing the β-configuration at positions 1 and 1′. The ether *XXXIX* was transformed into compound *XLIV* which is anomeric with the derivative of the fundamental sugar fragment of exotoxin *III*. The earlier proposed structure *III* was unequivocally confirmed by the synthesis starting from the diglycoside ether *XXV*.

Some years ago[1,2], a toxin was isolated from the culture of *Bacillus thuringiensis*. This toxin exhibits remarkable insecticidal effects. In a short time, several groups of investigators have been engaged in isolation and purification of this substance[2-7].

Exotoxin has been paid special attention in our Institute[4,5]. Thus, *e.g.*, exotoxin was found to inhibit the synthesis of ribonucleic acids[8], particularly of the DNA-dependent RNA polymerase[9]. Up to the present time, there has been known only a low number of DNA-dependent RNA polymerase inhibitors the structure of which is analogous to the naturally occurring substrates. Thus, formycin 5′-triphosphate[10,11] and sangivamycin 5′-triphosphate[12] differ by the

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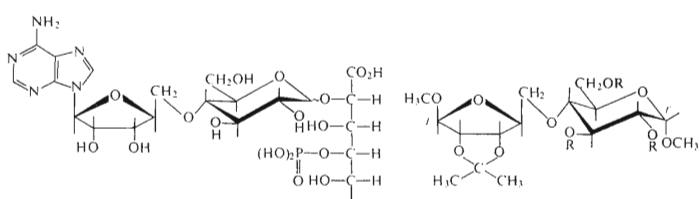
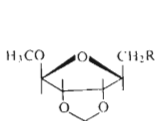
basic moiety from the naturally occurring adenosine 5'-triphosphate while cordycepin 5'-triphosphate[13] has a different sugar moiety. Furthermore, exotoxin has been observed to inhibit competitevely the polymerisation step of the DNA-dependent RNA polymerase reaction. Exotoxin is bound to the enzym by its adenosine moiety in accordance with the base pairing rule and is in turn displaced by adenosine 5'-triphosphate[14].

Our investigations on exotoxin culminated by determination of its chemical structure[15,16]. Exotoxin (*I*) is represented by an anomalous adenine nucleotide containing an unusual type of disaccharide the both components (ribose and glucose) of which are linked through an ethereal bond, and allaric acid bound to the disaccharide through a glycosidic linkage and carrying one phosphate group[15,16]. Both allaric acid and the above mentioned type of disaccharide have not been hitherto observed in Nature. The presence of allaric acid in exotoxin was also confirmed by other authors[6,7]. Acid-catalysed methanolysis of enzymatically dephosphorylated exotoxin afforded a dimethyl diglycoside which was successfully used in structural determinations. On the basis of NMR spectrum, mass spectrum, periodate oxidation, and optical rotatory dispersion (of both anomeric constituents of the original dimethyl diglycoside and the corresponding ribonolactone[15,16]), the structure of methyl 4-O-(methyl 5-deoxy-D-ribofuranosid-5-yl)-D-glucopyranoside was ascribed to the diglycoside. Recently, the anomeric diglycosides were converted to the acetonide *II* and the acetonide triacetate *III* which exhibited a satisfactorily resolved NMR spectrum[16]. The ethereal linkage localised at positions 5 and 4, resp., of the riboside and glucoside residues appears as the most important structural feature of the sugar fragment of exotoxin molecule. Such a type of compounds has not been hitherto reported. The present unequivocal synthesis of compound *III* confirms *inter alia* the structure proposed.

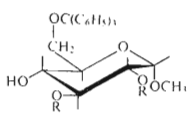
The ethereal linkage of compound *III* could be theoretically realised analogously to the formation of glycoside O-alkyl ethers. We prepared therefore methyl 5-O-*p*-toluenesulfonyl-2,3-O-benzylidene-β-D-ribofuranoside (*IV*) and methyl 5-iodo-5-deoxy-2,3-O-benzylidene-β-D-ribofuranoside (*V*) as ribofuranoside derivatives activated at position 5 and methyl 2,3-di-O-benzyl-6-O-triphenylmethyl-α-D-glucopyranoside (*VI*) as a glucopyranoside derivative possessing a free hydroxylic function at position



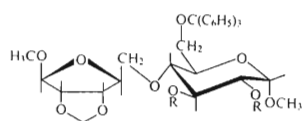
I *II*, R = H *III*, R = CH_3CO



IV, R = *p*-$CH_3C_6H_4SO_3$
V, R = I



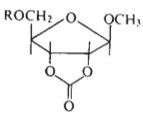
VI, R = $C_6H_5CH_2$



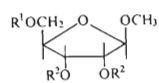
VII, R = $C_6H_5CH_2$

4. The tosyl derivative *IV* was prepared by benzylidenation of methyl ribofuranoside in the presence of zinc chloride and molecular sieve Potassit 3 (*cf.* the benzylidenation of D-ribose[17]). The resulting methyl 2,3-O-benzylidene-β-D-ribofuranoside was isolated by filtration through a column of neutral aluminum oxide and converted in a high yield into the crystalline tosyl derivative *IV* by the action of *p*-toluenesulfonyl chloride in pyridine. The 5-iodo derivative *V* was prepared by a known procedure[18], namely, on treatment of methyl 2,3-O-benzylidene-β-D-ribofuranoside with methyltriphenyloxyphosphonium iodide in anhydrous dimethylformamide. Compound *VI* has been prepared earlier[19] in an impure state contaminated with triphenyl carbinol as the usual by-product. Pure *VI* has been now obtained by column chromatography on silica gel in benzene and benzene–ethyl acetate. By the action of one equivalent of dimethyl sulfoxide sodium salt in dimethyl sulfoxide–dimethylformamide, the alcohol *VI* was converted to the corresponding sodium salt and this reacted at temperatures up to 60°C with the tosyl derivative *IV* or the 5-iodo derivative *V*. As shown by thin-layer chromatography on silica gel, the reaction mixtures contained the starting compounds (recovery, 40–70%) but the expected product *VII* was absent. The reaction failed probably for steric reasons.

Another approach might comprise the reaction of 2-O-substituted 1,6:3,4-dianhydro-β-D-galactopyranoses with ribosides carrying a free hydroxylic function

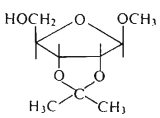


VIII, R = $(C_6H_5)_3C$
IX, R = $C_6H_5CH_2OCO$
X, R = H

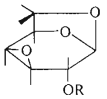


XI, $R^1 = (C_6H_5)_3C$; R^2 = H
XII, $R^1 = (C_6H_5)_3C$; $R^2 = C_6H_5CO$
XIII, $R^1 = (C_6H_5)_3C$; $R^2 = p$-$CH_3C_6H_4SO_2$
XIV, R^1 = H; $R^2 = C_6H_5CO$
XV, R^1 = H; $R^2 = p$-$CH_3C_6H_4SO_2$

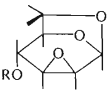
at position 5. As reported recently by Černý and coworkers[20], the reaction of 2-O-*p*-toluenesulfonyl-1,6:3,4-dianhydro-β-D-galactopyranose and benzyl alcohol catalysed by *p*-toluenesulfonic results acid in a *trans*-diaxial opening of the epoxide ring under



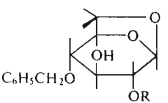
XVI



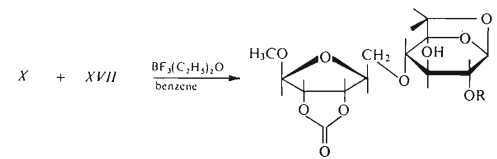
XVII, R = *p*-$CH_3C_6H_4SO_2$
XVIII, R = H
XIX, R = $C_6H_5CH_2$



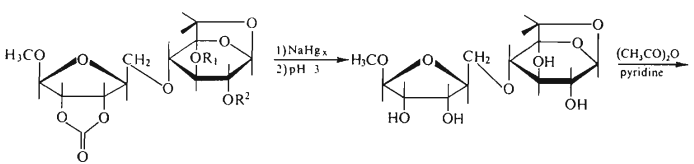
XX, R = $C_6H_5CH_2$



XXI, R = *p*-$CH_3C_6H_4SO_2$
XXII, R = $C_6H_5CH_2$

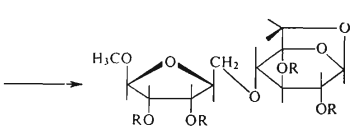


XXIII, R = *p*-$CH_3C_6H_4SO_2$



XXIV, R^1 =  , R^2 = *p*-$CH_3C_6H_4SO_2$

XXV,



XXVI, R = CH_3CO

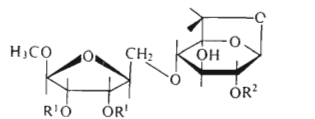
SCHEME 1

the formation of a single product, namely, 2-O-*p*-toluenesulfonyl-4-O-benzyl-1,6-anhydro-β-D-glucopyranose (*XXI*) in a very satisfactory yield. The earlier reported[21] epoxide-ring opening of the same compound *XVII* by the action of methanol in the presence of Dowex-50 (H^+) ion exchange resin proceeds similarly. The *trans*-diaxial epoxide-ring opening may be also effected under alkaline conditions. Thus, as reported by Höök and Lindberg[22], the tosyl epoxide *XVII* may be converted to the free 1,6:3,4-dianhydro-β-D-galactopyranose (*XVIII*) which was then pyranylated at position 2. Treatment of the product with sodium isopropoxide in isopropyl alcohol afforded 4-O-isopropyl-2-O-tetrahydropyranyl-1,6-anhydro-β-D-glucopyranose.

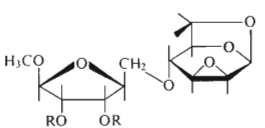
We focussed our attention to the *trans*-diaxial epoxide-ring opening of compound *XVII* in the presence of acids. Methyl 2,3-O-cyclocarbonyl-(*X*), methyl 2,3-di-O-benzoyl-(*XIV*), and methyl 2,3-di-O-*p*-toluenesulfonyl-β-D-ribofuranoside (*XV*) were used as the ribose component. The cyclic carbonate *X* was prepared by reaction of the known[23] methyl 5-O-triphenylmethyl-β-D-ribofuranoside (*XI*) with phosgene in pyridine and hydrolysis of the product *VIII* in refluxing 80% aqueous acetic acid. In contrast to the earlier method[24], the present procedure is shorter and gives satisfactory yields; 50–100 grams of pure compound *X* may be prepared in a single batch. The cyclic carbonate *X* was also prepared by hydrogenolysis of methyl 5-O-benzyloxycarbonyl-2,3-O-cyclocarbonyl-β-D-ribofuranoside (*IX*) over palladium on carbon catalyst in acetic acid as solvent. Compound *IX* was obtained by reaction of ribofuranoside with benzyl chloroformate in analogy to the reaction of methyl ribofuranoside with methyl chloroformate under alkaline conditions[25]. It is, however, more advantageous to prepare the compound *X* by the former method than by the latter. Methyl2,3-di-O-benzoyl-β-D-ribofuranoside (*XIV*) was obtained by benzoylation of the triphenylmethyl derivative *XI* and the subsequent removal of the triphenylmethyl group from compound *XII* in refluxing 80% aqueous acetic acid. Methyl 2,3-di-O-*p*-toluenesulfonyl-β-D-ribofuranoside (*XV*) was prepared analogously to the dibenzoate *XIV*, the intermediary triphenylmethyl derivative *XIII* being obtained by a simplified procedure (*cf.*[26]).

Reaction of the cyclic carbonate *X* with the tosyl epoxide *XVII* in refluxing benzene in the presence of 5–15 mol % of boron trifluoride etherate afforded (see Scheme 1) the expected 2-O-*p*-toluenesulfonyl-4-O-(methyl 5-deoxy-2,3-O-cyclocarbonyl-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXIII*). With lower concentrations of the Lewis acid, the reaction does not proceed at all or very slowly while higher concentrations lead to the degradation of the required product *XXIII*. The isolation of ester *XXIII* from a relatively complicated reaction mixture was performed by column chromatography over silica gel. Analogously, the reaction of the epoxide *XVII* with the dibenzoate *XIV* or the di-*p*-toluenesulfonate *XV* afforded the expected 2-O-*p*-toluenesulfonyl-4-O-(methyl 5-deoxy-2,3-di-O-benzoyl- (*XXVII*) and 2-O-*p*-toluenesulfonyl-4-O-(methyl 5-deoxy-2,3-di-O-*p*-toluenesulfonyl-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXVIII*) in a 21 and 54% yield, respectively.

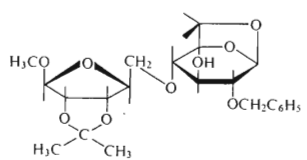
In the preparation of the fundamental fragment *III* of exotoxin, the above mentioned diglycoside ethers *XXIII*, *XXVII*, and *XXVIII* have to be subjected to several successive transformations, namely, removal of the protecting groups present, a suitable re-blocking, opening of the 1,6-anhydro ring of the D-laevoglucosan component, formation of two glycosidic linkages, protection of the *cis*-diol system by the isopropylidene group, and acetylation of the remaining free hydroxylic functions of the glucoside residue. Prior to the removal of the tosyl group from the ester *XXIII*, it is necessary to protect position 3 of the laevoglucosan residue by an alkali-stable group, *e.g.*, the tetrahydropyranyl group. Since position 3 is sterically hindered by the presence of the 1,6-anhydro ring, the protection is not quantitative. The tetrahydropyranyl derivative *XXIV* was not isolated but directly subjected to reduction with sodium amalgam under controlled conditions (pH 6·6 – 8·0) to remove simultaneously the tosyl group and the alkali-labile cyclocarbonyl group. Acidification of the reaction mixture (pH 3) resulted in removal of the tetrahydropyranyl group. The resulting free 4-O-(methyl 5-deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXV*) was converted by acetylation to the tetraacetate *XXVI* which was isolated by column chromatography on silica gel (see Scheme 1).



XXVII, $R^1 = C_6H_5CO$; R = *p*-$CH_3C_6H_4SO_2$
XXVIII, $R^1 = R^2$ = *p*-$CH_3C_6H_4SO_2$

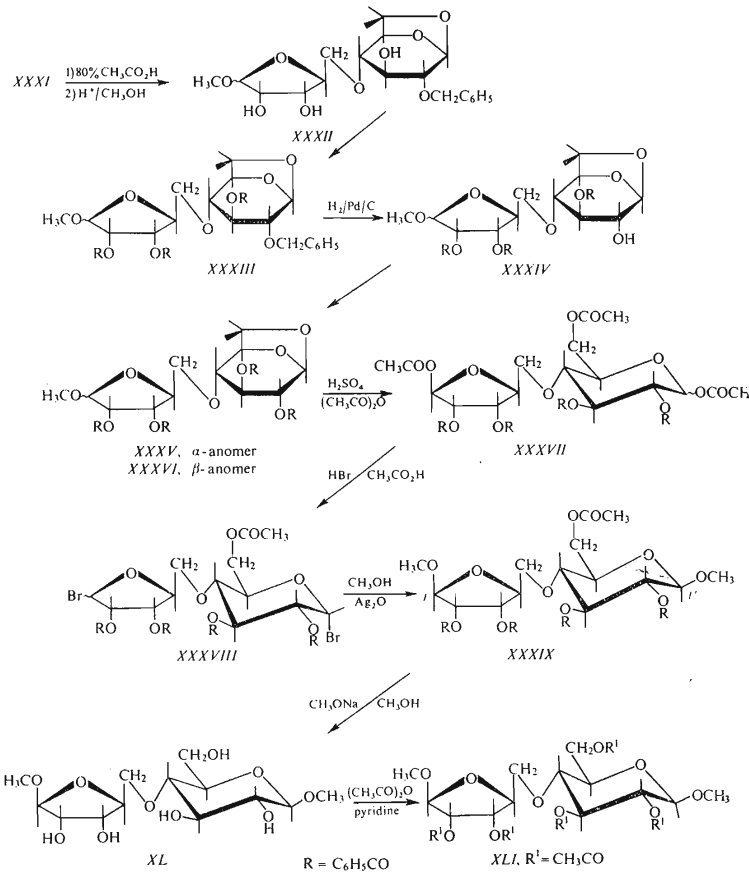


XXIX, R = H
XXX, R = C_6H_5CO



XXXI

Since the reaction sequence according to Scheme 1 gives very poor yields, another method was attempted for removal of protecting groups of the diglycoside ethers


XXXI
1) 80% CH3CO2H
2) H+/CH3OH
XXXII
H2/Pd/C
XXXIII
XXXIV
H2SO4
(CH3CO)2O
XXXV, α-anomer
XXXVI, β-anomer
XXXVII
HBr / CH3CO2H
XXXVIII
CH3OH
Ag2O
XXXIX
CH3ONa / CH3OH
XL
(CH3CO)2O
pyridine
XLI, R1 = CH3CO
R = C6H5CO

SCHEME 2

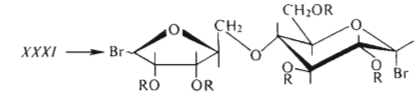
XXIII and *XXVII*, namely, the treatment with sodium methylate or sodium benzylate. The assumed reaction course was the following: removal of the cyclocarbonyl group or benzoyl groups, closure of the epoxide ring of 4-O-(methyl 5-deoxy-β-D-ribofuranosid-5-yl)-1,6:2,3-anhydro-β-D-mannopyranose (*XXIX*), and the subsequent ring opening. By this reaction sequence, the treatment of both esters *XXIII* and *XXVII* with sodium benzylate in benzyl alcohol should afford 2-O-benzyl-4-O-(methyl 5-deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose. An analogous reaction of the known[20] 4-O-benzyl-2-O-*p*-toluenesulfonyl-1,6-anhydro-β-D-glucopyranose (*XXI*) with sodium benzylate leads *via* the isolated 4-O-benzyl-1,6:2,3-dianhydro-β-D-mannopyranose (*XX*) (*cf.* ref.[27]) to the reported[28] 2,4-di-O-benzyl-1,6-anhydro-β-D-glucopyranose (*XXII*) through the *trans*-diaxial opening of the 2,3-anhydro ring of compound *XX*. Formation of the 2,3-anhydro ring by the action of alcoholates is much easier than the opening, the latter requiring higher temperatures (above 100°C). Unexpectedly, the analogous reaction of compound *XXIII* with sodium benzylate results in a complete destruction of the dianhydromannose *XXIX*. The latter compound *XXIX* was converted by benzoylation to the crystalline dibenzoate *XXX* which was readily isolated from the reaction mixture by filtration through a column of aluminum oxide.

For the related investigations on the attempted acid-catalysed opening of the epoxide ring of 1,6 : 3,4-dianhydro-β-D-galactopyranose protected at position 2 by the participating benzoyl or acetyl group see ref.[29]. No *trans*-diaxial epoxide-ring opening has been observed in these experiments.

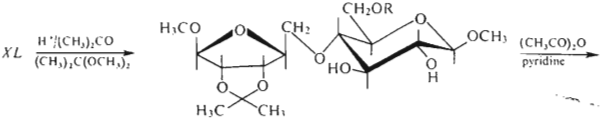
Another set of our experiments relates to the opening of the epoxide ring of 1,6:3,4-dianhydro-β-D-galactopyranoses under alkaline conditions. As the reaction components, 2-O-benzyl-1,6:3,4-dianhydro-β-D-galactopyranose (*XIX*) and methyl 2,3-O-isopropylidene-β-D-ribofuranoside (*XVI*) were used. Compound *XIX* was prepared by benzylation of the free dianhydrogalactopyranose *XVIII* by the action of excess benzyl bromide and silver oxide in dimethylformamide at an elevated temperature. The isopropylidene derivative *XVI* was prepared according to a procedure worked out in this Institute[30] (*cf.* ref.[31]). In a model reaction of the benzyl derivative *XIX* with one equivalent of sodium benzylate in dimethylformamide at an elevated temperature, an unequivocal *trans*-diaxial ring opening of the epoxide has been observed in a very satisfactory yield. The reaction of the benzyl derivative *XIX* with an excess of the isopropylidene ribofuranoside *XVI* was effected with at least one equivalent of dimethyl sulfoxide sodium salt (referred to compound *XIX*) in dimethyl sulfoxide as solvent at the temperature of 100 to 110°C. The reaction was accomplished within 1·5 to 2·5 hours. The reaction mixture was neutralised with carbon dioxide, the neutral suspension evaporated under diminished pressure, and the residue processed as usual to afford 2-O-benzyl-4-O-(methyl 5-deoxy-2,3-O-isopropylidene-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXXI*) which was isolated by column chromatography over silica gel. The analogous reaction of crystalline (m.p.

93 – 102°C; *cf.* ref.[22]) 2-O-tetrahydropyranyl-1,6:3,4-dianhydro-β-D-galactopyranose with the isopropylidene derivative *XVI* was accompanied by decomposition of the product.

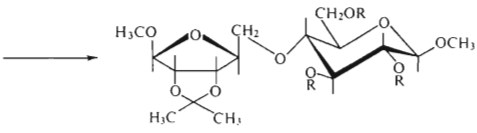
The diglycoside ether *XXXI* was converted in several steps to 1,6-di-O-acetyl-2,3-di-O-benzoyl-4-O-(1-O-acetyl-2,3-di-O-benzoyl-5-deoxy-α,β-D-ribofuranos-5-yl)-α,β-D-glucopyranose (*XXXVII*), as shown in Scheme 2. Thus, the ether *XXXI* was heated in 80% aqueous acetic acid to remove the isopropylidene group; the removal was accompanied in an extent of 20 – 30% by hydrolysis of the methyl riboside. In the subsequent step, anomeric 2-O-benzyl-4-O-(methyl 5-deoxy-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranoses (*XXXII*) were prepared on treatment with methanolic hydrogen chloride, and benzoylated to the anomeric tribenzoates *XXXIII*. Hydrogenolysis of the anomeric mixture *XXXIII* over palladium on carbon catalyst



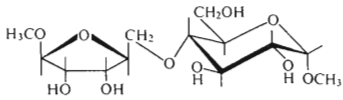
XLII. R = CH_3CO



XLIII



XLIV, R = CH_3CO



XLV

SCHEME 3

in glacial acetic acid resulted in removal of the benzyl group at position 2 of the laevoglucosan residue. The resulting anomeric benzoates *XXXIV* were converted into anomeric 2,3-di-O-benzoyl-4-O-(methyl 5-deoxy-2,3-di-O-benzoyl-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranoses *XXXV* and *XXXVI*. The predominating β-anomer *XXXVI* was quantitatively separated from the α-anomer *XXXV* by column chromatography over silica gel. Both anomers *XXXV* and *XXXVI* were converted by the action of acetic anhydride in the presence of sulfuric acid under controlled conditions to the triacetate *XXXVII*; the yields were very satisfactory.

The triacetate *XXXVII* was treated with hydrogen bromide in acetic acid and methylene chloride to afford the 1,1'-dibromo derivative *XXXVIII* which was not isolated but converted directly to methyl 2,3-di-O-benzoyl-6-O-acetyl-4-O-(methyl 2,3-di-O-benzoyl-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XXXIX*). Since the reaction of the dihalogenose *XXXVIII* proceeds under conditions of a steric control, both anomeric centers 1 and 1' of compound *XXXIX* possess the β-configuration. Alkali-catalysed methanolysis of compound *XXXIX* afforded the free methyl 4-O-(methyl 5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XL*), the acetylation of which with acetic anhydride in pyridine yielded the pentaacetate *XLI* (see Scheme 2). The identical pentaacetate *XLI* was obtained in a much lower yield and after a laborious isolation by reaction of the diglycoside ether *XXXI* with a 34% solution of hydrogen bromide in 10 : 1 acetic acid–acetic anhydride and treatment of the resulting complex reaction mixture (containing, *inter alia*, the 1,1'-dibromo derivative *XLII*) with methanol in the presence of silver oxide. Conversion of the diglycoside ether *XXXI* to the halogenose *XLII* thus comprises removal of the isopropylidene and benzyl group, acetylation of free hydroxylic functions, cleavage of the glycosidic linkage of the riboside residue, opening of laevoglucosan 1,6-anhydro ring, and the subsequent acetylation of the hydroxylic function at position 6 of the resulting glucosyl bromide. As shown by the reaction of the isopropylidene derivative *XVI*, the dibenzyl derivative *XXII*, and laevoglucosan with solutions of hydrogen bromide in the solvent mixture acetic acid–acetic anhydride (concentration of these solutions was varied), the bonds are cleaved in the order stated while the acid-catalysed acetylation obviously represents the fastest reaction.

The configuration at the anomeric centers 1 and 1' of the exotoxin diglycoside fragment *III* was found[16] β and α, respectively. It was therefore of interest to prepare the anomeric diglycoside ether the configuration of which at both anomeric centers would be β (see Scheme 3). Compound *XL* was converted by the action of 2,2-dimethoxypropane and acetone in dimethylformamide in the presence of an acid to the acetonide *XLIII* the mass spectrum of which was identical with that of the acetonide *II* obtained from the naturally occurring material[16]. The ether *XLIII* was acetylated as usual to afford a high yield of the crystalline methyl 2,3,6-tri-O-acetyl-4-O-(methyl 2,3-O-isopropylidene-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XLIV*). The NMR spectrum of the triacetate *XLIV* was different from that of compound *III*

obtained from the naturally occurring material especially in chemical shifts of H_1 and $H_{2'}$ protons and the $J_{1',2'}$ constant; for details see the Experimental Part.

The fundamental fragment *III* was finally prepared from compound *XXV*. The ring opening of laevoglucosan by the action of methanolic hydrogen chloride at an elevated temperature affords at least 70% of methyl α-D-glucopyranoside while the methyl ribofuranoside protected at position 5 yields under analogous conditions predominantly the β-anomer. Reaction of the crystalline diglycoside ether *XXV* containing the methyl riboside and the laevoglucosan residues with methanolic hydrogen chloride in a sealed tube at 100°C afforded an anomeric mixture in which methyl 4-O-(methyl 5-deoxy-β-D-ribofuranosid-5-yl)-α-D-glucopyranoside (*XLV*) predominated. This compound was finally converted to the fragment *III*. The NMR spectrum of the synthetic product and the degradation product of exotoxin both in deuteriochloroform and hexadeuteriobenzene was in every respect identical. Also the mass spectra were identical. The present synthesis thus represents the unequivocal confirmation of the structure of the fundamental and more complex fragment of exotoxin.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 20°C/0·1 Torr, unless stated otherwise. Infrared spectra were measured in chloroform.

Methyl 5-O-*p*-Toluenesulfonyl-2,3-O-benzylidene-β-D-ribofuranoside (*IV*)

A mixture of methyl β-D-ribofuranoside (5 g), benzaldehyde (27 ml), freshly fused zinc chloride (2·8 g), acetic acid (2 ml), and molecular sieve Potassit 3 (5 g) was mechanically shaken for 2 hours at room temperature. After 20 hours, the suspension was filtered, the molecular sieve washed with benzaldehyde (two 5 ml portions), and the filtrates poured into a mixture of water (100 ml) and ice (50 g). The aqueous layer was extracted with three 30 ml portions of chloroform, the organic solutions combined, dried, and evaporated under diminished pressure. The residue was applied to a column of 250 g of neutral aluminum oxide (Brockmann activity II—III) in benzene. The column was washed with benzene (750 ml) and then moist ethyl acetate (600 ml). The ethyl acetate eluate was dried and evaporated under diminished pressure to afford 5·9 g (77%) of the oily methyl 2,3-O-benzylidene-β-D-ribofuranoside. The product was dissolved in pyridine (100 ml), the solution evaporated under diminished pressure, the residue redissolved in pyridine (70 ml), and the solution treated with *p*-toluenesulfonyl chloride (7 g). The reaction mixture was heated at 50°C for 6 hours and allowed to stand at room temperature for 3 days. Water (0·5 ml) was then added, the suspension allowed to stand for 10 minutes, and evaporated under diminished pressure. The residue was diluted with benzene (80 ml) and the resulting solution washed successively with saturated aqueous sodium hydrogen sulfate (until the reaction was acidic to Congo Red), water (150 ml), and saturated aqueous potassium hydrogen carbonate (100 ml). After drying, the benzenic solution was filtered through a column of 120 g of neutral aluminum oxide (Brockmann activity II) in benzene. The eluate was evaporated under diminished pressure, the residue crystallised from ethanol (15 ml), and the mother liquors processed as usual to afford 6·5 g of compound *IV* (62%; 48%, referred to the starting methyl ribofuranoside), m.p. 100—105°C. Recrystallisation from ethanol did not change the melting point. Infrared spectrum: $\nu(SO_2)_{as}$ 1 367 cm^{-1} and $\nu(SO_2)_s$ 1 190 and 1 176 cm^{-1}. For $C_{20}H_{22}O_7S$ (406·4) calculated: 59·10% C, 5·46% H, 7·89% S; found: 59·21% C, 5·41% H, 7·73% S.

Methyl 5-Iodo-5-deoxy-2,3-O-benzylidene-β-D-ribofuranoside (*V*)

A mixture of the benzylideneribofuranoside from the preceding paragraph (11 g) and 0·80M-$[(C_6H_5O)_3P^+CH_3]I^-$ in dimethylformamide (70 ml) was allowed to stand at room temperature for 24 hours, diluted with benzene (700 ml), and washed successively with water (1 000 ml), 10% aqueous sodium thiosulfate (500 ml) and three 800 ml portions of water. The benzenic solution was dried, evaporated under diminished pressure, and the residue applied to a column of neutral aluminum oxide (1 000 g; Brockmann activity II). The column was eluted with benzene (3 000 ml; fractions 1—15) at the flow-rate of 600 ml per hour. The chromatographically homogeneous fractions 4—8 (R_F value 0·4 on a thin layer of silica gel with binder, in benzene) were combined and evaporated to afford 11·5 g (73%) of sirupous 5-iodo derivative, $[\alpha]_D^{25}$ −42·5° (chloroform, *c* 0·80). For $C_{13}H_{15}IO_4$ (362·2) calculated: 43·11% C, 4·18% H, 35·04% I; found: 43·39% C, 4·19% H, 35·24% I.

Methyl 2,3-Di-O-benzyl-6-O-triphenylmethyl-α-D-glucopyranoside (*VI*)

A mixture of methyl 2,3-di-O-benzyl-α-D-glucopyranoside[32] (29 g), recrystallised triphenylmethyl chloride (30 g), and pyridine (320 ml) was allowed to stand at room temperature for 10 days and then decomposed by the addition of water (1 ml). After 15 minutes at 20°C, the mixture was evaporated under diminished pressure and the residue dissolved in benzene (500 ml). The solution was washed with water (800 ml) and saturated aqueous potassium hydrogen carbonate, dried, and evaporated. The oily residue was applied to a column of silica gel (800 g; deactivated with 10% of water), packed in benzene. The column was eluted with benzene (5 400 ml); fractions 1—12) and the solvent mixture (15 : 1) benzene–ethyl acetate (4 500 ml; fractions 13—22). The chromatographically homogeneous fractions 9—19 were combined and evaporated to afford 45·1 g (95%) of compound *VI* in the form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ +9·2° (*c* 0·51; chloroform); reported[19], $[\alpha]_D^{26}$ +14·5° (*c* 3; chloroform). For $C_{40}H_{40}O_6$ (616·8) calculated: 77·90% C, 6·54% H; found: 77·84% C, 6·51% H.

Methyl 2,3-O-Cyclocarbonyl-β-D-ribofuranoside (*X*)

A. Via *methyl* 5-O-*triphenylmethyl*-β-D-*ribofuranoside* (XI). Triphenylmethyl chloride (14·2 g; 51 mmol) was added to a solution of methyl ribofuranoside (8·2 g; 50 mmol) in pyridine (80 ml), the reaction mixture allowed to stand at room temperature for 5 days, diluted with water (1 ml), and evaporated under diminished pressure (*cf.* ref.[23]). The residue was diluted with chloroform (120 ml) and the resulting chloroform solution washed successively with water (500 ml), 3% aqueous potassium hydrogen sulfate (500 ml), and water (200 ml) again. After drying, the solution was evaporated under diminished pressure and the residue dissolved in pyridine (100 ml). A solution of phosgene (14 g) in toluene (350 ml) was then added at 0°C under mechanical stirring, the reaction mixture allowed to stand at room temperature for one hour, and decomposed under cooling by the addition of water (total 500 ml). The toluene layer was washed successively with 0·1% aqueous sodium chloride (400 ml), 1% aqueous hydrochloric acid (400 ml), and 0·1% aqueous sodium chloride again (500 ml). The toluene solution was dried, evaporated under diminished pressure, and the residue refluxed for 25 minutes in 80% aqueous acetic acid (50 ml). The mixture was then kept at 0°C for 2 hours, the precipitate of triphenyl carbinol filtered off, and washed at 0°C with three 15 ml portions of 80% aqueous acetic acid. The filtrate and the washings were combined, evaporated under diminished pressure, the residue coevaporated with acetic acid and then two 100 ml portions of toluene. The final residue was dissolved in benzene (40 ml) and water (80 ml). The aqueous layer was filtered with active charcoal and the filtrate

evaporated under diminished pressure (bath temperature up to 23°C). The residue was coevaporated with two 70 ml portions of ethanol, recrystallised from carbon tetrachloride (60 ml) at 0°C, and the mother liquors processed as usual to afford total 7·8 g (82%) of the cyclic carbonate *X*, m.p. 49–50°C; reported[24], m.p. 50–51°C. For $C_7H_{10}O_6$ (190·2) calculated: 44·22% C, 5·30% H; found: 43·99% C, 5·19% H.

B. *With the use of benzyl chloroformate.* An emulsion of methyl ribofuranoside (16·4 g; 100 mmol), benzyl chloroformate (68 g; 400 mmol), and water (50 ml) was treated dropwise under vigorous stirring with 2M-NaOH (200 ml) at such a rate that the temperature did not exceed 5°C. After 60 minutes in the ice bath, the reaction mixture was extracted with benzene (100 ml) and the organic layer washed with water. After drying, the benzenic solution was evaporated under diminished pressure and the residue diluted with a mixture of ether–light petroleum to afford 9·5 g (29%) of methyl 5-O-benzyloxycarbonyl-2,3-O-cyclocarbonyl-β-D-ribofuranoside (*IX*), m.p. 122–124°C. Recrystallisation of this product from the same solvent mixture increased the melting point to the value of 124°C. Infrared spectrum of compound *IX*: ν(C=O) cyclic carbonate at 1814 cm^{-1} and carbonate at 1753 cm^{-1}. Optical rotation: $[\alpha]_D^{25}$ −55·4° (chloroform, *c* 0·50). For $C_{15}H_{16}O_8$ (324·3) calculated: 55·56% C, 4·97% H; found: 55·43% C, 4·79% H.

Hydrogenolysis of the ester IX. A mixture of the ester *IX* (5 g), glacial acetic acid (80 ml), and 10% palladium on carbon catalyst (200 mg) was hydrogenolysed at room temperature for 2 hours by introduction of hydrogen until the evolution of carbon dioxide ceased. The reaction mixture was filtered through Celite 545 (1 g), the filtrate evaporated under diminished pressure, and the residue crystallised from carbon tetrachloride to afford the cyclic carbonate *X*, m.p. 49–51°C, undepressed on admixture with a specimen obtained by procedure *A*. Yield, 96%.

Reaction of Methyl 2,3-O-Cyclocarbonyl-β-D-ribofuranoside (*X*) with 2-O-*p*-Toluenesulfonyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*XVII*)

A mixture of the carbonate *X* (250 mg; 1·31 mmol) and the epoxide[20] *XVII* (350 mg; 1·17 mmol) in benzene (20 ml) containing 0·1% of boron trifluoride etherate was refluxed for 3 hours. After cooling down, the reaction mixture was diluted with chloroform (50 ml) and water (20 ml). The organic layer was washed with two 50 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was applied to a column of silica gel (60 g; deactivated with 8% of water). The column was eluted successively with benzene (100 ml), benzene–ethyl acetate 19 : 1 (200 ml), benzene–ethyl acetate 9 : 1 (200 ml), benzene–ethyl acetate 5 : 1 (300 ml; fractions 1–18), benzene–ethyl acetate 2 : 1 (300 ml; fractions 19–34), and benzene–ethyl acetate 1 : 1 (400 ml; fractions 35–55). The chromatographically homogeneous fractions 38–43 (thin-layer chromatography on silica gel with binder: R_F value 0·1 in 7 : 3 benzene–ethyl acetate, R_F value 0·65 in ethyl acetate) were combined, evaporated under diminished pressure and coevaporated with three 30 ml portions of benzene. The residue (a solid foam) was dried for 2 days in an evacuated desiccator over sulfuric acid and potassium hydroxide. Yield, 208 mg (36%) of compound *XXIII*, $[\alpha]_D^{25}$ −63·6° (*c* 0·49; chloroform). Infrared spectrum: ν(O—H) (bonded) at 3593 cm^{-1}, 3530 cm^{-1}, ν(C=O) at 1809 cm^{-1}, $\nu(SO_2)_{as}$ at 1372 cm^{-1} and $\nu(SO_2)_s$ at 1190 and 1177 cm^{-1}. NMR spectrum in deuteriochloroform: δ 5·12 (s, 1-H, $J_{1,2}$ 0·5), 4·97 (d, 2-H, $J_{2,3}$ 6·5), 5·16 (d, 3-H, $J_{3,4} \neq 0$), 4·49 (q, 4-H, $J_{4,5a}$ 5·8 and $J_{4,5b}$ 7·5), 3·55–3·80 (m, 2 × 5-H), 5·33 (broad s, 1′-H, $J_{1',2'}$ 1·3 and $J_{1',3'} \neq 0$), 4·28 (dd, 2′-H, $J_{2',3'}$ 3·0), 3·90 (m, 3′-H), ~3·30 (m, 4′-H), 4·52–4·68 (m, 5′-H), 4·00 (d, $6'_{en}$-H, $J_{6'_{en},5'}$ 1·0 and $J_{6'_{en},6'_{ex}}$ 7·8), 3·64 (q, $6'_{ex}$-H, $J_{6'_{ex},5'}$ 3·0 c.p.s.), 3·34 (s, CH_3), 2·44 (s, CH_3 of the *p*-toluenesulfonyl group), and 2·80 p.p.m. (broad s, OH). For $C_{20}H_{24}O_{12}S$ (488·5) calculated: 49·17% C, 4·95% H, 6·56% S; found: 49·61% C, 4·98% H, 6·28% S.

4-O-(Methyl 5-Deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXV*)

A solution of the *p*-toluenesulfonate *XXIII* (0·9 g), ethereal 0·7M-HCl (0·5 ml), dihydropyran (2 ml), and dioxane (10 ml) was allowed to stand at room temperature for 40 hours and then treated under magnetical stirring with silver oxide (2·5 g). The neutral suspension was filtered, the filtrate diluted with 96% ethanol (85 ml), nad treated under vigorous stirring with ten portions of 2% sodium amalgam (total 100 g), the pH of the reaction mixture being held in the range of 6·5—8·0 by additions of acetic acid. After one hour of vigorous stirring, the reaction mixture was treated with water (30 ml), the aqueous-organic layer separated, and filtered with active charcoal. The filtrate was acidified (pH 2·5) with concentrated hydrochloric acid, kept at room temperature for 15 minutes, and neutralised with aqueous potassium hydrogen carbonate. The neutral solution was evaporated under diminished pressure, the residue diluted with water (40 ml), the aqueous solution washed with chloroform (50 ml), then at 0°C with a saturated solution of chlorine in chloroform (30 ml), and with chloroform again (50 ml). The aqueous layer containing compound *XXV* was evaporated under diminished pressure, the residue coevaporated with two 10 ml portions of acetic acid, and finally treated with acetic anhydride (20 ml) and fused sodium acetate (2 g). The reaction mixture was refluxed for 10 minutes, cooled, evaporated under diminished pressure, the residue diluted with water (20 ml), and extracted with chloroform (30 ml). The chloroform extract was washed with water (15 ml), dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (10 g; deactivated by the addition of 10% of water) in 1 : 1 benzene–ethyl acetate. The column was eluted in the course of 4 hours with 50 ml of the same solvent mixture (fractions 1—25). The chromatographically homogeneous fractions 11—17 (thin-layer chromatography on silica gel with binder: R_F value 0·12 in 7 : 3 benzene–ethyl acetate and R_F value 0·4 in 1 : 1 benzene–ethyl acetate) were combined and processed as usual to afford 95 mg (11%) of the tetraacetate *XXVI* in the form of a solid foam. The analytical sample was dried for 8 hours over phosphorus pentoxide at 50°C/0·1 Torr. NMR spectrum in deuteriochloroform: δ 4·91 (d, 1-H, $J_{1,2}$ 1·1), 5·21 (q, 2-H, $J_{2,3}$ 4·5), 5·37 (q, 3-H, $J_{3,4}$ 6·5), 4·15—4·35 (m, 4-H), 3·80—3·90 (m, 2 × 5-H), 4·45 (broad s, 1′-H, $J_{1',2'} \neq 0$), 4·61 (m, 2′-H), 4·91 (m, 3′-H), 3·45 (m, 4′-H), 4·60—4·75 (m, 5′-H), 3·96 (q, 6′$_{en}$-H, $J_{6'_{en},5'}$ 1·0 and $J_{6'_{en},6'_{ex}}$ 7·3), 3·78 (q, 6′$_{ex}$-H, $J_{6'_{ex},5'}$ 5·5 c.p.s.), 3·37 (s, CH_3) and 2·03,2·08, 2·10, 2·12 p.p.m. (s, 4 × CH_3 of acetyl groups). For $C_{20}H_{28}O_{13}$ (476·4) calculated: 50·42% C 5·93% H; found: 50·97% C, 5·98% H.

The acetate *XXVI* (120 mg) was deblocked by the action of 3 ml 0·01M-$NaOCH_3$ in methanol (3 hours at room temperature). The free glycoside *XXV* crystallised from a mixture methanol–ether after seeding with a specimen obtained by debenzoylation of the ester *XXXVI*. Yield, 52 mg (67%, referred to the tetraacetate *XXVI*); m.p. 132—133°C, undepressed on admixture with an authentic sample. For $C_{12}H_{20}O_9.H_2O$ (326·3) calculated: 44·17% C, 6·80% H; found: 44·39% C, 6·78% H.

4 O-(Methyl 2,3-Di-O-benzoyl-5-Deoxy-β-D-ribofuranosid-5-yl)-1,6 : 2,3-dianhydro-β-D-mannopyranose (*XXX*)

A mixture of the tosyl derivative *XXIII* (244 mg; 0·50 mmol) and methanolic 1M-$NaOCH_3$ (4 ml) was allowed to stand at room temperature for 2½ hours, neutralised by the addition of Amberlite-IRC 50 (H^+) ion exchange resin, filtered, and the filtrate evaporated under diminished pressure. The residue was coevaporated with two 10 ml portions of pyridine, and dissolved in pyridine (5 ml). Benzoyl chloride (0·5 ml) was added, the mixture allowed to stand at room temperature overnight, decomposed with water (0·1 ml), and after 10 minutes evaporated under diminished pressure. The residue was dissolved in chloroform (10 ml), the solution washed

successively with 1% aqueous hydrochloric acid, 0·1% aqueous sodium chloride, and saturated aqueous potassium hydrogen carbonate, dried, and evaporated under diminished pressure. The residue was filtered through a column of neutral aluminium oxide (20 g; Brockmann activity II—III) packed in benzene. The eluate (100 ml) was evaporated under diminished pressure, the residue applied to a column of silica gel (20 g; deactivated by the addition of 10% of water), and the column eluted successively with benzene (50 ml), 19 : 1 benzene–ethyl acetate (120 ml; fractions 1—5), and 9 : 1 benzene–ethyl acetate (120 ml; fractions 6—10). The chromatographically homogeneous fractions 6 and 7 (thin-layer chromatography on silica gel with binder: R_F value 0·3 in 9 : 1 benzene–ethyl acetate) were pooled, evaporated under diminished pressure, and the residue crystallised from ether to afford 85 mg (34%) of the dibenzoate *XXX*, m.p. 125·5 to 127°C; $[\alpha]_D^{25}$ +27·5° (*c* 0·50, chloroform). Infrared spectrum: ν(C=O) at 1727 cm^{-1}. NMR spectrum in deuteriochloroform: δ 5·17 (broad s, 1-H, $J_{2,1}$ 0), 5·55—5·75 (m, 2-H and 3-H), 4·45—4·60 (m, 4-H), 3·90—4·05 (m, 2 × 5-H), 5·65 (broad s, 1′-H, $J_{1',2'} \neq 0$ c.p.s.), 3·60—3·75 (m, 3′-H), 3·25 (m, 4·-H), 4′45—4·60 (m, 5′-H), 3·60—3·75 (m, 2 × 6′-H) and 3·48 p.p.m. (s, CH_3). For $C_{26}H_{26}O_{10}$ (498·5) calculated: 62·65% C, 5·26% H; found: 62·47% C, 5·28% H.

Methyl 2,3-Di-O-benzoyl-β-D-ribofuranoside (*XIV*)

A solution of methyl 5-O-triphenylmethyl-β-D-ribofuranoside (prepared from 8·2 g of methyl ribofuranoside, *vide supra*) in pyridine (80 ml) was treated dropwise under cooling (iced water) with benzoyl chloride (14 ml), the reaction mixture allowed to stand at room temperature for one day, decomposed with water (2 ml), and evaporated under diminished pressure. The residue was dissolved in benzene (100 ml), the benzenic solution washed successively with 0·1% aqueous sodium chloride (500 ml), 0·5% aqueous hydrochloric acid (500 ml), and saturated aqueous potassium hydrogen carbonate (100 ml). After drying, the benzenic solution was filtered through a column of neutral aluminum oxide (500 g; Brockmann actuvity II—III) packed in benzene. The eluate (3000 ml) was evaporated under diminished pressure, the residue (a solid foam) coevaporated under diminished pressure with two 250 ml portions of glacial acetic acid, and hydrogenolysed at 60°C/130 atm of hydrogen in glacial acetic acid (300 ml) over 10% palladium on carbon catalyst (4 g). After 5 hours, the mixture was cooled, filtered, and the filtrate evaporated under diminished pressure. The residue was coevaporated with three 500 ml portions of toluene and applied to a column of neutral aluminum oxide (300 g; Brockmann activity III). The column was eluted with 30 : 1 benzene-ethyl acetate (1000 ml; fraction 1) and 1 : 1 benzene–ethyl acetate (water content, 0·5%; 1200 ml; fraction 2). Work-up of fraction 2 afforded 7·1 g of the ester *XIV* in the form of a solid foam. Thin-layer chromatography on silica gel with binder: R_F value 0·08 in 19 : 1 benzene–ethyl acetate and R_F value 0·6 in 7 : 3 benzene–ethyl acetate. Infrared spectrum: ν(C=O) at 1720 cm^{-1} and ν(OH) bonded at 3593 and 3505 cm^{-1}. For $C_{20}H_{20}O_7$ (372·4) calculated: 64·52% C, 5·41% H; found: 64·20% C, 5·57% H.

Reaction of Methyl 2,3-Di-O-benzoyl-β-D-ribofuranoside (*XIV*) with 2-O-*p*-Toluenesulfonyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*XVII*)

A solution of the dibenzoate *XIV* (370 mg; 1·0 mmol) and the tosyl epoxide *XVII* (239 mg; 0·80 mmol) in 15 ml of benzene containing 0·1% of boron trifluoride etherate was refluxed for 140 minutes. After cooling, the reaction mixture was diluted with ethyl acetate (20 ml), washed with three 100 ml portions of water and saturated aqueous potassium hydrogen carbonate (20 ml), dried, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (50 g; deactivated by the addition of 10% of water). The column was eluted successively with benzene (250 ml), 9 : 1 benzene–ethyl acetate (300 ml; fractions 1—30), and 3 : 1 benzene–

ethyl acetate (300 ml; fractions 31—60). The chromatographically homogeneous fractions (thin-layer chromatography on silica gel with binder: R_t value 0·3 in 3 : 1 benzene–ethyl acetate) 34—39 were pooled, evaporated under diminished pressure, and the light yellow residue coevaporated with three 20 ml portions of anhydrous benzene to afford 21% of the amorphous ester *XXVII*. Infrared spectrum: $\nu(SO_2)_{as}$ at 1 370 cm^{-2} and $\nu(SO_2)_s$ at 1 190 cm^{-1}, $\nu(C{=}O)$ at 1 727 cm^{-1}, $\nu(O{-}H)$ bonded at 3 590 and 3 525 cm^{-1}. For $C_{33}H_{34}O_{13}S$ (670·7) calculated: 59·10% C, 5·11% H, 4·79% S; found: 59·52% C, 5·28% H, 4·50% S.

Treatment of compound *XXVII* with methanolic sodium methoxide and the subsequent benzoylation (for conditions see the ester *XXIII*) afforded the mannopyranose derivative *XXX* in a 38% yield; m.p. 125—127°C.

Methyl 2,3-Di-O-*p*-toluenesulfonyl-β-D-ribofuranoside (*XV*)

A solution of methyl 5-O-triphenylmethyl-β-D-ribofuranoside (prepared from 4·1 g, *i.e.* 25 mmol, of methyl ribofuranoside) in pyridine (50 ml) was treated in one lot with *p*-toluenesulfonyl chloride (11·3 g; 60 mmol), the mixture allowed to stand at room temperature for 2 days, and decomposed with water (1 ml). The suspension was evaporated under diminished pressure, the residue dissolved in benzene (200 ml), and the benzenic solution washed successively with water (500 ml), 0·1% aqueous hydrochloric acid (200 ml), and 5% aqueous potassium hydrogen carbonate (100 ml). After drying, the benzenic solution was filtered through a column of neutral aluminium oxide (300 g; Brockmann activity II) with the use of 30 : 1 benzene–ethyl acetate (1 200 ml). The eluate (*cf.* ref.[26]) was evaporated under diminished pressure, the residue coevaporated with two 100 ml portions of glacial acetic acid, and finally refluxed in 80% aqueous acetic acid (50 ml) for 20 minutes. The mixture was allowed to stand at 0°C for one hour, the precipitate of triphenylmethanol filtered off, washed at 5°C with three 10 ml portions of 80% aqueous acetic acid, the filtrates combined, and evaporated under diminished pressure. The residue was coevaporated with two 200 ml portions of toluene and applied to a column of neutral aluminum oxide (300 g; Brockmann activity II). The column was eluted with 30 : 1 benzene–ethyl acetate (1 200 ml; fraction 1) and 1 : 1 benzene–ethyl acetate (1 000 ml; fraction 2). Work-up of fraction 2 afforded 7·6 g (64%) of the di-*p*-toluenesulfonate *XV* in the form of a solid foam. The analytical sample was dried for 8 hours over phosphorus pentoxide at 50°C/0·1 Torr. Infrared spectrum: $\nu(SO_2)_{as}$ at 1 366 cm^{-1}, $\nu(SO_2)_s$ at 1 192 and 1 178 cm^{-1}, $\nu(O{-}H)$ bonded at 3 580—3 590 cm^{-1}. For $C_{20}H_{24}O_9S_2$ (472·5) calculated: 50·84% C, 5·13% H, 13·57% S; found: 51·23% C, 5·20% H, 13·20% S.

Reaction of Methyl 2,3-Di-O-*p*-toluenesulfonyl-β-D-ribofuranoside (*XV*) with 2-O-*p*-Toluenesulfonyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*XVII*)

A mixture of the tosyl epoxide *XVII* (447 mg; 1·50 mmol) and the di-*p*-toluenesulfonate *XV* (475 mg; 1·0 mmol) in 17·5 ml of benzene containing 0·1% of boron trifluoride etherate was refluxed for 160 min, cooled, washed with 5% aqueous potassium hydrogen carbonate (20 ml), dried over anhydrous magnesium sulfate, and applied to a column of silica gel (50 g; deactivated by the addition of 8% of water). The column was eluted successively with 19 : 1 benzene–ethyl acetate (300 ml), 9 : 1 benzene–ethyl acetate (300 ml), 17 : 3 benzene–ethyl acetate (300 ml; fractions 1—30), and 4 : 1 benzene–ethyl acetate (200 ml; fractions 31—50). The chromatographically homogeneous fractions 25—41 (thin-layer chromatography on silica gel with binder: R_F value 0·05 in 9 : 1 benzene–ethyl acetate and R_F value 0·40 in 7 : 3 benzene–ethyl acetate) were combined, evaporated under diminished pressure, and the residue coevaporated with three 10 ml portions of benzene to afford 416 mg (54%) of compound *XXVIII* in the form of a solid foam. The analytical sample was dried analogously to the dibenzoate *XXVII*. Infrared spectrum

of compound *XXVIII*: $\nu(SO_2)_{as}$ at 1365 cm^{-1}, $\nu(SO_2)_s$ 1193 and 1179 cm^{-1}, ν(O—H) bonded at about 3540 cm^{-1}. For $C_{33}H_{38}O_{15}S_3$ (770·8) calculated: 51·41% C, 4·97% H, 12·48% S; found: 51·82% C, 5·23% H, 12·12% S.

Methyl 2,3-O-Isopropylidene-β-D-ribofuranoside (*XVI*)

A mixture of methyl ribofuranoside (10 g), methyl orthoformate (9 g), ethereal 6M-HCl (0·25 ml), and acetone (200 ml; dried by distillation with calcium chloride) was stirred magnetically until the solid dissolved. The solution was allowed to stand at room temperature overnight, neutralised with concentrated aqueous ammonia, evaporated under diminished pressure, the residue dissolved in water (100 ml), and the product extracted with five 50 ml portions of ether. The ethereal extracts were combined, dried, evaporated under diminished pressure and the residue distilled to afford 9·7 g (78%) of compound *XVI*, b.p. 88—89°C/0·6 Torr, chromatographically homogeneous. Thin-layer chromatography on silica gel with binder: R_F value 0·5 in 7 : 3 benzene–ethyl acetate.

2-O-Benzyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*XIX*)

A mixture of the hydroxy epoxide *XVIII* (42 g; prepared by modification of a known procedure[21] consisting in reduction of the tosyl epoxide *XVII* with sodium amalgam at the pH range of 6—8), silver oxide (180 g), and dimethylformamide (500 ml) was gradually heated under stirring to 90°C and treated at this temperature dropwise over 30 minutes with freshly distilled benzyl bromide (80 ml). The stirring was continued for additional 90 minutes, the mixture filtered at 60°C through a layer of Celite 545 in benzene and the precipitate washed thoroughly with three 200 ml portions of benzene. The filtrates were combined, evaporated under diminished pressure, the oily residue dissolved in benzene (250 ml), the solution washed with three 500 ml portions of 0·1% aqueous sodium chloride, dried, and applied to a column of neutral aluminum oxide (500 g; Brockmann activity II). The column was eluted with benzene (3500 ml), the eluate evaporated under diminished pressure, and the residue distilled to afford 48 g (70%) of compound *XIX*, b.p. 160°C/0·8 Torr, $[\alpha]_D^{25}$ −39·2° (*c* 0·50; chloroform). NMR spectrum in deuteriochloroform: δ 5·29 (broad s, 1-H, $J_{1,2}$ 1·5 and $J_{1,3}$ 0·7), 3·14 (q, 2-H, $J_{2,3}$ 4·4), 3·54—3·65 (m, 3-H and 4-H), 4·79 (t, 5-H, $J_{5,4}$ 4·9), 3·94 (d, 6_{en}-H, $J_{6en,5}$ 4·9 and $J_{6en,6ex}$ 6·7), 3·85 (q, 6_{ex}-H, $J_{6ex,5}$ 1·0 c.p.s.) and 4·72 p.p.m. (s, CH_2 of benzyl group). For $C_{13}H_{14}O_4$ (234·3) calculated: 66·66% C, 6·02% H; found: 66·98% C, 6·07% H.

4-O-Benzyl-1,6 : 2,3-dianhydro-β-D-mannopyranose (*XX*)

2-O-*p*-Toluenesulfonyl-4-O-benzyllaevoglucosan[20] (10 g) was added in one lot to methanolic sodium methoxide (prepared from 3·0 g of sodium and 55 ml of methanol). The resulting solution was allowed to stand at room temperature for 45 min, poured into cold water (750 ml), and the product extracted with benzene (200 ml). The benzenic solution was washed with two 500 ml portions of 0·1% aqueous sodium chloride, dried, evaporated under diminished pressure, and the oily residue crystallised from ether–light petroleum to afford 89% of compound *XX*, m.p. 59 to 60°C (this melting point did not change on recrystallisation), $[\alpha]_D^{25}$ −28° (*c* 0·50, chloroform); reported[27]: m.p. 63—65°C, $[\alpha]_D$ −34° (chloroform). NMR spectrum in deuteriochloroform: δ 5·65 (d, 1-H, $J_{1,2}$ 3·0 and $J_{1,3}$ 0·5), 3·39 (t, 2-H, $J_{2,3}$ 0·8 and $J_{2,3}$ 3·8), 3·65—3·80 (m, 3-H), 3·14 (dd, 4-H, $J_{4,5}$ 1·6 and $J_{4,3}$ 0·7 c.p.s.), 4·47 (m, 5-H), 3·65—3·80 (m, 2 × 6-H), 7·86 (m, 5 × H of benzyl group) and 4·70 p.p.m. (s, CH_2 of benzyl group). For $C_{13}H_{14}O_4$ (234·3) calculated: 66·66% C, 6·02% H; found: 66·75% C, 6·05% H.

2,4-Di-O-benzyl-1,6-anhydro-β-D-glucopyranose (*XXII*)

A. *From* 2-O-*benzyl*-1,6 : 3,4-*dianhydro*-β-D-*galactopyranose* (XIX). A solution of the benzyl epoxide *XIX* (1·0 g) in dimethylformamide (30 ml) was added to the solution of sodium benzylate (prepared from 0·5 g of sodium hydride and 3 ml of benzyl alcohol) and the reaction mixture refluxed under stirring for 75 minutes. After cooling down, the mixture was diluted with water (200 ml) and chloroform (25 ml). The chloroform layer was washed with two 300 ml portions of water, dried, evaporated under diminished pressure, and the residue applied to a column of silica gel (80 g; deactivated by the addition of 10% of water). The column was eluted successively with benzene (300 ml), 19 : 1 benzene–ethyl acetate (400 ml; fractions 1—5), and 9 : 1 benzene–ethyl acetate (400 ml; fractions 6—10). The chromatographically homogeneous fractions 7—9 were pooled, evaporated under diminished pressure, and the residue crystallised from ether (5 ml) to afford 1·15 g (79%) of the pyranose *XXII*; m.p. 107°C, in accordance with literature[28]. For $C_{20}H_{22}O_5$ (342·4) calculated: 70·16% C, 6·48% H; found: 70·27% C, 6·33% H.

B. *From* 4-O-*benzyl*-1,6 : 2,3-*dianhydro*-β-D-*mannopyranose* (XX). A solution of the benzyl epoxide *XX* (1·0 g) in dimethylformamide (30 ml) was added to the solution of sodium benzylate (prepared from 0·48 g of sodium hydride and 3 ml of benzyl alcohol), the reaction mixture refluxed for 80 minutes under stirring, and processed similarly to paragraph *A*. Yield, 36% of 2,4-di-O benzyl-D-laevoglucosan, m.p. 107°C, undepressed on admixture with the specimen obtained in paragraph *A*. Optical rotation: $[\alpha]_D^{25}$ −30·5° (*c* 0·50, chloroform), in accordance with literature[28]. NMR spectrum of compound *XXII* in deuteriochloroform: δ 5·44 (s, 1-H, $J_{1,2}$ 0·8), (dd, 2-H, $J_{2,3}$ 4·2), 3·85 (m, 3-H, $J_{3,4}$ 4·2), 3·30 (dd, 4-H, $J_{4,5}$ 1·0), 4·53 (dd, 5-H, $J_{5,6en}$ 1·0 and $J_{5,6ex}$ 5·2), 3·78 (dd, 6_{en} -H, $J_{6en,6ex}$ 7·5 c.p.s.) and 3·60 p.p.m. (q, 6_{ex}-H). For $C_{20}H_{22}O_5$ (342·4) calculated: 70·16% C, 6·48% H; found: 70·25% C, 6·46% H.

Reaction of Methyl 2,3-O-Isopropylidene-β-D-ribofuranoside (*XVI*) with 2-O-Benzyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*XIX*)

The benzyl epoxide *XIX* (705 mg; 3·0 mmol) was added in one lot to a solution of the isopropylidene derivative *XVI* (2·05 g; 10·0 mmol) in 17 ml of 0·48M-$NaCH_2SOCH_3$ in dimethyl sulfoxide. The reaction mixture was heated at 100—105°C for 2 hours under exclusion of atmospheric moisture, cooled down, and neutralised with gaseous carbon dioxide. The suspension was evaporated under diminished pressure and the residue diluted with benzene (20 ml) and water (200 ml). The benzenic layer was washed with 0·1% aqueous sodium chloride (three 200 ml portions), dried over anhydrous magnesium sulfate, and applied to a column of silica gel (50 g; deactivated by the addition of 10% of water). The column was eluted successively with benzene (100 ml), 19 : 1 benzene–ethyl acetate (150 ml), 9 : 1 benzene–ethyl acetate (150 ml), 4 : 1 benzene–ethyl acetate (150 ml; fractions 1—10), and 7 : 3 benzene–ethyl acetate (150 ml; fractions 11—20). The chromatographically homogeneous fractions 12—17 (thin-layer chromatography on silica gel with binder: R_F value 0·20 in 7 : 3 benzene–ethyl acetate) were pooled, evaporated to dryness under diminished pressure, and the residue coevaporated with three 20 ml portions of benzene to afford 777 mg (59%) of the benzyl derivative *XXXI* in the form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ +30·4° (*c* 0·49; chloroform). Infrared spectrum: ν(O—H) bonded at 3593 and 3460 cm^{-1}. NMR spectrum in deuteriobenzene: δ 5·02 (s, 1-H, $J_{1,2} \neq 0$), 4·50—4·75 (m, 2-H and 3-H), 3·20 (m, 4-H), 3·60—3·75 (m, 2 × 5-H), 5·49 (broad s, 1′-H, $J_{1',2'} \neq 0$ and $J_{1',3'} \neq 0$), 3·31 (d, 2′-H, $J_{2',3'}$ 4·9), 3·91 (t, 3′-H, $J_{3',4'}$ 5·5), 3·35—3·48 (m, 4′-H), 4·41 (m, 5′-H, $J_{5',4'}$ 2·5), 4·54 (m, $6'_{en}$-H, $J_{6'en,5'}$ 1·0 and $J_{6'en,6'ex}$ 7·8), 3·53 (q, 6_{ex}-H, $J_{6'ex5'}$ 5·0 c.p.s.), 3·18 (s, OCH_3), 1·21 and 1·44 (s, 2 × CH_3 of isopropylidene group), 4·55 (s, CH_2 of benzyl group), and 2·75 p.p.m. (broad s, OH). For $C_{22}H_{30}O_9$ (438·5) calculated: 60·26% C, 6·90% H; found: 60·56% C, 6·79% H.

Methyl 2,3,6-Tri-O-acetyl-4-O-(methyl2,3-di-O-acetyl-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XLI*)

A mixture of compound *XXXI* (329 mg; 0·75 mmol) and 35 ml of a 34% solution of hydrogen bromide in 10 : 1 acetic acid–acetic anhydride was stirred at room temperature until the solid dissolved, the solution allowed to stand for 2 hours, diluted with toluene (100 ml), and evaporated under diminished pressure. The residue was coevaporated with two 50 ml portions of toluene, treated with methanol (25 ml) and then, after two minutes, with silver oxide (2 g). After 25 minutes, the suspension was filtered, the filtrate evaporated under diminished pressure, and the residue applied to a column of silica gel (40 g; deactivated by the addition of 10% of water). The column was eluted successively with benzene (130 ml), 9 : 1 benzene–ethyl acetate (250 ml), 4 : 1 benzene–ethyl acetate (350 ml; fractions 1—25), and 7 : 3 benzene–ethyl acetate (350 ml; fractions 26—50). The chromatographically homogeneous fractions 27—44 (thin-layer chromatography on silica gel with binder: R_F 0·20 in 7 : 3 benzene–ethyl acetate) were pooled, evaporated under diminished pressure, and the residue coevaporated with three 25 ml portions of benzene to afford 24 mg (6%) of compound *XLI*. The analytical sample was dried for 10 hours over phosphorus pentoxide at 50°C/0·1 Torr. Infrared spectrum: ν(C=O) at about 1730 cm^{-1}. NMR spectrum in deuteriochloroform: δ4·86 (s, 1-H, $J_{1,2} = 0$), 5·10—5·30 (m, 2-H and 3-H), 4·12 (m, 4-H), 3·62—3·75 (m, 2 × 5-H), 4·38 (d, 1′-H, $J_{1',2'}$ 8·0), 4·85 (q, 2′-H, $J_{2',3'}$ 9·0 c.p.s.), 5·10 to 5·25 (m, 3′-H), 3·50—3·65 (m, 4′-H and 5′-H), 4·30—4·40 (m, 2 × 6′-H), 3·35 and 3·47 (s, 2 × OCH_3) and 2·02, 2·03, 2·05, 2·07 and 2·08 p.p.m. (s, 5 × CH_3 of acetyl groups). For $C_{23}H_{34}O_{15}$ (550·5) calculated: 50·18% C, 6·23% H; found: 50·76% C, 6·44% H.

2-O-Benzyl-3-O-benzoyl-4-O-(methyl2,3-di-O-benzoyl-5-deoxy-α,β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXXIII*)

A mixture of the ether *XXXI* (21·9; 50 mmol) and 80% aqueous acetic acid (300 ml) was refluxed for 35 minutes, cooled down, evaporated under diminished pressure, and the residue dried by coevaporation with three 300 ml portions of an 1 : 2 mixture of dioxane and toluene. The final residue was dissolved in methanolic 0·2M-HCl (300 ml), the solution allowed to stand at room temperature for 100 minutes, neutralised with pyridine (50 ml), evaporated under diminished pressure, the residue coevaporated with three 20 ml portions of pyridine, and the final residue dissolved in pyridine (400 ml). Benzoyl chloride (25 ml) was added dropwise under stirring and cooling (iced water), the resulting mixture heated at 45°C for 2 hours, and allowed to stand at room temperature overnight. Water (2 ml) was then added, the mixture kept at room temperature for 10 minutes, evaporated under diminished pressure, and the residue diluted with benzene (400 ml). The benzenic solution was washed successively with water (1000 ml), 1% aqueous hydrochloric acid (1200 ml), and saturated aqueous potassium hydrogen carbonate (150 ml), dried and applied to a column of silica gel (1000 g; deactivated by the addition of 10% of water). The column was eluted successively with benzene (500 ml), 100 : 3 benzene–ethyl acetate (4000 ml; fractions 1—4), 25 : 1 benzene–ethyl acetate (1500 ml; fractions 5 and 6), 19 : 1 benzene–ethyl acetate (1500 ml; fractions 7 and 8), and 50 : 3 benzene–ethyl acetate (7000 ml; fractions 9—16). The chromatographically homogeneous fractions 8—15 (thin-layer chromatography on silica gel with binder: R_F value 0·25 in 10 : 1 benzene–ethyl acetate) were combined, evaporated under diminished pressure, and the residue coevaporated with three 400 ml portions of benzene to afford 21·3 g (64%) of sirupous tribenzoate *XXXIII*. The analytical sample was purified by chromatography on a thin layer (plate, 17 × 43 cm) of loose silica gel (30—45 micron) (deactivated previously by the addition of 12% of water) in 6 : 1 benzene–ethyl acetate. Infrared spectrum: ν(C=O) at 1725 cm^{-1}. For $C_{40}H_{38}O_{12}$ (710·9) calculated: 67·60% C, 5·39% H; found: 67·86% C, 5·54% H.

2,3-Di-O-benzoyl-4-O-(methyl 2,3-di-O-benzoyl-5-deoxy-α,β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranoses (*XXXV* and *XXXVI*)

Compound *XXXIII* (22 g) was hydrogenolysed at room temperature and ordinary pressure in acetic acid (400 ml) over 10% palladium on carbon catalyst (5 g). After 4 hours, the suspension was filtered, the filtrate evaporated under diminished pressure, and the residue coevaporated with three 500 ml portions of toluene to afford an almost quantitative yield of a solid foam, R_F value 0·30 on a thin layer of silica gel with binder in 4 : 1 benzene–ethyl acetate. The analytical sample of compound *XXXIV* was obtained by chromatography on a thin layer of loose silica gel (*cf.* the purification of compound *XXXIII*) in 3 : 1 benzene–ethyl acetate. Optical rotation: $[\alpha]_D^{25}$ −16·0° (*c* 0·50; chloroform). For $C_{33}H_{32}O_{12}$ (620·6) calculated: 63·82% C, 5·19% H; found: 64·38% C, 5·36% H.

The freshly distilled benzoyl chloride (10 ml) was added in one lot to a solution of compound *XXXIV* (19 g) in pyridine (200 ml), the mixture heated at 50°C for 4 hours and then allowed to stand at room temperature for 2 days. Water (1 ml) was added, the mixture evaporated under diminished pressure, and the residue dissolved in water (500 ml) and benzene (300 ml). The benzenic solution was washed with 2% aqueous hydrochloric acid until acidic to Congo Red paper, then 0·1% aqueous sodium sulfate, and finally with concentrated aqueous potassium hydrogen carbonate (50 ml). After drying, the benzenic solution was applied to a column of neutral alumina (350 g; Brockmann activity II–III) and the column eluted with 19 : 1 benzene–ethyl acetate (1400 ml). The eluate was evaporated under diminished pressure and the residue chromatographed on a column of silica gel (1200 g) deactivated previously by the addition of 10% of water. The column was washed successively with benzene (1000 ml), 100 : 1 benzene–ethyl acetate (3500 ml, fractions 1 and 2), 100 : 2 benzene–ethyl acetate (3500 ml; fractions 3–6), 100 : 3 benzene–ethyl acetate (6000 ml; fractions 7–13), and 100 : 4 benzene–ethyl acetate (8000 ml; fractions 14–21) at the flow rate 1000 ml per hour. The chromatographically homogeneous fractions 10–16 (thin-layer chromatography on silica gel with binder: R_F value 0·40 in 9 : 1 benzene–ethyl acetate) were processed as usual to afford 15·3 g (70%) of the β-anomer *XXXVI* in the form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ +46·9° (*c* 0·50; chloroform). Infrared spectrum: ν(C=O) at 1725 cm^{-1}. NMR spectrum in deuteriochloroform: δ 3·36 p.p.m. (s, OCH_3). For $C_{40}H_{36}O_{13}$ (724·9) caculated: 66·27% C, 5·01% H; found: 66·49% C, 5·10% H. The chromatographically homogeneous fractions 18–21 (thin-layer chromatography as above: R_F value 0·31) afforded 2·3 g (11%) of the α-anomer *XXXV* in the form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ +66° (*c* 0·50; chloroform). NMR spectrum in deuteriochloroform: δ 3·42 p.p.m. (s, OCH_3). For $C_{40}H_{36}O_{13}$ (724·9) calculated: 66·27% C, 5·01% H; found: 66·51% C, 5·00% H.

4-O-(Methyl 5-deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXV*)

A solution of the tetrabenzoate *XXXVI* (400 mg; 0·55 mmol) in 10 ml of methanolic 0·004M-$NaOCH_3$ was allowed to stand at room temperature for 15 hours and then treated with Amberlite-IRC 50 (H^+) ion exchange resin. After 5 minutes, the reaction mixture was filtered, the filtrate evaporated under diminished pressure, and the residue dissolved in water (10 ml) and benzene (30 ml). The aqueous solution was washed with benzene (20 ml) and evaporated under diminished pressure. The residue was crystallised from methanol–ether to afford substance *XXV*, m.p. 132–133°C. The mother liquors were processed as usual; overall yield, 91%. Optical rotation: $[\alpha]_D^{25}$ −52·8° (*c* 0·50; water). For $C_{12}H_{20}O_9.H_2O$ (326·3) calculated: 44·17% C, 6·80% H; found: 44·29% C, 6·69% H.

2,3-Di-O-acetyl-4-O-(methyl 2,3-di-O-acetyl-5-deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XXVI*)

Acetylation (3 days at room temperature) of the glycoside *XXV* (40 mg) with acetic anhydride (0·3 ml) in pyridine (2 ml) afforded 89% of compound *XXVI*, m.p. 75—78°C (ether–light petroleum), which was isolated by column chromatography on silicagel Woelm (5 g) previously deactivated by the addition of 12% of water, with the use of 1 : 1 benzene–ethyl acetate (25 ml) as eluant. The tetraacetate *XXVI* was chromatographically homogeneous and identical with the specimen obtained from the ester *XXIII*. For $C_{20}H_{28}O_{13}$ (476·4) calculated: 50·42% C, 5·93% H; found: 50·78% C, 6·09% H.

1,6-Di-O-acetyl-2,3-di-O-benzoyl-4-O-(1-O-acetyl-2,3-di-O-benzoyl-5-deoxy-β-D-ribofuranos-5-yl)-α,β-D-glucopyranose (*XXXVII*)

A solution of the tetrabenzoate *XXXVI* (10·3 g) in acet c anhydride (180 ml) was treated with concentrated sulfuric acid (2·5 ml), the reaction mixture allowed to stand at room temperature for 20 minutes, and poured onto ice (500 g). After 5 minutes, there was added sodium acetate (30 g), water (3 liters), and benzene (250 ml). The benzenic layer was washed with 0·1% aqueous sodium chloride (three 2 liter portions) and saturated aqueous potassium hydrogen carbonate (50 ml). After drying, the benzenic solution was applied to a column of silica gel (400 g) previously deactivated by the addition of 10% of water. The column was eluted successively with 100 : 3 benzene–ethyl acetate (1 500 ml; fractions 1 and 2), 14 : 1 benzene–ethyl acetate (2 500 ml; fractions 3—7), and 9 : 1 benzene–ethyl acetate (3 000 ml; fractions 8—14) at the flow rate of 500 ml per hour. The chromatographically homogeneous fractions 7—13 (thin-layer chromatography on silica gel with binder: R_F value 0·30 in 4 : 1 benzene–ethyl acetate) were processed as usual to afford 9·0 g (74%) of compound *XXXVII* in the form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ + 72·8° (*c* 0·50; chloroform). Infrared spectrum: ν(C=O) of benzoyl group at 1 729 and 1 739 cm^{-1} and of acetyl group (shoulder) at about 1 750 cm^{-1}. For $C_{45}H_{42}O_{17}$ (854·8) calculated: 63·22% C, 4·95% H; found: 63·41% C, 5·03% H.

Methyl 2,3-Di-O-benzoyl-6-O-acetyl-4-O-(methyl 2,3-di-O-benzoyl-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XXXIX*)

A mixture of the triacetate *XXXVII* (2·4 g), dichloromethane (2·5 ml), acetyl bromide (0·1 ml), and 35% hydrogen bromide in glacial acetic acid (2·5 ml) was allowed to stand at room temperature for 75 minutes, diluted with toluene (50 ml), and evaporated under diminished pressure. The residue was coevaporated with three 50 ml portions of toluene, dissolved in dichloromethane (35 ml), the solution treated under stirring with methanol (15 ml) and then, after 2 minutes, with 4 g of silver oxide (in one lot). The stirring was continued at room temperature for 3 hours, the mixture filtered, and the filtrate evaporated under diminished pressure. The residue was applied to a column of neutral aluminum oxide (100 g; Brockmann activity II—III) and eluted with 19 : 1 benzene–ethyl acetate. The eluate was evaporated under diminished pressure and the residue chromatographed on a column of silica gel (240 g) deactivated previously by the addition of 10% of water. The column was washed successively with benzene (500 ml), 50 : 1 benzene–ethyl acetate (800 ml), 100 : 3 benzene–ethyl acetate (800 ml; fractions 1—3), 25 : 1 benzene–ethyl acetate (1 000 ml; fraction 4—7), 20 : 1 benzene–ethyl acetate (1 500 ml; fractions 8—13), 50 : 3 benzene–ethyl acetate (1 500 ml; fractions 14—19), and 100 : 7 benzene–ethyl acetate (1 000 ml; fractions 20—23). The chromatographically homogeneous fractions 16—22 (thin-layer chromatography on silicagel with binder: R_F value 0·28 in 5 : 1 benzene–ethyl acetate) were processed as usual to afford 1·50 g (48%) of the dimethyl diglycoside ether *XXXIX* in the

form of a solid foam. Optical rotation: $[\alpha]_D^{25}$ +68° (*c* 0·50; chloroform). Infrared spectrum: ν(C=O) at about 1730 cm^{-1}. NMR spectrum in deuteriochloroform: δ 3·24 and 3·51 (s, 2 × × OCH_3) and 2·10 p.p.m. (s, CH_3 of the acetyl group). For $C_{43}H_{42}O_{15}$ (798·8) calculated: 64·64% C, 5·29% H; found: 64·76% C, 5·18% H.

Methyl 4-O-(Methyl 5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XL*)

Methanolysis of the ester *XXXIX* by the action of methanolic sodium methoxide afforded an almost quantitative yield of the free glycoside *XL* in the form of a solid foam. For $C_{13}H_{24}O_{10}$ (340·3) calculated: 45·88% C, 7·11% H; found: 45·26% C, 7·16% H. Acetylation of the free glycoside *XL* with acetic anhydride in pyridine afforded the pentaacetate *XLI* which was identical with the specimen obtained by reaction of the halogenose *XLII* with methanol. For $C_{23}H_{34}O_{15}$ (550·5) calculated: 50·18% C, 6·23% H; found: 50·56% C, 6·40% H.

Methyl 2,3,6-Tri-O-acetyl-4-O-(methyl-2,3-O-isopropylidene-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranoside (*XLIV*)

A mixture of the glycoside *XL* (132 mg), dimethylformamide (2 ml), acetone (1 ml), 2,2-dimethoxy propane (1·5 ml) and ethereal 1·8M-HCl (0·15 ml) was allowed to stand at room temperature for 3 days and evaporated under diminished pressure (bath temperature up to 60°C). The residue was applied to a column of silica gel Woelm (12 g) deactivated by the addition of 12% of water. The column was eluted with 5 : 3 ethyl acetate–acetone (50 ml; fractions 1—30) at the flow rate of 12 ml per hour. The chromatographically homogeneous fractions 10—19 (thin-layer chromatography on silica gel with binder: R_F value 0·45 in 5 : 3 ethyl acetate–acetone) were processed as usual to afford 93% of the oily isopropylidene derivative *XLIII*. Mass spectrum: (M-15) ion at *m/e* 365 and D-ribose[15] ions at *m/e* 173, 141, and 115 in accordance with compound *II*. The oily derivative *XLIII* was coevaporated with two 10 ml portions of pyridine and finally mixed with pyridine (5 ml) and acetic anhydride (0·5 ml). The mixture was allowed to stand at room temperature for 15 hours, evaporated under diminished pressure, the residue coevaporated with three 10 ml portions of toluene, and crystallised from toluene–light petroleum to afford 140·5 mg of the acetate *XLIV*, m.p. 117·5—118°C. Work-up of mother liquors afforded an additional crop. Overal yield, 172 mg (87%) of the acetate *XLIV* (referred to the glycoside *XL*). Optical rotation: $[\alpha]_D^{25}$ −55·9° (*c* 0·50; chloroform). NMR spectrum in deuteriochloroform: δ 4·92 (s, 1-H, $J_{1,2} = 0$), 4·55 (s, 2-H and 3-H, $J_{2,1} = J_{3,2} = 0$ and $J_{3,4} < 1$), 4·23 (q, 4-H, $J_{4,5}$ 8·5), 3·50—3·65 (m, 2 × 5-H), 4·41 (d, 1′-H, $J_{1',2'}$ 8·0), 4·87 (q, 2′-H, $J_{2',3'}$ 9·5), 5·19 (q, 3′-H, $J_{3',4'}$ 6·0 c.p.s.), 3·50—3·65 (m, 4′-H and 5′-H), 4·30—4·43 (m, 2 × 6′-H), 3·28 (s, CH_3 in $C_{(1)}$—OCH_3), 3·49 (s, CH_3 in $C_{(1')}$-OCH_3), 2·04, 2·07, and 2·11 (s, 3 × CH_3 of acetyl groups), and 1·30, 1·46 p.p.m. (s, 2 × CH_3 of isopropylidene group). NMR spectrum in hexadeuteriobenzene: δ 4·87 (s, 1-H, $J_{1,2} = 0$), 4·48 (s, 2-H and 3-H, $J_{2,1} = J_{3,2} = 0$ and $J_{3,4} < 1$), 4·30 (q, 4-H), 3·58—3·75 (m, 2 × 5-H), 3·99 (d, 1-H, $J_{1',2'}$ 7·5), 4·99 (q, 2′-H, $J_{2',3'}$ 9·2), 5·19 (q, 3′-H, $J_{3',4'}$ 8·3), 3·23 (q, 4′-H, $J_{4',5'}$ 9·3), 3·03 (m, 5′-H, $J_{5',6'} \sim 4{\cdot}0$ c.p.s.), 4·10 to 4·21 (m, 2 × 6′-H), 2·95 (s, CH_3 in $C_{(1)}$-OCH_3), 3·08 (s, CH_3 in $C_{(1')}$-OCH_3), 1·59, 1·64, and 1·72 (s, 3 × CH_3 of acetyl groups), and 1·08, 1·31 p.p.m. (s, 2 × CH_3 of isopropylidene group). For $C_{22}H_{34}O_{13}$ (506·5) calculated: 52·16% C, 6·77% H; found: 52·27% C, 6·77% H.

Methyl 2,3,6-Tri-O-acetyl-4-O-(methyl 2,3-O-isopropylidene-5-deoxy-β-D-ribofuranosid-5-yl) -α-D-glucopyranoside (*III*)

A mixture of the substituted laevoglucosan *XXV* (117 mg; 0·357 mmol) and methanolic 0·12M-HCl (10 ml) was heated in a sealed tube for 4 hours at 95°C. After cooling down, the reaction

mixture was evaporated under diminished pressure and the residue mixed with dimethylformamide (1 ml), acetone (1 ml), 2,2-dimethoxypropane (1 ml), and ethereal 1·8M-HCl (0·15 ml). The reaction mixture was allowed to stand at room temperature for one day and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel Woelm (12 g) previously deactivated by the addition of 12% of water. The column was eluted with 5 : 3 ethyl acetate–acetone (60 ml; fractions 1—30). Fractions 26—29 were chromatographically homogeneous, as shown by thin-layer chromatography in the solvent mixture stated (R_F value 0·45). Fractions 21—25 contained the product along with a faster migrating by-product (R_F value 0·52 under analogous conditions) and were therefore subjected to two rechromatographies. The homogeneous fractions from the chromatography 1 to 3 containing exclusively the compound possessing the R_F value 0·45 afforded 33·6 mg (27%) of the oily isopropylidene derivative *II*. Mass spectrum: (M — 15) ion at *m/e* 365 and D-ribose[15] ion at *m/e* 173, 141, and 115. The mass spectrum was identical with that of an authentic specimen[15]. A mixture of the above isopropylidene derivative *II* (26·5 mg), pyridine (1 ml), and acetic anhydride (0·4 ml) was allowed to stand at room temperature for 20 hours, evaporated under diminished pressure, the residue coevaporated with three 5 ml portions of toluene, and chromatographed on a column of silica gel Woelm (7·5 g) previously deactivated by the addition of water (12%) with the use of 7 : 3 benzene–ethyl acetate (45 ml; fractions 1—24). The chromatographically homogeneous fractions 12—22 (their mobility was identical with that of compound *XIV*) afforded 82% of the oily ester *III*. Optical rotation: $[\alpha]_D^{25} + 34°$ (chloroform; *c* 0·50). NMR spectrum (deuteriochloroform): δ 4·84 (s, 1-H, $J_{1,2} = 0$), 4·47 (s, 2-H and 3-H, $J_{2,1} = J_{2,3} = 0$ and $J_{3,4} < 1$), 4·15 (q, 4-H), 3·38—3·58 (m, 2 × 5-H), 4·78 (m, 1′-H, $J_{1',2'}$ 3·5), 4·74 (q, 2′-H, $J_{2',3'}$ 10·0), 5·42 (m, 3′-H, $J_{3',4'}$ 9·0), 3·41 (m, 4′-H), 3·81 (sext., 5′-H, $J_{5',4'}$ 9·8, $J_{5',6'} = J_{5',6''}$ 3·3), 4·27 (d, 2 × 6′-H, $J_{6',5'} = J_{6'',5'}$ 3·3 c.p.s.) 3·22 (s, CH_3 in $C_{(1')}$-OCH_3), 3·32 (s, CH_3 in $C_{(1')}$-OCH_3), 1·99, 2·00, and 2·04 (s, 3 × CH_3 of acetyl groups), and 1·23, 1·39 p.p.m. (s, 2 × CH_3 of isopropylidene group). NMR spectrum in hexadeuteriobenzene: δ 4·89 (s, 1-H, $J_{1,2} = 0$), 4·51 (s, 2-H and 3-H, $J_{2,1} = J_{2,3} = 0$, $J_{3,4} < 1$), 4·36 (q, 4-H, $J_{4,3} < 1$), 4·40—4·60 (m, 2 × 5-H), 4·70 (d, 1′-H, $J_{1',2'}$ 3·6), 4·86 (q, 2′-H, $J_{2',3'}$ 9·8), 5·69 (q, 3′-H, $J_{3',4'}$ 8·9), 3·28 (t, 4′-H, $J_{4',5'}$ 9·5), 3·61 (sext., 5′-H, $J_{5',6'} = J_{5',6''}$ 3·5 c.p.s.), 4·20 (d, 2 × 6′-H), 2·97 (s, CH_3 in $C_{(1')}$-OCH_3), 2·85 (s, CH_3 in $C_{(1')}$-OCH_3), 1·56, 1·61, and 1·72 (s, 3 × CH_3 of acetyl groups), and 1·09, 1·33 p.p.m. (s, 2 × CH_3 of isopropylidene group). Both NMR spectra were in every detail identical with those of a specimen obtained by transformation of the exotoxin fragment[16]. Mass spectrum: (M — 15) ion at *m/e* 491·5 and (M — 31) ion at *m/e* 475·5. The mass spectrum of the synthetic product was in every detail identical with that of the substance prepared from the exotoxin fragment[16]. For $C_{22}H_{34}O_{13}$ (506·5) calculated: 52·16% C, 6·77% H; found: 52·60% C, 6·91% H.

Degradation of Laevoglucosan

A mixture of laevoglucosan (100 mg) and methanolic 0·12M-HCl (10 ml) was heated in a sealed tube for 4 hours at 95°C, cooled down, evaporated under diminished pressure, and the residue crystallised from ethanol at 20°C. Usual work up afforded 78 mg (65%) of methyl α-D-glucopyranoside, m.p. 165—167°C, undepressed on admixture with an authentic specimen[33].

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