SYNTHESIS OF EXOTOXIN PRODUCED BY *Bacillus thuringiensis*. I. FORMATION OF THE ETHEREAL BOND BETWEEN RIBOSE AND GLUCOSE*

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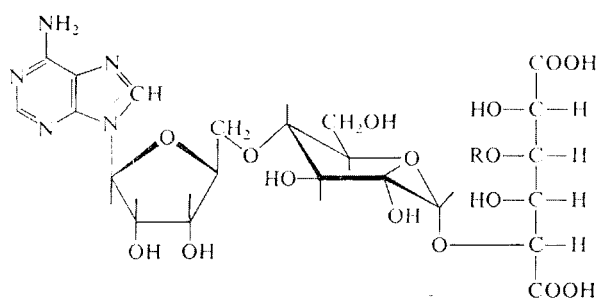
In connection with the synthesis of exotoxin (*I*) there have been prepared the ethers *VII* and *VIII*, the pentose anomeric centre of which is considerably less reactive than that one of the glucose moiety. This difference has been made use of in a further part (part II) of the synthesis in the selective α-glucosidation of allaric acid. In the synthesis of ethers *VII* and *VIII*, the pentose anomeric centre has been protected by the trichloroethyl group and a method has been developed for the preparation of 2,2,2-trichloroethyl glycosides. A method is also reported for conversion of the glycoside *XX* into the cyclic carbonate *VI* without any previous protection of the primary hydroxylic function.

The insecticidal exotoxin (*I*) produced by *Bacillus thuringiensis*, constitutes a complicated nucleotide which has been isolated almost simultaneously in several European institutes. The structure of exotoxin (without configuration of the glucosidic bond and allaric portion of the molecule) has been proposed on the basis of some degradations by workers of this Institute[1]. The proposed sequence adenine-ribose--glucose-allaric acid has been confirmed by synthesis of the basic sugar fragment[2].



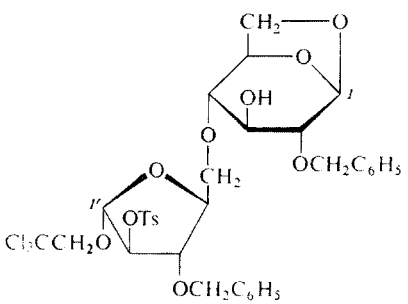
I, R = $PO(OH)_2$

* Part CLXXXII in the series Nucleic Acid Components and their Analogues; Part CLXXXI: This Journal *41*, 647 (1976).

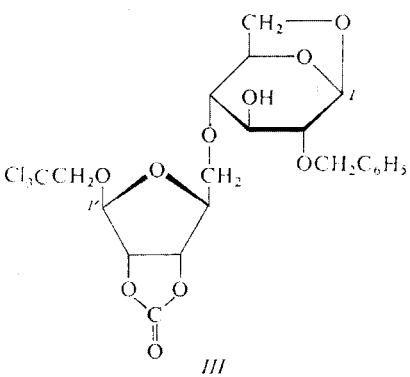
The structure of the allaric portion of the exotoxin molecule has been established by two independent chemical routes[3,4] and the whole structure has been now confirmed by total synthesis.

As shown by preliminary investigations on the synthesis of exotoxin, it appears advantageous to begin with the realisation of the relatively stable ethereal bond between glucose and ribose and to continue with the formation of the similarly stable α-glucosidic bond to allaric acid. The relatively labile nucleosidic bond should be formed and the phosphoryl group introduced in the last synthetic steps.

The formation of an ethereal bond between the $C_{(5)}$ atom of ribose and the $C_{(4)}$ atom of glucose is rather difficult. In the literature, only a few preparations of glucose 4-alkyl ethers have been reported using 1,6 : 3,4-dianhydro-β-D-galactopyranose as the starting material, the epoxide ring of which is subjected to a *trans*-diaxial opening by the action of alcohol under an acidic or basic catalysis[5-7]. This method of the preparation of glucose 4-alkyl ethers has also been successfully used in the synthesis of the basic sugar fragment of exotoxin, namely, in the formation of the ethereal bond between ribose and glucose[2].

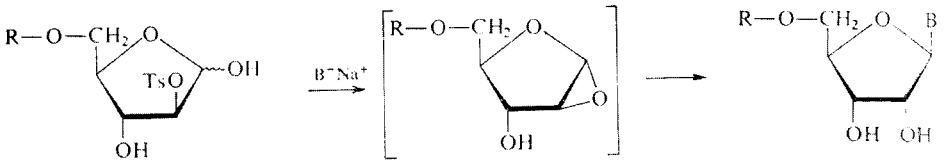


II



III

In order to create first the α-glucosidic bond to allaric acid and then the relatively labile nucleosidic bond between ribose and adenine, such a ribosyl glucosyl ether was required which would exhibit a considerably less reactive anomeric centre of the ribose moiety when compared with the glucose anomeric centre. The required intermediates of this type have been found in ethers *II* and *III*. In the ether *II*, the ribose portion of the exotoxin molecule is preformed in a 2-O-*p*-toluenesulfonyl-D-arabinose derivative from which nucleosides of the β-D-ribose series may be readily obtained by the action of alkali metal salts of nucleic bases[8,9] (Scheme 1). The requirements on a suitable intermediate in the synthesis of exotoxin (*I*) are fulfilled in the ether *II* as follows. The 2'-O-*p*-toluenesulfonyl group protects owing to its electronegative



SCHEME 1

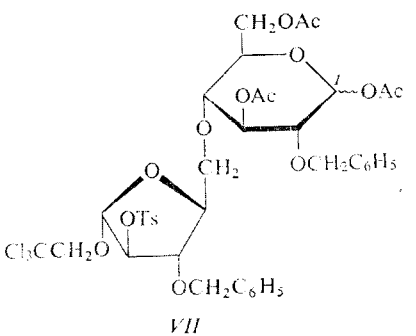
R = triphenylmethyl
B = nucleic base

nature the adjacent glycosidic centre of D-arabinose against an electrophilic attack to such an extent that the subsequent acid-catalysed reactions may be selectively performed on the glucose portion of the molecule. The 3'-O-benzyl group and the glycosidic 2,2,2-trichloroethyl group stabilise the vicinal *p*-toluenesulfonyl group against removal in alkaline media by the formation of a 2',3'- or 1',2'-epoxide. The trichloroethyl group makes possible an easy reductive liberation of the glycosidic centre of arabinose prior to the intended nucleosidation. The 2-O-benzyl group on the glucose portion of the molecule protects position 2 against acylation which would lower reactivity on the anomeric centre of glucose. Owing to a very poor participation in reactions on the anomeric centre, the benzyloxy group is also useful in that respect that glucosidation of a suitable allaric acid derivative results in the predominant formation of the energetically more advantageous α-anomer.

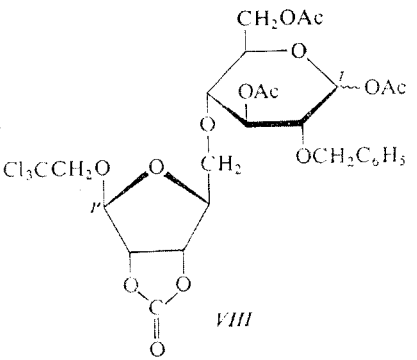
In the analogous ether *III* synthesised as an intermediate in the preparation of exotoxin, the ribose anomeric centre is deactivated by the cyclocarbonate bond. This bond does not lower reactivity of the ribose anomeric centre to such an extent as the *p*-toluenesulfonyl group in ether *II* but, notwithstanding, the presence of this grouping makes possible a selective attachment of allaric acid to the glucose portion of the molecule. The ethers *II* and *III* were prepared by an acid-catalysed *trans*-diaxial epoxide ring opening of 2-O-benzyl-1,6 : 3,4-dianhydro-β-D-galactopyranose[2] (*IV*) by 2,2,2-trichloroethyl 3-O-benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinofuranoside (*V*) and 2,2,2-trichloroethyl 2,3-O-carbonyl-β-D-ribofuranoside (*VI*), resp. The free hydroxylic function of the ether *II* or *III* (though considerably less reactive than the primary hydroxylic function of glycosides *V* and *VI*) may undergo a further reaction with the epoxide *IV* with the formation of polycondensation products. These undesired side reactions may be suppressed by the use of a molar excess of glycosides *V* and *VI* with respect to the epoxide *IV*. Such a requirement is unpleasant especially in the case of the less accessible glycoside *V*. On the other hand, the synthesis of the starting glycoside *VI* is not as difficult and moreover, the resulting ether *III* is readily obtained in crystalline form and is poorly soluble in benzene. Under suitable reaction conditions, the ether *III* is directly deposited from the reaction mixture in crystalline form in view of a higher conversion of the glycoside *IV*.

In ethers *II* and *III*, the glycosidic centre of the pentose portion of the molecule has been protected by the trichloroethyl group. In spite of its use in protection of the carboxyl[10], phosphate[11], hydroxyl[12], and amine[12] group, the trichloroethyl residue has not been to our knowledge used to protect the glycosidic centre. With respect to the latter purpose, the trichloroethyl group exhibits excellent properties. Thus, trichloroethyl glycosides are highly stable both in acidic and basic media. The trichloroethyl group is stable towards oxidants or towards conditions of the hydrogenolytical removal of the benzyloxy group over palladium. Another advantageous feature consists in the ready reductive fission with zinc in the presence of hydrochloric acid, acetic acid or ammonium chloride. The method developed for the preparation of trichloroethyl glycosides is a modification of the Helferich preparation of phenolic glycosides and is based on condensation of acetylated saccharides with trichloroethanol under catalysis of boron trifluoride etherate. Since this condensation proceeds in fair yields, the trichloroethyl group could find application also in other syntheses of sugars.

By a selective acetolysis of the 1,6-anhydro ring in the glucose portion of the molecule, the disaccharidic ethers *II* and *III* were converted to the triacetates *VII* and *VIII*, resp., which were then made use of in the α-glucosidic attachment of allaric acid.



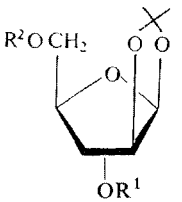
VII

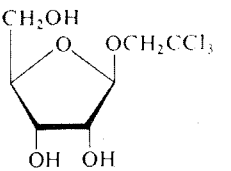


VIII

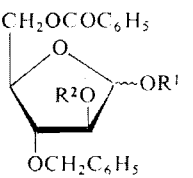
In the synthesis of the starting 2,2,2-trichloroethyl 3-O-benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinoside (*V*), 1,2-O-isopropylidene-5-O-*p*-toluenesulfonyl-β-D-arabinofuranose (*IX*) was used as the key intermediate. Since the product obtained from D-arabinose diethylmercaptal[13] is strongly contaminated with sulfur-containing compounds which are difficult to remove, another method has been developed starting from 2,2,2-trichloroethyl α-D-arabinofuranoside (*X*). Thus, the glycoside *X* was converted by partial *p*-toluenesulfonylation into 2,2,2-trichloroethyl 5-O-*p*-toluenesulfonyl-α-D-arabinoside (*XI*) which was reduced with zinc in acetone to afford directly 1,2-O-isopropylidene-5-O-*p*-toluenesulfonyl-α-D-arabinofuranose (*IX*). In

the subsequent steps, the isopropylidene derivative *IX* was benzylated with benzyl bromide in dimethylformamide in the presence of sodium hydride and the resulting 3-O-benzyl-1,2-O-isopropylidene-2-O-*p*-toluenesulfonyl-α-D-arabinofuranose(*XII*)reduced with sodium amalgam to remove the *p*-toluenesulfonyl group. The thus-obtained 3-O-benzyl-1,2-O-isopropylidene-α-D-arabinofuranose (*XIII*) was benzoylated with benzoyl chloride in pyridine to afford 5-O-benzoyl-3-O-benzyl-1,2-O-isopropylidene-α-D-arabinofuranose (*XIV*). The benzoate *XIV* was subjected to an acid-catalysed hydrolysis to remove the isopropylidene group. Acetylation of the resulting 5-O--benzoyl-3-O-benzyl-D-arabinose (*XV*) afforded a mixture of anomeric 1,2-di-O-acetyl-5-O-benzoyl-3-O-benzyl-D-arabinofuranoses *XVI* which was condensed with 2,2,2-trichloroethanol under catalysis of boron trifluoride etherate with the formation





IX, $R^1 = H$; $R^2 = Ts$
XII, $R^1 = CH_2C_6H_5$; $R^2 = Ts$
XIII, $R^1 = CH_2C_6H_5$; $R^2 = H$
XIV, $R^1 = CH_2C_6H_5$; $R^2 = COC_6H_5$

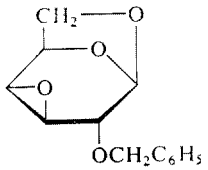


XV, $R^1 = R^2 = H$
XVI, $R^1 = R^2 = Ac$

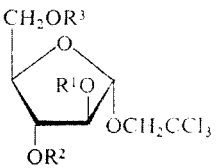
Ts = *p*-toluenesulphonyl

of 2,2,2-trichloroethyl 2-O-acetyl-5-O-benzoyl-3-O-benzyl-α-D-arabinofuranoside (*XVII*). Controlled methanolysis of the crude reaction mixture yielded crystalline 2,2,2-trichloroethyl-5-O-benzoyl-3-O-benzyl-α-D-arabinofuranoside (*XVIII*). Configuration at the anomeric centre of compound *XVIII* was assigned on the basis of the absence of a band due to an intramolecular hydrogen bonding between the hydroxylic function at position 5 and the glycosidic oxygen atom. Reaction of compound *XVIII* with *p*-toluenesulfonyl chloride in pyridine and removal of the protecting

benzoyl group from the resulting 2,2,2-trichloroethyl 5-O-benzoyl-3-O-benzyl-2-O--*p*-toluenesulfonyl-α-D-arabinofuranoside (*XIX*) by hydrolysis afforded the required glycoside *V*.

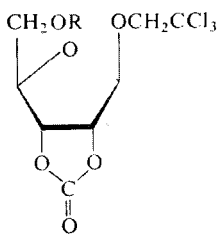


IV



V, R^1 = Ts; R^2 = $CH_2C_6H_5$; R^3 = H
X, R^1 = R^2 = R^3 = H
XI, R^1 = R^2 = H; R^3 = Ts
XVII, R^1 = Ac; R^2 = $CH_2C_6H_5$; R^3 = COC_6H_5
XVIII, R^1 = H; R^2 = $CH_2C_6H_5$; R^3 = $OCOC_6H_5$
XIX, R^1 = Ts; R^2 = $CH_2C_6H_5$; R^3 = COC_6H_5

2,2,2-Trichloroethyl 2,3-O-carbonyl-β-D-ribofuranoside (*VI*) was prepared from 2,2,2-trichloroethyl β-D-ribofuranoside (*XX*) which was obtained analogously to the arabinoside *X* by means of an acid-catalysed condensation of anomeric tetra-O-acetyl-D-ribofuranoses with 2,2,2-trichloroethanol in the presence of boron trifluoride etherate. After the alkaline removal of acetyl groups, the ribofuranoside *XX* was isolated from the reaction mixture in a fair yield. By the action of excess phosgene on the glycoside *XX* in ethyl acetate in the presence of N,N-dimethylaniline, a product



VI, R = H
XXI, R = COCl

was obtained (presumably the 2,2,2-trichloroethyl 5-O-chloroformyl-2,3-O-carbonyl--β-D-ribofuranoside *XXI*) which was subjected to a mild hydrolysis to afford the required carbonate *VI*. In the case of compounds *VI*, *XX*, and *XXI*, the configuration

at the anomeric centre has been assigned on the basis of the IR spectrum of the carbonate *VI* which exhibits an intensive band of an intramolecular hydrogen bonding between the hydroxylic function at position 5 and the glycosidic oxygen atom.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 20°C/0·1 Torr for 10 h. The ^{1}H-NMR spectra were recorded by means of the spectrometer Varian HA 100. The IR spectra were measured on a double-beam Zeiss UR 10 spectrometer. Thin-layer chromatography was performed on the Merck Kieselgel GF_{254} (Type 60) silica gel; spots were detected by viewing under ultraviolet light or by spraying with 10% sulfuric acid in methanol and heating (carbonisation). Solutions were dried over anhydrous magnesium sulfate and the filtrates taken down on a rotatory evaporator at the bath temperature of 40°C under diminished pressure.

2,2,2-Trichloroethyl β-D-Ribofuranoside (*XX*)

The anomeric mixture of 1,2,3,5-tetra-O-acetyl-D-ribofuranoses was prepared according to ref.[14]. Thus, ribose (32·0 g; 0·21 mol) was dissolved in methanol (500 ml) and the solution treated with conc. sulfuric acid (2·5 ml). The mixture was kept at 0—3°C for 12—14 h, neutralised with pyridine (100 ml), and evaporated under diminished pressure to remove methanol and excess pyridine. The residual sirupous mixture of methyl D-ribofuranosides was dissolved in pyridine (250 ml) and the solution treated portionwise with acetic anhydride (100 ml) under cooling. The mixture was kept at room temperature for 2 days and concentrated under diminished pressure. The concentrate was diluted with water (250 ml) and extracted with two 150 ml portions of chloroform. The extract was washed with dilute hydrochloric acid and water, dried, and evaporated. To the residue there was added acetic acid (300 ml), acetic anhydride (70 ml) and then portionwise with cooling conc. sulfuric acid (15 ml). The whole mixture was kept at room temperature for 12 h, diluted with pyridine (50 ml) and evaporated under diminished pressure. The residue was diluted with water (250 ml) and extracted with two 150 ml portions of chloroform. The extract was washed with dilute hydrochloric acid, water, and dilute aqueous ammonia, dried, and evaporated. The thus-obtained mixture of anomeric tetra-O-acetyl-D-ribofuranoses was dissolved in dichloromethane (230 ml) and the solution treated with 2,2,2-trichloroethanol (85 g; 0·57 mol) and boron trifluoride etherate (85 ml). The whole mixture was kept at room temperature for 30 min and poured into water (500 ml). The organic layer was washed with water and dilute aqueous ammonia, dried, and evaporated. The residue was coevaporated with four 150 ml portions of xylene to remove excess trichloroethanol and the final residue was dissolved in methanol (250 ml). The solution was treated with 1M methanolic sodium methoxide (10 ml) until the reaction was permanently alkaline. After the saponification (2 h; thin-layer chromatography on silica gel in 2 : 1 benzene–acetone; compound *XX*, R_F 0·4), the methanol was evaporated, the residue diluted with water (150 ml), and extracted with three 150 ml portions of ethyl acetate. The extract was dried and evaporated. The residue was triturated with ether to deposit a solid which was collected with suction and washed with ether. Yield: 25·0 g (39%) of compound *XX*, m.p. 114 to 115°C, $[\alpha]_D^{25}$ −60·9° (*c* 1 in ethanol). For $C_7H_{11}Cl_3O_5$ (281·5) calculated: 29·86% C, 3·94% H, 37·78% Cl; found: 30·18% C, 4·01% H, 37·71% Cl.

2,2,2-Trichloroethyl α-D-Arabinofuranoside (*X*)

To a suspension of D-arabinose (32·0 g; 0·21 mol) in methanol (800 ml) there was added conc. sulfuric acid (3·0 ml), the mixture stirred at room temperature for 7 h, diluted with pyridine (100 ml), and evaporated. The residual mixture of methyl D-arabinofuranosides was transformed to the glycoside *X* analogously to the preparation of the riboside *XX*. Yield, 30·5 g (51%) of the arabinoside *X*, m.p. 130·0—132·5°C, $[\alpha]_D^{25}$ +94·4° (*c* 1 in water). For $C_7H_{11}Cl_3O_5$ (281·5) calculated: 29·86% C, 3·94% H, 37·78% Cl; found: 29·94% C, 3·88% H, 37·65% Cl.

1,2-Isopropylidene-5-O-toluenesulfonyl-α-D-arabinofuranose (*IX*)

To a precooled (0°C) solution of the arabinoside *X* (30·0 g; 0·107 mol) in pyridine (150 ml) there was added with stirring *p*-toluenesulfonyl chloride (21·0 g; 1·1 mol), the mixture kept at room temperature for 20 h, and evaporated under diminished pressure. The residue was diluted with benzene and water (150 ml each), the benzene layer separated, washed with 10% hydrochloric acid and water, dried, and evaporated. The residual sirupous 2,2,2-trichloroethyl 5-O-*p*-toluenesulfonyl-α-D-arabinofuranoside (*XI*; 45·4 g) was dissolved in acetone (600 ml) and zinc chloride (60 g) along with powdered zinc was added (25 g) to the solution; conc. sulfuric acid (15 ml) was then added dropwise with cooling and stirring. The whole mixture was stirred at room temperature for 2 h, treated portionwise with pyridine (100 ml), filtered, and the material on the filter washed with acetone. The filtrate and washings were combined and evaporated, the residue diluted with water (100 ml) and chloroform (200 ml), and the mixture acidified with hydrochloric acid. The chloroform layer was separated, dried, and evaporated. Ether was added to the residue until turbid and the mixture allowed to crystallise. Yield 20·0 g (58%, based on compound *X*) of compound *IX*, m.p. 127—130°C (reported[11], m.p. 129—130°C).

3-O-Benzyl-1,2-O-isopropylidene-5-O-*p*-toluenesulfonyl-α-D-arabinofuranose (*XII*)

To a solution of the isopropylidene derivative *IX* (20·0 g; 0·058 mol) in dimethylformamide (50 ml) there was added benzyl bromide (1·8 ml) and then portionwise with ice-cooling a 50% oil suspension of sodium hydride (2·4 g). The whole mixture was stirred at room temperature overnight, diluted with saturated methanolic ammonia (50 ml), stirred additional 2 h, poured into water, and extracted with benzene. The extract was washed with dilute hydrochloric acid (when an emulsion is formed, the material is filtered through a Celite layer) and water, dried, and evaporated. The residual sirup may be directly used in the preparation of compound *XIII* or, otherwise, ethanol is added and the solution allowed to deposit crystals. Yield, 13·2 g (52%) of compound *XII*, m.p. 60°C, $[\alpha]_D^{25}$ +20·2° (*c* 0·5 in chloroform). For $C_{22}H_{26}O_7S$ (434·4) calculated: 60·82% C, 6·03% H, 7·36% S; found: 60·87% C, 6·02% H, 7·40% S.

3-O-Benzyl-1,2-O-isopropylidene-α-D-arabinofuranose (*XIII*)

The benzyl ether *XII* obtained by benzylation of the isopropylidene derivative *IX* (20·0 g) was dissolved in ethanol (250 ml) and 2% sodium amalgam (350 g) was gradually added to the stirred solution. The whole mixture was stirred at room temperature for 4 h, the mercury separated, and the supernatant evaporated. The residue was diluted with water (100 ml) and extracted with two 75 ml portions of chloroform. The extract was dried, evaporated, and the residue triturated with ether to deposit a solid which was collected with suction and washed with ether. Yield, 12·8 g (79%, referred to compound *IX*) of the derivative *XIII*, m.p. 79—80°C, $[\alpha]_D^{25}$ −23·2° (*c* 0·5 in chloroform). For $C_{15}H_{20}O_5$ (280·3) calculated: 64·27% C, 7·19% H; found: 64·56% C, 7·42% H.

5-O-Benzoyl-3-O-benzyl-1,2-O-isopropylidene-α-D-arabinofuranose (*XIV*)

To an ice-cooled solution of compound *XIII* (12·8 g; 0·045 mol) in pyridine (50 ml) there was added dropwise benzoyl chloride (10·0 g; 0·07 mol), the mixture stirred at room temperature for 2 h, diluted with water (50 ml), and extracted with two 50 ml portions of benzene. The extract was washed with dilute hydrochloric acid, dilute aqueous ammonia and water, dried, and evaporated. After the addition of ethanol, the residue deposited crystals. Yield, 14·4 g (83%) of compound *XIV*, m.p. 78–79°C, $[\alpha]_D^{25}$ +20·1° (*c* 0·5 in chloroform). For $C_{22}H_{24}O_6$ (384·4) calculated: 68·74% C, 6·29% H; found: 68·53% C, 6·46% H.

2,2,2-Trichloroethyl 5-O-Benzoyl-3-O-benzyl-α-D-arabinofuranoside (*XVIII*)

A stirred suspension of compound *XIV* (8·0 g; 20·8 mmol) in 50% aqueous formic acid (25 ml) was heated at 100°C for 30 min, cooled down, and evaporated. The residual crude dihydroxy derivative *XV* was converted to the acetate *XVI* on heating (100°C) with acetic anhydride (30 ml) and anhydrous sodium acetate (3·0 g) for 30 min. The mixture was then decomposed with water (100 ml) and extracted with two 50 ml portions of benzene. The extract was washed with dilute aqueous ammonia and water, dried, and evaporated. The residual diacetate *XVI* was condensed (10 min at room temperature) with 2,2,2-trichloroethanol (3·6 g; 25 mmol) in benzene (10 ml) under catalysis of boron trifluoride etherate (1·6 ml). The reaction mixture was poured into water (50 ml), and extracted with two 30 ml portions of benzene. The extract was washed with dilute aqueous ammonia, dried, and evaporated. The residual crude glycoside *XVII* was dissolved in saturated methanolic ammonia (100 ml), the solution kept at room temperature for 75 min, evaporated, the residue diluted with water (100 ml), and extracted with two 50 ml portions of benzene. The extract was dried, evaporated, the residue diluted with cyclohexane until just turbid, seeded with crystals of compound *XVIII* (obtained by chromatography of the crude product on a layer of loose silica gel in 95 : 5 benzene–ethyl acetate; R_F of compound *XVIII*, 0·35), and the whole allowed to deposit crystals which were collected with suction and washed with cyclohexane. Yield, 5·2 g (53%, referred to compound *XIV*) of compound *XVIII*, m.p. 94–95°C, $[\alpha]_D^{22}$ +91·51 (*c* 0·5 in chloroform). For $C_{21}H_{21}Cl_3O_6$ (475·8) calculated: 53·01% C, 4·44% H, 22·35% Cl; found: 53·22% C, 4·58% H, 21·86% Cl.

2,2,2-Trichloroethyl 5-O-Benzoyl-3-O-benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinofuranoside (*XIX*)

To a solution of compound *XVIII* (5·0 g; 10·5 mmol) in pyridine (30 ml) there was added *p*-toluenesulfonyl chloride (5·4 g; 27·5 mmol), the mixture kept at room temperature for 3 days, decomposed with water (100 ml), and extracted with benzene (100 ml). The extract was washed with dilute hydrochloric acid and water, dried, and evaporated to afford 6·2 g (94%) of sirupous glycoside *XIX* which solidified after a longer period of time; m.p. 65–67°C (ether–light petroleum) $[\alpha]_D^{25}$ +87·7° (*c* 0·6 in chloroform). For $C_{28}H_{27}Cl_3OS$ (629·95) calculated: 53·38% C, 4·32% H, 16·82% Cl, 5·09% S; found: 53·11% C, 4·29% H, 17·11% Cl, 5·07% S.

2,2,2-Trichloroethyl 3-O-Benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinofuranoside (*V*)

To a solution of the benzoate *XIX* (12·6 g; 20 mmol) in ether (30 ml) there was added 1M solution of sodium hydroxide in 80% aqueous methanol (22 ml), the whole kept at room temperature for 2 h, diluted with water (100 ml), and extracted with two 50 ml portions of benzene. The extract was dried and evaporated to afford 10·0 g (95%) of the crude glycoside *V* which was directly used without any purification in the preparation of the ether *II*. For analytical purposes, a small

amount of contaminating methyl benzoate was removed by chromatography on a layer of loose silica gel in the solvent system 95 : 5 benzene–ethyl acetate (R_F of *V*, 0·35). Optical rotation: $[\alpha]_D^{25}$ +90·2° (*c* 1·2 in chloroform). IR spectrum (*c* 3 . 10^{-3}M; CCl_4): ν(OH) free 3613 cm^{-1} (very intensive), ν(OH) bound 3510 cm^{-1} (weak) belonging to hydrogen bonding towards the furanose ring oxygen atom[15]. For $C_{21}H_{23}Cl_3OS$ (525·8) calculated: 47·96% C, 4·40% H, 20·23% Cl, 6·10% S; found: 48·18% C, 4·33% H, 20·29% Cl, 6·10% S.

2,2,2-Trichloroethyl 2,3-O-Carbonyl-β-D-ribofuranoside (*VI*)

To a suspension of 2,2,2-trichloroethyl β-D-ribofuranoside (*XX*; 28·1 g; 0·10 mol) in ethyl acetate (300 ml) there was added phosgene (50 ml). The stirred and cooled (5°C) mixture was then treated dropwise with N,N-dimethylaniline (40 g; 0·33 mol), the whole stirred at 0—5°C or 1 h and poured onto ice (evolution of carbon dioxide). The organic layer was separated, washed with water, and evaporated. The residue was dissolved in acetone (750 ml) and the solution was diluted with water (250 ml). Pyridine was then added portionwise (30 ml) at 10°C, the whole mixture kept at 10°C for 15 min, acidified with hydrochloric acid, and evaporated. The residue was extracted with two 150 ml portions of benzene, the extract washed with dilute hydrochloric acid, dried, and evaporated to afford 29·1 g (95%) of compound *VI* (almost chromatographically homogeneous) which was directly used in the preparation of the ether *III* without any previous purification. For analytical purposes, the contaminants were removed by chromatography on a layer of loose silica gel in the solvent system 8 : 2 benzene–ethyl acetate (R_F value of compound *VI*, 0·42). Optical rotation: $[\alpha]_D^{25}$ −85·9° (*c* 1 in ethanol). IR spectrum (*c* 3 . 10^3M; CCl_4): ν(C=O) 1844, 1824 cm^{-1}; ν(OH) free 3636 cm^{-1} (weakly intensive); ν(OH) bound 3559 cm^{-1} (very intensive). For $C_8H_9Cl_3O_6$ (307·5) calculated: 31·25% C, 2·95% H, 34·59% Cl; found: 30·89% C, 3·01% H, 34·13% Cl.

2-O-Benzyl-4-O-(2,2,2-trichloroethyl-3-O-benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinosid-5-yl)--1,6-anhydro-β-D-glucopyranose (*II*)

To a solution of compound *V* (5·3 g; 10·0 mmol) in chloroform (10 ml) there were added the epoxide *IV* (1·17 g; 5·0 mmol) and boron trifluoride etherate (0·2 ml). The mixture was kept at room temperature until the epoxide *IV* disappeared (as shown by thin-layer chromatography on silica gel), diluted with chloroform (50 ml), and washed with saturated aqueous sodium hydrogen carbonate (50 ml). The organic layer was separated, dried, evaporated, and the residue chromatographed on a 3·8 × 30 cm column of silica gel. Elution with 9 : 1 benzene–ethyl acetate yielded 2·0 g (37%) of the unreacted compound *V*. With 85 : 15 benzene–ethyl acetate as eluant there was obtained compound *II* (thin-layer chromatography on silica gel in the solvent system 85 : 15 benzene–ethyl acetate: *IV*, R_F 0·5; *V*, R_F 0·35; *II*, R_F 0·18). The sirupous ether *II* was diluted with ether (2 ml) and light petroleum until turbid, and allowed to crystallise. The solid was collected with suction and washed with ether and light petroleum. Yield, 1·9 g (25%, referred to compound *V*) of the ether *II*, m.p. 108—109°C, $[\alpha]_D^{25}$ +38·85° (*c* 0·5 in chloroform. For $C_{34}H_{37}Cl_3O_{11}S$ (760·0) calculated: 53·74% C, 4·91% H, 14·00% Cl, 4·21% S; found: 53·81% C, 4·73% H, 13·81% Cl, 3·97% S.

2-O-Benzyl-4-O-(2,2,2-trichloroethyl-2,3-O-carbonyl-β-D-ribofuranosid-5-yl)-1,6-anhydro--β-D-glucopyranose (*III*)

To a solution of the carbonate *VI* (30·7 g; 0·1 mol) in benzene (75 ml) there were added the epoxide *IV* (23·4 g; 0·1 mol) and boron trifluoride etherate (0·75 ml). The mixture was kept in an ice--box at 0°C until the epoxide *IV* disappeared (about 44 h; thin-layer chromatography on silica gel

in the solvent system 85 : 15 benzene–ethyl acetate; R_F value of compound *IV*, 0·5). The mixture which solidified during this period of time was diluted with chloroform (200 ml), washed with 5% aqueous sodium acetate and water, dried, and evaporated. The residue was triturated with benzene to deposit crystals which were collected with suction and washed with benzene. Yield, 22·5 g (42·5%) of compound *III*, m.p. 117–118·5°C, $[\alpha]_D^{25}$ −78·26° (*c* 1 in chloroform). Chromatography of mother liquors on silica gel recovered 14·0 g (0·045 mol) of the carbonate *VI*. For $C_{21}H_{23}Cl_3O_9$ (529·8) calculated: 47·61% C, 4·39% H, 20·07% Cl; found: 47·40% C, 4·27% H, 19·55% Cl.

1,3,5-Tri-O-acetyl-2-O-benzyl-4-O-(2,2,2-trichloroethyl-2,3-carbonyl-β-D-ribofuranosid-5-yl)-α,β-D-glucopyranose (*VIII*)

To a suspension of the ether *III* (530 mg; 1 mmol) in acetic anhydride (1·0 ml) there was added at 0°C boron trifluoride etherate (0·04 ml) and the mixture stirred at 0°C for 5 min. After the addition of 5% aqueous sodium acetate (10 ml), the product was extracted with two 10 ml portions of xylene, the extract dried, evaporated, and the residue coevaporated with two 10 ml portions of xylene. As indicated by ^{1}H-NMR spectrum, the final product represents a 20 : 80 mixture of the β- and α-anomer; this mixture may be used in subsequent glycosylations without any previous purification. By crystallisation, there may be obtained *VIII*-α-anomer, m.p. 149 to 151°C (benzene–ether), $[\alpha]_D^{25}$ +13·1° (*c* 0·5 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 2·08 (2 s; $OCOCH_3$), 2·16 (s, $OCOCH_3$), 4·06, 4·21 (2 d, 2 H; J = 11·5 Hz; OCH_2CCl_3), 6·34 p.p.m. (d, 1 H, $C_{(1)}$–H; $J_{1,2}$ = 3·5 Hz). For $C_{27}H_{31}Cl_3O_{14}$ (685·9) calculated: 47·28% C, 4·55% H, 15·50% Cl; found: 47·37% C, 4·64% H, 15·57% Cl.

1,3,5-Tri-O-acetyl-2-O-benzyl-4-O-(2,2,2-trichloroethyl-3-O-benzyl-2-O-*p*-toluenesulfonyl-α-D-arabinosid-5-yl)-α,β-D-glucopyranose (*VII*)

The acetolytical fission of the 1,6-anhydro ring in the ether *II* was performed analogously to the preparation of the triacetates *VIII*. A sirupous anomeric mixture *VII* was obtained containing 80% of the α-anomer and 20% of the β-anomer; this mixture may be used in subsequent glycosylations without any previous purification. ^{1}H-NMR spectrum of the *VII*-α-anomer ($CDCl_3$): δ 1·98, 2·07; 2·19 (s, 3 $OCOCH_3$), 2·44 (s, CH_3 of *p*-toluenesulfonyl), 6·32 p.p.m. (d, 1 H; $C_{(1)}$–H; $J_{1,2}$ = 3·2 Hz). For $C_{40}H_{45}Cl_3O_{15}S$ (904·2) calculated: 53·13% C, 5·02% H, 11·76% Cl; found: 53·63% C, 4·94% H, 11·33% Cl.

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