ALTERNATIVE SYNTHESIS OF EXOTOXIN FROM *Bacillus thuringiensis** **

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The *Bacillus thuringiensis* exotoxin (*I*) synthesis is presented as a general approach to preparation of exotoxin analogoues. The ethereal bond between the glucopyranose and ribofuranose unit was formed by *trans*-diaxial opening of the epoxide ring of compound *XV* with the use of 2,2,2-trichloroethyl 2,3-di-O-benzoyl-β-D-ribofuranoside (*XIII*) under acidic conditions. The α-glucosidic bond connecting the allaric acid residue was realised by a stereoselective reaction of the intermediate *XVIII* with allaric acid lactone ester *XXIX* protected at position 3 by a non-participating benzyl group. The intermediate *XXXIX* was transformed to the nucleoside *XLIV*, the γ-lactone of which was opened by methanolysis with the formation of the alcohol *LIX*. Phosphorylation of this alcohol and the subsequent alkaline hydrolysis yielded exotoxin (*I*) identical with the naturally occurring toxin. The stereoselectivity of the glucosidation of the lactone ester *XXIX* with diacetates *XXXII* and *XXXIII* has been examined with a special respect to the formation of by-products derived from the intermediate *XVIII*.

Investigations performed in the field of the insecticidal exotoxin (*I*) from *Bacillus thuringiensis* elucidated mechanism of its biological activity[1], the chemical structure[2-4], and approach to the total synthesis[5]. The strategy of the whole synthesis of exotoxin (*I*) consists in a right order of the following steps: *1.* formation of the nucleosidic bond, *2.* formation of the anomalous type of the ethereal bond, *3.* formation of the α-glucosidic bond, and *4.* selective phosphorylation. Twenty four combinations are possible but, as shown by preliminary experiments, only two routes appear promising that are based on the formation of an ethereal bond in the first step and the phosphorylation in the last step. One of them, with the step sequence (*2*)-(*1*)-(*3*)-(*4*) and synthesis of the adenine-ribofuranose-glucopyranose portion of the molecule could not be realised to a full extent. The other route is based on the step sequence (*2*)-(*3*)-(*1*)-(*4*), *i.e.*, on the formation of the whole sugar moiety of exotoxin, the subsequent selective nucleosidation, and the final phosphorylation. The

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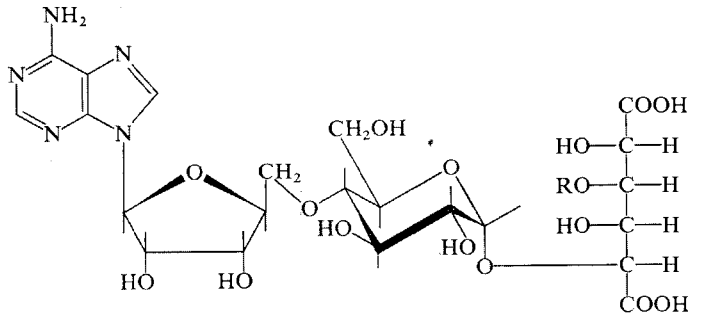
** For a preliminary communication see Prystaš M., Kalvoda L., Šorm F.: Nucl. Acids Res., Special Publication No 1, p. s77 (1975).

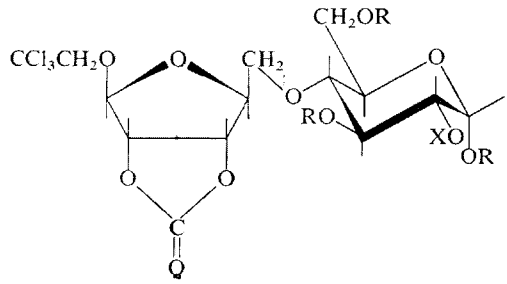


I, R = $PO(OH)_2$

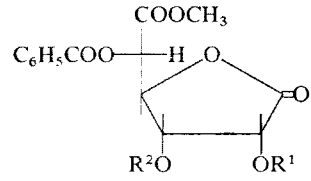
latter route was performed in two variants: one of them represents the total synthesis[5] of exotoxin while the other variant may also be used in the preparation of exotoxin analogues.

In variant 2, the drawbacks of variant 1 were removed and the whole synthetic process was shortened. The key intermediate *II* of variant 1 is shaped as follows: the ribofuranose portion is deactivated by trichloroethyl and cyclocarbonyl protecting groups while the glucopyranose portion is activated by a benzyl group which favours the formation of the α-glucosidic bond. For a sterically controlled nucleosidation however, the cyclocarbonyl group is of no use since its exchange for an acetyl group is accompanied by a preferential opening of the lactone ring in compound *VII* (this ring protects position 4 for the final phosphorylation step). In glucosidation which affords a complex mixture containing the α-glucoside *VI*, the allaric acid lactone ester *III* was used. Position 3 of compound *III* was protected by the participating benzoyl group which undergoes migration with the formation of the isomer *IV*. Under the reaction conditions, the equilibrium is markedly shifted in favour of the isomer *IV* from which the lactone *III* is recovered in weakly acidic medium (*e.g.*, by chromatography on silica gel). The lactone *IV* was therefore obtained in admixture with the lactone *III*. The structure *IV* was inferred from ^{1}H-NMR spectrum: the signal of the proton at position 2 forms a doublet of a highest chemical shift. A further evidence was supplied by conversion to the tribenzoate *V* which was identical with the specimen obtained by benzoylation of allaric acid lactone ester *III*.

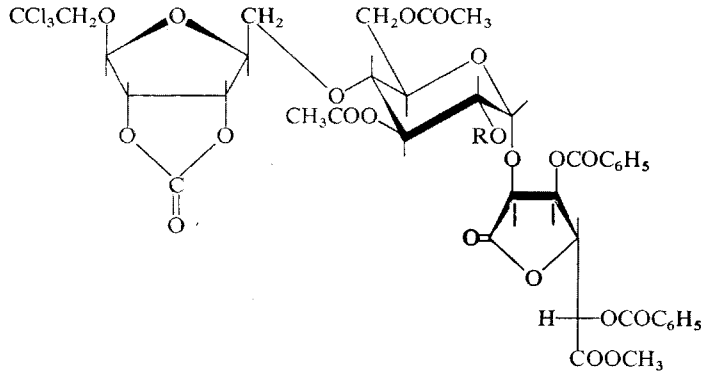
In the synthesis of the key intermediate *XVIII*, compound *XIII* was used as the starting material (the *cis*-diol system of 2,2,2-trichloroethyl β-D-ribofuranoside is protected by benzoyl groups). Reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose with 2,2,2-trichloroethanol in the presence of 4·5 equivalents of ethereal boron trifluoride etherate afforded a fair yield of the anomeric protected ribofuranosides *VIII* and *X* which were separated by chromatography on silica gel. The anomeric pair of compounds *VIII* and *X* has been earlier prepared by Dr J. V. P. Verheyden[6]



II, R = CH_3CO, X = $C_6H_5CH_2$



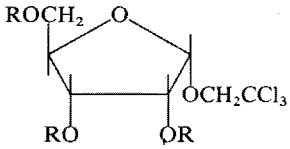
III, R^1 = H; R^2 = C_6H_5CO
IV, R^1 = C_6H_5CO; R^2 = H
V, R^1 = R^2 = C_6H_5CO



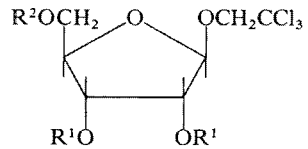
VI, R = $C_6H_5CH_2$
VII, R = CH_3CO

from the Institute of Molecular Biology, Palo Alto, California, U.S.A. The structure of the highly predominating β-anomer *VIII* and the α-anomer *X* was inferred from ^{1}H-NMR spectra. The earlier reported[5] 2,2,2-trichloroethyl β-D-ribofuranoside (*IX*)

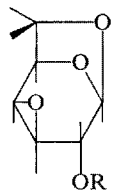
was obtained by methanolysis of the protected β-D-ribofuranoside *VIII* or directly from the anomeric mixture *VIII* + *X*. Compound *IX* was converted to the corresponding dibenzoate *XIII* by triphenylmethylation of position 5, benzoylation of the diol *XI*, and partial hydrolysis of the triphenylmethyl dibenzoyl derivative *XII* without isolation of intermediates in the total yield of 70%.



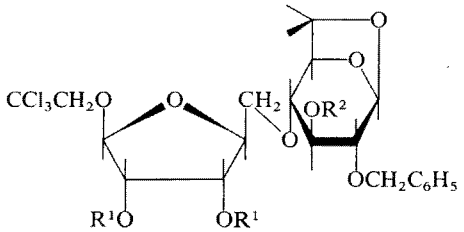
X, R = C_6H_5CO



VIII, $R^1 = R^2 = C_6H_5CO$
IX, $R^1 = R^2 = H$
XI, $R^1 = H$; $R^2 = (C_6H_5)_3C$
XII, $R^1 = C_6H_5CO$; $R^2 = (C_6H_5)_3C$
XIII, $R^1 = C_6H_5CO$; $R^2 = H$



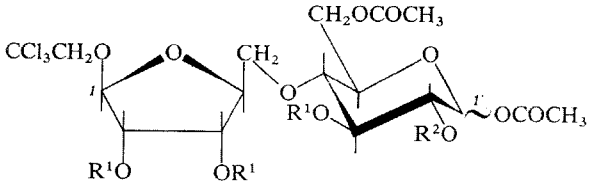
XIV, R = H
XV, R = $C_6H_5CH_2$



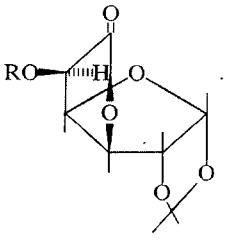
XVI, $R^1 = C_6H_5CO$; $R^2 = H$
XVII, $R^1 = R^2 = C_6H_5CO$

In step (*2*), use has been made of the earlier observations on the *trans*-diaxial opening of the epoxide ring in protected 1,6 : 3,4-dianhydro-β-D-galactopyranoses[4,5]. The starting 1,6 : 3,4-dianhydro-2-O-benzyl-β-D-galactopyranose (*XV*) was prepared by an improved procedure. The stannic chloride catalysed reaction of equimolar amounts of 2,2,2-trichloroethyl 2,3-di-O-benzoyl-β-D-ribofuranoside (*XIII*) and the benzoyl epoxide *XV* was performed in benzene at room temperature; the reaction was advantageously interrupted when 50% of the starting compounds was present in the reaction mixture. Chromatography was then used to isolate a fair yield of the diglycoside ether *XVI* and recover the reactants *XIII* and *XV*. Compound *XVI* was benzoylated and the resulting tribenzoate *XVII* (not isolated) subjected to acetolytic opening of the 1,6-anhydro ring by the action of acetic anhydride under catalysis of

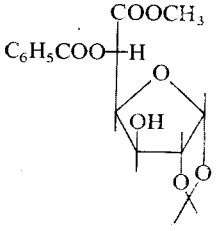
sulfuric acid to afford a crystalline mixture of the anomeric diacetates *XVIIIα'* and *XVIIIβ'*. This mixture represents the key intermediate in the synthesis of exotoxin. The acetolysis must be performed under controlled conditions to prevent removal of the benzyl group which would occur in excess sulfuric acid. As shown by preliminary experiments, the ribofuranose portion of the intermediate *XVIII* is deactivated enough to allow a selective formation of the glucosidic bond.



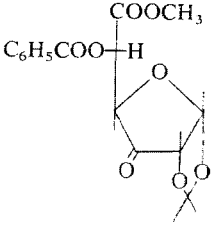
XVIII, $R^1 = C_6H_5CO$; $R^2 = C_6H_5CH_2$
XIX, $R^1 = C_6H_5CO$; $R^2 = H$
XX, $R^1 = C_6H_5CO$; $R^2 = CH_3CO$



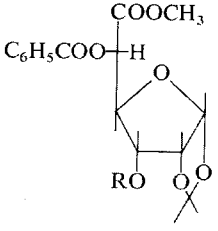
XXI, R = H
XXII, R = C_6H_5CO



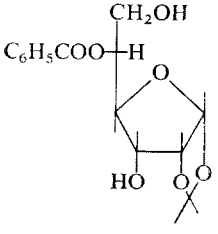
XXIII



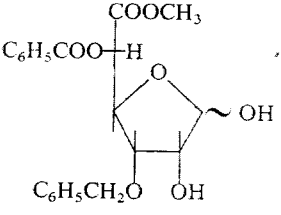
XXIV



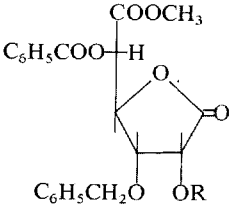
XXV, R = H
XXVI, R = $C_6H_5CH_2$



XXVII



XXVIII

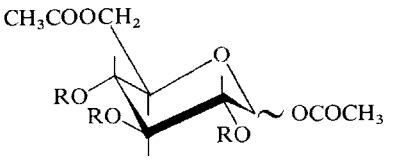


XXIX, R = H
XXX, R = CH_3CO
XXXI, R = C_6H_5CO

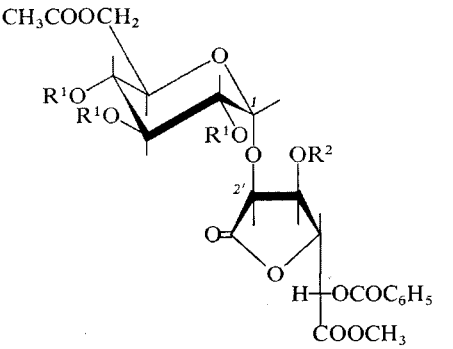
On the basis of our earlier experience with intermediates derived from allaric acid, the lactone ester dibenzoate *III* was replaced by the lactone ester *XXIX*, the position 3 of which is protected by the nonparticipating benzyl group. Compound *XXIX* was prepared by two routes. The shorter one consists in transformation of the structurally related substituted methyl D-glucuronate *XXIII*. Compound *XXIII* was prepared by benzoylation of the known[7] 3,6-lactone of 1,2-O-isopropylidene-α-D-glucofuranuronic acid (*XXI*) and the subsequent methanolysis of the resulting lactone *XXII* by the action of methanolic triethylamine. The epimerisation of the alcohol *XXIII* was accomplished by oxidation with ruthenium tetraoxide in aqueous acetone in the presence of sodium periodate and the subsequent highly stereoselective reduction of the keto sugar *XXIV* with tri(tert-butyloxy)lithium aluminium hydride in tetrahydrofuran with the formation of the alcohol *XXV*. On the other hand, the sodium borohydride reduction of the ulose *XXIV* is accompanied by a simultaneous reduction of the activated methoxycarbonyl group affording 5-O-benzoyl-1,2-O-isopropylidene--α-D-allofuranose (*XXVII*) as the single product. The alcohol *XXV* was converted to the benzyl derivative *XXVI* by the action of benzyl bromide and silver oxide in benzene at room temperature and in the presence of Potassite 3 molecular sieves. In this case, the use of dimethylformamide cannot be recommended, especially at elevated temperatures. A brief reflux of compound *XXVI* in 50% aqueous formic acid resulted in removal of the isopropylidene group. The thus-obtained anomeric mixture of hemiacetals *XXVIII*α and *XXVIII*β was oxidized with bromine in aqueous dioxane and in the presence of a continual excess of sodium hydrogen carbonate to afford the allaric acid lactone ester *XXIX* in an overall yield above 60%. All the intermediates in the synthesis of the lactone *XXIX* were obtained in crystalline state and their structure was confirmed on the basis of ^{1}H-NMR spectra. The lactone ester *XXIX* was also characterised by conversion into the acetate *XXX* and the benzoate *XXXI*.

The steric course of glucosidations of the lactone ester *XXIX* was examined with the use of the anomeric 1,6-di-O-acetyl-2,3,4-tri-O-methyl-D-glucopyranose (*XXXII*) and the anomeric (*cf.*[8]) 1,6-di-O-acetyl-2,3,4-tri-O-benzyl-D-glucopyranose (*XXXIII*) as model substances. Thus under optimum conditions (1 equivalent of boron trifluoride etherate in benzene, 16 h, 20°C) an equimolar mixture of the diacetate *XXXII* and the lactone ester *XXIX* afforded a fair yield of the α-glucoside *XXXIV*, the structure of which was confirmed by ^{1}H-NMR spectrum ($J_{1,2}$ = 3·5 Hz) and by hydrogenolysis leading to the alcohol *XXXV*. Also the reaction of the diacetate *XXXIII* with the lactone ester *XXIX* was highly stereoselective and yielded the α-glucoside *XXXVI*. In both glucosidations there was recovered 20–25% of the starting lactone *XXIX*. Furthermore, the polyfunctional α-glucosides *XXXIV* and *XXXVI* were used to verify the suitability of methanolysis (one equivalent of 0·01M sodium acetate in methanol; a brief treatment at room temperature) for the selective opening of the lactone ring without affecting the acetoxy group. By this process, the corresponding dimethyl esters of (2*R*)-2-O-α-D-glucopyranosylallaric acid (*XXXVII* or *XXXVIII*)

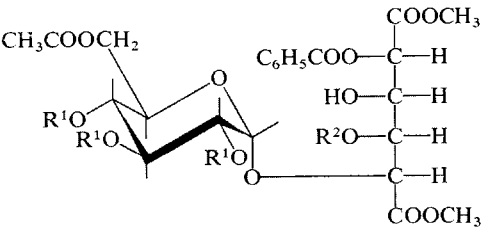
were obtained. The selective opening of the lactone ring is indispensable for the realisation of the phosphorylation step (*4*).



XXXII, R = CH_3
XXXIII, R = $C_6H_5CH_2$



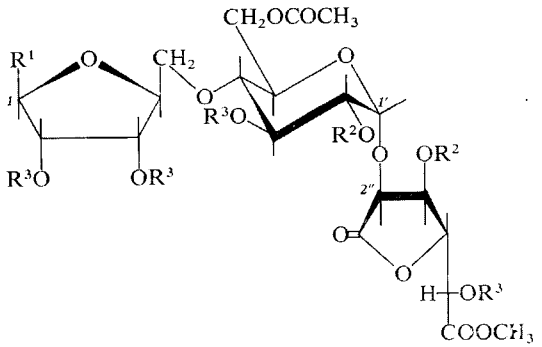
XXXIV, $R^1 = CH_3$; $R^2 = C_6H_5CH_2$
XXXV, $R^1 = CH_3$; $R^2 = H$
XXXVI, $R^1 = R^2 = C_6H_5CH_2$



XXXVII, $R^1 = CH_3$; $R^2 = C_6H_5CH_2$
XXXVIII, $R^1 = R^2 = C_6H_5CH_2$

As shown by preliminary experiments, step (*3*), *i.e.*, connection of the intermediate *XVIII* with the lactone ester *XXIX* by means of an α-glucosidic bond, requires strongly acidic conditions (10–15 equivalents of boron trifluoride etherate in benzene, 30 to 40 min at 20°C) which, nevertheless, are milder than those required by variant 1 (20 equivalents of boron trifluoride etherate in chloroform, 120 min at 20°C). It is highly probable that a mixture of anomeric glucosides is formed under such conditions. The glucosidation mixture resulting from reactants *XVIII* and *XXIX* (25% molar excess) was uncompletely separated by column chromatography on silica gel into the starting substances (20–25%), the anomeric bis(2,2,2-trichloroethyl)diglycoside ethers *XLVII* and *L* as by-products (total 4%), and a fraction containing a mixture of anomeric glucosides *XXXIX* (13%) and *XLV* (2·3%) along with the remaining

starting substance *XVIII* (5%). The latter fraction was then hydrogenolysed over activated palladium on charcoal catalyst in glacial acetic acid and the product subjected to chromatography to afford the α-glucoside *XL*, m.p. 182–185°C, the corresponding β-anomer, and the alcohol *XIXα'*, derived from starting component *XVIII* and characterised as the acetate *XXα'*. The structure of the α-glucoside *XL* was inferred from the ^{1}H-NMR spectrum of the acetyl derivative *XLI* exhibiting the anomeric proton of the glucosidic bond as a doublet with the coupling constant $J_{1,2}$ equal to 3·5 Hz. The β-anomer was converted to the acetyl derivative *XLVI*, m.p. 119–123°C. The ^{1}H-NMR spectrum of compound *XLVI* of the β-series is similar to that of compound *XLI* of the α-series, but the anomeric proton of the β-glucosidic bond (overlapping with further sugar protons) exhibits a lower chemical shift as expected. The above acetates were prepared by the action of 0·15M-CF_3CO_2H in acetic anhydride at 40°C.

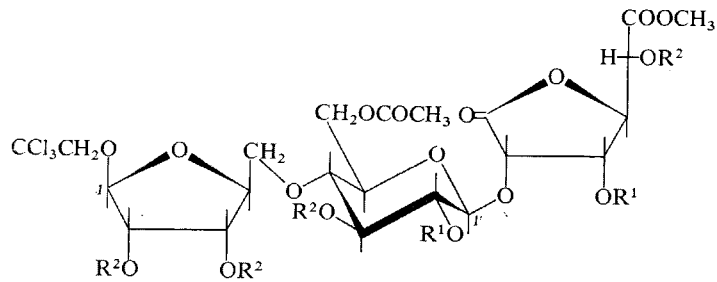


XXXIX, $R^1 = CCl_3CH_2O$; $R^2 = C_6H_5CH_2$
XL, $R^1 = CCl_3CH_2O$; $R^2 = H$
XLI, $R^1 = CCl_3CH_2O$; $R^2 = CH_3CO$
XLII, $R^1 = CH_3COO$; $R^2 = CH_3CO$
XLIII, $R^1 = Br$; $R^2 = CH_3CO$
XLIV, R^1 = N^6-benzoyladenine; $R^2 = CH_3CO$

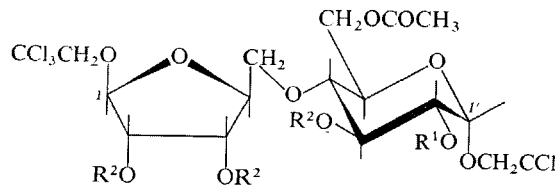
In formulae *XXXIX–XLIV*, $R^3 = C_6H_5CO$

The by-products *XLVII* and *L* were examined in detail. The intermediate *XVIII* was assumed to react with acetic acid formed in the glucosidation. The liberated 2,2,2-trichloroethanol can then compete with the component *XXIX* for the anomeric centre of the glucopyranose moiety of the intermediate *XVIII*. Reaction of an equimolar mixture of compound *XVIII* under glucosidation conditions virtually yielded 8% of an anomeric mixture of the diglycoside ethers *XLVII* and *L*. Furthermore,

reaction of compound *XVIII* with excess 2,2,2-trichloroethanol and column chromatography on silica gel afforded an anomeric pair of diglycoside ethers *XLVII* (29%) and *L* (15·5%) along with a pair of compounds *LII* (23%) and *LV* (9%). Hydrogenolysis of the anomeric pair *XLVII* and *L* yielded the alcohols *XLVIII* and *LI* which were separated by chromatography. The ^{1}H-NMR spectrum of the alcohol *XLVIII* is in accordance with configurations β (at position 1 of the ribofuranoside moiety) and α′ (at position 1 of the glucopyranoside moiety). The configuration of the alcohol *LI* is β,β′. The analogous pair of anomers *LII* and *LV* afforded the alcohols *LIII* (of the α,α′ configuration as inferred from the ^{1}H-NMR spectrum of the acetate *LIV*) and *LVI* (of the α,β′ configuration). The structure of the alcohol *XLVIII* is supported by the ^{1}H-NMR spectrum of the acetate *XLIX*. The ^{1}H-NMR spectra of all the above substances indicate the Cl conformation of their glucopyranoside moiety. The strongly acidic conditions thus obviously result in an easy anomerisation of position 1 in compound *XVIII* and in a poorly stereoselective formation of the glucosidic bond as otherwise expected from the formation of anomeric ribofuranosides *VIII* and *X* and anomeric glucopyranosides *XXXIX* and *XLV*.

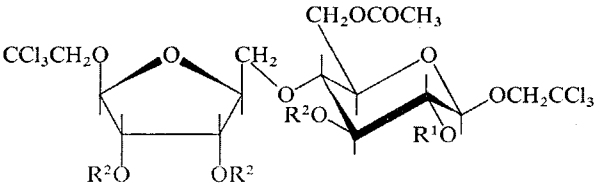


XLV, $R^1 = C_6H_5CH_2$; $R^2 = C_6H_5CO$
XLVI, $R^1 = CH_3CO$; $R^2 = C_6H_5CO$

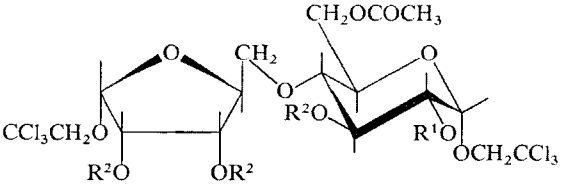


XLVII, $R^1 = C_6H_5CH_2$
XLVIII, $R^1 = H$
XLIX, $R^1 = CH_3CO$

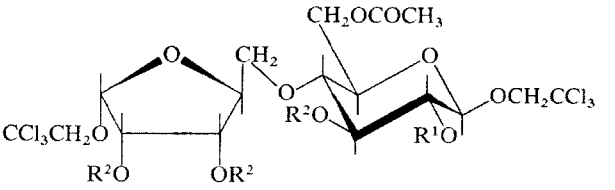
Prior to realisation of step (*l*), attention has been paid to transformation of the α-glucoside *XL* into such an intermediate which would be advantageous for the preparation of the corresponding halogenose. Since the protection of the *cis*-diol system by benzoyl groups is considered as advisable for a sterically controlled nucleosidation, an attempt was made to replace in one step the trichloroethyl group by the acetyl group without affecting the benzoyl groups. Thus, by reaction with activated zinc in acetic anhydride and in the presence of trifluoroacetic acid, the protected 2,2,2-trichloroethyl β-D-ribofuranoside *VIII* was converted into the known[9] 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose. A similar treatment was used to transform the intermediate *XVIII* and its analogue *XXα'* into the anomeric acetates *LVII* and



L, $R^1 = C_6H_5CH_2$
LI, $R^1 = H$



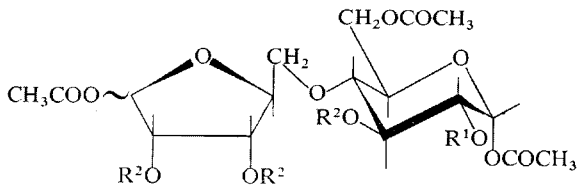
LII, $R^1 = C_6H_5CH_2$
LIII, $R^1 = H$
LIV, $R^1 = CH_3CO$



LV, $R^1 = C_6H_5CH_2$
LVI, $R^1 = H$

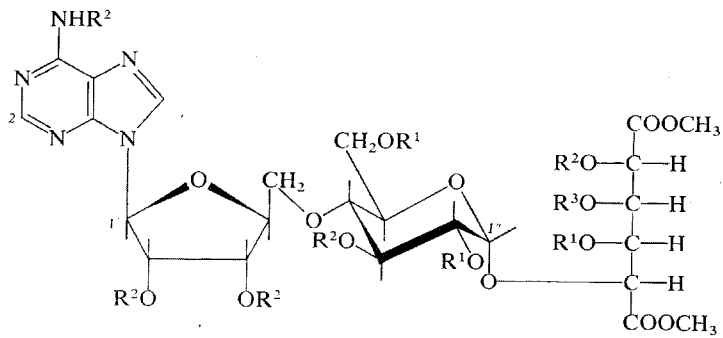
LVIII, resp., in 60–70% yield (recovery, 20–25% of the starting material). An analogous conversion (under simultaneous acetylation of the free hydroxylic function) of the α-glucoside *XL* to anomeric acetates *XLII* required a longer reaction time and a higher concentration of trifluoroacetic acid. The acetates *XLII* were also obtained from the glycoside *XLI* in 80% yield. Compound *XLII* may be readily isolated by chromatography on silica gel and converted to the halogenose *XLIII* by the action of dry hydrogen bromide in toluene.

The nucleosidation step (*1*) was realised with the use of the halogenose *XLIII* and N^6-benzoyladenine chloromercuri salt in refluxing acetonitrile. The resulting nucleoside *XLIV* was isolated by chromatography. Prior to the final phosphorylation step (*4*), the lactone system of the nucleoside *XLIV* was opened by the action of two equivalents of 0·01M-CH_3COONa in methanol with the formation of the allarate *LIX* (conversion, 75%). The phosphorylation of compound *LIX* was performed analogously to the earlier alternative, namely, by the action of excess phosphorus oxychloride in benzene and in the presence of pyridine followed by alkaline hydrolysis



LVII, $R^1 = C_6H_5CH_2$
LVIII, $R^1 = CH_3CO$

In formulae *XLVII*–*LX*, $R^2 = C_6H_5CO$



LIX, $R^1 = CH_3CO$; $R^3 = H$
LX, $R^1 = CH_3CO$; $R^3 = Cl_2PO$

of the phosphodichloridate *LX* in aqueous dioxane. The thus-obtained exotoxin (*I*) was identical with the naturally occurring toxin of *Bacillus thuringiensis*.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Unless stated otherwise, the analytical samples were dried at 20°C/0·1 Torr for 10 h. The ^{1}H-NMR spectra were recorded in deuteriochloroform on a Varian HA-100 spectrometer. Optical rotations were measured in chloroform on a Perkin-Elmer MC-141 polarimeter. The chromatography was performed on silica gel (particle size, 60—120 micron) partially deactivated by the addition of water (12—14%) and on neutral alumina (Brockmann activity II—III).

1,4-Lactone of (2*R*,5*S*)-2,5-Di-O-benzoylallaric Acid 6-Methyl Ester (*IV*)

A solution of the lactone ester *III* (104 mg; 0·25 mmol) and boron trifluoride etherate (0·30 ml; Lachema, Brno, Czechoslovakia) in chloroform (3 ml) was stirred at 20°C for 2 h, washed with two 20 ml portions of water and 3% aqueous potassium hydrogen carbonate (5 ml), and evaporated under diminished pressure. The residue was chromatographed on a thin layer of loose silica gel (2 plates, 17 × 44 cm) in chloroform to recover compound *III* (45%; R_F 0·45) and to obtain (yield, 50%) the crude lactone ester *IV*, m.p. 172—182°C (ether), R_F 0·40. ^{1}H-NMR spectrum: δ 5·87 (d, 2-H, $J_{2,3}$ = 6·0), 4·82 (dd, 3-H, $J_{3,4}$ = 1·0), 5·01 (dd, 4-H), 5·67 (d, 5-H, $J_{5,4}$ = 2·8 Hz), 4·30 (bs, OH), 3·82 (s, $COOCH_3$), 7·30—7·75 and 7·95—8·20 p.p.m. (m, 2 × C_6H_5); the spectrum exhibits the $COOCH_3$ signal (δ 3·87 p.p.m.) of the starting lactone ester *III* (about 15%). For $C_{21}H_{18}O_9$ (414·4) calculated: 60·87% C, 4·38% H; found: 60·85% C, 4·61% H. An analogous isolation of the lactone ester *IV* (27%) was performed after the interaction of compounds *II* and *III*.

Benzoyl derivative V. A mixture of the crude lactone *IV* (25 mg), benzoyl chloride (20 mg), and pyridine (1 ml) was kept at 20°C for 3 h, evaporated, and the residue dissolved in benzene (5 ml). The solution was washed with 1M-HCl (2 ml) and 2% aqueous potassium hydrogen carbonate (5 ml), and evaporated. The residue was chromatographed on a thin layer of loose silica gel (one plate, 17 × 35 cm) in 20 : 1 benzene–ethyl acetate to afford compound *V* (60%), m.p. 166—167°C (chloroform–ether), undepressed on admixture with the benzoylation product of the lactone ester *III*. For $C_{28}H_{22}O_{10}$ (518·5) calculated: 64·86% C, 4·28% H; found: 64·53% C, 4·41% H.

Anomeric 2,2,2-Trichloroethyl 2,3,5-Tri-O-benzoyl-D-ribofuranosides (*VIII* and *X*)

A mixture of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose[9] (25·2 g; 50 mmol), 2,2,2-trichloroethanol (16 ml), boron trifluoride etherate (28 ml), and ether (30 ml) was stirred at 20°C for 40 min, poured onto ice (500 g), the oil washed with three 500 ml portions of water, and dissolved in benzene (500 ml). The solution was washed with two 500 ml portions of water, dried over anhydrous sodium sulfate, passed through a column of alumina (500 g), and the effluent evaporated. The residue was chromatographed on a column of silica gel (1000 g) in 200 : 1 benzene–ethyl acetate (5000 ml; fractions 1—50). Fractions 20—36 (R_F 0·35 in thin-layer chromatography on silica gel with binder in the same solvent system) yielded 61% of the β-anomer *VIII* (dried at 80°C/0·1 Torr for 6 h). ^{1}H-NMR spectrum: δ 5·52 (d, 1-H, $J_{1,2} < 0·5$), 5·77—6·02 (m, 2-H and 3-H), 4·50—4·88 (m, 4-H and 2 × 5-H), 4·14 and 4·34 (d, CCl_3CH_2, J_{gem} 11·5 Hz), 7·20—7·60 and 7·78—8·10 p.p.m. (m, 3 × C_6H_5). For $C_{28}H_{23}Cl_3O_8$ (593·8) calculated: 56·63%

C, 3·90% H, 17·92% Cl; found: 56·96% C, 4·19% H, 17·78% Cl. The homogeneous fractions 39—45 (R_F 0·20 under the above conditions) yielded 9% of the α-anomer *X* (dried as compound *VIII*). ^{1}H-NMR spectrum: δ 5·70 (d, 1-H, $J_{1,2}$ = 4·8), 5·38 (qu, 2-H, $J_{2,3}$ = 7·0), 5·84 (qu, 3-H, $J_{3,4}$ = 2·5), 4·60—4·90 (m, 4-H and 2 × 5-H), 4·14 and 4·46 (d, CCl_3CH_2, J_{gem} = 11·5 Hz), 7·10—7·63 and 7·80—8·20 p.p.m. (m, 3 × C_6H_5). For $C_{28}H_{23}Cl_3O_8$ (593·8) calculated: 56·63% C, 3·90% H, 17·92% Cl; found: 56·94% C, 4·08% H, 17·67% Cl.

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

A. A solution of the tribenzoate *VIII* (11·9 g; 20·0 mmol) in 0·006M-CH_3ONa in methanol (150 ml) was kept at room temperature for 15 h, neutralised with Dowex 50W (H^+) ion exchange resin, filtered, the filtrate evaporated under diminished pressure, and the residue diluted with benzene (90 ml). The solid was collected with suction, washed with three 15 ml portions of benzene, and crystallised from 1 : 20 methanol–benzene to afford 5·24 g (93%) of the ribofuranoside *IX*, m.p. 117—118°C, undepressed on admixture with a specimen obtained by another procedure[5]. Optical rotation: $[\alpha]_D^{25}$ −47·8° (*c* 0·51; water). ^{1}H-NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 5·01 (s, 1-H, $J_{1,2} < 0·5$), 3·87—4·20 (m, 2-H—4-H), 3·62 (m, 2 × 5-H), 4·06 and 4·33 p.p.m. (d, CCl_3CH_2, J_{gem} = 13·0 Hz).

B. Solvolysis of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (100 g) with 2,2,2-trichloroethanol yielded a mixture of anomeric tribenzoates *VIII* and *X* which was passed through a column of alumina, and the effluent methanolysed with methanolic 0·01M-CH_3ONa (600 ml) for 8 h at room temperature. The mixture was neutralised, evaporated under diminished pressure, and the residue diluted with benzene (250 ml) to afford 33 g (59%) of the ribofuranoside *IX*, m.p. 112—114°C (resolidification) and then 116—118°C.

2,2,2-Trichloroethyl 2,3-Di-O-benzoyl-β-D-ribofuranoside (*XIII*)

A mixture of the ribofuranoside *IX* (14·05 g; 50·0 mmol), triphenylmethyl chloride (14·6 g; 52·5 mmol), and pyridine (100 ml) was kept at room temperature for 15 h, then heated at 100°C for 1 h, and poured onto ice. The solid was collected with suction, washed with water, and dissolved in chloroform (500 ml). The solution was washed with 5% aqueous sodium hydrogen sulfate (100 ml) and water (500 ml), dried, and evaporated. A sample of the residual triphenylmethyl derivative *XI* was chromatographed on a thin layer of loose silica gel in 4 : 1 benzene–ethyl acetate, dried at 80°C/0·1 Torr for 6 h, and analysed. For $C_{26}H_{25}Cl_3O_5$ (523·8) calculated: 59·61% C, 4·81% H; found: 59·32% C, 4·95% H. The remaining crude triphenylmethyl derivative *XI* was dissolved in pyridine (250 ml) and the benzoyl chloride (17 ml) was added dropwise at −50°C to the solution. The reaction mixture was kept at 0°C for 15 h, decomposed with water (10 ml), evaporated, and the residue dissolved in benzene (500 ml). The solution was washed successively with water (1 500 ml), 5% aqueous sodium hydrogen sulfate (1 000 ml) and saturated aqueous potassium hydrogen carbonate (300 ml), dried, filtered, and the filtrate passed through a column of alumina (300 g). The effluent was evaporated and a sample of the residual ester *XII* chromatographed on a thin layer of loose silica gel in 200 : 1 benzene–ethyl acetate, dried at 50°C/0·1 Torr for 8 h, and analysed. ^{1}H-NMR spectrum: δ 5·52 (s, 1-H), 5·80 (m, 2-H and 3-H), 4·64 (m, 4-H), 3·44 (d, 2 × 5-H), 4·10 and 4·22 (d, CCl_3CH_2, J_{gem} = 11·0 Hz), 7·25—7·60 and 7·80—8·10 p.p.m. (m, 5 × C_6H_5). For $C_{40}H_{33}Cl_3O_7$ (732·0) calculated: 65·63% C, 4·55% H; found: 66·01% C, 4·82% H. The remaining crude ester *XII* was then refluxed in 80% aqueous acetic acid (75 ml) for 30 min, the suspension kept at 0°C for 15 h, filtered, the filtrate evaporated under diminished pressure, and the residue coevaporated with three 200 ml portions of toluene. The

final residue was chromatographed on a column of silica gel (700 g) in 50 : 1 benzene–ethyl acetate (3000 ml; fractions 1—7) and then in 19 : 1 benzene–ethyl acetate (4000 ml; fractions 8—24). Fractions 7—19 yielded 17·2 g (70%) of the dibenzoate *XIII*, m.p. 120·5—122·0°C (chloroform–light petroleum). Optical rotation: $[\alpha]_D^{25}$ −13·2° (*c* 0·49). ^{1}H-NMR spectrum: δ 5·54 (s, 1-H), 5·82 (m, 2-H and 3-H), 4·54 (m, 4-H), 3·94 (m, 2 × 5-H), 4·23 and 4·42 (d, CCl_3CH_2, J_{gem} = 11·0), 2·33 (bt, OH, *J* = 6 Hz), 7·20—7·62 and 7·80—8·10 p.p.m. (m, 2 × C_6H_5). For $C_{21}H_{19}Cl_3O_7$ (489·7) calculated: 51·50% C, 3·91% H, 21·72% Cl; found: 51·57% C, 3·91% H, 21·53% Cl.

1,6 : 3,4-Dianhydro-2-O-benzyl-β-D-galactopyranose (*XV*)

Silver oxide (295 g) was added portionwise over 45 min at 0°C to a vigorously stirred mixture of the epoxide *XIV* (70 g), benzyl bromide (130 ml), and dimethylformamide (820 ml). The whole mixture was stirred at room temperature for additional 30 min, heated at 90°C for 1½ h, and filtered while hot. The filtrate was evaporated and the residue distilled to afford two fractions boiling at 140—160°C/0·6 Torr (8 g) and 160—164°C/0·6 Torr (104 g). The main fraction was diluted with 1 : 5 ether–light petroleum to afford 93 g of compound *XV*, m.p. 46—47°C. After recrystallisation from the same solvent mixture the melting point was 47—48°C. The low-boiling fraction was processed similarly to afford additional 1·5 g of compound *XV*; overall yield, 87·5%. Optical rotation: $[\alpha]_D^{25}$ −55° (*c* 0·50). For $C_{13}H_{14}O_4$ (234·3) calculated: 66·66% C, 6·02% H; found: 66·69% C, 6·03% H.

1,6-Anhydro-2-O-benzyl-4-O-(2,2,2-trichloroethyl-2,3-di-O-benzoyl-5-deoxy-β-D-ribofuranosid-5-yl)-β-D-glucopyranose (*XVI*)

A mixture of the dibenzoate *XIII* (14·68 g; 30·0 mmol), the benzyl epoxide *XV* (7·03 g; 30·0 mmol), and 0·0012M-$SnCl_4$ in benzene (300 ml) was stirred at room temperature for 7 h, decomposed with saturated aqueous sodium hydrogen carbonate (50 ml), dried, and chromatographed on a column of silica gel (700 g) in 16 : 1 benzene–ethyl acetate (4500 ml; fraction 1—9), in 11 : 1 benzene–ethyl acetate (2000 ml; fractions 10—14), in 9 : 1 benzene–ethyl acetate (2000 ml; fractions 15—18), and finally in 7 : 1 benzene–ethyl acetate (1500 ml; fractions 19—21). Fractions 6—10 were rechromatographed on a column of alumina (200 g) in benzene (700 ml) and in 3 : 1 benzene–ethyl acetate (500 ml) to recover the epoxide *XV* (49%) and the dibenzoate *XIII* (48%). Homogeneous fractions 13—20 yielded 8·25 g (38%; 75% with respect to the recovered reactants *XIII* and *XV*) of compound *XVI*. A sample was rechromatographed on a thin layer of loose silica gel in 4 : 1 benzene–ethyl acetate, dried at 75°C/0·1 Torr for 2 h, and analysed. Optical rotation: $[\alpha]_D^{25}$ −19·8° (*c* 0·49). For $C_{34}H_{33}Cl_3O_{11}$ (724·0) calculated: 56·40% C, 4·60% H, 14·69% Cl; found: 56·63% C, 4·69% H, 14·33% Cl.

Anomeric 1,6-Di-O-acetyl-3-O-benzoyl-2-O-benzyl-4-O-(2,2,2-trichloroethyl 2,3-di-O-benzoyl--5-deoxy-β-D-ribofuranosid-5-yl)-D-glucopyranoses (*XVIIIα′* and *XVIIIβ′*)

A mixture of the alcohol *XVI* (15 g; 20·7 mmol), benzoyl chloride (3 ml), and pyridine (100 ml) was kept at 0°C for 18 h, decomposed with water (2 ml), evaporated under diminished pressure, and the residue dissolved in benzene (250 ml). The solution was successively washed with 3% aqueous hydrochloric acid (100 ml), water (500 ml), and saturated aqueous sodium hydrogen carbonate (100 ml), dried, and applied to a column of alumina (250 g). The tribenzoate *XVII* was eluted with 40 : 1 benzene–ethyl acetate. For purposes of analysis, a sample was dried at 80°C/0·1 Torr for 10 h. For $C_{41}H_{37}Cl_3O_{12}$ (828·1) calculated: 59·46% C, 4·50% H; found:

59·81% C, 4·78% H. A mixture of the remaining tribenzoate *XVII*, acetic anhydride (100 ml), and concentrated sulfuric acid (0·1 ml) was then kept at room temperature for 40 min, neutralised with solid potassium hydrogen carbonate (1 g), and evaporated under diminished pressure. The residue was dissolved in benzene (300 ml), the solution washed with water (500 ml), dried, evaporated, and the residue crystallised from ether to afford a mixture (14·9 g; m.p. 130—132°C) of anomeric diacetates *XVIIIα′* and *XVIIIβ′*. The mother liquors were rechromatographed on a column of silica gel (100 g) in 9 : 1 benzene–ethyl acetate to afford additional 2·1 g of the above mixture; overall yield, above 88%. ^{1}H-NMR spectrum: δ 6·37 (d, 1′-H of compound *XVIIIα′*, $J_{1',2'}$ = = 3·5), 6·42 (d, 1′-H of compound *XVIIIβ′*, $J_{1',2'}$ = 5·5 Hz), 2·02 and 2·18 (s, 2 × CH_3CO), 7·12, 7·20—7·60, and 7·75—8·10 p.p.m. (m, 4 × C_6H_5). For $C_{45}H_{43}Cl_3O_{15}$ (930·2) calculated: 58·10% C, 4·66% H, 11·44% Cl; found: 57·98% C, 4·50% H, 11·25% Cl.

Methyl 5-O-Benzoyl-1,2-O-isopropylidene-α-D-glucofuranuronate (*XXIII*)

To a solution of the lactone *XXI* (6·48 g; 30·0 mmol) in pyridine (25 ml) there was added dropwise at −70°C benzoyl chloride (4·2 ml), the suspension kept at 0°C for 15 h, decomposed with water (0·5 ml), evaporated, and the residue dissolved in chloroform (100 ml). The solution was successively washed with water (200 ml), 2% aqueous hydrochloric acid (80 ml), water (100 ml), and saturated aqueous sodium hydrogen carbonate (50 ml), dried, and evaporated. The residue was coevaporated with methanol (75 ml) and the benzoate *XXII* added into a mixture of methanol (25 ml) and triethylamine (0·2 ml). The solution was kept for 30 min at 20°C and for 1 h at 0°C to deposit crystals which were collected with suction and washed at 0°C with three 8 ml portions of methanol. Yield, 8·36 g of compound *XXIII*. Mother liquors were chromatographed on a column of silica gel (50 g) in 9 : 1 benzene–ethyl acetate (500 ml; fractions 1—5) and fractions 2—4 worked-up to afford additional 0·73 g. Overall yield, 86% of compound *XXIII*, m.p. 147—151°C (methanol), $[\alpha]_D^{25}$ +18·3° (*c* 0·50). For $C_{17}H_{20}O_8$ (352·3) calculated: 57·95% C, 5·72% H; found: 57·94% C, 5·92% H.

Methyl 5-O-Benzoyl-1,2-O-isopropylidene-α-D-allofuranuronate (*XXV*)

To a solution of the glucofuranuronate *XXIII* (17·6 g; 50 mmol) in acetone (1 000 ml) there was successively added with stirring at 20° sodium periodate (85 g), water (430 ml), acetic acid (8 ml) and a liquor[10] (25 ml) containing ruthenium dioxide. The whole mixture was kept at room temperature for 9 h, decomposed with ethanol (50 ml), and concentrated to the volume of 500 ml. The concentrate was diluted with water (1 000 ml) and extracted with two 500 ml portions of benzene. The extract was washed with saturated aqueous sodium hydrogen carbonate (200 ml) and the required amount of aqueous sodium thiosulfate, dried, and evaporated. The residue was chromatographed on a column of silica gel (400 g) in 50 : 1 benzene–ethyl acetate (3 500 ml) and 6 : 1 benzene–ethyl acetate (2 300 ml). Work-up of the more polar fraction afforded the starting material *XXIII* (recovery, 6%). The less polar fraction was evaporated, the residual crude ulose *XXIV* dissolved in tetrahydrofuran (150 ml) and to this solution there was added under cooling with ice tri(tert-butoxy) lithium aluminium hydride in three portions (total 19 g). The mixture was stirred at room temperature for 30 min, evaporated under diminished pressure, and the residue diluted with chloroform (500 ml) and 3·5% aqueous hydrochloric acid (380 ml). The aqueous layer was extracted with chloroform (300 ml), the organic phases combined, washed with 1% aqueous sodium chloride, dried, and evaporated. The residue was crystallised from ether–light petroleum to afford 11·0 g of the allofuranuronate *XXV*, m.p. 94—96°C. The mother liquors were chromatographed on a column of silica gel (40 g) in 4 : 1 benzene–ethyl acetate to afford additional 1·5 g; overall yield, 71% of compound *XXV*. Optical rotation: $[\alpha]_D^{25}$ +46·9° (*c* 0·51).

^{1}H-NMR spectrum: δ 5·82 (d, 1-H, $J_{1,2}$ = 3·5), 4·64 (t, 2-H, $J_{2,3}$ = 4·0), 4·38 (dd, 3-H, $J_{3,4}$ = = 9·0), 4·29 (dd, 4-H, $J_{4,5}$ = 2·1 Hz), 5·66 (d, 5-H), 1·39 and 1·61 (s, 2 × CH_3 of the isopropylidene group), 3·80 (s, $COOCH_3$), 7·32—7·65 and 8·04—8·17 p.p.m. (m, C_6H_5). For $C_{17}H_{20}O_8$ (352·3) calculated: 57·95% C, 5·72% H; found: 58·02% C, 5·55% H.

5-O-Benzoyl-1,2-O-isopropylidene-α-D-allofuranose (*XXVII*)

To a solution of the ulose *XXIV* (obtained from 1 mmol of the glucofuranuronate *XXIII*) in chloroform (30 ml) there was added a solution of sodium borohydride (100 mg) in ethanol (20 ml) and water (0·5 ml). The mixture was kept at 20°C for 25 min, decomposed with acetic acid (1 ml), washed with saturated aqueous sodium hydrogen carbonate (200 ml), dried, and evaporated. The residue was chromatographed on a column of silica gel (40 g) in 5 : 1 benzene–ethyl acetate (300 ml) and 7 : 3 benzene–ethyl acetate (400 ml; fractions 1—5). Fractions 3—5 yielded 216 mg (67%) of the diol *XXVII*, m.p. 94—95° (ether–light petroleum); $[\alpha]_D^{25}$ +26·7°C (*c* 0·50). For C_{16}·$.H_{20}O_7$ (324·3) calculated: 59·25% C, 6·22% H; found: 59·14% C, 6·17% H.

Methyl 5-O-Benzoyl-3-O-benzyl-1,2-O-isopropylidene-α-D-allofuranuronate (*XXVI*)

A mixture of the alcohol *XXV* (8·80 g; 25·0 mmol), benzyl bromide (14·1 ml), benzene (200 ml), molecular sieve Potassite 3 (35 g), and silver oxide (31 g) was stirred at 20°C for 5·5 h, filtered, the filtrate applied to a column of silica gel (250 g), and the column eluted with benzene (1 500 ml) and 40 : 1 benzene–ethyl acetate (1 500 ml). The more polar fraction yielded 10·6 g (96%) of compound *XXVI*, m.p. 107·0—107·5°C (ether–light petroleum); $[\alpha]_D^{25}$ +115·2° (*c* 0·50). ^{1}H-NMR spectrum: δ 5·74 (d, 1-H, $J_{1,2}$ = 3·5) 4·53 (dd, 2-H, $J_{2,3}$ = 4·0), 4·15 (dd, 3-H, $J_{3,4}$ = 9·0), 4·63 (dd, 4-H, $J_{4,5}$ = 2·5), 5·65 (d, 5-H), 1·38 and 1·64 (s, 2 × CH_3 of the isopropylidene group), 3·80 (s, $COOCH_3$), 4·69 (d, CH_2, J_{gem} = 8·0 Hz), 7·30—7·70 and 7·90—8·10 p.p.m. (m, 2 × × C_6H_5). For $C_{24}H_{26}O_8$ (442·5) calculated: 65·15% C, 5·92% H; found: 65·30% C, 5·98% H.

Anomeric Methyl 5-O-Benzoyl-3-O-benzyl-D-allofuranuronates (*XXVIIIα* and *XXVIIIβ*)

The isopropylidene derivative *XXVI* (8·84 g; 20·0 mmol) was refluxed in 50% aqueous formic acid (120 ml) for 15 min, the solution evaporated, and the residue coevaporated with 1 : 3 ethanol–toluene (250 ml) and then with toluene (200 ml). Crystallisation of the final residue from toluene (0°C) yielded 93% of the anomeric mixture, m.p. 116·0—118·5°C. ^{1}H-NMR spectrum: δ 5·25 (d, 1-H of compound *XXVIIIα*, $J_{1,2}$ = 4·0), 5·29 (d, 1-H of compound *XXVIIIβ*, $J_{1,2}$ < 1), 4·18 (m, 2-H), 3·99 (m, 3-H), 4·40—4·60 (m, 4-H), 5·43 (d, 5-H, $J_{5,4}$ = 3·0 Hz), 2·20—3·0 (m, 2 × OH), 3·64 (s, $COOCH_3$), 4·54 (m, CH_2), 7·0—7·70 and 7·90—8·10 p.p.m. (m, 2 × C_6H_5). For $C_{21}H_{22}O_8$ (402·4) calculated: 62·68% C, 5·51% H; found: 62·39% C, 5·32% H.

1,4-Lactone of (5*S*)-5-O-Benzoyl-3-O-benzylallaric Acid 6-Methyl Ester (*XXIX*)

To a stirred mixture of the diol *XXVIII* (4·02 g; 10·0 mmol), sodium hydrogen carbonate (4·50 g), dioxane (80 ml), and water (30 ml) there was added at 20°C bromine (2·4 ml), the whole kept at 20°C for 18 min and concentrated to the volume of 35 ml under diminished pressure. The concentrate was decolourised with solid sodium thiosulfate, extracted with two 50 ml portions of chloroform, the extract dried, and evaporated. Crystallisation of the residue from ether–light petroleum yielded 92% of the lactone ester *XXIX*, m.p. 129·5—131°C; $[\alpha]_D^{25}$ −3·8° (*c* 0·50). ^{1}H-NMR spectrum: δ 4·50—4·80 (m, 2-H, $J_{2,3}$ = 6·0 and $J_{2,OH}$ = 9·0), 4·29 (dd, 3-H, $J_{3,4}$ = = 1·0), 4·97 (dd, 4-H, $J_{4,5}$ = 3·1 Hz), 5·59 (d, 5-H), 2·95 (d, OH), 3·70 (s, $COOCH_3$), 4·65

(m, CH_2), 7·20—7·60 and 7·80—8·0 p.p.m. (m, 2 × C_6H_5). For $C_{21}H_{20}O_8$ (400·4) calculated: 63·00% C, 5·03% H; found: 63·10% C, 4·98% H.

Acetyl derivative XXX. A solution of the lactone ester *XXIX* (40·0 mg; 0·10 mmol) in 0·15M-CF_3COOH in acetic anhydride (3 ml) was heated at 40°C for 45 min, evaporated, and the residue coevaporated with two 15 ml portions of xylene. The final residue was chromatographed on a thin layer of loose silica gel (one plate, 17 × 44 cm) in 20 : 1 benzene–ethyl acetate. The R_F 0·3 band yielded 41 mg (75%) of the amorphous acetate *XXX* which was dried at 75°C/0·05 Torr for 3 h. For $C_{23}H_{22}O_9$ (442·4) calculated: 62·44% C, 5·01% H; found: 62·78% C, 5·16% H.

Benzoyl derivative XXXI. A mixture of the lactone ester *XXIX* (60 mg; 0·15 mmol), benzoyl chloride (0·035 ml), and pyridine (2 ml) was kept at 20°C for 4 h, evaporated, the residue dissolved in benzene (10 ml), the solution washed with 1M-HCl (5 ml) and saturated aqueous sodium hydrogen carbonate (5 ml), dried, and evaporated. Chromatography (see above) of the residue yielded 64% of the dibenzoate *XXXI*, m.p. 96—98°C (ether–light petroleum). ^{1}H-NMR spectrum: δ 5·91 (d, 2-H, $J_{2,3} = 6·0$), 4·64 (dd, 3-H, $J_{3,4} = 1·8$), 5·11 (qu, 4-H, $J_{4,5} = 3·0$ Hz), 5·81 (d, 5-H), 3·75 (s, $COOCH_3$), 4·58 (s, CH_2), 7·25—7·70 and 8·0—8·25 p.p.m. (m, 3 × C_6H_5). For $C_{28}H_{24}O_9$ (504·5) calculated: 66·66% C, 4·80% H; found: 66·74% C, 4·75% H.

1,4-Lactone of (2*R*)-2-O-(6-O-acetyl-2,3,4-tri-O-methyl-α-D-glucopyranosyl)-5-O-benzoyl-3-O-benzylallaric Acid 6-Methyl Ester (*XXXIV*)

A mixture of anomeric diacetates[4] *XXXII* (0·48 mmol), the lactone ester *XXIX* (200 mg; 0·50 mmol), and 0·05M boron trifluoride etherate in benzene (10 ml) was kept at 20°C for 16 h, washed with saturated aqueous potassium hydrogen carbonate (10 ml), dried, and chromatographed on a thin layer of loose silica gel (two plates, 17 × 44 cm) in 4 : 1 benzene–ethyl acetate. The R_F 0·33 band yielded 176 mg (66%) of the α-glucoside *XXXIV* (dried at 75°C/0·1 Torr for 3 h). ^{1}H-NMR spectrum: δ 5·46 (d, 1-H, $J_{1,2} = 3·5$), 3·24 (dd, 2-H, $J_{2,3} = 9·5$), 3·60 (m, 3-H and 5-H), 3·0 (qu, 4-H, $J_{4,3} = 10·0$ and $J_{4,5} = 8·5$), 4·03 (dd, 6-H, $J_{6,5} = 6·0$ and $J_{gem} = 12·0$), 4·30 (m, 6*-H, $J_{6*,5} = 2·5$), 3·50 and 3·58 (s, 3 × OCH_3), 4·83 (d, 2′-H, $J_{2',3'} = 6·0$), 4·28 (dd, 3′-H, $J_{3',4'} = 1·7$), 5·0 (dd, 4′-H, $J_{4',5'} = 3·0$ Hz), 5·59 (d, 5′-H) and 3·70 (s, $COOCH_3$). For $C_{32}H_{38}O_{14}$ (646·6) calculated: 59·43% C, 5·92% H; found: 59·68% C, 5·94% H. Work-up of the R_F 0·5 band afforded the starting lactone ester *XXIX* (recovery, 19%).

Alcohol XXXV. The benzyl derivative *XXXIV* (65 mg; 0·10 mmol) in acetic acid (5 ml) was hydrogenated in the presence of 10% palladium on charcoal catalyst (50 mg) at 20°C for 30 min. The filtrate was evaporated under diminished pressure and the residue coevaporated with two 10 ml portions of xylene. The final residue was chromatographed on a thin layer of loose silica gel (one plate, 17 × 35 cm) in 1 : 1 benzene–ethyl acetate. Work-up of the R_F 0·3 band yielded 83% of the alcohol *XXXV* which was dried at 90°C/0·05 Torr for 5 h. Optical rotation: $[\alpha]_D^{25}$ +108·6° (*c* 0·27). For $C_{25}H_{32}O_{14}$ (556·5) calculated: 53·95% C, 5·79% H; found: 53·92% C, 5·77% H.

Allarate XXXVII. A solution of the lactone *XXXIV* (65 mg; 0·10 mmol) in methanolic 0·01M-CH_3COONa (10 ml) was stirred at 20°C for 45 min, and diluted with benzene (15 ml) and water (50 ml). The dry benzene layer was evaporated and the residue chromatographed analogously to the preceding paragraph. Work-up of the R_F 0·4 band yielded 82% of the allarate *XXXVII* which was dried at 95°C/0·05 Torr for 3 h. For $C_{33}H_{42}O_{15}$ (678·7) calculated: 58·40% C, 6·24% H; found: 58·46% C, 6·41% H.

Anomeric 1,6-Di-O-acetyl-2,3,4-tri-O-benzyl-D-glucopyranoses (*XXXIIIα* and *XXXIIIβ*)

A mixture of 1,6-anhydro-2,3,4-tri-O-benzyl-β-D-glucopyranose (2·0 g), boron trifluoride etherate (0·25 ml), and acetic anhydride (6 ml) was stirred at 20°C for 10 min, poured into 2% aqueous sodium acetate (40 ml), and extracted with xylene (25 ml). The extract was washed with two 50 ml portions of water and saturated aqueous sodium hydrogen carbonate (50 ml), dried, and evaporated. The residue was chromatographed on a column of silica gel (150 g) in 19 : 1 benzene–ethyl acetate (1 000 ml; fractions 1—50). Fractions 22—40 yielded 1·81 g of the anomeric mixture which was dried at 80°C/0·05 Torr for 10 h). In this mixture, the anomer *XXXIIIα* predominated (*cf.*[8]). ^{1}H-NMR spectrum of compound *XXXIIIα*: δ 6·27 (d, 1-H, $J_{1,2}$ = 3·5), 3·59 (dd, 2-H, $J_{2,3}$ = 9·5), 3·92 ('t', 3-H, $J_{3,4}$ = 8·5), 3·48 ('t', 4-H, $J_{4,5}$ = 9·5), 3·90 (m, 5-H), 4·20 (d, 2 × × 6-H, $J_{6,5}$ = 3·5 Hz), 1·95 and 2·08 p.p.m. (s, 2 × CH_3CO). ^{1}H-NMR spectrum of compound *XXXIIIβ*: δ 5·58 p.p.m. (d, 1-H, $J_{1\ 2}$ = 8·0 Hz), For $C_{31}H_{34}O_8$ (534·6) calculated: 69·65% C, 6·41% H; found: 69·70% C, 6·34% H.

1,4-Lactone of (2*R*)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-5-O-benzoyl--3-O-benzylallaric Acid 6-Methyl Ester (*XXXVI*)

A mixture of diacetates *XXXIII* (267 mg; 0·50 mmol), the lactone ester *XXIX* (200 mg; 0·50 mmol), boron trifluoride etherate (0·50 ml), and benzene (10 ml) was kept at 20°C for 50 min, washed with saturated aqueous potassium hydrogen carbonate (15 ml), dried, and evaporated. The residue was chromatograohed on a thin layer of loose silica gel (2 plates, 18 × 48 cm) in 9 : 1 benzene–ethyl acetate to afford 25% recovery of the lactone ester *XXIX* (R_F 0·28) and 61% yield of compound *XXXVI* (R_F 0·5) which was dried at 90°C/0·05 Torr for 4 h. Optical rotation: $[\alpha]_D^{25}$ +93·4° (*c* 0·30). ^{1}H-NMR spectrum: δ 5·52 (d, 1-H, $J_{1,2}$ = 3·8), 3·61 (dd, 2-H, $J_{2,3}$ = 9·5), 3·99 ('t', 3-H, $J_{3,4}$ = 9·5), 3·39 ('t', 4-H, $J_{4,5}$ = 10·0), 3·95 (m, 5-H and 2 × 6-H), 1·78 (s, CH_3. .CO), 4·72 (d, 2'-H, $J_{2',3'}$ = 5·5), 4·31 (dd, 3'-H, $J_{3',4'}$ = 1·8), 5·03 (qu, 4'-H, $J_{4',5'}$ = 3·0 Hz), 5·63 (d, 5'-H) and 3·72 p.p.m. (s, $COOCH_3$). For $C_{50}H_{50}O_{14}$ (874·9) calculated: 68·64% C, 5·76% H; found: 68·80% C, 5·86% H.

Allarate XXXVIII. A solution of the lactone *XXXVI* (175 mg; 0·20 mmol) in methanolic 0·01M-CH_3COONa (20 ml) was kept at 20°C for 40 min, poured into a mixture of 2% aqueous sodium chloride (300 ml) and benzene (25 ml), the benzene layer dried, evaporated, and the residue chromatographed on a thin layer of loose silica gel (one plate, 18 × 48 cm) in 9 : 1 benzene–-ethyl acetate to afford 79% of the allarate *XXXVIII* which was dried at 80°C/0·05 Torr for 3 h. Optical rotation: $[\alpha]_D^{25}$ +89·7° (*c* 0·51). For $C_{51}H_{54}O_{15}$ (906·9) calculated: 67·54% C, 6·00% H; found: 67·38% C, 6·08% H.

Reaction of the Key Intermediate *XVIII* with Allaric Acid Lactone Ester *XXIX*

A mixture of the intermediate *XVIII* (1·86 g; 2·0 mmol), the lactone ester *XXIX* (1·00 g; 2·50 mmol), boron trifluoride etherate (4·0 ml), and benzene (75 ml) was kept at 20°C for 40 min, washed with water (200 ml) and saturated aqueous potassium hydrogen carbonate (50 ml), dried, and chromatographed on a column of silica gel (150 g) in 19 : 1 benzene–ethyl acetate (1 300 ml; fractions 1—65) and in 9 : 1 benzene–ethyl acetate (700 ml; fractions 66—100). Fractions 15—18 afforded a mixture of anomeric diglycoside ethers *XLVII* and *L* (79 mg; 4%), m.p. 144—148°C (ether). For $C_{45}H_{42}Cl_6O_{14}$ (1019·6) calculated: 53·01% C, 4·15% H; found: 53·29% C, 4·24% H. Fractions 32—55 were pooled, evaporated, and the residue crystallised from ether to afford 402 mg (recovery, 22%) of the starting reactant *XVIII*, m.p. 133—136°C. The other starting reactant *XXIX* (222 mg; recovery, 22%) was obtained from fractions 87—97.

Mother liquors of fractions 32—55 and fractions 56—81 (containing the remaining portion of the intermediate *XVIII* along with the anomeric glucosides *XXXIX* and *XLV*) were pooled, evaporated under diminished pressure, and the residue subjected to hydrogenolysis in acetic acid (25 ml) in the presence of 10% palladium on charcoal catalyst (0·6 g) and 10% aqueous palladium chloride (50 mg) for 35 min at 20°C, the mixture filtered, the filtrate evaporated, and the residue coevaporated with two 30 ml portions of toluene and with benzene (50 ml). The final residue was chromatographed on a column of silica gel (50 g) in 4 : 1 benzene–ethyl acetate (600 ml; fractions 1—36) and in 7 : 3 benzene–ethyl acetate (400 ml; fractions 37—60). Fractions 17—23 yielded 88 mg (5%) of the alcohol *XIXα'*, m.p. 150—153°C (ether). ^{1}H-NMR spectrum: δ 5·39 (s, 1-H), 5·50—5·72 (m, 2-H, 3-H and 3'-H), 4·30—4·50 (m, 4-H and 2 × 5-H), 6·22 (d, 1'-H, $J_{1',2'} = 3{\cdot}5$), 3·90 (dd, 2'-H, $J_{2',3'} = 10{\cdot}0$), 5·30 (m, 3'-H), 3·72 (t, 4'-H, $J_{4',3'} = J_{4',5'} = 9{\cdot}0$ Hz), 3·82—4·10 (m, 5'-H, 2 × 6'-H and CCl_3CH_2), 2·50 (bs, OH), 2·04 and 2·18 (s, 2 × CH_3CO) and 7·20—8·10 p.p.m. (m, 3 × C_6H_5). For $C_{38}H_{37}Cl_3O_{15}$ (840·1) calculated: 54·32% C, 4·44% H, 12·66% Cl; found: 53·98% C, 4·34% H, 12·38% Cl. Fractions 25—35 yielded 226 mg (10·4% or 13·4% with respect to the recovered reactants *XVIII* and *XXIX*) of the α-glucoside *XL*, m.p. 182—185°C (ether–light petroleum). ^{1}H-NMR spectrum: δ 5·34 (s, 1-H), 5·47 to 5·74 (m, 2-H, 3-H, 1'-H, and 3'-H), 3·82 (s, $COOCH_3$), 1·97 (s, CH_3CO) and 7·20—8·05 p.p.m. (m, 4 × C_6H_5). For $C_{50}H_{47}Cl_3O_{21}$ (1090) calculated: 55·08% C, 4·34% H, 9·76% Cl; found: 55·08% C, 4·40% H, 9·58% Cl. Fractions 38—47 were evaporated, the residue (140 mg) heated at 40°C with trifluoroacetic acid (0·15 ml) and acetic anhydride (10 ml) for 50 min, the resulting solution evaporated under diminished pressure, the residue coevaporated with two 20 ml portions of xylene, and finally chromatographed on a thin layer of loose silica gel (one plate, 17 × × 45 cm) in 5 : 1 benzene–ethyl acetate. The R_F 0·27 band yielded 21 mg (1·8% or 2·3% with respect to the recovered reactants) of the acetylated β-glucoside *XLVI*, m.p. 119—123°C (ether). ^{1}H-NMR spectrum: δ 5·33 (s, 1-H), 3·80—5·67 (unresolved multiplets of total 19 protons), 3·84 (s, $COOCH_3$), 1·90, 1·98, and 2·08 (s, 3 × CH_3CO) and 7·20—8·10 p.p.m. (m, 4 × C_6H_5). For $C_{54}H_{51}Cl_3O_{23}$ (1174) calculated: 55·23% C, 4·38% H, 9·04% Cl; found: 55·48% C, 4·52% H, 8·97% Cl.

Acetyl derivatives XXα' *and* XL1. A mixture of the alcohol *XIXα'* (84 mg; 0·10 mmol) and 0·15M-CF_3COOH in acetic anhydride (5 ml) was heated at 40°C for 50 min, evaporated, the residue coevaporated with two 10 ml portions of xylene, and finally crystallised from chloroform–ether to afford 91% of the acetyl derivaitve *XXα'*, m.p. 177—181°C. ^{1}H-NMR spectrum: δ 5·34 (s, 1-H), 6·31 (d, 1'-H, $J_{1',2'} = 3{\cdot}5$), 5·19 (dd, 2'-H, $J_{2',3'} = 10{\cdot}0$), 5·82 (dd, 3'-H, $J_{3',4'} = 9{\cdot}0$), 3·76 ('t', 4'-H, $J_{4',5'} = 9{\cdot}0$ Hz), 1·87, 2·06, and 2·18 p.p.m. (s, 3 × CH_3CO). For $C_{40}H_{39}Cl_3O_{16}$ (882·1) calculated: 54·46% C, 4·46% H; found: 54·57% C, 4·50% H.

An analogous acetylation of the alcohol *XL* (0·10 mmol) and chromatography on a thin layer of loose silica gel (one plate, 17 × 44 cm) in 5 : 1 benzene–ethyl acetate yielded 72% of the acetyl derivative *XLI* which was dried at 100°C/0·1 Torr for 5 h. ^{1}H-NMR spectrum: δ 5·37 (d, 1-H, $J_{1,2} = 1{\cdot}0$), 5·70 (d, 1'-H, $J_{1',2'} = 3{\cdot}5$), 5·88 (t, 3'-H, $J_{3',2'}$ m 10·0), 3·74 (t, 4'-H, $J_{4',3'} = J_{4',5'} = 9{\cdot}5$ Hz), 3·87 (s, $COOCH_3$), 1·97, 2·01, and 2·30 p.p.m. (s, 3 × CH_3CO). For $C_{54}H_{51}Cl_3O_{23}$ (1174·3) calculated: 55·23% C, 4·38% H; found: 54·95% C, 4·57% H.

Reactions of the Intermediate *XVIII* with Acetic Acid and 2,2,2-Trichloroethanol

A. A mixture of the intermediate *XVIII* (186 mg; 0·20 mmol), boron trifluoride etherate (0·4 ml), and 0·025M-CH_3COOH in benzene (8 ml) was kept at 20°C for 1 h, washed with saturated aqueous potassium hydrogen carbonate (15 ml), dried, and evaporated. The residue was chromatographed on a thin layer of loose silica gel (one plate, 17 × 44 cm) in 9 : 1 benzene–ethyl

acetate to recover 57% of the starting material *XVIII* (R_F 0·35) and to obtain (8·5%) of the anomeric pair (R_F 0·5) of glucosides *XLVII* and *L*; the melting point of the mixture was 145–149°C (ether), without depression on admixture with the by-product of the reaction of compound *XVIII* with the lactone ester *XXIX*.

B. A mixture of the intermediate *XVIII* (930 mg; 1·00 mmol), 2,2,2-trichloroethanol (0·75 g; 5·0 mmol), boron trifluoride etherate (2·0 ml), and benzene (38 ml) was kept at 20°C for 50 min, washed with water (150 ml) and saturated aqueous potassium hydrogen carbonate (50 ml), dried, evaporated, and the residue chromatographed on a thin layer of loose silica gel (5 plates, 17 × × 44 cm) in 13 : 1 benzene–ethyl acetate to afford 47% of the anomeric pair (R_F 0·47; m.p. 133–139°C) of compounds *XLVII* and *L*, and 34% (dried at 90°C/0·1 Torr for 4 h) of the anomeric pair (R_F 0·39) of compounds *LII* and *LV*. For $C_{45}H_{42}Cl_6O_{14}$ (1019·6) calculated: 53·01% C, 4·15% H, 20·87% Cl; found: 53·28% C, 4·20% H, 20·96% Cl.

Alcohols XLVIII, LI, LIII, *and* LVI. The anomeric pair of compounds *XLVII* and *L* (m.p. 133–139°C; 204 mg; 0·20 mmol) was hydrogenolysed in glacial acetic acid (25 ml) in the presence of 10% palladium on charcoal catalyst (0·4 g) and 10% aqueous palladium chloride (50 mg) for 1 h at room temperature. The mixture was filtered, the filtrate evaporated under diminished pressure, and the residue coevaporated with two 20 ml portions of xylene and with benzene (20 ml). The final residue was chromatographed on a thin layer of loose silica gel (two plates, 17 × 44 cm) in 9 : 1 benzene–ethyl acetate to afford 61% of the alcohol *XLVIII*, R_F 0·22, m.p. 150–153°C (chloroform–ether), and 33% of the alcohol *LI*, R_F 0·33, m.p. 151–155°C (ether). ^{1}H-NMR spectrum of the alcohol *XLVIII*: δ 5·35 (s, 1-H, $J_{1,2}$ = 0·5), 5·50–5·75 (m, 2-H and 3-H), 5·27 (d, 1′-H, $J_{1',2'}$ = 3·5), 3·78 (dd, 2′-H, $J_{2',3'}$ = 10·0), 5·50–5·75 (m, 3′-H), 3·67 (t, 4′-H, $J_{4',3'}$ = = $J_{4',5'}$ = 9·0 Hz) and 2·05 p.p.m. (s, CH_3CO). For $C_{38}H_{36}Cl_6O_{14}$ (929·4) calculated: 49·10% C, 3·90% H; found: 49·37% C, 3·88% H. ^{1}H-NMR spectrum of the alcohol *LI*: δ 5·34 (bs, 1-H, $J_{1,2}$ = 0·5), 5·45–5·70 (m, 2-H and 3-H), 4·72 (d, 1′-H, $J_{1',2'}$ = 7·5), 3·77 (dd, 2′-H, $J_{2',3'}$ = = 9·0 Hz) and 2·06 p.p.m. (s, CH_3CO). For $C_{38}H_{36}Cl_6O_{14}$ (929·4) calculated: 49·10% C, 3·90% H; found: 49·36% C, 3·93% H.

Hydrogenolysis of the anomeric pair of compounds *LII* and *LV* and an analogous work-up yielded 67% of the alcohol *LIII* (R_F 0·13) and 27% of the anomer *LVI* (R_F 0·20). Compound *LIII* was dried at 100°C/0·1 Torr for 5 h. For $C_{38}H_{36}Cl_6O_{14}$ (929·4) calculated: 49·10% C, 3·90% H; found: 49·50% C, 4·09% H. ^{1}H-NMR spectrum of compound *LVI*: δ 5·46 (d, 1-H, $J_{1,2}$ = 4·0), 5·11 (qu, 2-H, $J_{2,3}$ = 6·5), 4·75 (d, 1′-H, $J_{1',2'}$ = 7·5), 3·78 (t, 2′-H, $J_{2',3'}$ = 8 Hz) and 2·15 p.p.m. (s, CH_3CO). For $C_{38}H_{36}Cl_6O_{14}$ (929·4) calculated: 49·10% C, 3·90% H; found: 49·39% C, 4·19% H.

Acetyl derivatives XLIX *and* LIV. A mixture of the alcohol *XLVIII* (60 mg), trifluoroacetic acid (0·10 ml), and acetic anhydride (5 ml) was heated at 40°C for 1 h, evaporated, and the residue coevaporated with two 10 ml portions of xylene to afford 96% of the acetyl derivative *XLIX*, m.p. 171–174°C (chloroform–ether). ^{1}H-NMR spectrum: δ 5·30 (bs, 1-H, $J_{1,2}$ = 1·0), 5·65 (dd, 2-H, $J_{2,3}$ = 5·0), 5·51 (qu, 3-H, $J_{3,4}$ = 6·5), 5·38 (d, 1′-H, $J_{1',2'}$ = 3·5), 5·0 (dd, 2′-H, $J_{2',3'}$ = = 10·0), 5·90 (bt, 3′-H, $J_{3',4'}$ = 9·0), 3·73 (t, 4′-H, $J_{4',5'}$ = 9·0 Hz), 1·94 and 2·07 p.p.m. (s, 2 × CH_3CO). For $C_{40}H_{38}Cl_6O_{15}$ (971·5) calculated: 49·45% C, 3·94% H, 21·90% Cl; found: 49·34% C, 3·81% H, 22·01% Cl. The acetyl derivative *LIV* was prepared (yield, 98%) analogously, isolated by chromatography on a thin layer of loose silica gel in 9 : 1 benzene–ethyl acetate, and dried at 100°C/0·1 Torr for 5 h. ^{1}H-NMR spectrum: δ 5·46 (d, 1-H, $J_{1,2}$ = 4·5), 5·11 (qu, 2-H, $J_{2,3}$ = 6·5), 5·41 (d, 1′-H, $J_{1',2'}$ = 3·5), 5·02 (dd, 2′-H, $J_{2',3'}$ = 10·0), 5·91 (bt, 3′-H, $J_{3',4'}$ = 9·0), 3·70 (t, 4′-H, $J_{4',5'}$ = 9·0 Hz), 1·96 and 2·15 p.p.m. (s, 2 × CH_3CO). For $C_{40}H_{38}Cl_6O_{15}$ (971·5) calculated: 49·45% C, 3·94% H; found: 49·71% C, 4·20% H.

Exchange of the 2,2,2-Trichloroethyl Group in Glycosides *XVIII*, *XXα'*, *XL*, and *XLI* for an Acetyl Group

A mixture of compound *XVIII* (100 mg), powdered zinc (1·0 g), and 0·3M-CF_3COOH in acetic anhydride (20 ml) was heated with stirring for 90 min at 40°C, evaporated, the residue coevaporated with two 20 ml portions of xylene, and dissolved in benzene (25 ml). The solution was washed with water (50 ml), dried, evaporated, and the residue chromatographed on a thin layer of loose silica gel (one plate, 17 × 44 cm) in 5 : 1 benzene–ethyl acetate. The R_F 0·25 band yielded 67% of the anomeric acetates *LVII* (dried at 85°C/0·1 Torr for 7 h). For $C_{45}H_{44}O_{16}$ (840·8) calculated: 64·28% C, 5·28% H; found: 63·89% C, 5·30% H. Work-up of the minor band afforded the starting material *XVIII* (recovery, 10%).

The glycoside *XXα'* was converted analogously to the anomeric acetates *LVIII* in 63% yield (recovery, 26% of compound *XXα'*). For $C_{40}H_{40}O_{17}$ (792·7) calculated: 60·60% C, 5·08% H; found: 61·00% C, 5·24% H.

A mixture of compound *XL* (100 mg), powdered zinc (1·0 g), trifluoroacetic acid (0·75 ml), and acetic anhydride (10 ml) was stirred at 40°C for 3 h and processed as above to afford 65% of compound *XLII* (dried at 100°C/0·1 Torr for 5 h) along with 7% of the acetyl derivative *XLI*. For $C_{54}H_{52}O_{24}$ (1085) calculated: 59·78% C, 4·83% H; found: 59·41% C, 5·07% H. The acetyl derivative *XLI* was analogously converted into compound *XLII* in 80% yield (recovery, 5% of the starting acetate *XLI*).

Reaction of N^6-Benzoyladenine Chloromercuri Salt with the Halogenose *XLIII*

A solution of anomeric acetates *XLII* (126 mg; 0·10 mmol) in toluene was saturated with dry hydrogen bromide at 0°C for 5 min and kept at 20°C for 10 min. The mixture was evaporated under diminished pressure and the residue coevaporated with two 10 ml portions of toluene. The residual halogenose *XLIII*, acetonitrile (20 ml), and N^6-benzoyladenine chloromercuri salt (previously dried by coevaporation with toluene; 150 mg; 0·33 mmol) were refluxed for 75 min, the solvent evaporated, and the residue dissolved in a mixture of benzene (25 ml) and 20% aqueous potassium iodide (10 ml). The benzene layer was washed with saturated aqueous potassium hydrogen carbonate (5 ml), dried, evaporated, and the residue chromatographed on a thin layer of loose silica gel (one plate, 17 × 38 cm) in 1 : 1 benzene–ethyl acetate. The R_F 0·4 band yielded 42% of the amorphous nucleoside *XLIV* which was dried at 100°C/0·1 Torr for 4 h. ^{1}H-NMR spectrum: δ 6·54 (d, 1-H, $J_{1,2} = 6$), 5·63 (d, 1'-H, $J_{1',2'} = 4$ Hz), 3·80 (s, $COOCH_3$), 1·95, 2·02, and 2·40 p.p.m. (s, 3 × CH_3CO). For $C_{64}H_{57}N_5O_{23}$ (1264·1) calculated: 60·80% C, 4·55% H, 5·54% N; found: 60·50% C, 4·80% H, 5·21% N.

Allarate LIX. A solution of the lactone *XLIV* (37·9 mg; 0·030 mmol) in methanolic 0·01M-CH_3COONa (6·0 ml; 0·060 mmol) was kept at 20°C for 1 h and diluted with a mixture of 2% aqueous sodium chloride (100 ml) and benzene (30 ml). The benzene layer was washed with water (100 ml), dried, evaporated, and the residue chromatographed on a thin layer of loose silica gel (one plate, 17 × 34 cm) in 1 : 1 benzene–ethyl acetate to afford the allarate *LIX* (dried at 100°C/0·1 Torr for 4 h) in 58% yield (recovery, 22% of the lactone *XLIV*). ^{1}H-NMR spectrum: δ 8·34 and 8·67 (s, 8-H and 2-H of adenine), 6·34 (d, 1'-H, $J_{1',2'} = 5$), 5·62 (d, 1''-H, $J_{1'',2''} = 3·5$ Hz), 3·82, 3·84 (s, 2 × $COOCH_3$), 1·92, 1·96, 2·32 (s, 3 × CH_3CO) and 7·15–8·10 p.p.m. (m, 5 × × C_6H_5). For $C_{65}H_{61}N_5O_{24}$ (1296·2) calculated: 60·23% C, 4·74% H, 5·40% N; found: 60·53% C, 4·90% H, 5·18% N.

Exotoxin (*I*)

To a solution of the allarate *LIX* (26 mg; 0·020 mmol) in benzene (5 ml) there was added 0·1M-$-C_5H_5N$ in benzene (0·5 ml) and 0·1M-$POCl_3$ in benzene (0·4 ml). The mixture was kept at 20°C for 100 min, decomposed with water (0·5 ml), and the aqueous phase extracted with two 10 ml portions of chloroform. The organic layers were combined, filtered, the filtrate evaporated, and the residue hydrolysed at 20°C for 22 h in a mixture of methanolic 1M-CH_3ONa (3 ml), water (1 ml), and dioxane (20 ml). The solution was neutralised with Dowex 50 (pyridinium form) ion exchange resin, filtered, the filtrate concentrated under diminished pressure to the volume of 3 ml. The concentrate was chromatographed on paper Whatman No 3 MM in the solvent system 55 : 10 : 35 1-propanol–conc. aqueous ammonia–water to afford 42% of exotoxin (*I*), identical[5] on chromatography and electrophoresis with a specimen obtained from a naturally occurring material[1].

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