SYNTHESIS OF EXOTOXIN PRODUCED BY *Bacillus thuringiensis*. II. FORMATION OF THE α-GLUCOSIDIC BOND, NUCLEOSIDATION, PHOSPHORYLATION*

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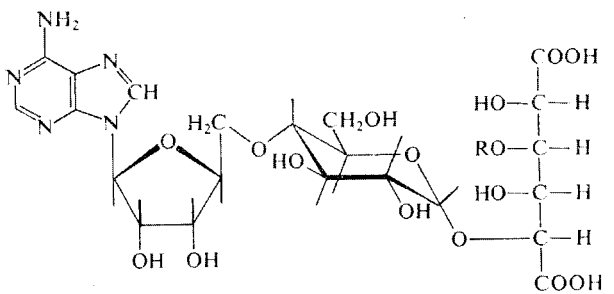
An optically active ester-lactone *IV* has been synthesised and attached through an α-glucosidic bond to ethers *II* and *III*. The resulting α-glucoside *XX* has been transformed by a sequence of reactions to the nucleoside *XXXI* which has furnished (opening of the lactone ring, phosphorylation, and hydrolysis of protecting groups) the exotoxin *I*. Oxidation of the primary hydroxylic function of the alcohol *VII* to the carboxylic group has been affected under very mild conditions with sodium periodate under catalysis of ruthenium tetraoxide. α-Glucosylation of the ester-lactone *IV* with ethers *II* or *III* has been catalysed by boron trifluoride etherate. The general applicability of the present method to the synthesis of disaccharides has been demonstrated by the preparation of the 5-O-α-D-glucopyranosyl-D-glucose derivative *XV*.

In the first part of the exotoxin *I* synthesis, the preparation of the intermediary ethers *II* and *III* has been accomplished[1]. In the present part, we wish to report attachment of these ethers to allaric acid by an α-glucosidic bond, nucleosidation of the pentose portion of the molecule, and finally, phosphorylation of the allaric portion of the molecule at the appropriate position.



I, R = $PO(OH)_2$

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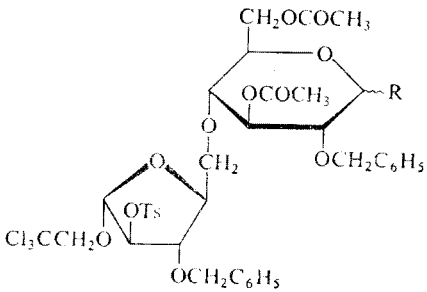
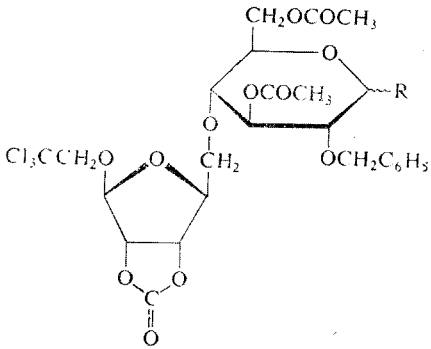
Since the bond between glucose and allaric acid in exotoxin *I* is formed by an α-glucosidic attachment[2,3] to a hydroxylic function possessing[3,4] the absolute configuration *R*, a suitable optically active derivative of allaric acid was required as intermediate. It appeared advisable to prepare such an allaric acid derivative, the hydroxylic function (*i.e.*, that hydroxyl bound to a phosphoryl group in the naturally occurring exotoxin *I*) of which would be protected simultaneously with the carbo-

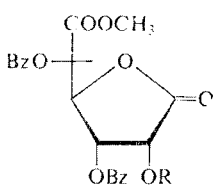


II, R = $OCOCH_3$
XVI, R = Br

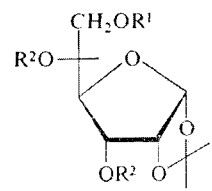


III, R = $OCOCH_3$
XVII, R = Br

xylic group at the γ-position by the formation of a lactone ring. On the basis of a series of preliminary model experiments, the ester-lactone *IV* was selected as the most suitable substance. The synthesis of the ester-lactone *IV* was started from 1,2-O-isopropylidene-α-D-allofuranose[5] (*V*) which was triphenylmethylated and benzoylated to afford 5,6-di-O-benzoyl-1,2-O-isopropylidene-6-O-triphenylmethyl-α-D-allofuranose (*VI*) from which (without isolation) the protecting triphenylmethyl group was removed by the action of hydrochloric acid in acetone. The thus-obtained 3,5-di-O-benzoyl-1,2-O-isopropylidene-α-D-allofuranose (*VII*) was transformed by the

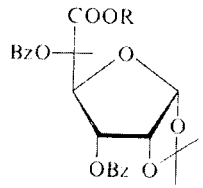


IV, R = H
XXXII, R = Bz

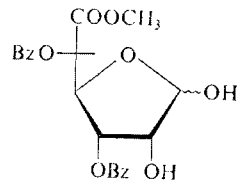


V, $R^1 = R^2 = H$
VI, $R^1 = C(C_6H_5)_3$; $R^2 = Bz$
VII, $R^1 = H$; $R^2 = Bz$

ruthenium tetraoxide catalysed oxidation with sodium periodate[6] to the uronic acid *VIII*, the esterification of which with diazomethane yielded methyl 3,5-di-O-benzoyl--1,2-O-isopropylidene-α-D-alluronate (*IX*). Acidic hydrolysis of the protecting isopropylidene group in the ester *IX* afforded methyl 3,5-di-O-benzoyl-D-alluronate (*X*) which was converted by oxidation with bromine in aqueous dioxane into the required ester-lactone *IV*.

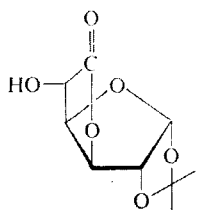


VIII, R = H
IX, R = CH_3

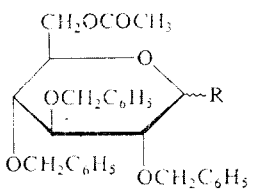


X

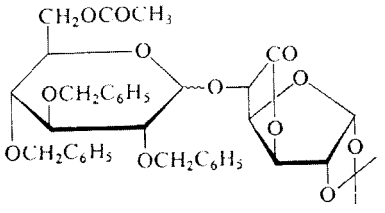
The attachment of glucose by means of an α-glucosidic bond to an aglycone bearing a γ-lactone ring in the neighbourhood of the hydroxylic function has been examined on model glucosylations of 1,2-O-isopropylidene-α-D-glucurono-1,4-lactone (*XI*) with 6-O-acetyl-2,3,4-tri-O-benzyl-D-glucopyranosyl bromide (*XII*) or with 1,6-di-O-acetyl--2,3,4-tri-O-benzyl-D-glucopyranose (*XIII*). Satisfactory yields were obtained by the Koenigs–Knorr condensation of the lactone *XI* with the bromide *XII* in nitromethane in the presence of mercuric cyanide and by the direct condensation of the diacetate *XIII* with the lactone *XI* in the presence of stannic chloride or boron trifluoride etherate. In all three cases an anomeric mixture of the 5-O-glucosyl derivatives *XIVα* and *XIVβ* was obtained always in the anomeric ratio of 4 : 1. The predominating anomer could be isolated by crystallisation. Since the anomeric portion of the ^{1}H-NMR spectrum of glycosyl derivatives *XIVα,β* is rather complicated, the assignment of the α-configuration to the predominating anomer *XIVα* was performed on the basis of the well resolved spectrum of the corresponding alcohol *XV* obtained from the anomer *XIVα* by lithium aluminium hydride reduction.



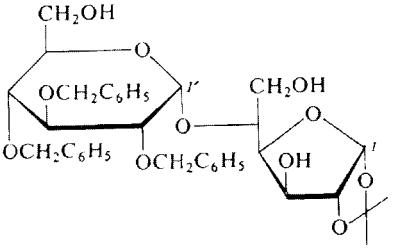
XI



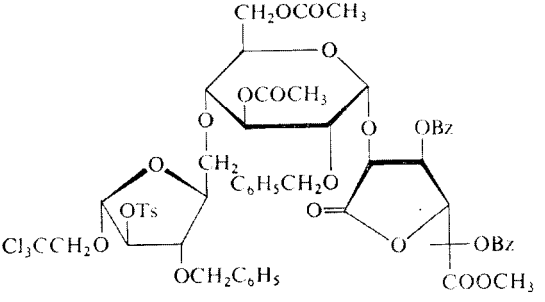
XII, R = Br
XIII, R = $COCH_3$



XIVα,β



XV

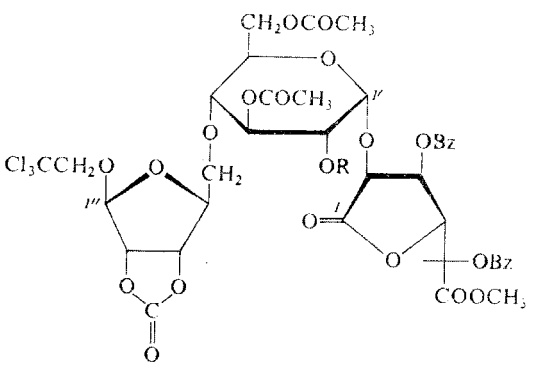


XVIII

In syntheses of disaccharides, the acid-catalysed glycosylation has not been so far widely used. Thus, the literature reports the use of stannic chloride in the preparation of glycosides of some simple alcohols[7] and the application of boron trifluoride etherate to the preparation of glucosides from the benzyl-type alcohols and 2,3,4,6--tetra-O-acetyl-D-glucopyranose under mild conditions (15 min at −20°C; *cf.*[8,9]). In the latter case, the sugar component does not represent the active electrophilic particle but figures as the passive component which is alkylated by an electrophilic particle arisen from the alcohol and boron trifluoride etherate. It has ben found that glycosides may be prepared from 2,3,4,6-tetra-O-acetyl-D-glucopyranose under those conditions only with the use of considerably reactive alcohols of the benzyl alcohol type; the other alcohols (*e.g.*, 2,2,2-trichloroethanol) do not react except for much more vigorous conditions (elevated temperatures). To our opinion, the acid-catalysed glycosylation may be preferably used in the synthesis of disaccharides (a simple technique and often considerably higher yields when compared with the Koenigs–Knorr method) in those cases when the sugar component to be glycosylated readily forms an active electrophilic particle by the action of acidic catalysts. Such cases are suitable for glycosylation of as sensitive substances as the lactone *XI* and the ester-lactone *IV*.

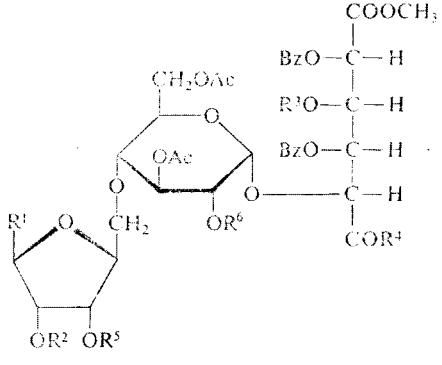
On the basis of the above successful model experiment, the α-glucosylation of the ester-lactone *IV* with ethers *II* or *III* was attempted. These two α-glucosylations markedly depend on the condensation methods. Thus, stannic chloride as catalyst did not prove successful. Somewhat better yields were obtained when the ethers *II* or *III* were converted by the action of gaseous hydrogen bromide in toluene into the corresponding halogenoses *XVI* and *XVII* which were then condensed with the ester-lactone *IV* in nitromethane in the presence of mercuric cyanide. In the best case (condensation of the halogenose *XVII* with the ester-lactone *IV*) however, the yield of the α-glucoside *XXI* was 9% only. Better yields (25%) were obtained when condensations of ethers *II* and *III* with the ester-lactone were catalysed by boron trifluoride etherate. The chromatographic behaviour of the glycoside *XVIII* (condensation product of the ether *II* with the ester-lactone *IV*) is identical with that of the starting ether *II*. Consequently, the chromatographic fraction corresponding by its mobility to the ether *II* was subjected to the action of ammonia and acetylation; the resulting amide *XIX* was then readily isolated by chromatography. From the ^{1}H-NMR spectrum of the amide *XIX* there was not possible to determine the configuration at the anomeric centre of the glucose residue. As suggested however by numerous analogous cases, the amide *XIX* represents an α-glucoside which could be contaminated only with traces of the β-anomer.

On the other hand, the chromatographic mobility of the glycoside *XX* (obtained from glycosylation of the ester-lactone *IV* with the ether *III* or the corresponding bromide *XVII*) is very similar to that of the starting ester-lactone *IV*. The chromatographic fraction corresponding to the mobility of the ester-lactone *IV* was therefore hydrogenolysed; the resulting debenzylated glycoside *XXI* travels much more slowly and may be thus isolated by chromatography. Acetylation of the glycoside *XXI* afforded the acetate *XXII* in the form of a solvate with two molecules of *m*-xylene. In the glycosylation of the ester-lactone *IV* with the ether *III* or the bromide *XVII* (prepared from *III*) no β-anomer was detected by preparative or spectral (^{1}H-NMR) methods.



XX, R = $CH_2C_6H_5$
XXI, R = H
XXII, R = $COCH_3$

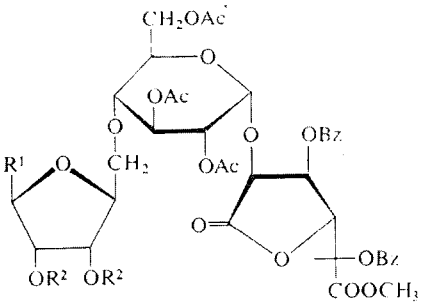
The further steps of the exotoxin *I* synthesis required attachment of adenine to the pentose portion of the glycoside *XIX* and *XXII* molecule and phosphorylation of the allaric acid residue. The attempted conversion of the pentose portion of the ester-amide *XIX* to the required adenosine derivative comprised reductive removal of the trichloroethyl group and treatment of the resulting hydroxy derivative *XXIII* with the sodium salt of adenine. Unfortunately, the allaric portion of the molecule was to a considerable extent destructed during this procedure owing to a relatively high basicity of the sodium salt of adenine. A complicated mixture of products was obtained from which the required compound *XXIV* (as confirmed by ^{1}H-NMR spectrum) was isolated in a low yield and insufficient purity. This route was therefore abandoned in favour of the synthesis starting from the glycoside *XXII*.



XIX, R = α-OCH_2CCl_3
XXIII, R = OH

Prior to nucleosidation of the glycoside *XXII*, it was necessary to activate the ribose anomeric centre by hydrolysis of the 2″,3″-cyclocarbonate bond, reductive removal of the trichloroethyl group, and their replacement by acetyl groups. The glycoside *XXII* was therefore subjected to a base-catalysed hydrolysis. In this step, the hydrolysis of the cyclocarbonate bond was accompanied by opening of the 1,4-lactone system on the allaric portion of the molecule. With respect to the future introduction of a phosphoryl group into the allaric moiety, the lactone ring of the hydroxy acid *XXV* had to be recyclised. Recyclisation of the lactone ring by heating in formic acid did not afford satisfactory yields. Considerably better yields were obtained with the use of the Woodward[10] method, especially with ethoxyacetylene as the cyclisation agent. The thus-obtained ester-lactone *XXVI* was acetylated with acetic anhydride under catalysis of trifluoroacetic acid to afford the pentaacetate *XXVII*, the trichloroethyl group of which was then removed by the action of zinc in acetone in the presence of hydrochloric acid. The resulting hydroxy derivative *XXVIII* was

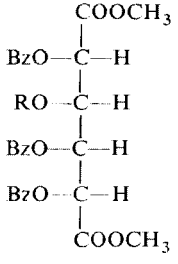
converted by acetylation to a mixture of anomeric hexaacetates *XXIX* and then (by the action of gaseous hydrogen bromide) to the halogenose *XXX* which afforded the nucleoside *XXXI* on treatment with N^6-benzoyladenine chloromercuri salt.



XXVI, $R^1 = OCH_2CCl_3$; $R^2 = H$
XXVII, $R^1 = OCH_2CCl_3$; $R^2 = Ac$
XXVIII, $R^1 = \alpha,\beta$-OH; $R^2 = Ac$
XXIX, $R^1 = \alpha,\beta$-OAc; $R^2 = Ac$
XXX, $R^1 = \alpha,\beta$-Br; $R^2 = Ac$
XXXI, $R^1 = 9$-N^6Bz-adenyl; $R^2 = Ac$

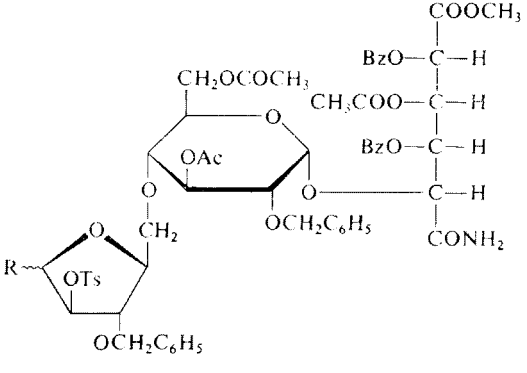
The opening of the lactone ring in the nucleoside *XXXI* and phosphorylation of the free hydroxylic function has been first examined with the use of the ester-lactone *XXXII* as the model substance. Compound *XXXII* was obtained by benzoylation of the free hydroxyl in compound *IV*. In the model methanolysis of the ester-lactone *XXXII*, the lactone ring could be opened by the action of methanol under acidic catalysis of boron trifluoride etherate as well as under basic catalysis of pyridine. In both cases there was obtained the identical dimethyl ester *XXXIII* without any perceptible migration of benzoyl groups. In the choice of a suitable phosphorylating agent for the phosphorylation of the free hydroxylic function in the dimethyl ester *XXXIII*, the following factors had to be taken into consideration: considerable inclination of the non-ionised phosphoryl group in β-position with respect to the ester function towards elimination by the action of basic agents, potential migration of benzoyl groups, and the relatively low accessibility of the secondary hydroxylic function. In this respect, phosphorus oxychloride appears as the single suitable agent. Phosphorylation with this agent is rapid and practically quantitative even in the presence of one equivalent of pyridine. The resulting ester-dichloride *XXXIV* may be hydrolysed under very mild conditions to the phosphate *XXXV*; there is no danger any more of an elimination of the phosphoryl group from the phosphate *XXXV* by the action of basic substances. For purposes of isolation, the phosphate *XXXV* was treated with ethereal diazomethane to obtain compound *XXXVI* which was characterised by elemental analysis and ^{1}H-NMR spectrum.

On the basis of the above successful model experiment, methanolysis of the lactone ring in the nucleoside *XXXI* was undertaken. With the use of an acidic catalyst,



XXXIII, R = H
XXXIV, R = $POCl_2$
XXXV, R = $PO(OH)_2$
XXXVI, R = $PO(OCH_3)_2$

the methanolysis of the lactone ring was accompanied by a partial removal of protecting acetyl groups. Considerably better results were obtained with pyridine as catalyst. The resulting dimethyl ester *XXXVII* was phosphorylated with excess phosphorus oxychloride to afford the ester-dichloride *XXXVIII* which was then subjected to alkaline hydrolysis. The thus-obtained exotoxin *I* was isolated from the reaction mixture by chromatography on DEAE cellulose.



XXV, $R^1 = OCH_2CCl_3$;
$R^2 = R^3 = R^5 = H$; $R^4 = OH$
$R^6 = Ac$;
XXXVII, R^1 = 9-N^6Bz-adenyl;
$R^2 = R^5 = R^6 = Ac$
$R^3 = H$; $R^4 = OCH_3$
XXXVIII, R^1 = 9-N^6Bz-adenyl;
$R^2 = R^5 = R^6 = Ac$;
$R^3 = POCl_2$; $R^4 = OCH_3$
XXIV, R^1 = 9-adenyl; $R^2 = H$
$R^3 = Ac$; $R^4 = NH_2$;
$R^5 = R^6 = CH_2C_6H_5$

Characterisation of the synthetic exotoxin *I* is rather dificult since its isolation in crystalline form has failed. Consequently, some comparisons were performed between the naturally occurring and the synthetic exotoxin with respect to the biological activity, chromatography on ion exchange resins, and electrophoresis at different pH values. All these comparisons demonstrated identity of the two specimens of different origin.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Unless stated otherwise, analytical samples were dried at 20°C/0·1 Torr for 10 h. The ^{1}H-NMR spectra were measured on a Varian HA 100 apparatus. The IR spectra were recorded on Zeiss UR 10 apparatus. Thin-layer chromatography was performed on the Merck Kieselgel CF_{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 taken down under diminished pressure on a rotatory evaporator at the bath temperature of 40°C.

3,5-Di-O-benzoyl-1,2-O-isopropylidene-α-D-allofuranose (*VII*)

To a solution of 1,2-O-isopropylidene-α-D-allofuranose[5] (*V*; 28·0 g; 0·128 mol) in pyridine (140 ml) there was added triphenylmethyl chloride (39·6 g; 0·142 mol). The stirred mixture was kept at room temperature for 24 h, cooled down with ice, and treated dropwise with benzoyl chloride (56 g; 0·40 mol) at the temperature up to 25°C. The stirring at room temperature was continued for 1 h, the mixture poured onto ice (100 g), and extracted with benzene. The extract was washed with dilute hydrochloric acid, water, and dilute aqueous ammonia, dried, and evaporated. The residue was dissolved in acetone (280 ml), the solution kept with conc. hydrochloric acid (14 ml) for 1 h at room temperature, neutralised with aqueous ammonium acetate, and evaporated. The residue was diluted with water (100 ml) and extracted with two 150 ml portions of benzene. The extract was dried and concentrated under diminished pressure to the volume of 200 ml.

Sudan Orange (10 mg) was added to the concentrate and the mixture applied to a column of silica gel (500 g). The Sudan Orange was eluted along with triphenylmethanol by 95 : 5 benzene–ether. With 85 : 15 benzene–acetone solvent mixture, the dibenzoate *VII* was eluted (R_F of compound *VII*, 0·45 on thin-layer chromatography in the same solvent mixture). Fractions containing the dibenzoate *VII* were pooled and evaporated. The residue (49·0 g; sufficiently pure for the preparation of the ester *X*) was diluted with ether and then light petroleum until slightly turbid and kept at 0°C to deposit crystals. The solid was collected with suction and washed with 1 : 2 ether–light petroleum to afford 29·0 g (53%) of the dibenzoate *VII*, m.p. 106–108°C, $[\alpha]_D^{25}$ +125·0° (*c* 0·5 in chloroform). For $C_{23}H_{24}O_8$ (428·4) calculated: 64·48% C, 5·65% H; found: 64·40% C, 5·70% H.

Methyl 3,5-Di-O-benzoyl-1,2-O-isopropylidene-α-D-alluronate (*IX*)

To a solution of the dibenzoate *VII* (21·6 g; 0·05 mol) in 70% aqueous acetone (400 ml) there were added sodium periodate (42·6 g; 0·20 mol) and 5% aqueous ruthenium trichloride trihydrate (0·5 ml). The mixture was stirred at room temperature until the dibenzoate *VII* disappeared (3·5 h; thin-layer chromatography in 85 : 15 benzene–acetone, R_F 0·45), filtered, and the material on the filter washed with acetone. The filtrate and washings were combined, evaporated,

and the residue extracted with two 100 ml portions of ethyl acetate. The extract was treated with excess ethereal diazomethane, dried, and evaporated. The residual sirupous ester *IX* was directly used in the preparation of the dihydroxy derivative *X* without any further purification. For purposes of analysis, the crude *IX* was chromatographed on a column of silica gel in 95 : 5 benzene–ethyl acetate (thin-layer chromatography on silica gel in the same solvent system, R_F *IX* 0·30). Optical rotation: $[\alpha]_D^{25}$ +140·8° (*c* 0·4 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 1·53 and 1·39 (2 s, CH_3 of the isopropylidene group), 3·61 (s, $COOCH_3$), 5·87 (d, 1 H, $C_{(1)}$—H; $J_{1,2}$ = = 3·7), 4·96 (q, 1 H, $C_{(2)}$—H; $J_{2,3}$ = 4·7), 5·35 (q, 1 H, $C_{(3)}$—H; $J_{3,4}$ = 9·0), 4·78 (dd, 1 H, $C_{(4)}$—H; $J_{4,5}$ 3·0 c.p.s.), 5·62 p.p.m. (d, 1 H, $C_{(5)}$—H). For $C_{24}H_{24}O_9$ (456·5) calculated: 63·13% C, 5·30% H; found: 63·35% C, 5·23% H.

Methyl 3,5-Di-O-benzoyl-D-alluronate (*X*)

A. To the ester *IX* (obtained from 21·6 g of the dibenzoate *VII* on oxidation) there was added 50% aqueous formic acid (108 ml), the stirred mixture heated at 100°C for 45 min, and cooled down to deposit a solid which was collected with suction and washed with ether. Yield, 15·5 g (75%, referred to the starting alcohol *VII*) of the methyl ester *X*.

B. To a solution of the crude dibenzoate *VII* (49·0 g) in 70% aqueous acetone (1000 ml) there were added sodium periodate (97 g) and 5% aqueous ruthenium trichloride trihydrate (1·2 ml). The whole mixture was stirred at room temperature for 3·5 h, filtered, and the material on the filter washed with acetone. The filtrate and washings were combined and evaporated. The residue was extracted with two 100 ml portions of ethyl acetate, the extract treated with excess ethereal diazomethane, dried, filtered with active charcoal, and the filtrate evaporated. The residue was heated with stirring in 50% aqueous formic acid (250 ml) for 45 min at 100°C, the solution cooled down, and kept at 0°C overnight to deposit crystals which were collected with suction and washed with ether. Yield, 31·0 g (58%, referred to the isopropylidene derivative *V*) of the methyl ester *X*, m.p. 124–130°C, $[\alpha]_D^{25}$ +74·6° (*c* 0·5 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 3·75 (s, $COOCH_3$), 4·51 (m, 1 H, $C_{(4)}$—H), 4·85 (m, 1 H, $C_{(2)}$—H), 5·40–5·70 (complex m, 3 H, $C_{(1)}$ + $C_{(3)}$ + $C_{(5)}$—H), 7·25–8·15 p.p.m. (m, 10 H, H aromatic). For $C_{21}H_{20}O_9$ (416·4) calculated: 60·58% C, 4·84% H; found: 60·68% C, 4·86% H.

Methyl 3,5-Di-O-(2*R*)-allaro-1,4-lactone-6-ate (*IV*)

To a solution of the dihydroxy derivative *X* (15·5 g; 37 mmol) in dioxane (150 ml) and water (77·5 ml) there was added bromine (7·8 ml) followed by (portionwise) sodium hydrogen carbonate (15·5 g). The mixture was stirred at room temperature for 30 min and then treated dropwise with water (300 ml). The precipitate was collected with suction and washed with water, methanol, and ether. Yield, 13·8 g (90%) of compound *IV*, m.p. 191–193°C, $[\alpha]_D^{25}$ −34·0° (*c* 1 in chloroform). ^{1}H-NMR spectrum: $CDCl_3$ δ 3·86 (s, 3 H, $COOCH_3$), 4·95 (dd, 1 H, $C_{(2)}$—H; $J_{2,3}$ = 5·02; J_{H_2OH} = 7·5), 5·07 (dd, 1 H, $C_{(4)}$—H; $J_{4,5}$ = 3; $J_{4,3}$ = 1·5 c.p.s.), 5·68 (d, 1 H, $C_{(5)}$—H), 5·72 (dd, 1 H, $C_{(3)}$—H), 6·32 (d, 1 H, OH), 7·30–8·20 p.p.m. (m, 10 H, H aromatic). For $C_{21}H_{18}O_9$ (414·4) calculated: 60·87% C, 4·38% H; found: 60·85% C, 4·20% H.

1,6-Di-O-acetyl-2,3,4-tri-O-benzyl-D-glucopyranose (*XIII*)

To a suspension of 2,3,4-tri-O-benzyl-1,6-anhydro-β-D-glucopyranose (432 mg; 1·0 mmol) in acetic anhydride (1·0 ml) there was added boron trifluoride etherate (0·04 ml), the mixture stirred at room temperature for 5 min, diluted with water, and extracted with two 10 ml portions of benzene. The extract was washed with dilute aqueous ammonia and water, dried, and evaporated.

The residual sirup, $[\alpha]_D^{25}$ +55·1° (*c* 0·5 in $CHCl_3$), representing a 4 : 1 mixture of the α- and β-anomer (as determined by ^{1}H-NMR spectrum) was directly used in the glucosylation of the lactone *XI* without an additional purification. For $C_{31}H_{34}O_8$ (534·6) calculated: 69·65% C, 6·41% H; found: 69·38% C, 6·37% H.

5-O-(6-O-Acetyl-2,3,4-tri-O-benzyl-α,β-D-glucopyranosyl)-1,2-O-isopropylidene-α-D--glucurono-1,4-lactone (*XIV*α and *XIV*β)

A. To a solution of the diacetate *XIII* (1·068 mg; 2·0 mmol) in benzene (20 ml) there was added the lactone *XI* (864 mg; 4·0 mmol) and boron trifluoride etherate (2·0 ml). The mixture was kept at room temperature for 20 min, diluted with water (20 ml), the benzene layer separated, washed with aqueous sodium hydrogen carbonate, dried, and evaporated. The residue was chromatographed on a 2·5 × 20 cm column of silica gel in 9 : 1 benzene–ethyl acetate (thin-layer chromatography on silica gel in the same solvent system: R_F *XIII* 0·45; R_F *XIV*α,β 0·19; R_F *XI* 0·085) to afford 1·220 mg (88%) of the anomeric mixture of compounds *XIV*α and *XIV*β. The sirupous mixture was dissolved in ether, the solution treated with light petroleum until slightly turbid, and allowed to deposit crystals which were collected with suction and washed with ether. Yield, 450 mg (32·5%) of the anomer *XIV*α, m.p. 151—153°C, $[\alpha]_D^{25}$ +73·76° (*c* 0·5 in chloroform).

B. To a solution of the diacetate *XIII* (1·068 mg; 2·0 mmol) in benzene (20 ml) there was added the lactone *XI* (864 mg; 4·0 mmol) along with 1M stannic chloride in benzene (2·0 ml). The mixture was kept at room temperature for 30 min, decomposed with water (5 ml), the benzene layer separated, dried, and evaporated. The residue was processed analogously to paragraph *A* to afford 950 mg (69%) of a mixture of the anomers *XIV*α and *XIV*β.

C. A solution of the diacetate *XIII* (1·058 mg; 2·0 mmol) in benzene (20 ml) was saturated with gaseous hydrogen bromide. After the saturation, the hydrogen bromide was introduced for additional 5 min and the mixture evaporated under diminished pressure at 40°C. The residue was coevaporated with two 20 ml portions of toluene. To the final residue there was added nitromethane (20 ml; distilled over phosphorus pentoxide), the lactone *XI* (864 mg; 4·0 mmol), and mercuric cyanide (1·0 g). The whole mixture was stirred at room temperature overnight, filtered through Celite, the filtrate evaporated, and the residue processed analogously to paragraph *A*. Yield, 1·000 mg (72·5%) of the mixture of anomers *XIV*α and *XIV*β. For $C_{38}H_{42}O_{12}$ (690·75) calculated: 66·07% C, 6·12% H; found: 65·88% C, 5·96% H.

5-O-(2,3,4-Tri-O-benzyl-α-D-glucopyranosyl)-1,2-O-isopropylidene-α-D-glucopyranose (*XV*)

A solution of the anomer *XIV*α (690 mg; 1·0 mmol) in tetrahydrofuran (10 ml) was added dropwise to a suspension of lithium aluminium hydride (200 mg) in tetrahydrofuran (5·0 ml). The mixture was stirred at room temperature for 2 h, decomposed with several drops of water, filtered through Celite, and the filtrate evaporated. The residue was chromatographed on a 2·3 × 13 cm column of silica gel in 9 : 1 benzene–acetone (thin-layer chromatography on silica gel in the same solvent system: R_F *XV* 0·39). The product-containing fractions were pooled, evaporated, and the residue diluted with ether to deposit a solid which was collected with suction and washed with ether. Yield, 590 mg (92%) of compound *XV*, m.p. 134—135°C, $[\alpha]_D^{25}$ +28·44° (*c* 0·5 in chloro form). ^{1}H-NMR spectrum ($CDCl_3$): δ 1·41 and 1·23 (2 s, isopropylidene group), 5·79 (d, 1 H, $C_{(1)}$—H; $J_{1,2}$ = 3·8), 4·41 (d, 1 H, $C_{(2)}$—H; $J_{2,3}$ = 0·5), 4·89 (d, 1 H, C_1H; $J_{1',2'}$ = 3·6), 3·44 (dd, 1 H, $C_{(2')}$—H; $J_{2',3'}$ = 9·5 c.p.s.), 3·90 (t, 1 H, $C_{(3')}$—H), 7·25 p.p.m. (broad s, 15 H, aromatic H). For $C_{36}H_{44}O_{11}$ (644·7) calculated: 66·24% C, 6·79% H; found: 66·46% C, 6·67% H.

Methyl 4-O-Acetyl-3,5-di-O-benzoyl-2-O-[3,6-di-O-acetyl-2-O-benzyl-4-O-2,2,2-trichloroethyl--(3-O-benzyl-2-*o*-*p*-toluenesulfonyl-α-D-arabinofuranosid-5-yl)-α-D-glucopyranosyl]-(2*R*)-allar--6-ate 1-Amide (*XIX*)

To a solution of the anomeric acetates *II* (904 mg; 1·0 mmol) in chloroform (15 ml) there was added the lactone *IV* (455 mg; 1·1 mmol) and boron trifluoride etherate (1·5 ml). The mixture was stirred at room temperature for 2 h, washed with water and aqueous sodium hydrogen carbonate, dried, and evaporated. The residue was kept with ether in an ice-box overnight to deposit crystals which were collected with suction and washed with ether; yield, 180 mg of the lactone *IV*. The mother liquor was chromatographed on a 2·6 × 20 cm column of silica gel in 85 : 15 benzene–ethyl acetate to afford fractions (770 mg) corresponding by its mobility to the starting acetate *II* (thin-layer chromatography on silica gel in the same solvent mixture: R_F *II* 0·55). These fractions were evaporated, the residue dissolved in ether (3·0 ml), and the solutions treated with liquid ammonia (10 ml). The ammonia was allowed to evaporate gradually (1 h) and the remaining ether removed under diminished pressure. The residue was kept at room temperature with acetic anhydride (2·0 ml) and pyridine (2·0 ml) overnight, the mixture treated with methanol (10 ml), kept at room temperature for 30 min, evaporated, and the residue coevaporated with two 20 ml portions of xylene. The final residue was chromatographed on a 2·6 × 20 cm column of silica gel in 8 : 2 benzene–acetone (thin-layer chromatography on silica gel in the same solvent system: R_F *XIX* 0·33) to afford 372 mg (28%) of the amide *XIX*, $[\alpha]_D^{25}$ + 71·0° (*c* 0·5 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 1·98, 1·94, 1·85 (3 s, CH_3COO), 2·35 (s, CH_3 of the *p*-toluenesulfonyl residue), 3·48 p.p.m. (s, $COOCH_3$). For $C_{61}H_{64}Cl_3NO_{23}S$ (1 318) calculated: 55·60% C, 4·90% H, 8·07% Cl, 1·06% N; 2·43% S; found: 55·65% C, 4·95% H, 8·18% Cl, 0·91% N, 2·74% S.

Methyl 3,5-Di-O-benzoyl-2-O-[3,6-di-O-acetyl-4-O-2,2,2-trichloroethyl-(2,3-O-carbonyl--β-D-ribofuranosid-5-yl-α-D-glucopyranosyl]-(2*R*)-allaro-1,4-lactono-6-ate (*XXI*)

A. To a solution of anomeric acetates *III* (686 mg; 1 mmol) in chloroform (15 ml) there was added the lactone *IV* (455 mg; 1·1 mmol) and boron trifluoride etherate (1·5 ml). The mixture was stirred at room temperature for 2 h, washed with water (15 ml) and saturated aqueous sodium hydrogen carbonate (15 ml), dried, and evaporated. Ether (5 ml) was added to the residue and the mixture kept in an ice-box overnight to deposit crystals of the lactone *IV* (150 mg) which were filtered off and washed with ether. The mother liquor was concentrated under diminished pressure and the concentrate chromatographed on a 2·6 × 15 cm column of silica gel in 9 : 1 benzene–ethyl acetate to afford fractions travelling similarly to the starting lactone *IV* (thin-layer chromatography on silica gel in the same solvent system: R_F *IV* 0·32; R_F *XX* 0·36). These fractions were evaporated and the residue (400 mg) dissolved in acetic acid (5·0 ml). Palladium on charcoal (10%; 50 mg) and 10% aqueous palladium chloride (0·05 ml) were then added and the whole mixture shaken under hydrogen for 30 min at ordinary pressure (hydrogen uptake, 5·3 ml). The catalyst was filtered off, the filtrate evaporated, and the residue coevaporated with two 10 ml portions of toluene. The final residue was chromatographed on a 2·6 × 15 cm column of silica gel in 7 : 3 benzene–ethyl acetate (thin-layer chromatography on silica gel in the same solvent system: R_F *XXI* 0·32) to afford 250 mg (26%) of compound *XXI*.

B. Dry gaseous hydrogen bromide was introduced at room temperature for 5 min into a solution of the anomeric acetates *III* (686 mg; 1 mmol) in toluene (10 ml). The mixture was then evaporated and the residue coevaporated with two 10 ml portions of toluene. To the final residue there was added nitromethane (10 ml), the lactone *IV* (455 mg; 1·1 mmol), and mercuric cyanide (500 mg). The whole mixture was stirred at room temperature for 20 h, filtered, and the

filtrate evaporated. Ether (5 ml) was added to the residue and the mixture kept at 0°C to deposit crystals of the lactone *IV* which were filtered off and washed with ether. The mother liquor was concentrated under diminished pressure and the concentrate processed analogously to paragraph *A*. Yield, 87 mg (9·1%) of compound *XXI*, $[\alpha]_D^{25}$ +10·4° (*c* 1 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 2·02, 2·14 (2 s, $OCOCH_3$), 3·90 (s, $COOCH_3$), 4·04 and 4·17 (2d, 2 H, OCH_2CCl_3; J_{gem} = 11·0), 5·05 (d, 1 H, $C_{(2)}$—H; $J_{2,3}$ 6·5), 5·18 (dd, 1 H, $C_{(4)}$—H; $J_{4,5}$ = 2·5), 5·80 (dd, 1 H, $C_{(3)}$—H; $J_{3,4}$ = 1·5), 5·31 (d, 1 H, $C_{(1')}$—H; $J_{1',2'}$ = 3·5 c.p.s.), 5·42 (s, 1 H, $C_{(1'')}$—H), 7·40—7·75 (m, 6 H, H aromatic), 7·90—8·20 p.p.m. (m, 4 H, H aromatic). For $C_{39}H_{43}Cl_3O_{21}$ (954·1) calculated: 49·09% C, 4·54% H, 11·44% Cl; found: 49·32% C, 4·12% H, 11·18% Cl.

Methyl 3,5-Di-O-benzoyl-2-O-[2,3,6-tri-O-acetyl-4-O-2,2,2-trichloroethyl(2,3-O-carbonyl--β-D-ribofuranosid-5-yl)-α-D-glucopyranosyl]-(2*R*)-allaro-1,4-lactono-6-ate (*XXII*)

To compound *XXI* (2·5 g; 2·6 mmol) there was added acetic anhydride (10 ml) and trifluoroacetic acid (0·20 ml). The mixture was heated at 60°C for 1 h and evaporated to remove excess acetic anhydride. The residue was coevaporated with two 25 ml portions of *m*-xylene. *m*-Xylene (2·5 ml) was then added to the final residue and the solution kept at 0°C to deposit crystals which were collected with suction and washed with a little *m*-xylene. Yield, 2,0 g (65%) of the solvate (with two molecules of *m*-xylene) of compound *XXII*, m.p. 64°C, $[\alpha]_D^{25}$ + 25·85° (*c* 0·5 in chloroform). For purpose of analysis, the product was dried at 20°C/1 Torr for 30 min. For $C_{57}H_{65}Cl_3O_{22}$ (1208·5) calculated: 56·65% C, 5·42% H, 8·80% Cl; found: 56·62% C, 5·26% H, 8·92% Cl.

Methyl 3,5-Di-O-benzoyl-2-O-[(2,3,6-tri-O-acetyl-4-O-2,2,2-trichloroethyl-β-D-ribofuranosid--5-yl)-α-D-glucopyranosyl]-(2*R*)-allaro-1,4-lactono-6-ate (*XXVI*)

A. A mixture containing the acetate *XXII* (2·0 g; 1·65 mmol), pyridine (30 ml), and water (30 ml) was heated at 100°C for 45 min and evaporated. Water (50 ml) and ethyl acetate (50 ml) were then added to the residue and the mixture adjusted to a strongly acidic reaction by the addition of conc. hydrochloric acid. The upper layer was separated, washed with water, dried, and evaporated. Ethyl acetate (10·0 ml) and 1M N,N'-dicyclohexylcarbodiimide in ethyl acetate (5·0 ml) were added to the residue, the mixture kept at room temperature of 10 min, treated with methanol (8·0 ml) and acetic acid (2·0 ml), kept for additional 20 min, and filtered. The filtrate was evaporated and the residue coevaporated with two 10 ml portions of toluene. The final residue was chromatographed on a column of silica gel (50 g); the column was washed with a 8 : 2 benzene–ethyl acetate mixture (200 ml) and then eluted with 1 : 1 benzene–ethyl acetate (thin-layer chromatography on silica gel in the same solvent mixture: R_F *XXVI* 0·31) to afford 1·1 g (68%) of compound *XXVI*.

B. A mixture of the acetate *XXII* (2·4 g; 1·97 mmol), pyridine (47 ml), and water (47 ml) was heated at 100°C for 45 min and evaporated under diminished pressure. Water (70 ml) and ethyl acetate (70 ml) were then added to the residue and the mixture was brought to a strongly acidic reaction by the addition of conc. hydrochloric acid. The upper layer was separated, washed with water, dried, and evaporated. To the residue there was added chloroform (24 ml) and ethoxyacetylene (4·0 ml). The mixture was kept at room temperature for 2 h, evaporated, and the residue processed analogously to paragraph *A* to afford 1·5 g (78%) of compound *XXVI*, $[\alpha]_D^{25}$ +43·5° (*c* 0·5 in chloroform). IR spectrum ($CHCl_3$): ν(C=O) lactone 1804 cm^{-1}, ν(C=O) ester 1735 cm^{-1}. ^{1}H-NMR spectrum ($CDCl_3$): δ 2·06; 2·04 and 2·01 (3 s, $OCOCH_3$), 3·89 p.p.m. (s, $COOCH_3$). For $C_{40}H_{42}Cl_3O_{21}$ (966·1) calculated: 49·72% C, 4·83% H, 11·01% Cl; found: 49·54% C, 4·57% H; 10·91% Cl.

Methyl 3,5-Di-O-benzoyl-2-O-[2,3,6-tri-O-acetyl-4-O-2,2,2-trichloroethyl(2,3-di-O-acetyl -β-D-ribofuranosid-5-yl)-α-D-glucopyranosyl]-(2*R*)-allaro-1,4-lactono-6-ate (*XXVII*)

To a solution of the hydroxy derivative *XXVI* (1·50 g; 1·52 mmol) in acetic anhydride (15 ml) there was added trifluoroacetic acid (0·3 ml). The mixture was heated at 60°C for 1 h, evaporated, and the residue coevaporated with two 10 ml portions of xylene. ^{1}H-NMR spectrum ($CDCl_3$): δ 2·01, 2·02, 2·023, 2·05, 2·1 (5 s, CH_3COO), 3·92 (s, $COOCH_3$), 7·40—7·70, and 7·95—8·2 p.p.m. (m, 10 H, 2 $OCOC_6H_5$).

Methyl 3,5-Di-O-benzoyl-2-O-[2,3,6-tri-O-acetyl-4-O-(N^6-benzoyl-2′,3′-di-O-acetyladenosin--5-yl)-α-D-glucopyranosyl]-(2*R*)-allaro-1,4-lactono-6-ate (*XXXI*)

To a solution of the pentaacetate *XXVII* (obtained from 1·5 g *i.e.* 1·52 mmol of the hydroxy derivative *XXVI* by acetylation) in acetone (25 ml) there was added zinc powder (4·0 g) and then under vigorous stirring conc. hydrochloric acid (drop by drop; 1·5 ml). The stirring was continued for 15 min, the mixture filtered, the filtrate evaporated, the residue diluted with water (15 ml), and extracted with two 15 ml portions of ethyl acetate (the emulsion, if any, was destroyed by the addition of a few drops of acetic acid). The ethyl acetate extract was dried, evaporated, and the residual chromatographically homogeneous hydroxy derivative *XXVIII* (thin-layer chromatography on silica gel in 7 : 4 benzene–ethyl acetate: R_F *XXVII* 0·58; R_F *XXVIII* 0·24) dissolved in acetic anhydride (10 ml). The solution was treated with trifluoroacetic acid (0·2 ml) and heated at 60°C for 1 h. Excess acetic anhydride was then evaporated and the residue coevaporated with two 5 ml portions of xylene. The thus-obtained mixture of anomeric acetates *XXIX* (thin-layer chromatography on silica gel in 7 : 4 benzene–ethyl acetate: R_F *XXIX* 0·39 and 0·45) was dissolved in chloroform (5·0 ml) and toluene (5·0 ml), the solution saturated with a rich stream of gaseous hydrogen bromide, kept at room temperature for 5 min, evaporated under diminished pressure at the bath temperature of 40°C, and the residue coevaporated with toluene (10 ml). To the final residue there was added acetonitrile (5·0 ml) and N^6-benzoyladenine chloromercuri salt (1·0 g; dried at 130°C/0·1 Torr for 2 h), the whole mixture refluxed with stirring for 2 h, cooled down, and filtered. The filtrate was evaporated and the residue heated with acetic anhydride (20 ml) and trifluoroacetic acid (0·4 ml) for 1 h at 60°C. Excess acetic anhydride was evaporated, the residue coevaporated with two 20 ml portions of xylene, and the final residue chromatographed on a 3 × 30 cm column of silica gel in 8 : 2 chloroform–acetone (thin-layer chromatography on silica gel in the same solvent system: R_F *XXXI* 0·37) to afford 495 mg (29%, referred to compound *XXVI*) of compound *XXXI*, $[\alpha]_D^{25}$ +34·1° (*c* 0·5 in chloroform). UV spectrum (methanol): λ_{max} 232 nm (log ε 4·16), λ_{min} 256 nm (log ε 3·99), and λ_{max} 278 nm (log ε 4·08). ^{1}H-NMR spectrum ($CDCl_3$): δ 1·92, 1·99, 2·00, 2·08, 2·15 (s, 5 $OCOCH_3$), 3·79 (s, $COOCH_3$), 8·71, and 8·87 p.p.m. (s, 2 H and 8 H of adenine). For $C_{54}H_{53}N_5O_{23}$ (1140) calculated: 56·94% C, 4·60% H, 6·14% N; found: 57·09% C, 4·72% H, 5·96% N.

Methyl 2,3,5-Tri-O-benzoyl-(2*R*)-allaro-1,4-lactono-6-ate (*XXXII*)

To an ice-cooled suspension of the lactone *IV* (1·5 g; 3·57 mmol) in pyridine (15 ml) there was added dropwise benzoyl chloride (1·4 g; 10 mmol). The mixture was then stirred at 0°C for 30 min and treated with water (10 ml). After 10 min, methanol (20 ml) was added, the precipitate collected with suction, and washed with methanol and ether. Yield, 1·6 g (86%) of compound *XXXII*, m.p. 159—160°C, $[\alpha]_D^{25}$ −14·1° (*c* 0·5 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 3·92 (s, 3 H, $COOCH_3$), 6·26 (d, 1 H, $C_{(2)}$—H; $J_{2,3}$ 6·5), 6·0 (dd, 1 H, $C_{(3)}$—H; $J_{3,4}$ 0·8), 5·24 (broad d, 1 H, $C_{(4)}$—H; $J_{4,5}$ = 2·5 c.p.s.), 5·78 p.p.m. (d, 1 H, $C_{(5)}$—H). For $C_{28}H_{22}O_{10}$ (518·5) calculated: 64·86% C, 4·28% H; found: 64·68% C, 4·30% H.

Dimethyl 2,3,5-Tri-O-benzoyl-(2*R*)-allarate (*XXXIII*)

A. To a solution of the ester-lactone *XXXII* (1·5 g; 2·9 mmol) in dichloromethane (60 ml) and methanol (60 ml) there was added boron trifluoride etherate (0·70 ml). The mixture was kept at room temperature for 24 h and then poured into aqueous 0·1M sodium acetate (150 ml). The organic layer was separated, dried, and evaporated. The residual dimethyl ester *XXXIII* was contaminated with a small amount of the ester-lactone *XXXII* only (thin-layer chromatography on silica gel in 9 : 1 benzene–ethyl acetate; R_F *XXXII* 0·69; R_F *XXXIII* 0·20) and was used directly in the phosphorylation step without any previous purification.

B. A mixture of the ester-lactone *XXXII* (1·5 g; 0·29 mmol), methanol (25 ml), and pyridine (2·5 ml) was refluxed for 45 min, cooled down, and diluted with chloroform (50 ml) and water (50 ml). The chloroform layer was separated, washed with dilute hydrochloric acid and water, dried, and evaporated. The residue was directly used in the phosphorylation step. ^{1}H-NMR spectrum ($CDCl_3$): δ 3·35 and 3·72 (s, 2 $COOCH_3$), 5·01 (dd, 1 H, $C_{(4)}$—H; $J_{4,5}$ = 2·4; $J_{4,3}$ = = 9·8), 5·63 (d, 1 H, $C_{(5)}$—H), 5·86 (d, 1 H, $C_{(2)}$—H; $J_{2,3}$ = 2·2 c.p.s.), 7·20—7·70 and 7·80 to 8·15 p.p.m. (m, 15 H, 3 $OCOC_6H_5$).

Dimethyl 2,3,5-Tri-O-benzoyl-4-O-(di-O-methylphosphoryl)-(2*R*)-allarate (*XXXVI*)

To the dimethyl ester *XXXIII* (550 mg; 1 mmol) in benzene (5·0 ml) there was added an 1M solution of phosphorus oxychloride in benzene (4·0 ml) and an 1M solution of pyridine in benzene (4·0 ml). The mixture was kept at room temperature for 2 h, treated with water (5·0 ml), and the stirring continued for 20 h at room temperature. The benzene layer was then separated, dried, and evaporated. The residue was dissolved in methanol and the solution applied to a 2 × 12 cm column of Dowex 1X4 (acetate) ion exchange resin (prewashed with methanol). The column was washed with methanol (150 ml) and then the phosphate *XXXV* eluted with 94 : 6 methanol–conc. hydrochloric acid. The eluate was evaporated, the residue esterified with excess ethereal diazomethane, and the resulting ester *XXXVI* chromatographed on a 2 × 20 cm column of silica gel in 7 : 3 benzene–ethyl acetate (thin-layer chromatography on silica gel in the same solvent system: R_F *XXXVI* 0·31) to afford 320 mg (48%) of compound *XXXVI*, $[\alpha]_D^{25}$ +7·78° (*c* 0·4 in chloroform). ^{1}H-NMR spectrum ($CDCl_3$): δ 6·02 (d, 1 H, $C_{(5)}$—H; $J_{5,4}$ = 1·8), 5·72 (sept. 1 H, $C_{(4)}$—H; $J_{3,4}$ = 9·8; $J_{4,P}$ = 8·0), 6·33 (dd, 1 H, $C_{(3)}$—H; $J_{3,2}$ = 1·8), 5·87 (d, 1 H, $C_{(2)}$—H), 3·33 and 3·80 (s, 2 $COOCH_3$), 3·71 (d, 3 H, OCH_3; J_{P,OCH_3} = 8·0), 3·82 p.p.m. (d, 3 H, OCH_3; J_{P,OCH_3} = 8·0 c.p.s.). For $C_{31}H_{31}OP$ (657·6) calculated: 56·62% C, 4·75% H, 4·70% P; found: 56·73% C, 4·74% H, 4·35% P.

Exotoxin *I*

To a solution of the nucleoside *XXXI* (50 mg; 0·044 mmol) in methanol (6·0 ml) there was added pyridine (0·60 ml), the mixture refluxed for 45 min, cooled down, diluted with water (10 ml), made acid by the addition of hydrochloric acid, and extracted with two 15 ml portions of ethyl acetate. The extract was dried, evaporated, and the residue coevaporated with four 10 ml portions of chloroform to afford the dimethyl ester *XXXVII*, ^{1}H-NMR spectrum ($CDCl_3$): δ 3·73, 3·24 (s, 2 $COOCH_3$), 1·87, 1·90, 2·02, 2·05, 2·11 (s, 5 CH_3COO), 8·78, and 8·50 p.p.m. (s, 2 H and 8 H of adenine). This final residue was dissolved in chloroform (5·0 ml) and the solution treated with an 1M solution of phosphorus oxychloride in chloroform (2·0 ml) and an 1M solution of pyridine in chloroform (2·0 ml). The mixture was kept at room temperature for 2 h, treated with water (10 ml), shaken at room temperature for 5 min, the chloroform layer separated, and the remaining aqueous layer extracted with ethyl acetate (10 ml). The organic layers were combined, dried,

and evaporated. The residue was kept with pyridine (2·0 ml) and water (2·0 ml) for 15 min at room temperature; 1M aqueous sodium hydroxide (10 ml) was then added, the whole mixture heated at 60°C for 2 h, and evaporated. The residue was diluted with water (20 ml) and the solution passed through a 2 × 20 cm column of Dowex 50X8 (NH_4^+) ion exchange resin. The column was then washed with dilute aqueous ammonia, the effluent and washings combined, and evaporated. Exotoxin *I* was isolated from the residue by chromatography on DEAE-cellulose: 2 × 25 cm, column, gradient, water (500 ml) —0·5M aqueous triethylammonium carbonate, pH 7·5—8·0; flow rate, 1·3 ml per min; 13 ml fractions. Fractions 41—54 (exhibiting maximum at 260 nm) were pooled and evaporated. The residue was dissolved in water and passed through a 2 × 2 cm column of Dowex 50X8 (H^+) ion exchange resin. Excess aqueous ammonia was added to the effluent, the mixture filtered, the filtrate evaporated, and the residue freeze-dried to afford 10 mg (0·0124 mmol on the basis of optical density measurements; 28%, referred to the nucleoside *XXXI*) of exotoxin *I*; adenine to phosphorus ratio, 1·00 : 1·18.

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