STRUCTURE PROOF OF EXOTOXIN FROM *Bacillus thuringiensis**

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Structure proof of exotoxin (*I*) from *Bacillus thuringiensis* was presented. The α-configuration of the glucosidic bond was inferred from comparison of NMR spectra of the permethylated sequence *V* with those of the permethylated adenosine *III* and α-D-glucopyranosylallitol *XII*. The structure of the allaric portion of exotoxin was established by methanolysis of the sequence *V* leading to (2*S*)-1,3,4,5,6-penta-O-methylallitol (*XXVI*) in accordance with an alternative process.

In this Institute, mechanism of the action of the insecticidal exotoxin has been elucidated[1] and the structure of exotoxin proposed[2]. The original proposal did not however contain a rigorous structural proof of the pentofuranose component and the glucopyranose–allaric acid sequence.

In connection with the work on the total synthesis of the complicated exotoxin it appeared desirable to complete the original structural proposal. The unambiguous synthesis of the fundamental sugar fragment has confirmed the earlier idea and the *ribo* configuration of the pentofuranose component[3]. Configuration and location of the glucosidic bond on the allaric acid residue including its configuration has been now examined stepwise.

Determination of the glucosidic bond configuration has been attempted by Bond and coworkers[4] on comparison of the NMR spectra of exotoxin (*I*) with those of the dephosphorylated exotoxin *II*. The NMR spectrum (hexadeuteriodimethyl sulfoxide) signal (δ 5·17 p.p.m.) of compound *II* was tentatively assigned to the anomeric proton of the glucosidic bond with the α-configuration. According to the experience[5] obtained in this Institute, however, it is hardly possible to perform an unambiguous assignment of mutually overlapping signals in the δ 3·0–5·5 p.p.m. region.

As inferred from the rich experimental material, the configuration of the glucosidic bond could be preferably determined on comparison of NMR spectra of a suitably modified dephosphorylated exotoxin with those of the corresponding model compounds. As model substances, pentamethyladenosine (*III*) and permethylated (5*S*)-5-O-(α-D-glucopyranosyl)allitol (*XII*) have been used since they exhibit distinct

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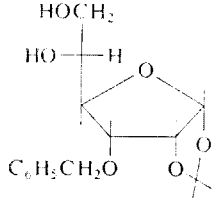
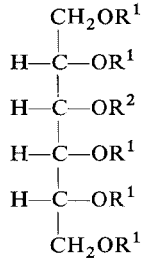
I, R = PO(OH)2
II, R = H
III, R = CH3
IV, R = H
V, R = CH3
VI
VII, R = >C(CH3)2
VIII, R = H
IX
X
XI, R = CH3CO
XII, R = CH3

NMR spectra. The dephosphorylated exotoxin *II* was successively converted to the dimethyl ester by the action of diazomethane, the free hydroxylic functions and the amino group protected by silylation with bis(trimethylsilyl)acetamide and the ester functions reduced with lithium aluminium hydride. The free allitol *IV* was methylated by the action of a mixture of methyl iodide and sodium hydride in dimethylformamide to obtain a permethylated adenine–ribofuranose–glucopyranose–allitol sequence (*V*). By comparison of the fairly resolved NMR spectrum of the sequence *V* with those of model compounds *III* and *XII* the perfectly isolated signal (δ 5·25 p.p.m.) was unequivocally assigned to the anomeric proton of the glucosidic bond; from the coupling constant value ($J_{1,2}$ = 3·3 c.p.s.), the configuration α was inferred[6].

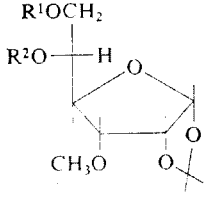
The dephosphorylated exotoxin was assumed to possess the structure of one of the four allaric acid O-glycosyl derivatives. Methanolysis of the permethylated sequence *V* should afford penta-O-methylallitol bearing a free hydroxylic function at position



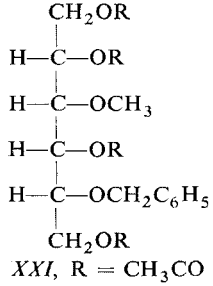
XIII



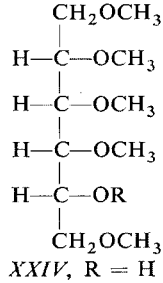
XIV, $R^1 = CH_3CO$; $R^2 = C_6H_5CH_2$
XV, $R^1 = H$; $R^2 = C_6H_5CH_2$
XVI, $R^1 = CH_3$; $R^2 = C_6H_5CH_2$
XVII, $R^1 = CH_3$; $R^2 = H$



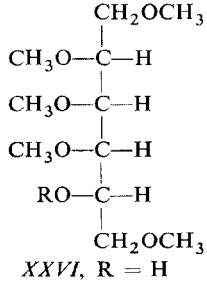
XVIII, $R^1 = R^2 = H$
XIX, $R^1 = (C_6H_5)_3C$; $R^2 = H$
XX, $R^1 = (C_6H_5)_3C$; $R^2 = C_6H_5CH_2$



XXI, R = CH_3CO
XXII, R = H
XXIII, R = CH_3



XXIV, R = H
XXV, R = CH_3CO



XXVI, R = H
XXVII, R = CH_3CO

of the original glucosidic bond to allaric acid. In this connection, (3*S*)-1,2,4,5 6-penta--O-methylallitol (*XVII*) and (5*R*)-1,2,3,4,6-penta-O-methylallitol (*XXIV*) were therefore prepared by unequivocal procedures. Acidic methanolysis of the permethylated sequence *V* yielded penta-O-methyllallitol the mass spectrum of which was identical with that of the authentic allitol *XXIV*. On the other hand, the CD spectrum of the fragment acetate exhibited in the 200–225 nm region an opposite course to that of the allitol acetate *XXV*. Consequently, the fragment is unequivocally the enantiomer of the allitol *XXIV* and therefore possesses the structure of (2*S*)-1,3,4,5,6--penta-O-methylallitol (*XXVI*). On the basis of this result, the dephosphorylated exotoxin was assigned[7] the structure of (2*R*)-2-O-α-glycosylallaric acid *II*. As inferred from the different course of the alkaline periodate oxidation[2] of compound *II* and exotoxin, the latter substance was ascribed the structure of the 4-phosphate *I*. By an independent procedure consisting in oxidation of substance *II* with alkaline periodate, an identical conclusion on the structure of the allaric portion of exotoxin was obtained[7,8].

Pentamethyladenosine was prepared by methylation of adenosine with methyl iodide and sodium hydride in dimethylformamide[9] and its structure *III* was confirmed by methylation of $N^{6,6}$-dimethyladenosine under similar conditions.

Glucopyranosyllallitol *XII* was prepared by reaction of 1,6-di-O-acetyl-2,3,4-tri--O-methyl-D-glucopyranose (*VI*) with 3,5,6-tri-O-methyl-D-allonolactone (*IX*) in the presence of boron trifluoride etherate and lithium aluminium hydride reduction of the highly predominating α-glucosylalonolactone *X*. The product was isolated as the triacetate *XI* which was then transformed into the allitol *XII*. The starting diacetate *VI* was obtained by acidic acetolysis of 1,6-anhydro-2,3,4-tri-O-methyl-β--D-glucopyranose. The starting lactone *IX* was prepared by hydrolysis of 3,5,6-tri--O-methyl-1,2-O-isopropylidene-α-D-allopyranose (*VII*) and the subsequent oxidation of the hemiacetal *VIII* with bromine in the presence of sodium hydrogen carbonate. (3*S*)-1,2,4,5,6-Penta-O-methylallitol (*XVII*) was prepared by the following sequence of reactions. The known[10,11] 3-O-benzyl-1,2-O-isopropylidene-α-D-allofuranose (*XIII*) was subjected to acidic hydrolysis and then reduced with sodium borohydride. The resulting benzylallitol *XV* was methylated and the benzyl ether *XVI* hydrogenolysed with the formation of the required allitol *XVII* in a high overall yield. The isomeric penta-O-methyllalitol *XXIV* was obtained by a multistep transformation of 3-O--methyl-1,2-O-isopropylidene-α-D-allofuranose (*XVIII*). The terminal hydroxylic function was protected by triphenylmethylation and the vicinal hydroxylic group of compound *XIX* was benzylated. The triphenylmethyl and isopropylidene groups of the allofuranose *XX* were removed by acidic hydrolysis and the free sugar was reduced. The allitol *XXII* (isolated in the form of the tetraacetate *XXI*) was methylated in the usual manner and the resulting ether *XXIII* was hydrogenolytically cleaved to afford the alcohol *XXIV*.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 20°C/0·1 Torr for 10 h unless stated otherwise. The NMR spectra were recorded on a Varian HA-100 spectrometer in deuteriochloroform. Mass spectra were measured on a MS·902 apparatus and CD spectra were taken on a Model CD-185 II Roussel-Jouan Dichrograph. Chromatographies were performed on neutral alumina (Brockmann activity II—III) and silica gel (particle size, 60—120 micron) partially deactivated by 12—14% water.

$N^{6,6}$-Dimethyladenosine

A mixture of 6-chloronebularine[12] (1·15 g; 4·0 mmol) and 2·6M methanolic dimethylamine (20 ml) was heated in a pressure vessel at 100°C for 1 h, cooled down, the product collected, and the mother liquors worked up. Yield, 85% of the title substance, m.p. 184—185°C (methanol); reported[13] for an authentic specimen prepared by another route, m.p. 182—183°C.

2′,3′,5′,$N^{6,6}$-Pentamethyladenosine (*III*)

A mixture of $N^{6,6}$-dimethyladenosine (591 mg; 2·00 mmol), sodium hydride (0·8 g), and dimethylformamide (20 ml) was stirred at room temperature for 20 min, treated with methyl iodide (2·5 ml), kept for 16 h, decomposed with methanol (3 ml), and evaporated. The residue was chromatographed on a column of alumina (120 g) in 7 : 3 benzene–ethyl acetate (400 ml). The effluent was evaporated, the residue coevaporated with two 100 ml portions of xylene, and finally rechromatographed on a column of silica gel (particle size, 30—60 micron; 50 g) in 1 : 40 ethanol–ethyl acetate (300 ml) to obtain fractions 1—23. Work-up of the homogeneous fractions 11—17 afforded 74% of the nucleoside *III*, m.p. 73°C (1 : 2 ether–light petroleum), undepressed on admixture with a substance obtained by permethylation of adenosine[9]. NMR spectrum: δ 6·14 (d, 1′-H, $J_{1',2'}$ = = 3·0), 4·20 (dd, 2′-H, $J_{2',3'}$ = 4·5), 4·0 (qu, 3′-H, $J_{3',4'}$ = 6·4), 4·2 (m, 4′-H), 3·55 (dd, 5′-H, $J_{5',4'}$ = 3·0, $J_{5',5''}$ = 11·0), 3·75 (dd, 5″-H, $J_{5'',4'}$ = 2·8 c.p.s.), 3·38, 3·40, 3·48, 3·52 (s, 2 × × CH_3N and 3 × CH_3O), 8·12 (s, 2-H), and 8·28 p.p.m. (s, 8-H). For $C_{15}H_{23}N_5O_4$ (337·4) calculated: 53·40% C, 6·87% H, 20·76% N; found: 53·23% C, 6·73% H, 20·57% N.

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

To a solution of 1,6-anhydro-2,3,4-tri-O-methyl-β-D-glucopyranose (3·65 g; 18·0 mmol) in acetic anhydride (15 ml) there was added under cooling with ice-cold water boron trifluoride etherate (0·5 ml), the mixture kept at room temperature for 15 min, and poured into 2% aqueous sodium acetate (100 ml). The aqueous solution was filtered with active charcoal, the filtrate extracted with four 100 ml portions of chloroform, the extracts combined, washed with water (100 ml) and saturated aqueous potassium hydrogen carbonate (100 ml), dried, and evaporated to afford the anomers *VI* in an almost quantitative yield. The anomeric mixture *VI* (0·60 g) was chromatographed on a column (100 g) of silicagel in 3 : 1 benzene–ethyl acetate (640 ml; fractions 1—80). The homogeneous fractions 46—51 (R_F 0·45 on a thin layer of silica gel, gypsum as binder) yielded 100 mg (17%) of the β-anomer *VI*β (dried for 2·5 h at 80°C/0·1 Torr). NMR spectrum: δ 5·49 (d, 1-H, $J_{1,2}$ = 7·5 c.p.s.), 3·0—3·3 (m, 2-H, 3-H, 4-H), 3·5 (m, 5-H), 4·22—4·32 (m, 2 × 6-H), 2·09 and 2·15 (s, 2 × CH_3CO), and 3·52, 3·56, and 3·65 p.p.m. (s, 3 × CH_3O). For $C_{13}H_{22}O_8$ (306·3) calculated: 50·98% C, 7·24% H; found: 51·10% C, 7·25% H. Fractions 53—79 (R_F 0·37) yielded 450 mg (75%) of the α-anomer *VI*α (dried for 3 h at 80°C/0·1 Torr). NMR spectrum:

δ 6·31 (d, 1-H, $J_{1,2}$ = 3·5), 3·27 (dd, 2-H, $J_{2,3}$ = 9·5), 3·45 (d, 3-H, $J_{3,4}$ = 9·5), 3·13 (qu, 4-H, $J_{4,5}$ = 10·0), 3·80 (sext., 5-H, $J_{5,6} = J_{5,6'}$ = 3·0 c.p.s.) and 4·27 p.p.m. (d, 2 × 6-H). For $C_{13}H_{22}O_8$ (306·3) calculated: 50·98% C, 7·24% H; found: 50·92% C, 7·32% H.

1,2-O-Isopropylidene-3,5,6-tri-O-methyl-α-D-allofuranose (*VII*)

To a mixture of 1,2-O-isopropylidene-3-O-methyl-α-D-allofuranose[14,15] (5·0 g; 21·4 mmol), sodium hydride (2·3 g), and dimethylformamide (125 ml) which was stirred for 10 min, there was added methyl iodide (12·5 ml) and the stirring continued for additional 3 h under cooling with tap water. The mixture was then decomposed with methanol (100 ml), poured into water (100 ml), and the aqueous solution extracted with five 60 ml portions of chloroform. The extracts were combined, washed with water (150 ml), dried, evaporated, the residue coevaporated with two 100 ml portions of xylene, and finally passed in 500 ml of benzene–ethyl acetate (50 : 1) through a column of alumina (150 g) to yield 96% of compound *VII*, b.p. 172°C/12 Torr. NMR spectrum: δ 5·78 (d, 1-H, $J_{1,2}$ = 3·6), 4·68 (broad t, 2-H, $J_{2,3}$ = 4·3), 3·81 (qu, 3-H, $J_{3,4}$ = 8·6), 4·10 (dd, 4-H, $J_{4,5}$ = 2·0 c.p.s.), 3·69 (m, 5-H), 3·45 (m, 2 × 6-H), 3·37, 3·48, and 3.52 (s, 3 × CH_3O), 1·39, and 1·60 p.p.m. (s, CH_3 of the isopropylidene group). For $C_{12}H_{22}O_6$ (262·3) calculated: 54·95% C, 8·45% H; found: 55·22% C, 8·45% H.

3,5,6-Tri-O-methyl-D-allonolactone (*IX*)

A mixture of the isopropylidene derivative *VII* (2·10 g; 8·0 mmol) and 50% aqueous formic acid (60 ml) was refluxed for 25 min and evaporated under diminished pressure. The residue was coevaporated with two 100 ml portions of water and then dissolved in water (30 ml). Sodium hydrogen carbonate (1 g) was added to the aqueous solution which was then treated dropwise over 3 min at room temperature with bromine (1·7 ml). The mixture was kept for 25 min, concentrated under diminished pressure to the volume of 15 ml, the concentrate decolourised with sodium thiosulfate and extracted with six 20 ml portions of chloroform. The extracts were combined, dried, and evaporated. The residue was chromatographed on a column of silica gel (30 g) in 7 : 3 benzene–ethyl acetate (280 ml; fractions 1—40). The homogeneous fractions 15—36 yielded 920 mg (52%) of the oily lactone *IX* (dried for 7 h at 75°C/0·1 Torr). For $C_9H_{16}O_6$ (220·2) calculated: 49·09% C, 7·32% H; found: 48·72% C, 7·48% H.

2-O-(2,3,4-Tri-O-methyl-6-O-acetyl-α-D-glucopyranosyl)-3,5,6-tri-O-methyl-D-allonolactone (*X*)

To a mixture of the diacetate *VI* (612 mg; 2·00 mmol) and lactone *IX* (385 mg; 1·75 mmol) in benzene (18 ml) there was added boron trifluoride etherate (0·22 ml; 1·75 mmol), the whole mixture kept at room temperature for 40 min, treated with acetic anhydride (0·1 ml), kept for additional 10 min, decomposed with water (5 ml) and saturated aqueous sodium hydrogen carbonate (5 ml), and extracted with eight 35 ml portions of chloroform. The extracts were combined, dried, and evaporated. The residue was chromatographed on a column of silica gel (50 g) in 7 : 3 benzene–ethyl acetate (300 ml; fractions 1—2) and moist ethyl acetate (500 ml; fractions 3—10). Fractions 2—6 yielded 344 mg (42%) of compound *X*, m.p. 114—117°C (ether–light petroleum). NMR spectrum: δ 5·46 (d, 1-H, $J_{1,2}$ = 3·5), 3·25 (qu, 2-H, $J_{2,3}$ = 10·0), 3·50 (m, 3-H), 3·06 (qu, 4-H, $J_{4,3}$ = 8·5, $J_{4,5}$ = 10·0), 3·83 (sext., 5-H, $J_{5,6} = J_{5,6'}$ = 1·5), 4·29 (m, 2 × 6-H), 4·62 (d, 2′-H, $J_{2',3'}$ = 6·0), 4·12 (dd, 3′-H, $J_{3',4'}$ = 1·0 c.p.s.), 4·56 (m, 4′-H), 3·55—3·65 (m, 5′-H and 2 × 6′-H), 2·10 (s, CH_3CO), 3·38—3·63 p.p.m. (s, 6 × CH_3O). For $C_{12}H_{22}O_7$ (278·3) calculated: 51·79% C, 7·97% H; found: 51·97% C, 8·07% H.

(5*S*)-5-O-(2,3,4,6-Tetra-O-methyl-α-D-glucopyranosyl)-1,2,3,4,6-penta-O-methylallitol (*XII*)

To a solution of the lactone *X* (150 mg; 0·321 mmol) in tetrahydrofuran (15 ml) there was added dropwise at room temperature over 10 min a solution of lithium aluminium hydride (86 mg; 2·26 mmol) in tetrahydrofuran (4 ml), the whole mixture kept overnight, decomposed with ethyl acetate, and evaporated. The residue was coevaporated with acetic acid (20 ml) and then refluxed for 40 min in acetic anhydride (5 ml) in the presence of sodium acetate (50 mg). The resulting solution was evaporated, the residue coevaporated with three 20 ml portions of xylene, extracted with five 5 ml portions of boiling benzene, the extracts combined, filtered, and evaporated. The residue was chromatographed on a column of silica gel (13 g) in 4 : 1 benzene–ethyl acetate (50 ml), 7 : 3 benzene–ethyl acetate (80 ml; fractions 1—11), and ethyl acetate (50 ml; fractions 12—18). The homogeneous fractions 7—13 yielded 118 mg (100%) of the triacetate *XI* (dried for 6 h at 90°C/0·1 Torr). NMR spectrum: δ 5·12 (d, 1-H, $J_{1,2}$ = 3·2 c.p.s.), 5·20 (dd, 3′-H), 2·14, 2·16, 2·18 (s, 3 × CH_3CO), 3·35, 3·42, 3·44, 3·47, 3·52, and 3·62 p.p.m. (s, 6 × CH_3O). For $C_{24}H_{42}O_{14}$ (554·6) calculated: 51·98% C, 7·63% H; found: 51·76% C, 7·61% H.

A solution of the triacetate *XI* (139 mg; 0·25 mmol) in 0·02M methanolic sodium methoxide (4 ml) was kept at room temperature for 2 h, evaporated, and the residue coevaporated with two 20 ml portions of dimethylformamide. The residue was then dissolved in dimethylformamide (20 ml), the solution stirred with sodium hydride (200 mg) for 10 min, and the mixture treated with methyl iodide (2 ml) in one lot. After 15 h at room temperature, the mixture was decomposed with methanol (1 ml), evaporated, the residue coevaporated with xylene (two 20 ml portions), and finally passed through a column of alumina (15 g) in benzene (120 ml). The effluent was evaporated and chromatographed on a column of silica gel (10 g) in 7 : 3 benzene–ethyl acetate (80 ml; fractions 1—10) and ethyl acetate (80 ml; fractions 11—20). Fractions 12—14 were combined, evaporated, and the residue coevaporated with benzene to yield 81 mg (67%) of the permethyl derivative *XII* (dried for 6 h at 90°C/0·1 Torr). NMR spectrum: δ 5·32 (d, 1-H, $J_{1,2}$ = 3·6), 3·18 (dd, 2-H, $J_{2,3}$ = 8·5 c.p.s.), 3·34—3·64 (m, 3 × CH_2), 3·34—3·61 p.p.m. (s, 9 × CH_3O). For $C_{21}H_{42}O_{11}$ (470·6) calculated: 53·60% C, 9·00% H; found: 53·82% C, 9·13% H.

Permethylated Adenine-Ribofuranose-Glucopyranose-Allitol Sequence (*V*)

To a solution of the dephosphorylated exotoxin (50 mg) in 50% aqueous methanol (5 ml) there was added ethereal diazomethane (distilled in a stream of nitrogen) until the yellow colour was persistent. After 2 min, the emulsion was evaporated, the residue coevaporated with two 20 ml portions of dimethylformamide, and finally dissolved in dimethylformamide (5 ml). The solution was treated with N,O-bis(trimethylsilyl)acetamide (0·20 ml), the mixture kept at room temperature for 2·5 h, evaporated, and the residue coevaporated with three 20 ml portions of xylene. The final residue was dissolved in tetrahydrofuran (5 ml) and the solution treated with lithium aluminium hydride (60 mg) in one portion. After 8 min at room temperature, the mixture was successively decomposed with ethyl acetate (1 ml) and acetic acid (1 ml), and evaporated. The residue was coevaporated with two 10 ml portions of water and applied to a column of Dowex 50 WX4(H^+) ion exchange resin (15 ml). The column was washed at 0°C with water (100 ml) and eluted with 3M aqueous ammonia (90 ml). The basic effluent was evaporated, the residue applied to a column of Dowex 1 ($CH_3CO_2^-$) ion exchange resin (10 ml) and the column washed with water (100 ml). The ultraviolet-light extinguishing (at 255 nm) effluent was evaporated to afford the allitol *IV* in 73% yield; λ_{max} 261 nm and λ_{min} 233 nm (pH 6).

A dry solution of allitol *IV* (25 mg) in dimethylformamide (5 ml) was treated with sodium hydride (100 mg) and then, after 20 min of stirring, methyl iodide (0·5 ml) was added. The mixture was kept at room temperature for 15 h, the residue coevaporated with three 10 ml portions of

xylene and passed through a column of alumina (10 g) in 7 : 2 benzene–ethyl acetate (70 ml). The effluent was evaporated and the residue chromatographed on a thin layer of loose alumina (18 × 48 cm) in the above solvent mixture. The absorbing band yielded 48% of compound *V* (the analytical sample was dried at 80°C/0·1 Torr for 4 h); λ_{max} 275 nm and λ_{min} 235 nm (pH 6 and 13). Mass spectrum: $M^+ = 761$ and a characteristic change of the maximum at *m/e* 57, 71, 45, 101, 163 (B + 1) and 164 (B + 2). NMR spectrum: δ 8·13 (s, 2-H), 8·29 (s, 8-H), 6·15 (d, 1′-H, $J_{1',2'} = 2{\cdot}9$) and 5·25 p.p.m. (d, 1″-H, $J_{1'',2''} = 3{\cdot}3$ c.p.s.). For $C_{34}H_{59}N_5O_{14}$ (761·8) calculated: 53·60% C, 7·81% H, 9·20% N; found: 53·38% C, 8·05% H, 9·03% N.

Methanolysis of the Sequence *V*

A solution of the sequence *V* (20 mg) in 0·1M methanolic hydrogen chloride (5 ml) was heated in a sealed tube at 100°C for 1 h. The cooled content was evaporated under diminished pressure, the residue coevaporated with two 10 ml portions of benzene, and chromatographed on a column of alumina (5 g) in 20 : 1 benzene–ethyl acetate (40 ml) and 7 : 3 benzene–ethyl acetate (40 ml). The more polar eluate yielded 72% of the fragment *XXVI*. Mass spectrum: $M^+ + 1 = 253$ and a characteristic change of the maximum at *m/e* 45, 101, 89, 71, 59, 87, 145, and 175.

Acetyl derivative XXVII. The fragment *XXVI* (2 mg) was dissolved in pyridine (1 ml) and acetic anhydride (50 mg), the solution kept at 20°C for 3 h, evaporated, and the residue coevaporated with two 5 ml portions of toluene and one 5 ml portion of ethanol. CD spectrum (water): $\lambda([\Theta])$ 240 (0), 204 (−850°), and 195 nm (−750°).

(3*S*)-3-O-Benzyl-1,2,4,5,6-penta-O-acetylallitol (*XIV*)

The isopropylidene derivative *XIII* (3·10 g; 10·0 mmol) was refluxed in 50% aqueous formic acid (30 ml) for 25 min, the resulting solution evaporated, and the residue coevaporated with two 100 ml portions of water. The final residue was dissolved in water (12 ml), the aqueous solution neutralised with 1M aqueous ammonia and treated at room temperature over 7 min with a solution of sodium borohydride (1·5 g) in water (30 ml). After 15 min, the excess reagent was decomposed with acetic acid, the mixture evaporated under diminished pressure, the residue coevaporated with two 100 ml portions of acetic acid, and finally refluxed in acetic anhydride (20 ml) for 45 min. The mixture was evaporated and the residue taken into a mixture of chloroform (50 ml) and water (100 ml). The chloroform layer was washed with saturated aqueous sodium hydrogen carbonate (50 ml), dried, evaporated, the residue coevaporated with three 50 ml portions of toluene, and finally chromatographed on a column of silica gel (200 g) in the solvent mixtures 9 : 1 benzene–ethyl acetate (1 500 ml; fractions 1–62) and 6 : 1 benzene–ethyl acetate (1 000 ml; fractions 63–105). The homogeneous fractions 42–80 yielded 85% of the pentaacetate *XIV* (dried at 70°C/0·02 Torr for 7 h). For $C_{23}H_{30}O_{11}$ (482·5) calculated: 57·25% C, 6·26% H; found: 57·41% C, 6·35% H.

(3*S*)-O-Benzylallitol (*XV*)

A solution of the pentaacetate *XIV* (4·0 g) in 0·025M methanolic sodium methoxide (20 ml) was kept at room temperature for 30 min, neutralised with the Amberlite IRC-50 (H^+) ion exchange resin, and the filtrate evaporated. The residue was crystallised from ethyl acetate to yield 92% of the allitol *XV*, m.p. 76–78°C. NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 3·35 to 3·87 (m, 1-H–6-H), 4·58 (s, CH_2 of the benzyl group), and 7·14–7·32 p.p.m. (m, C_6H_5). For $C_{13}H_{20}O_6$ (272·3) calculated: 57·34% C, 7·40% H; found: 57·44% C, 7·28% H.

(3*S*)-3-O-Benzyl-1,2,4,5,6-penta-O-methylallitol (*XVI*)

A mixture of the benzylallitol *XV* (1·43 g; 5·25 mmol), sodium hydride (1·24 g; 50 mmol), and dimethylformamide (25 ml) was treated with methyl iodide (3 ml), the whole kept at 20°C for 15 h, treated with methanol (5 ml), and evaporated. The residue was taken up into a mixture of water (25 ml) and chloroform (20 ml), and the aqueous layer extracted with three 20 ml portions of chloroform. The chloroform solutions were combined, dried, and evaporated. The residue was passed through a column of alumina (10 g) in 50 : 1 benzene–ethyl acetate and the effluent distilled to yield 87% of the ether *XVI*, b.p. 168°C/0·05 Torr (Hickman flask). For $C_{18}H_{30}O_6$ (342·4) calculated: 63·14% C, 8·83% H; found: 63·27% C, 9·03% H.

(3*S*)-1,2,4,5,6-Penta-O-methylallitol (*XVII*)

The benzyl ether *XVI* (1·03 g; 3·0 mmol) in acetic acid (40 ml) was hydrogenolysed over 10% Pd/C (0·50 g) at room temperature for 2·5 h, the mixture filtered, the filtrate evaporated, the residue coevaporated with two 25 ml portions of toluene, and distilled under diminished pressure to yield 97% of the allitol *XVII*, b.p. 122°C/0·05 Torr. Mass spectrum: $M^+ + 1 = 253$ and characteristic maxima at *m/e* 45, 59, 71, 101, 131, 89, 75, 133, and 163. NMR spectrum: δ 3·4–3·7 (m, 8H), 3·85 (broad s, OH) and 3·32–3·38 (s, 5 × CH_3O); after the addition of CCl_3CONCS: δ 5·33 p.p.m. (t, 3-H, $J_{2,3} = J_{3,4} = 4·5$ c.p.s.). CD spectrum (water): λ ([Θ]) 205 (0) and 195 nm (−515°). For $C_{11}H_{24}O_6$ (252·3) calculated: 52·37% C, 9·59% H; found: 52·57% C, 9·39% H.

1,2-O-Isopropylidene-3-O-methyl-6-O-triphenylmethyl-α-D-allofuranose (*XIX*)

A mixture of the allofuranose *XVIII* (2·34 g; 10·0 mmol) and triphenylmethyl chloride (2·79 g; 10·0 mmol) in pyridine (50 ml) was kept at room temperature for 60 h, evaporated, the residue coevaporated with three 60 ml portions of xylene, and finally dissolved in benzene (100 ml). This solution was washed with three 200 ml portions of water, dried, evaporated, and the residue chromatographed on a column of silica gel (75 g) in the solvent mixtures 20 : 1 benzene–ethyl acetate (400 ml) and 3 : 1 benzene–ethyl acetate (450 ml). The more polar eluate yielded 57% of the triphenylmethyl derivative *XIX* (dried at 75°C/0·1 Torr for 2 h). For $C_{29}H_{32}O_6$ (476·6) calculated: 73·09% C, 6·77% H; found: 72·82% C, 6·49% H.

5-O-Benzyl-1,2-O-isopropylidene-3-O-methyl-6-O-triphenylmethyl-α-D-allofuranose (*XX*)

To a solution of compound *XIX* (3·58 g; 7·50 mmol) in dimethylformamide (25 ml) there was added sodium hydride (480 mg; 20 mmol) and then, after 15 min at 20°C, benzyl chloride (1·65 g; 13 mmol). The mixture was stirred at room temperature for 16 h, treated with methanol (4 ml), evaporated, the residue coevaporated with two 100 ml portions of xylene, and passed through a column of alumina (50 g) in 50 : 1 benzene–ethyl acetate. The effluent was evaporated and the residue chromatographed on a column of silica gel (65 g) in benzene (400 ml) and then 9 : 1 benzene–ethyl acetate (500 ml). The more polar eluate was evaporated to yield 47% of the protected allofuranose *XX* (the analytical sample was dried at 70°C/0·01 Torr for 8 h). For $C_{36}H_{38}O_6$ (566·7) calculated: 76·30% C, 6·76% H; found: 76·33% C, 6·99% H.

(5*R*)-5-O-Benzyl-3-O-methyl-1,2,4,6-tetra-O-acetylallitol (*XXI*)

The triphenylmethyl derivative *XX* (1·70 g; 3·0 mmol) was hydrolysed in refluxing 50% aqueous formic acid (30 ml) for 25 min, the solution evaporated, the residue neutralised with 7M aqueous ammonia, and taken up into a mixture of benzene (50 ml) and water (35 ml). The benzene layer

was extracted with two 35 ml portions of water. Sodium borohydride (0·40 g) was added to the combined aqueous phases, the whole kept at 20°C for 20 min, the excess reagent decomposed with acetic acid, the solution evaporated under diminished pressure, the residue coevaporated with acetic acid (30 ml), and finally refluxed in acetic anhydride (25 ml) for 40 min. The mixture was evaporated, the residue dissolved in water (20 ml) and the aqueous solution extracted with four 25 ml portions of chloroform. The extracts were combined, dried, evaporated, and the residue chromatographed on a column of silica gel (50 g) in 6 : 1 benzene–ethyl acetate (800 ml; fractions 1—45). The homogeneous fractions 11—19 were evaporated and the residue coevaporated with chloroform to yield 57% of the tetraacetate *XXI* (dried at 70°C/0·05 Torr for 8 h). For $C_{22}H_{30}O_{10}$ (454·5) calculated: 58·14% C, 6·65% H; found: 58·07% C, 6·52% H.

(5*R*)-5-O-Benzyl-1,2,3,4,6-penta-O-methylallitol (*XXIII*)

A solution of the tetraacetate *XXI* (0·57 g; 1·25 mmol) in 0·04M methanolic sodium methoxide (10 ml) was kept at room temperature for 20 min, neutralised with Amberlite IRC-50 (H^+) ion exchange resin, filtered, the filtrate evaporated, the residue coevaporated with dimethylformamide (20 ml), and finally dissolved in dimethylformamide (20 ml). Sodium hydride (0·48 g; 20 mmol) was added to this solution, the mixture kept at 20°C for 30 min, and treated with methyl iodide (6 ml) under stirring. After 10 h, methanol (5 ml) was added, the mixture evaporated, the residue coevaporated with two 20 ml portions of xylene, and finally dissolved in water (8 ml). The aqueous solution was extracted with chloroform (five 20 ml portions), the extracts combined, dried, evaporated, and the residue passed through a column of alumina (10 g) in 50 : 1 benzene–ethyl acetate (70 ml). The effluent was evaporated and the residue chromatographed on a column of silica gel (25 g) in 9 : 1 benzene–ethyl acetate (350 ml; fractions 1—35). The homogeneous fractions 9—13 were evaporated and the residue distilled to yield 84% of the ether *XXIII*, b.p. 160°C/0·05 Torr. For $C_{18}H_{30}O_6$ (342·4) calculated: 63·14% C, 8·83% H; found: 63·20% C, 8·76% H.

(5*R*)-1,2,3,4,6-Penta-O-methylallitol (*XXIV*)

The benzyl ether *XXIII* (0·85 g; 2·50 mmol) in acetic acid (30 ml) was hydrogenolysed at room temperature over 10% Pd/C (0·20 g) for 3 h, the mixture filtered, the filtrate evaporated, the residue coevaporated with benzene, and finally distilled to yield 85% of compound *XXIV*, b.p. 135°C/0·2 Torr. CD spectrum (water): $\lambda([\Theta])$ 210 (0), 200 (+50°), and 195 nm (+30°). Mass spectrum: $M^+ + 1 = 253$ and characteristic maxima at *m/e* 45, 101, 89, 71, 59, 87, 145, and 175. For $C_{11}H_{24}O_6$ (252·3) calculated: 52·37% C, 9·59% H; found: 52·67% C, 9·49% H.

Acetyl derivative XXV. A mixture of compound *XXIV* (100 mg), acetic anhydride (0·5 ml), and pyridine (5 ml) was kept at 20°C for 2 h, evaporated under diminished pressure, the residue coevaporated with two 5 ml portions of xylene, and finally chromatographed on a column of silica gel (5 g) in 7 : 3 benzene–ethyl acetate (50 ml; fractions 1—15). Fractions 4—9 were evaporated and the residue distilled under diminished pressure to yield 94% of the acetate *XXV*, b.p. 105°C/0·3 Torr. Mass spectrum: characteristic maxima at *m/e* 45, 101, 129, 145, 89, 161, 71, 75, and 59. CD spectrum (water): $\lambda([\Theta])$ 240 (0), 204 (+870°), and 195 nm (+735°). NMR spectrum: δ 3·4—3·55 (m, 1-H—4-H), 5·21 (qu, 5-H, $J_{5,4} = 10·0$, $J_{5,6} = 4·5$), 3·57 (d, 2 × 6-H, $J_{6,5} =$ = 4·5 c.p.s.), 2·00 (s, CH_3CO) and 3·28—3·37 p.p.m. (s, 5 × CH_3O). For $C_{13}H_{26}O_7$ (294·3) calculated: 53·05% C, 8·90% H; found: 53·07% C, 8·62% H.

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