NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CXXXIV.*

SYNTHESIS OF THE NUCLEOSIDIC MOIETY OF EXOTOXIN FROM *Bacillus thuringiensis*

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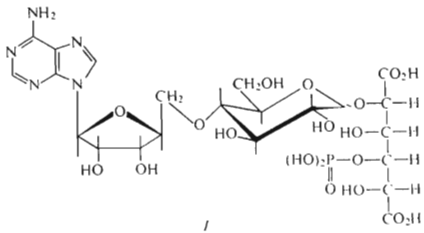
Reaction of an equimolecular mixture of the ribose derivative *XV* and the glucose derivative *XVI* with hydrogen bromide in acetic acid and the subsequent treatment of resulting halogenoses with the chloromercuri salt *XVII* in the molar ratio 1 : 1 : 0·5 afforded as the principal product the ribosyl derivative *XVIII* along with traces of the glucosyl derivative *XIX*. An analogous reaction of the dihalogenose *X* with 0·4 equivalent of the salt *XVII* occurred exclusively on the more reactive center of the ribose moiety under the formation of the monohalogenose *XXII* which was converted to the corresponding methyl glycoside *XXIII* possessing the β-configuration at the anomeric center 1′. Reaction of the dihalogenose *X* with an excess of the chloromercuri salt *XVII* afforded a new type of the nucleoside *XXIV* with two basic residues attached by the nucleosidic linkage to the sugar moiety.

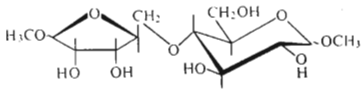
In an earlier paper of this Series[2], we have reported the synthesis of the fundamental sugar fragment of exotoxin from *Bacillus thuringiensis*. The structure *I* of this very complex insecticidal toxin was proposed by investigators of our Institute[3,4] on the basis of a study on its cleavage products with the use of physicochemical methods. According to the proposal, exotoxin contains *inter alia* adenosine attached to glucose by an ethereal linkage. The glucose is in turn attached by a glycosidic linkage to allaric acid which bears one phosphate grouping. Acidic methanolysis of exotoxin afforded a complex fragment the structure of which is represented by the formula *II* (*cf.*[3,4]). This anomeric mixture was successfully converted into the homogeneous diglycoside ether *III* the *cis*-diol grouping of which is protected by the isopropylidene group, the remaining hydroxylic functions being blocked by acetyl groups. The structure of the fragment *III*, the NMR spectrum of which is satisfactorily resolved[4], was confirmed[1,2] by an unequivocal synthesis (Scheme 1).

* Part CXXXIII: This Journal *36*, 1448 (1971).

** For a preliminary communication see reference[1].



I



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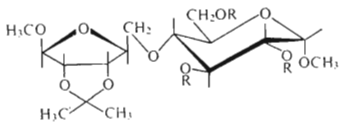
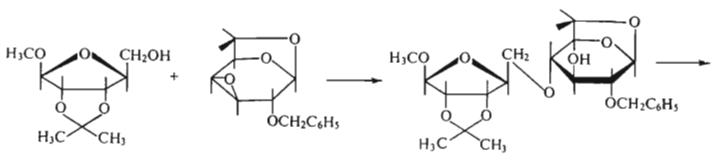
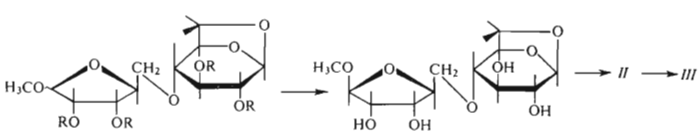


III, R = CH_3CO



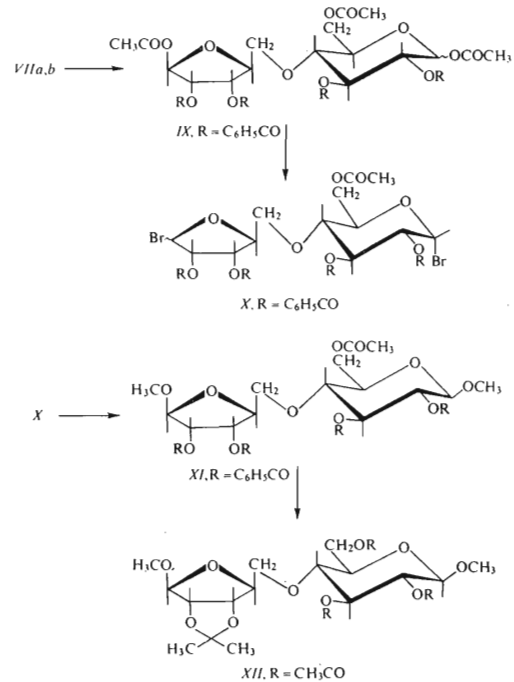
IV *V* *VI*



VIIa, α-anomer R = C_6H_5CO
VIIb, β-anomer, R = C_6H_5CO

SCHEME 1

The ethereal linkage connecting two sugar residues was realised by the diaxial opening of the epoxide ring of 2-O-benzyl-1,6:3,4-dianhydro-β-D-galactopyranose (*V*) with methyl 2,3-O-isopropylidene-β-D-ribofuranoside (*IV*) under alkaline conditions. The product *VI* was in several steps converted to the diglycoside ether *VIII*, the acid-catalysed methanolysis of which followed by acetylation and isopropylidenation afforded the ether *III* identical in every respect with that obtained by transformation[3,4] of the fragment *II*. The compound *XII* differring from the fragment *III* only by the β-configuration at the anomeric center 1′ was prepared by the reaction sequence shown in Scheme 2.

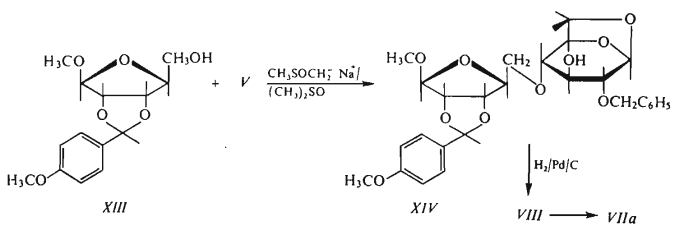


SCHEME 2

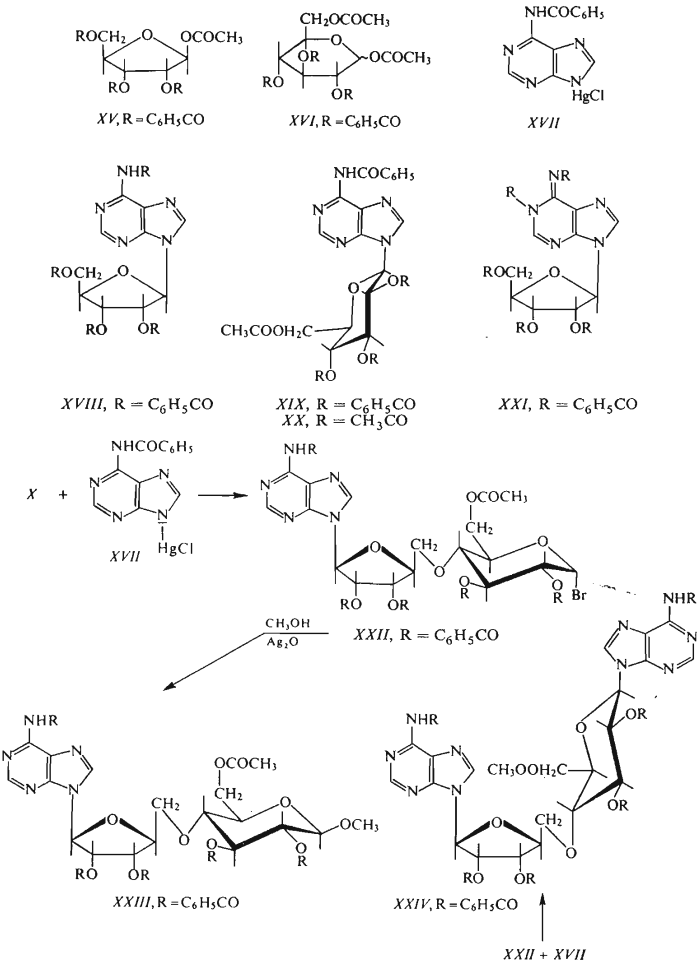
In the present paper, we wish to report the synthesis of a model of the dephosphorylated exotoxin containing a methyl glucoside residue (*cf. XXIII*) instead of allaric acid attached to glucose by a glycosidic linkage. As the starting material in this synthesis, we used the recently reported[1,2] dihalogenose *X*. Since the dihalogenose *X* must be used in the glycosylation reaction directly without any isolation, a suitable intermediate was required which would generate the dihalogenose *X* readily and without any by-products. Such an intermediate is represented by compound *IX* which was earlier successfully used[2] in the synthesis of compound *XII*. The triacetate *IX* was prepared by acetolysis of tribenzoates *VIIa* and *VIIb*. The earlier procedure[2] (Scheme 1) for the preparation of compound *VIIa* was shortened and modified as follows in Scheme 3.

Instead of the isopropylidene ribofuranoside *IV*, methyl 2,3-O-*p*-anisylidene-β-D-ribofuranoside (*XIII*) was used. Reaction of the latter compound *XIII* with benzyl epoxide *V* under alkaline conditions afforded the crystalline 2-O-benzyl-4-O-(methyl 2,3-*p*-anisylidene-5-deoxy-β-D-ribofuranosid-5-yl)-1,6-anhydro-β-D-glucopyranose (*XIV*). Hydrogenolysis of compound *XIV* over palladium on carbon catalyst in acetic acid led to the diglycoside ether *VIII* identical with the earlier specimen[2]. The *p*-anisylidene group of compound *XIV* may also be removed by heating at 60°C in 80% aqueous acetic acid for 20 minutes (the simultaneous hydrolysis of the methyl riboside residue occurs only to a negligible extent).

The dihalogenose *X* contains two anomeric centers (at positions 1 and 1'). The reactivity of these centers should differ analogously to those of the halogenoses derived from a ribofuranose and a glucopyranose. As a model of the dihalogenose *X*, an equimolecular mixture of the known[5] 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (*XV*) and 1,6-di-O-acetyl-2,3,4-tri-O-benzoyl-D-glucopyranose (*XVI*) was used. The latter mixture was converted on treatment with hydrogen bromide in acetic acid and methylene chloride to a mixture of the correspondiug halogenoses which was subjected to the reaction with N^6-benzoyladenine chloromercuri salt[6] (*XVII*)



SCHEME 3


XV, R = C6H5CO
XVI, R = C6H5CO
XVII
XVIII, R = C6H5CO
XIX, R = C6H5CO
XX, R = CH3CO
XXI, R = C6H5CO
X +
XVII
XXII, R = C6H5CO
CH3OH
Ag2O
XXIII, R = C6H5CO
XXIV, R = C6H5CO
XXII + XVII

SCHEME 4

in refluxing acetonitrile for the period of 1 to 5 minutes. It has been found that the equimolecular mixture of compound *XV*, compound *XVI*, and the chloromercuri salt *XVII* (molar ratio 1 : 1 : 1) affords a mixture containing 53% of the ribosyl derivative *XVIII* and 18% of the glucosyl derivative *XIX*. With the molar ratio 1 : 1 : 0·7, there is obtained 48% of compound *XVIII* and 4% of compound *XIX* while 50% of the ribosyl derivative *XVIII* and less than 1% of the glucosyl derivative *XIX* results in the case of the molar ratio 1 : 1 : 0·5. The mixture of compounds *XVIII* and *XIX* was quantitatively separated by chromatography on a thin layer of loose silica gel.

The glucose derivative *XVI* was prepared by an acid-catalysed acetolysis of laevoglucosan tribenzoate analogously to compound *IX*. Ribosylation of excess N^6-benzoyladenine chloromercuri salt led to the amorphous adenosine tetrabenzoyl derivative (*XVIII*) in a 57% yield (*cf.* ref.[7]). The corresponding glucosylation was performed under otherwise the same conditions (*cf.* ref.[8]) to afford the crystalline glucosyl derivatives *XIX* and *XX* in yields of 28 and 29%, respectively. Compound *XVIII* was converted to adenosine pentabenzoyl derivative *XXI*, identical with that obtained by perbenzoylation of adenosine[9,10].

The halogenose *X* was subjected to the reaction with 0·4 equivalent of N^6-benzoyladenine chloromercuri salt (*XVII*) in refluxing anhydrous acetonitrile for 4 minutes. The reaction mixture containing the halogenose *XXII* was without delay decomposed by the addition of methanol and silver oxide to afford the expected product *XXIII* (see Scheme 4) along with the known dimethyl diglycoside ether *XI*. Compound *XXIII* necesserily possesses the β-configuration at the anomeric center 1′ because of its formation under conditions of a sterically controlled glycosidation.

Attachment of an additional adenine residue to the less reactive anomeric center 1′ of the halogenose *XXII* was accomplished by the use of a considerable excess of the chloromercuri salt *XVII*. The resulting compound *XXIV* was isolated in a satisfactory yield by a repeated chromatography on silica gel. This novel nucleoside type with two basic residues attached to the sugar moiety by means of a nucleosidic linkage has not been hitherto reported, *inter alia* because of the earlier inaccessibility of the dihalogenose *X* in the chemistry of sugars. Recently, some nucleosides have been prepared the sugar moiety of which carries two basic residues, only one being of course attached by means of the nucleosidic linkage[11].

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 20°C/0.1 Torr for 10 hours unless stated otherwise. Infrared spectra and optical rotations were measured in chloroform.

Methyl 2,3-O-*p*-Anisylidene-β-D-ribofuranoside (*XIII*)

A mixture of methyl β-D-ribofuranoside (8·2 g; 50 mmol), methyl orthoformate (15 ml), freshly distilled anisaldehyde (25 ml), ethereal 0·7M-HCl (2 ml), and dioxane (100 ml) was allowed to

stand at room temperature for 18 hours, neutralised with 25% aqueous ammonia (0·3 ml), and evaporated under diminished pressure. The residue was coevaporated with two 150 ml portions of benzene and applied to a column of neutral aluminum oxide (750 g; Brockmann activity II). The column was washed with benzene (3000 ml) and then eluted with an 1 : 1 mixture of benzene and ethyl acetate presaturated with water (3500 ml; fractions 1—7). The chromatographically homogeneous fractions 4—7 (thin layer chromatography on silica gel with binder: R_F values 0·25 and 0·5 in benzene–ethyl acetate 10 : 7 and 7 : 3, resp.) were combined, evaporated under diminished pressure, the residue coevaporated with three 100 ml portions of benzene, and then dried at 60°C/0·2 Torr for 8 hours. Yield, 12·5 g of compound *XIII*, $[\alpha]_D^{25}$ −61° (*c* 0·50). For $C_{14}H_{18}O_6$ (282·3) calculated: 59·57% C, 6·43% H; found: 60·01% C, 6·67% H.

Reaction of 2-O-Benzyl-1,6 : 3,4-dianhydro-β-D-galactopyranose (*V*) with Methyl 2,3-O-*p*-Anisylidene-β-D-ribofuranoside (*XIII*)

A mixture of the riboside *XIII* (1·0 g), the benzyl epoxide *V* (0·06 g), 2M-$NaCH_2SOCH_3$ in dimethyl sulfoxide (1·2 ml), and dimethyl sulfoxide (3 ml) was heated at 100°C for 2 hours, cooled, and diluted with water (100 ml). The emulsion was extracted with three 5 ml of benzene, the extract washed with three 100 ml portions of water, dried, and applied to a column of silica gel (80 g; deactivated by the addition of 10% water). The column was eluted successively with benzene (600 ml), 10 : 1 benzene–ethyl acetate (600 ml), 7 : 1 benzene–ethyl acetate (600 ml), 4 : 1 benzene–ethyl acetate (300 ml; fraction 1—21), and 13 : 4 benzene–ethyl acetate (600 ml; fractions 22—63). The chromatographically homogeneous fractions 26—54 (thin-layer chromatography on silica gel with binder: R_F value 0·2 in 7 : 3 benzene–ethyl acetate) were combined and processed as usual to afford 354 mg (27%) of an oil which was crystallised from ether. The final product *XIV* melted at 127—133°C. Optical rotation: $[\alpha]_D^{25}$ −53·4° (*c* 0·51). Infrared spectrum: ν(O—H) bonded at 3595 and 3462 cm^{-1}. For $C_{27}H_{32}O_{10}$ (516·6) calculated: 62·78% C, 6·24% H; found: 62·81% C, 6·29% H.

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

A solution of compound *XIV* (110 mg) in methanol (40 ml) was hydrogenolysed at room temperature and ordinary pressure for $2^1/_2$ hours over 10% palladium on carbon catalyst. The filtrate was evaporated under diminished pressure and the residue crystallised from 95% methanol and ether to afford compound *VIII*, m.p. 132—133°C, undepressed on admixture with an authentic specimen[1,2]; yield, 68%. For $C_{12}H_{20}O_9.H_2O$ (326·3) calculated: 44·17% C, 6·80% H; found: 44·42% C, 6·85% H.

2′,3′,5′,N^6-Tetrabenzoyladenosine (*XVIII*)

A mixture of the chloromercuri salt *XVII* (475 mg; 1·00 mmol) and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared as follows: a mixture of 450 mg of the acetate *XV*, 1 ml of 35% hydrogen bromide in acetic acid and 1 ml of methylene chloride was allowed to stand at room temperature for 70 minutes, evaporated, and coevaporated with 40 ml of toluene) was refluxed in acetonitrile (5 ml) for 8 minutes, cooled, evaporated under diminished pressure, and the residue dissolved in chloroform (20 ml). The solution was washed with 10% aqueous potassium iodide (10 ml) and 5% aqueous potassium hydrogen carbonate (15 ml), dried, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (25 g; deactivated by the addition of 9% water) with the use of benzene (100 ml), 9 : 1 benzene–ethyl acetate (150 ml), 4 : 1 benzene–ethyl acetate (150 ml; fractions 1—11), and 7 : 3 benzene–ethyl acetate (200 ml; fractions 12—26). The chromatographically homogeneous fractions 16—23 afforded

346 mg (57%) of the ester *XVIII* in the form of a solid foam. The analytical sample was dried at room temperature for 2 days in an evacuated desiccator (1 Torr) over concentrated sulfuric acid and potassium hydroxide pellets. Infrared spectrum: ν(N—H) at 3 401 cm^{-1} and ν(C=O) at 1 726 and 1 710 cm^{-1} (shoulder, benzamide). For $C_{38}H_{29}N_5O_8$ (683·7) calculated: 66·76% C, 4·28% H, 10·24% N; found: 66·58% C, 4·33% H, 10·31% N.

A mixture of compound *XVIII* (300 mg), benzoyl chloride (300 mg), and pyridine (10 ml) was allowed to stand at room temperature for 15 hours and decomposed with one drop of water. After 10 minutes, the mixture was evaporated under diminished pressure, the residue dissolved in chloroform (20 ml), the solution washed with two 50 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (25 g; deactivated by the addition of 10% water). The column was eluted with benzene (200 ml) and 9 : 1 benzene–ethyl acetate (200 ml; fractions 1—5). The chromatographically homogeneous fractions 2—4 (thin-layer chromatography on silica gel with binder: R_F value 0·30 in 9 : 1 benzene–ethyl acetate) afforded a 90% yield of adenosine pentabenzoyl derivative *XXI*, m.p. 189—190°C (toluene), undepressed on admixture with a specimen obtained by perbenzoylation of adenosine[9,10]. Optical rotation: $[\alpha]_D^{25}$ −10·4° (*c* 0·50). For $C_{45}H_{33}N_5O_9$ (787·8) calculated: 68·61% C, 4·22% H, 8·89% N; found: 68·65% C, 4·38% H, 8·92% N.

9-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-N^6-benzoyladenine (*XX*)

A mixture of the thoroughly dried chlormercuri salt *XVII* (475 mg; 1·00 mmol), acetobromoglucose (411 mg; 1·00 mmol), and acetonitrile (10 ml) was refluxed for 2 hours and the resulting solution evaporated under diminished pressure. The residue was dissolved in chloroform (15 ml), the solution washed with 30% aqueous potassium iodide (5 ml) and water, dried, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (20 g; deactivated by the addition of 9% water) with the use of benzene (100 ml), 7 : 3 benzene–ethyl acetate (200 ml), 1 : 1 benzene–ethyl acetate (120 ml), and 1 : 3 benzene–ethyl acetate (200 ml; fractions 1—14). The chromatographically homogeneous fractions 3—11 (thin-layer chromatography on silica gel with binder: R_F value 0·25 in 1 : 1 benzene–ethyl acetate) were combined, evaporated under diminished pressure, and the residue crystallised from ether to afford 165 mg (29%) of compound *XX*, m.p. 176—179°C. NMR spectrum (in deuteriochloroform): δ 6·02 (d, 1′-H, $J_{1',2'}$ 9·0), 5·68 (t, 2′-H, $J_{2',3'}$ 9·0), 5·50 (t, 3′-H, $J_{3',4'}$ 9·0), 5·31 (t, 4′-H, $J_{4',5'}$ 9·0 c.p.s.), 4·18 (m, 5′-H), 4·10—4·50 (m, 2 × 6′-H), 8·79 (s, 2-H), 8·27 (s, 8-H), 1·79, 2·06, and 2·10 (s, 4 × CH_3 of acetyl groups), and 9·39 p.p.m. (broad s, N^6-H). For $C_{26}H_{27}N_5O_{10}$ (569·5) calculated: 54·83% C, 4·78% H, 12·30% N; found: 55·01% C, 4·80% H, 12·34% N.

The alkali-catalysed methanolysis of compound *XX* afforded the free 9-β-D-glucopyranosyladenine, m.p. 242—243°C in accordance with the literature[8].

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

A lukewarm solution of 1,6-anhydro-β-D-glucopyranose tribenzoate[12] (40 g) in acetic anhydride (1 000 ml) was treated over 1 minute under vigorous stirring with concentrated sulfuric acid (13·5 ml) and the reaction mixture then cooled without delay to the room temperature. The stirring was continued for additional 15 minutes, the mixture poured onto ice, after 10 minutes diluted with water (2000 ml), neutralised by the addition of sodium acetate trihydrate, and the product extracted with benzene (1 000 ml). The extract was washed with 0·2% aqueous sodium chloride (three 3 500 ml portions) and then saturated aqueous potassium hydrogen carbonate until the evolution of carbon dioxide ceased, dried over anhydrous magnesium sulfate, and

evaporated under diminished pressure. The residue was dried for 5 hours at 80°C/12 mm Hg in a rotatory evaporator to afford the compound *XVI* in an almost quantitative yield. The analytical sample was dried for 2 days at room temperature in an evacuated desiccator (1 Torr) over concentrated sulfuric acid and potassium hydroxide pellets. For $C_{31}H_{28}O_{11}$ (576·6) calculated: 64·57% C, 4·89% H; found: 64·99% C, 5·01% H.

9-(2,3,4-Tri-O-benzoyl-6-O-acetyl-β-D-glucopyranosyl)-N^6-benzoyladenine (*XIX*)

A mixture of the chloromercuri salt *XVII* (475 mg; 1·00 mmol) and the halogenose (prepared as follows: a mixture of 320 mg *i.e.* 0·55 mmol of the diacetate *XVI*, 1 ml of 35% hydrogen bromide in acetic acid, and 1 ml of methylene chloride is allowed to stand at room temperature for 90 minutes, evaporated under diminished pressure, and the residue coevaporated with 40 ml of toluene) in acetonitrile (3·5 ml) was refluxed for 90 min, the suspension cooled, and evaporated under diminished pressure. The residue was dissolved in chloroform (20 ml), the solution washed with 30% aqueous potassium iodide (5 ml) and water, dried, and evaporated under diminished pressure. The residue was chromatographed on a thin layer (one plate, 17 × 43 cm) of loose silica gel (deactivated by the addition of 10% water) in 1 : 1 benzene–ethyl acetate. The extinguishing band (R_F value 0·4) was eluted with ethyl acetate, the eluate evaporated under diminished pressure, and the residue crystallised from ether to afford 107 mg (26%) of the nucleoside *XIX*, m.p. 165—166°C. Optical rotation: $[\alpha]_D^{25}$ −51·9° (*c* 0·51). Infrared spectrum: ν(N—H) at 3402 cm^{-1}. For $C_{41}H_{33}N_5O_{10}$ (755·7) calculated: 65·16% C, 4·41% H, 9·27% N; found: 65·25% C, 4·28% H, 9·20% N.

The alkali-catalysed methanolysis of compound *XIX* afforded the free 9-β-D-glucopyranosyladenine, m.p. 241—243°C, in accordance with a specimen obtained by alcoholysis of the ester *XX*.

Mixed Glycosylation of N^6-Benzoyladenine Chloromercuri Salt (*XVII*)

A solution of the acetate *XV* (250 mg; 0·5 mmol) and the diacetate *XVI* (300 mg; 0·5 mmol) in methylene chloride (1 ml) was treated with 1 ml of 35% hydrogen bromide in acetic acid, the mixture allowed to stand at room temperature for 70 min, diluted with benzene (20 ml), evaporated under diminished pressure, and the residue coevaporated with three 20 ml portions of benzene, and finally dissolved in acetonitrile (6 ml). The solution was added to the thoroughly dried chloromercuri salt *XVII* (240 mg; 0·5 mmol), the whole refluxed for 4 min, cooled, and processed as above to afford a mixture of nucleosides *XVIII* and *XIX* which was separated on a thin layer (one plate, 17 × 43 cm) of loose silica gel (deactivated by the addition of 10% water) in 1 : 1 benzene–ethyl acetate. The band of the R_F value 0·50 afforded 53% of the ribosyl derivative *XVIII*. The glucosyl derivative *XIX*, m.p. 165—167°C (ether), was obtained (yield, 18%) from the band of the R_F value 0·4.

Reaction of the Dihalogenose *X* with N^6-Benzoyladenine Chloromercuri Salt (*XVII*)

A. With 0·4 equivalent of the salt XVII. A solution of the dihalogenose *X* (obtained[2] from 600 mg *i.e.* 0·70 mmol of the triacetate *IX*) in acetonitrile (7 ml) was added to the azeotropically dried chloromercuri salt *XVII* (132 mg; 0·28 mmol), the reaction mixture refluxed for 4 minutes, the resulting solution cooled down, and diluted with methanol (2 ml). After one minute, silver oxide (1·5 g) was added in one lot under stirring. The stirring at room temperature was continued for 4 hours, the suspension filtered, and the filtrate evaporated under diminished pressure. The residue was dissolved in chloroform (20 ml), the solution washed with 30% aqueous potassium iodide (5 ml) and then saturated aqueous potassium hydrogen carbonate (5 ml), dried, and

evaporated under diminished pressure. The residue was chromatographed on a thin layer (two plates, 17 × 43 cm) of loose silica gel (deactivated by the addition of 10% water) in 1 : 1 benzene–ethyl acetate. Bands possessing the R_F value 0·43 were combined and rechromatographed under the same conditions to afford 56 mg (20%) of the protected nucleoside *XXIII*. Infrared spectrum: ν(N—H) at 3402 cm^{-1} and ν(C=O) at 1733 and 1712 cm^{-1} (shoulder, benzamide). For $C_{54}H_{47}N_5O_{15}$ (1006·0) calculated: 6·95% N; found: 6·72% N. The bands possessing R_F values higher than 0·7 were combined, eluted with ethyl acetate and chromatographed on a column of silica gel (40 g; deactivated by the addition of 10% water) in benzene–ethyl acetate. Usual[2] work-up afforded the dimethyl diglycoside ether *XI* (27%), identical with the earlier prepared[2] specimen.

B. With 4 equivalents of the chloromercuri salt XVII. A mixture of the dihalogenose *X* (prepared from 130 mg *i.e.* 0·15 mmol of the triacetate *IX*), the chloromercuri salt *XVII* (285 mg; 0·60 mmol), and acetonitrile (3 ml) was refluxed for one hour, the suspension evaporated under diminished pressure, and the residue processed similarly to paragraph *A*. The product *XXIV* was isolated by chromatography on a thin layer (one plate, 18 × 43 cm) of loose silica gel (deactivated by the addition of 10% water) in 1 : 3 benzene–ethyl acetate. The band of R_F value 0·37 was rechromatographed under identical conditions to afford 41 mg (22%) of compound *XXIV*. Infrared spectrum: ν(N—H) at 3402 cm^{-1} and ν(C=O) at 1734 cm^{-1} (acetate and benzoate) and 1712 cm^{-1} (shoulder, benzamide). For $C_{65}H_{52}N_{10}O_{15}$ (1213·2) calculated: 64·35% C, 4·35% H, 11·52% N; found: 63·58% C, 4·71% H, 11·17% N.

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