NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CXLIII.*
NUCLEOSIDES DERIVED FROM 2-DEOXY-2(*R*)-C-METHYL-*erythro*-D-PENTOSE

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Derivatives of stereoisomeric 2-deoxy-2-C-methyl-*erythro*-D-pentoses, namely, 2′(*R*)-C-methylthymidine and 2′(*R*)-C-methyl-2′-deoxyadenosine, have been prepared and their configuration at $C_{(2)}$ determined.

In an earlier paper[1] of this Series, the synthesis of 2-C-methyl-D-ribose and its derivatives has been reported. Some intermediates of the same sugar have been used in the synthesis of 2-C-methyladenosine by Walton and coworkers[2,3]. As a continuation of these investigations, we wish to report now the synthesis of 2′-deoxy nucleosides derived from 2-deoxy-2-C-methyl-*erythro*-D-pentoses (2-C-methyl-2-deoxyribese). Two stereoisomeric 2-deoxy-2-C-methyl-*erythro*-D-pentoses differing in the absolute configuration at carbon $C_{(2)}$ are theoretically possible. We have focussed our attention to the isomer and its derivatives possessing the absolute configuration *R* at $C_{(2)}$, since this sugar appears isosteric with D-ribose.

In our synthesis, 2-C-methyl-D-ribonolactone (*I*) has been used as the starting compound. Since the reactivity of the tertiary carbon atom $C_{(2)}$ is sufficiently different, the partial toluylation of the lactone *I* with a calculated amount of *p*-toluyl chloride in pyridine leads to a homogeneous product, namely, 2-C-methyl-3,5-di-O-*p*-toluyl-D-ribonolactone (*II*). Replacement of hydroxylic function at position $C_{(2)}$ by bromine is readily accomplished on treatment with hydrogen bromide in acetic acid under the formation of 2-bromo-2-deoxy-2-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentonolactone (*III*). Configuration at the carbon atom $C_{(2)}$ of the latter derivative was not determined, since a mixture of isomers is obtained in the next step.

The removal of bromine from position $C_{(2)}$ is rather difficult. Thus, hydrogenation in ethanol over the palladium on active charcoal catalyst is unexpectedly accompanied by a high uptake of hydrogen. From the product, two crystalline substances

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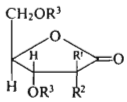
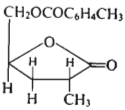
IV and *V* were isolated. As shown by elemental analysis, these substances did not contain any bromo atom and differred from each other by two hydrogen atoms. The analytical data indicate also the hydrogenolytical removal of one toluyloxyl group. The structure of 2,3-dideoxy-2-C-methyl-5-O-*p*-toluyl-*glycero*-D-pent-2-enono-1,4-lactone for compound *IV* and the structure of 2,3-dideoxy-2-C-methyl-5-O-*p*-toluyl-*glycero*-D-pentono-1,4-lactone for compound *V* was confirmed by an independent synthesis. Thus, when refluxed with zinc in ethanol, the bromo lactone *III* afforded compound *IV*, the hydrogenation of which led to compound *V*.

The lactones *IV* and *V* result also in that case, when the hydrogenation of the bromo lactone *III* is performed in ethyl acetate or dimethylformamide or acetic acid instead of ethanol, or, over palladium on barium sulfate instead of palladium on active charcoal. The required isomeric 2-deoxy-2-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentono-1,4-lactones *VI* and *VII* were finally obtained with the use of palladium oxide on barium sulfate as catalyst and dioxane as solvent even though in admixture with the hydrogenolytical by-products *IV* and *V*. Since the hydrogen bromide set free in the course of the reaction deactivates the catalyst, it is necessary to work up repeatedly the hydrogenation mixture and then continue in the hydrogenation with a fresh catalyst. The attempted neutralisation of hydrogen bromide by the addition of an organic or inorganic base led to the undesired formation of compounds *IV* and *V*. The complex mixture of products obtained by the hydrogenation has to be separated by column chromatography on silica gel to remove the by-products *IV* and *V* from the mixture of deoxy lactones *VI* and *VII*. Crystallisation of the latter mixture afforded the isomer *VII*, m.p. 137°C. The other isomer *VI*, m.p. 122°C, was obtained in a small quantity by a repeated chromatography of mother liquors.

The protecting groups of the isomers *VI* and *VII* were removed on alcoholysis. The resulting free 2-deoxy-2-C-methyl-*erythro*-D-pentonolactones *VIII* and *IX* were used to determine the absolute configuration at $C_{(2)}$.





V

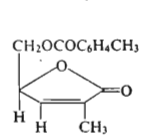
I, $R^1 = CH_3$, $R^2 = OH$, $R^3 = OH$,
II, $R^1 = CH_3$, $R^2 = OH$, $R^3 = 4\text{-}CH_3C_6H_4CO$
III, R^1, $R^2 = CH_3$, Br, $R^3 = 4\text{-}CH_3C_6H_4CO$
VI, $R^1 = CH_3$, $R^2 = H$, $R^3 = 4\text{-}CH_3C_6H_4CO$
VII, $R^1 = H$, $R^2 = CH_3$, $R^3 = 4\text{-}CH_3C_6H_4CO$
VIII, $R^1 = CH_3$, $R^2 = H$, $R^3 = H$
XI, $R^1 = H$, $R^2 = CH_3$, $R^3 = H$

As observed by Okuda and coworkers[4], the sugar 1,4-lactones bearing the hydroxylic function at position $C_{(2)}$ in the *R*-configuration exhibit a negative first extremum of the Cotton effect while the *S*-configuration results in a positive one. For circular dichroism, this relation was confirmed by Beecham[5]. On the basis of our investigations on the Cotton effect in lactones of sugar acids and their 2-C-methyl analogues (these results will be published later[6]) we have found that the same rules are qualitatively valid for the methyl group as for the hydroxylic function with respect to the relation between the configuration at $C_{(2)}$ and the sign of the first extremum. Since the ORD curve of the deoxy lactone *IX* (prepared from the protected derivative *VII*, m.p. 137°C) exhibited a negative sign of the first extremum, the isomer *IX* is ascribed the *R*-configuration. Because of the positive first extremum, the *S*-configuration is proposed for the lactone *VIII* (prepared from compound *VI*, m.p. 122°C).

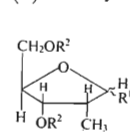
The subsequent synthetic steps were performed with the protected lactone *VII* because of its C-2(*R*) configuration. Thus, the disiamylborane[7] reduction of the latter lactone afforded 2-deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranose (*X*). The replacement of the glycosidic hydroxylic function by the chloro atom was performed in two steps. Compound *X* was converted to the *p*-nitrobenzoyl derivative *XI* which on treatment with hydrogen chloride afforded the halogenose *XII*. Without isolation, the latter halogenose was used in the preparation of nucleosides.

The reaction of 5-methyl-2,4-bis(trimethylsilyloxy)pyrimidine and the halogenose *XII* afforded a mixture of anomeric protected nucleosides from which crystalline 1-(2-deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranosyl)thymine(*XIII*) was isolated. Alcoholysis of the latter led to the free nucleoside, namely, 1,2-deoxy-2-C-methyl-*erythro*-D-pentofuranosyl)thymine (2′-methylthymidine, *XIV*). The structure of the nucleoside *XIV* was confirmed by ultraviolet, infrared, and mass spectra.

The reaction of N-benzoyladenine chloromercuri salt and the halogenose *XIII* afforded a semicrystalline mixture of anomeric 9-(2-deoxy-2(*R*)-C-methyl-3,5-di-



IV



X, R^1 = OH, R^2 = 4-$CH_3C_6H_4CO$
XI, R^1 = 4-$NO_2C_6H_4COO$, R^2 = 4-$CH_3C_5H_4CO$
XII, R^1 = Cl, R^2 = 4-$CH_3C_6H_4CO$
XIII, R^1 = 1-thyminyl, R^2 = 4-$CH_3C_6H_4CO$
XIV, R^1 = 1-thyminyl, R^2 = H
XV, R^1 = 9-adenyl, R^2 = 4-$CH_3C_6H_4CO$
XVI, R^1 = 9-adenyl, R^2 = H

O-*p*-toluyl-*erythro*-D-pentofuranosyl)-N-benzoyladenines (*XV*). Both the crystalline and the sirupous portion was deblocked on alcoholysis. The crystalline portion afforded crystalline 9-(2-deoxy-2(*R*)-C-methyl-*erythro*-D-pentofuranosyl)adenine(*XVI*), while the sirupous portion failed to give any crystalline product (as shown by mass spectrum, the sirup is probably the anomer of compound *XVI*). The calf intestine deaminase degradation of the above nucleoside in a phosphate buffer solution has a different course. As shown by ultraviolet spectrum (position of maximum) and electrophoresis, the crystalline derivative *XIII* does not suffer any deamination. On the other hand with the sirupous nucleoside a rapid shift of the maximum to shorter wavelengths may be observed; furthermore, the electrophoreogram exhibits a spot of the same mobility as deoxyinosine. The deamination is not quantitative: even after 48 hours the reaction mixture contains a compound of the same mobility as the crystalline nucleoside *XVI* and deoxyadenosine. To our opinion, the crystalline derivative *XVI* represents the α-anomer while the sirupous portion is a mixture of the α-anomer (unchanged by the action of deaminase) and the β-anomer which is deaminated by a similar rate as the naturally occurring adenine nucleosides.

The thymidine analogue *XIV* and the sirupous adenine nucleoside *XVI* inhibit completely the growth of *Escherichia coli* B in synthetic medium (glucose, inorganic salts) at concentrations of 1 mg and 0·01 mg per 1 ml, respectively.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Thin-layer chromatography was performed on silica gel (gypsum as binder) in the solvent systems S_1, chloroform, and S_2, 1-butanol saturated with water. Spots were detected in iodine vapours. Compounds containing aromatic and heterocyclic systems were detected by viewing under ultraviolet light. The paper chromatography of nucleosides was performed on paper Whatman No 1 in the solvent system S_3, 1-butanol–ethanol–water (40 : 11 : 19). The electrophoresis was carried out on the same paper in a citrate buffer solution (pH 4·2) at 20 Volt per cm. The ORD curves were taken on a JASCO ORD(UV) 5 apparatus. Mass spectra were recorded on a MCH 1303 apparatus.

2-C-Methyl-3,5-di-O-*p*-toluyl-D-ribonolactone (*II*)

p-Toluyl chloride (1·6 g; 10·3 mmol) was added at 0°C to a solution of 2-C-methyl-D-ribonolactone[1] (0·80 g; 5 mmol) in pyridine (6 ml) and the whole allowed to stand at 20°C for 24 hours. Methanol (1 ml) and toluene (10 ml) were then added to the mixture and the volatile portion evaporated at 40°C/15 Torr. This procedure was repeated three times. The final residue was dissolved in chloroform (30 ml), the solution washed with 10% aqueous hydrochloric acid, water, aqueous sodium hydrogen carbonate, and water, dried over anhydrous magnesium sulfate, and evaporated. Yield, 1·6 g (80%) of the di-*p*-toluyl derivative *II*, m.p. 123°C (diisopropyl ether). Optical rotation: $[\alpha]_D^{20}$ +99·3° (*c* 0·15, chloroform). Infrared spectrum: 1794 cm^{-1} (lactone). For $C_{22}H_{22}O_7$ (398·4) calculated: 66·32% C, 5·57% H; found: 66·42% C, 5·52% H.

2-Bromo-2-deoxy-2-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentonolactone (*III*).

A solution of the protected lactone *II* (1·0 g; 2·5 mmol) in glacial acetic acid (2 ml) was treated at 20°C with a 32% solution (2 ml) of hydrogen bromide in glacial acetic acid. After 24 hours, the mixture was poured onto ice (10 g) and extracted with chloroform. The extract was washed

three times with iced water and aqueous sodium hydrogen carbonate until neutral. Evaporation of chloroform afforded 0·9 g (77%) of the bromo lactone *III*, m.p. 93—95°C (ethanol). Optical rotation: $[\alpha]_D^{20}$ −13·5° (*c* 0·49, chloroform). Infrared spectrum: 1791 cm^{-1} (lactone). For $C_{22}H_{21}BrO_6$ (461·3) calculated: 57·27% C, 4·59% H, 17·32% Br; found: 57·76% C, 4·82% H, 17·43% Br.

Hydrogenation. A vigorously stirred solution of the bromo lactone *III* (2·3 g; 5 mmol) in dioxane (40 ml) was hydrogenated over the 5% $PdO/BaSO_4$ catalyst (2 g) at 20°C/1 atm. The hydrogen uptake ceased after 24 hours. The catalyst was filtered off, the filtrate diluted with three volume parts of chloroform, washed with iced water and aqueous sodium hydrogen carbonate, dried, and evaporated under diminished pressure. The residue was redissolved in dioxane and hydrogenated over fresh catalyst (the ratio of reactants was the same as above). This procedure was repeated three times in total to the disappearance of the starting bromo lactone *III* (tested by thin-layer chromatography in the solvent S_1). Overall hydrogen uptake, 120%. The crude product (1·5 g) was chromatographed on a column of silica gel (300 g) with the use of benzene (4500 ml) as eluant to afford 1·0 g (53%) of isomeric 2-deoxy-2-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentonolactones *VI* and *VII*. Elution with chloroform (1500 ml) afforded 0·1 g of the unsaturated lactone *IV*, m.p. 86°C (diisopropyl ether), $[\alpha]_D^{20}$ −65·9° (*c* 0·50, chloroform). For $C_{14}H_{14}O_4$ (246·3) calculated: 68·28% C, 5·73% H; found: 68·47% C, 5·64% H. Elution with additional 1000 ml of chloroform afforded 0·1 g of the saturated lactone *V*, m.p. 98°C (methanol), $[\alpha]_D^{20}$ +54·1° (*c* 0·51, chloroform); infrared spectrum: 1765 cm^{-1} (lactone). For $C_{14}H_{16}O_4$ (248·4) calculated: 67·73% C, 6·50% H; found: 67·79% C, 6·69% H. The above mixture of deoxylactones *VI* and *VII* (1 g) was recrystallised three times from diisopropyl ether to afford 0·2 g (10·5%) of the isomer *VII*, m.p. 137°C. Optical rotation: $[\alpha]_D^{20}$ +44·4° (*c* 0·068, chloroform). Infrared spectrum: 1788 cm^{-1} (lactone). For $C_{22}H_{22}O_6$ (382·4) calculated: 69·10% C, 5·80% H; found: 69·37% C, 6·02% H. The mother liquors remaining after the crystallisation of the lactone *VII* were evaporated and the residue (0·75 g) rechromatographed on deactivated silica gel (150 g) with the use of benzene as eluant (15 ml fractions). Fractions 32—65 contained the lactones as shown by detection under ultraviolet light. Fractions 32—36 were evaporated, the residues combined and recrystallised from diisopropyl ether to afford 0·1 g of the isomer *VI*, m.p. 122°C, $[\alpha]_D^{20}$ −14·5° (*c* 0·50, chloroform). For $C_{22}H_{22}O_6$ (382·4) calculated: 69·10% C, 5·80% H; found: 69·07% C, 5·80% H. Optical rotation of the subsequent fractions gradually increased up to the value of $[\alpha]_D^{20}$ equal to +36·0°. On active silica gel and with chloroform as eluant, the lactones are eluted in the opposite order.

2,3-Dideoxy-2-C-methyl-5-O-*p*-toluyl-*glycero*-D-pent-2-enono-1,4-lactone (*IV*)

A solution of the bromo lactone *III* (0·92 g; 2 mmol) in ethanol (10 ml) was treated with zinc powder (activated with hydrochloric acid) and the mixture refluxed under stirring until the starting lactone *III* was absent (shown by thin-layer chromatography in S_1). The inorganic material was filtered off, the filtrate evaporated under diminished pressure, and the residue extracted with chloroform. The extract was washed with 10% aqueous hydrochloric acid, water, and aqueous sodium hydrogen carbonate, dried, and evaporated. The product (0·35 g; 80%) is identical with the unsaturated lactone *IV* obtained by the above hydrogenation, as shown by the mixed melting point and optical rotation value.

2,3-Dideoxy-2-C-methyl-5-O-*p*-toluyl-*glycero*-D-pentono-1,4-lactone (*V*)

The unsaturated lactone *IV* (0·25 g; 1 mmol) was hydrogenated in methanol (5 ml) over 5% palladium on active charcoal catalyst (0·1 g) at 20°C/1 atm. Usual work up afforded an almost

theoretical yield of the saturated lactone *V*, identical on mixed melting point determination and optical rotation value with the specimen obtained by the above hydrogenation of the bromo lactone *III*.

2-Deoxy-2(*R*)-C-methyl-*erythro*-D-pentonolactone (*VIII*)

A solution of the protected lactone *VII* (m.p. 137°C; 0·75 g; 2 mmol) in methanol (5 ml) was treated dropwise with saturated methanolic barium methoxide until the mixture was definitively alkaline. The next day, the mixture was neutralised with dry ice and evaporated under diminished pressure. The residue was shaken with Dowex 50 (H^+) ion exchange resin (2 ml) and 10 ml of water for 2 hours, the resin filtered off, and washed with water. The filtrate and washings were combined, evaporated under diminshied pressure, and the residue heated at 70°C/0·05 Torr for 6 hours. The crude lactone was dissolved in water and the solution passed through a column of weakly basic Zerolite G (OH^-) ion exchange resin (10 ml). The effluant was evaporated under diminished pressure to dryness and the residue dried at 40°C/0·05 Torr for 6 hours. Yield, 0·2 g (66%) of the lactone *VIII*, chromatographically homogeneous in the solvent system S_2; $[\Phi]_{227}$ −860° (water). For $C_6H_{10}O_4$ (146·1) calculated: 49·31% C, 6·90% H; found: 49·79% C, 7·25% H.

2-Deoxy-2(*S*)-C-methyl-*erythro*-D-pentonolactone (*IX*)

The procedure was analogous to the preparation of compound *VIII*. Compound *VI* (0·2 g; 0·5 mmol) gave 41 mg (56%) of the lactone *IX*, $[\Phi]_{227}$ +540° (water). For $C_6H_{10}O_4$ (146·1) calculated: 49·31% C, 6·90% H; found: 48·85% C, 7·23% H.

2-Deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranose (*X*)

A 1·25M tetrahydrofuran solution (60 ml) of disiamylborane was added dropwise under stirring in the atmosphere of nitrogen to a solution of the protected lactone *VII* (7 g; 18 mmol) in tetrahydrofuran (50 ml). After 48 hours, the reaction mixture was treated cautiously drop by drop with water (15 ml) and refluxed for 30 minutes. The mixture was cooled down to −5°C and treated dropwise under stirring with 30% aqueous hydrogen peroxide (20 ml) at max. 5°C. The pH was maintained at the value of 7—8 by a simultaneous addition of 1M-NaOH. The volatile portions were evaporated under diminished pressure, the residue dissolved in chloroform, the solution washed with water, dried over anhydrous magnesium sulfate, and evaporated. Crystallisation of the residue from diisopropyl ether afforded 5·2 g (75%) of the furanose *X*, m.p. 117—119°C. Optical rotation: $[\alpha]_D^{20}$ −16·5° (*c* 0·37, chloroform). For $C_{22}H_{24}O_6$ (384·4) calculated: 68·73% C, 6·29% H; found: 68·57% C, 6·50% H.

2-Deoxy-2(*R*)-C-methyl-1-*p*-nitrobenzoyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranose (*XI*)

A solution of *p*-nitrobenzoyl chloride (2·6 g; 16 mmol) in pyridine (10 ml) was added to a solution of the furanose *X* (3 g; 8 mmol) in pyridine (5 ml), the whole mixture allowed to stand at 20°C for 3 days, decomposed with ice, and extracted with chloroform. The extract was evaporated under diminished pressure to remove the main portion of pyridine. The residue was redissolved in chloroform, the solution washed with 10% aqueous hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried, and evaporated to afford 2·6 g (65%) of the *p*-nitrobenzoyl derivative *XI*, m.p. 148°C. Optical rotation: $[\alpha]_D^{20}$ +75·3° (*c* 0·62, chloroform). For $C_{29}H_{27}NO_9$ (533·5) calculated: 65·28% C, 5·10% H, 2·62% N; found: 65·29% C, 5·21% H, 2·66% N.

1-Chloro-2-deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranose (*XII*)

The *p*-nitrobenzoyl derivative *XI* (2·6 g; 5 mmol) was dissolved in methylene chloride (5 ml), the solution treated with 0·277M-HCl in methylene chloride (19 ml), and the whole kept in a well stoppered flask in a refrigerator overnight. Under exclusion of atmospheric moisture, the crystalline *p*-nitrobenzoic acid was filtered off, and the filtrate evaporated under diminished pressure without any heating. The residue was dissolved in acetonitrile (25 ml) to afford a 0·2M solution of the halogenose *XII* which was used in the subsequent experiments.

1-(2-Deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranosyl)thymine (*XIII*)

A solution of the halogenose *XII* (2 mmol) in 10 ml of acetonitrile was added to a solution of 2,4-trimethylsilyloxy-5-methylpyrimidine (0·6 g; 2·2 mmol) in 10 ml of acetonitrile and the whole allowed to stand at room temperature for 24 hours. After this period of time, the *p*-nitrobenzylpyridine test for the alkylating agent was negative. The acetonitrile was then evaporated and the residue extracted with chloroform. The extract was filtered, the filtrate washed with aqueous sodium hydrogen carbonate, dried, and evaporated. The residue (0·8 g) was chromatographed on a column of silica gel (200 g), previously deactivated by the addition of water (20%). The column was washed with benzene (2000 ml) and then eluted with chloroform (1000 ml). Evaporation of the chloroform eluate afforded 0·4 g (42%) of the protected nucleoside *XIII*, m.p. 203—205°C (ethanol). Optical rotation: $[\alpha]_D^{20}$ +18·8° (*c* 0·82, chloroform). For $C_{27}H_{28}N_2O_7$ (492·5) calculated: 65·84% C, 5·73% H, 5·69% N; found: 65·41% C, 5·69% H, 5·76% N.

1-(2-Deoxy-2(*R*)-C-methyl-*erythro*-pentofuranosyl)thymine (*XIV*)

Saturated methanolic barium methylate (0·05 ml) was added to a solution of the protected nucleoside *XIII* (5 mg; 0·01 mmol) in methanol (1 ml), the mixture allowed to stand at 20°C overnight, neutralised with dry ice, and evaporated. The residue was extracted with water, the aqueous extract concentrated under diminished pressure, and the concentrate chromatographed on a preparative scale on paper Whatman No 3 MM. The broad band corresponding to the free nucleoside was eluted with water, the eluate concentrated, and rechromatographed on a layer of loose silica gel in the solvent system S_2. The product was eluted with methanol and the eluate evaporated to dryness to afford 2 mg of the nucleoside *XIV* as an amorphous residue. Optical rotation: $[\alpha]_D^{20}$ +25·7° (*c* 0·12, water). Mass spectrum[8]: 256 (M), 167 (M-89), 131 (sugar component), 126 and 127 (thymine + H, thymine + 2 H). Ultraviolet spectrum: λ_{max} 268 nm and λ_{min} 234 nm (pH 7); λ_{max} 267 nm and λ_{min} 246 nm (pH 12). Infrared spectrum: 1715 cm^{-1}, 1692 cm^{-1} (carbonyls), 3394 cm^{-1} ($N_{(3)}H$).

9-(2-Deoxy-2(*R*)-C-methyl-3,5-di-O-*p*-toluyl-*erythro*-D-pentofuranosyl)-N-benzoyladenine (*XV*)

A solution of the halogenose *XII* (2 mmol) in acetonitrile (10 ml) was stirred under exclusion of atmospheric moisture with N-benzoyladenine chloromercuri salt (1·1 g; 2·3 mmol) at 20°C for 24 hours (after this period of time the halogenose test was negative). The acetonitrile was then evaporated, the residue extracted with chloroform, the extract washed with saturated aqueous potassium iodide and water, dried, and evaporated. The residue (0·9 g) was chromatographed on silica gel (100 g) deactivated previously by the addition of water (20%). The column was washed with benzene (1000 ml) and then eluted with chloroform (1000 ml). Evaporation of the eluate afforded 0·45 g (37%) of a sirupous mixture of the anomeric nucleosides *XV*. The mixture was kept overnight to deposit crystals of compound *XV*, m.p. 119°C (diisopropyl ether). Optical rotation: $[\alpha]_{20}^{D}$ −38·5° (*c* 0·43, chloroform). For $C_{34}H_{31}N_5O_6$ (605·6) calculated: 67·42% C, 5·16% H, 11·56% N; found: 57·56% C, 5·28% H, 11·44% N.

9-(2-Deoxy-2(*R*)-C-methyl-*erythro*-D-pentofuranosyl)adenine (*XVI*)

Saturated methanolic barium methoxide (0·05 ml) was added to a solution of the crystalline protected nucleoside *XV* (0·12 g; 2 mmol) in methanol (5 ml), the mixture kept at 20°C overnight, neutralised with dry ice, and evaporated. The residue was repeatedly triturated with water and filtered off. The aqueous extracts were evaporated under diminished pressure to dryness and chromatographed on a loose layer of silica gel in the solvent system S_2 (detection under ultraviolet light). The band of the nucleoside *XVI* was eluted with water, the eluate evaporated, the residue dissolved in methanol, the solution filtered with active charcoal, and the clear filtrate concentrated to deposit 35 mg (66%) of the nucleoside *XVI*, m.p. 202°C. Optical rotation: $[\alpha]_{20}^{D}$ +44·1° (*c* 0·034, water). Mass spectrum[8]: 265 (M), 179 (M-89), 136, and 135 (adenine +2 H, adenine + H), 131 (sugar component). Ultraviolet spectrum: λ_{max} 260 nm and λ_{min} 228 nm (pH 7); λ_{max} 260 nm and λ_{min} 228 nm (pH 11).

The sirupous portions of the protected nucleoside *XV* and the corresponding mother liquors were combined and deblocked analogously to the crystalline portion to afford a sirup, $[\alpha]_{20}^{D}$ +5·9° (0·092, water), the mass and ultraviolet spectrum of which was identical with that of the crystalline nucleoside *XVI*.

Deamination of the Nucleoside *XVI* with Calf Intestine Deaminase

The crystalline nucleoside *XVI* was incubated at 37°C with calf intestine deaminase in a phosphate buffer solution (pH 7·4). Samples withdrawn after 2–48 hours did not show any shift of the ultraviolet absorption maximum when compared with the starting material. Consequently, no deamination occurred, as shown also by electrophoresis in a citrate buffer solution at pH 4·2.

The sirupous portion of the nucleoside was incubated similarly to the crystals. The ultraviolet absorption maximum shifted to the value of λ_{max} 256 nm after 2 hours and remained at the value λ_{max} 255 nm (pH 7) or 257 nm (pH 12) after 4 h. No change occurred then even after additional 44 h. The electrophoresis of the deaminated specimen in a citrate buffer solution (pH 4·2) revealed two spots. One of them migrated as fastly as the crystalline nucleoside *XVI*, the other spot remained at the start line similarly to the authentic specimen of deoxyinosine.

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