NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLV.*
MECHANISM OF ANOMALOUS OPENING OF THE $O^{2,2'}$-ANHYDRO BOND IN URACIL CYCLONUCLEOSIDES

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3′,5′-Di-O-benzoyl-$O^{2,2'}$-anhydro-L-uridine (*IV*) reacts with boron trifluoride etherate in methanol to give a mixture of 2′,5′- and 3′,5′-dibenzoates (*V*) of L-uridine. Alkaline deblocking of this mixture affords the free L-uridine (*III*). 3′,5′-Di-O-benzoyl-$O^{2,2'}$-anhydro-1-(α-D-xylofuranosyl)-uracil (*VI*) is converted to a mixture of 2′,5′- and 3′,5′-dibenzoates (*VII*) of 1-(α-D-lyxofuranosyl)-uracil and then, on removal of the protecting groups, to 1-(α-D-lyxofuranosyl)uracil (*VIII*). On the other hand, neither $O^{2,2'}$-anhydro-L-uridine (*I*) nor 3′,5′-di-O-benzoyl-$O^{2,2'}$-anhydro-α-uridine (*IX*) are affected by boron trifluoride etherate. These findings indicate participation of the trans-3′-benzoyl group which causes inversion of the anhydro bond opening.

The $O^{2,2'}$-cyclonucleosides derived from pyrimidine bases and various sugar components[1,4] became available especially owing to the simple and stereospecific reaction of aldoses with cyanamide[1]. It was therefore of interest to make use of these anhydro compounds in the synthesis of otherwise less accessible nucleoside derivatives.

The $O^{2,2'}$-cyclonucleosides are for example readily converted into 2′-deoxyribonucleosides as shown in the series of 2′-deoxy-L-ribonucleosides[2], 2′-deoxy-α-ribonucleosides[3], and 2′-deoxy-α-D-lyxofuranosides as well as 2′-deoxy-α-L-lyxofuranosides[4]. The hydrolytical cleavage of the anhydro bond in $O^{2,2'}$-anhydronucleosides is known to proceed stereospecifically in alkaline media. The hydroxylic function attacks the carbon atom at position 2 of the pyrimidine ring under the exclusive formation of such a nucleoside derivative the 2′-hydroxylic function of which is in *cis*-configuration with respect to the heterocyclic base (for a review see ref.[5]). On the other hand, the acidic hydrolysis may be also accompanied (due to protonation at position N^3) by a S_N2 reaction. Thus *e.g.*, $O^{2,2'}$-anhydro-L-uridine (*I*) is converted in aqueous ammonia or aqueous-ethanolic triethylamine to 1-(β-L-arabinofuranosyl)uracil[2] (*II*) as the single product while in 50% aqueous acetic acid, a small amount (5—10%) of L-uridine (*III*) is also formed.

The reaction of 3′,5′-di-O-benzoyl-$O^{2,2'}$-anhydro-L-uridine[2] (*IV*) with boron trifluoride etherate in methanol is quite anomalous. A mixture of 2′,5′- and 3′,5′-dibenzoates (*V*) of L-uridine is formed; deblocking of this mixture with sodium alkoxide affords L-uridine (*III*) as the single product. The anomalous reaction course

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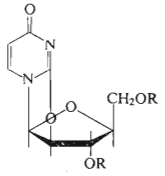
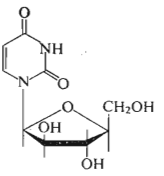
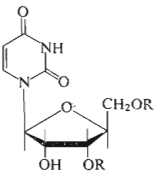
is obviously due to participation of the 3′-benzoyl group which forms a cyclic intermediate by an attack on the $C_{(2')}$ carbon atom of the anhydro bond which is activated by complex formation with a Lewis acid at position N^3. The simultaneous or subsequent hydrolysis in acidic media affords a mixture of 2′- and 3′-benzoyl derivatives; this mixture cannot be separated by chromatography. This idea is corroborated by the observation that the anhydro derivative *I* alone does not react with boron trifluoride etherate at all.



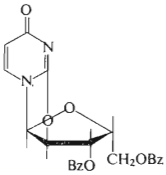
I, R = H
IV, R = C_6H_5CO



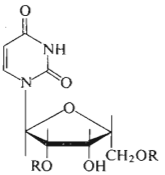
II



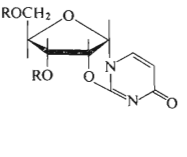
III, R = H
V, R = C_6H_5CO
(+2′,5′-isomer)



VI



VII, R = C_6H_5CO
VIII, R = H
(+3′,5′-isomer)



IX, R = C_6H_5CO

A similar treatment of 3′,5′-di-O-benzoyl-$O^{2,2'}$-anhydro-1-(α-D-xylofuranosyl)-uracil[4] (*VI*) affords a mixture of 2′,5′- and 3′,5′-dibenzoates (*VII*) in the ratio of about 1 : 1. Alkaline hydrolysis of this mixture leads to a single product, namely, 1-(α-D-lyxofuranosyl)uracil (*VIII*), identical with an authentic specimen[4]. On the other hand, 3′,5′-di-O-benzoyl-$O^{2,2'}$-anhydro-α-uridine[3] (*IX*) is not affected on treatment with boron trifluoride etherate even for a longer period of time. Both these findings are in accordance with the assumed participation of the benzoyl group in hydrolysis of the anhydro bond. In compound *VI*, the neighbouring 3′-benzoyl group is in configuration *trans* with respect to the anhydro bond at position 2′ and

can consequently attack the $C_{(2')}$ carbon atom under the formation of the corresponding cyclic intermediate. On the other hand, the 3'-benzoyl group of the cyclonucleoside *IX* is *cis* with respect to the anhydro bond.

The present cleavage of $O^{2,2'}$-anhydronucleoside 3',5'-dibenzoates is of preparative value for the pyrimidine L-ribonucleosides which have been accessible only by multistep syntheses starting from L-arabinose or L-ribose[6,7]. The novel procedure is exemplified by the preparation of L-uridine (*III*) as well as by conversion of the intermediary dibenzoate *V* to 2',3',5'-tri-O-benzoyl-L-uridine (*X*), thiation of the latter tribenzoate with phosphorus pentasulfide, and the subsequent ammonolysis to afford a high yield of L-cytidine (*XI*).

EXPERIMENTAL

Unless stated otherwise, the solutions were taken down on a rotatory evaporator at 40°C/15 Torr. The substances were dried over phosphorus pentoxide at 0·1 Torr. Paper chromatography was performed by the descending technique on paper Whatman No 3 MM in the solvent systems S_1, 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2), and S_2, 1-butanol–ethanol–water (40 : 11 : 19). Paper electrophoresis was carried out by the technique of Markham and Smith[8] on the same paper in E_1, 0·1M triethylammonium borate (pH 7·5) at 20 V/cm for 90 min (the mobilities refer to uridine). Thin-layer chromatography on silica gel was performed on ready-for-use Silufol UV_{254} plates (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system S_3, ethanol–chloroform (5 : 95). R_F values and electrophoretical mobilities, uridine: S_1, 0·50; S_2, 0·32; E_1, 1·00; *I*: S_1, 0·60; S_2, 0·27; E_1, —0·10; *II*: S_1, 0·60; S_2 0·40; *III*: S_1, 0·50; S_2, 0·32, E_1 1·00; *IV*: S_3, 0·12; *V*: S_3, 0·43; *VI*: S_3, 0·14; *VII*: S_3, 0·20; *VIII*: S_1, 0·46; S_2, 0·27; E_1, 1·12; *IX*: S_3, 0·12.

L-Uridine (*III*)

A suspension of compound[2] *IV* (8·7 g; 20 mmol) in methanol (400 ml) was treated with 8 ml of boron trifluoride etherate (Lachema, Brno) and the whole refluxed for 2 h. The clear solution was evaporated under diminished pressure, the residue dissolved in chloroform (500 ml), the solution washed with two 100 ml portions of aqueous sodium hydrogen carbonate and water, dried over magnesium sulfate, filtered, the material on the filter washed with chloroform, the filtrates combined, and evaporated to dryness under diminished pressure. The residual compound *V* (8·5 g) was chromatographically (S_1) homogeneous and identical with an authentic specimen[2]. The residue was heated in 0·1M methanolic sodium methoxide (100 ml) for 2 h at 50°C, the solution kept at room temperature overnight, evaporated to dryness under diminished pressure, the residue dissolved in water (200 ml), the solution neutralised by the addition of moist Dowex 50 (H^+) ion exchange resin, filtered, and the resin washed with water. The filtrates were combined, washed with two 50 ml portions of ether, the aqueous phase evaporated to dryness under diminished pressure, and the residue crystallised from ethanol (100 ml). Yield, 4·0 g (82%) of compound *III*, m.p. 162—163°C. For $C_9H_{12}N_2O_6$ (244·2) calculated: 44·26% C, 4·95% H, 11·47% N; found: 44·60% C, 4·95% H, 11·58% N.

2',3',5'-Tri-O-benzoyl-L-uridine (*X*)

Compound *IV* (20 mmol) was converted to the dibenzoate *V* (*cf.* the preceding paragraph), the residue dried *in vacuo*, dissolved in acetonitrile (50 ml), the solution treated first with benzoyl cyanide (3·3 g; 25 mmol) and then dropwise with 4 ml of triethylamine (exothermic reaction). As shown by chromatography in the solvent system S_3, the reaction was quantitative after 15 min. The reaction mixture was evaporated under diminished pressure, the residue dissolved in ethanol (50 ml), and the solution poured into water (500 ml). The precipitate was collected with suction,

washed with water, dried, and recrystallised from benzene. Yield, 6·4 g (58%) of compound *X*, m.p. 138—139°C. For $C_{30}H_{24}N_2O_9$ (556·5) calculated: 64·74% C, 4·34% H, 5·03% N; found: 65·16% C, 4·39% H, 5·01% N.

L-Cytidine (*XI*)

Phosphorus pentasulfide (2·5 g) was added to a solution of compound *X* (5·6 g; 10 mmol) in dioxane (200 ml), the whole refluxed for 30 min, treated with additional 2·5 g of phosphorus pentasulfide, refluxed for 30 min more, filtered while hot, and the material on the filter washed with dioxane (50 ml). The filtrate and washings were combined, evaporated to dryness under diminished pressure, and the residue dissolved in chloroform (500 ml). The solution was washed with three 100 ml portions of saturated aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate, and evaporated to dryness under diminished pressure. The residue was heated in 30% methanolic ammonia for 7 h at 110°C, the reaction mixture evaporated, the residue diluted with water (200 ml), and washed with two 50 ml portions of ether. The aqueous phase was concentrated under diminished pressure to the volume of about 50 ml, the concentrate adjusted with concentrated hydrochloric acid to pH 2·5—3·0, and applied to a column of Dowex 50 (H^+) ion exchange resin (200 ml). The column was washed with water (3000 ml) to the loss of the ultraviolet absorption and then eluted with 5% aqueous ammonia. The ultraviolet-absorbing portion of the eluate was evaporated under diminished pressure, the residue coevaporated with ethanol, and finally precipitated from methanol (20 ml) with ether (500 ml). The product was collected with suction, washed with ether, and dried. Yield, 2·0 g (83·5%) of compound *XI*, m.p. 162°C. For $C_9H_{13}N_3O_5$ (243·3) calculated: 44·43% C, 5·38% H, 17·27% N; found: 44·68% C, 5·54% H, 17·68% N.

1-(α-D-Lyxofuranosyl)uracil (*VIII*)

Boron trifluoride etherate (1 ml) was added to a solution of compound[4] *VI* (2·0 g; 4·6 mmol) in methanol (50 ml) and the whole refluxed under exclusion of atmospheric moisture for 2 h. The reaction mixture was processed analogously to the preparation of compound *III* to afford a mixture (1·8 g) of the isomeric dibenzoates *VII* (ratio, about 1 : 1), identical with authentic specimens[4] as shown by thin-layer chromatography in the solvent system S_3. The isomeric mixture was heated in 0·1M methanolic sodium methoxide (100 ml) for 6 h at 50°C and then processed analogously to compound *III*. Yield (after crystallisation from ethanol), 0·90 g (81%) of the chromatographically (S_1, S_2) and electrophoretically (E_1) pure compound *VIII*, m.p. 202—203°C. For $C_9H_{12}N_2O_6$ (244·2) calculated: 44·26% C, 4·95% H, 11·47% N; found: 44·12% C, 4·98% H, 11·68% N.

Reactions of Compounds *I* and *IX*

A. Reaction of compound *IX* with boron trifluoride etherate (time, 4 h) was performed analogously to that of compound *VI*. The unchanged starting compound *IX* was isolated in 85% yield, characterised by thin-layer chromatography in the solvent system S_3, and subjected to alkaline hydrolysis under standard conditions (*vide supra*) to afford α-uridine as the single product (electrophoresis in E_1).

B. A mixture of compound[2] *I* (1·0 g; 4·4 mmol), methanol (50 ml), and boron trifluoride etherate (2 ml) was refluxed for 2 h under exclusion of atmospheric moisture and then evaporated under diminished pressure. The residue was dissolved in 100 ml of 0·4M triethylammonium hydrogen carbonate, pH 7·5. Analysis of the resulting solution (pH 7·5) in S_1, S_2 and E_1 indicated the presence of the starting *I* as the single ultraviolet-absorbing material.

C. A mixture of compound[2] *I* (0·5 g; 2·2 mmol) and 50% aqueous acetic acid (25 ml) was kept at room temperature for 3 days. As shown by analysis in systems S_2 and E_1, the starting compound *I* was absent and the mixture contained 8% of compound *III* and 92% of compound *II* (spectrophotometry of spot eluates and electrophoresis in the buffer solution E_1).

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