PREPARATION OF 9-(β-D-RIBOFURANOSYL)-2-HYDROXYPURINE*

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Reaction of 5-aminocytosine (*VI*) with ethyl orthoformate afforded 2-hydroxypurine (*I*) which on acid-catalysed fusion with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, followed by methanolysis, gave the 1-ribofuranosyl derivative of *I* (*II*). Reaction of the mercuric salt of *I* with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride in toluene in the presence of mercuric bromide, or reaction of the monosodium salt of *I* with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide in acetonitrile, afforded 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-hydroxypurine (*VII*) which was methanolysed to 9-(β-D-ribofuranosyl)-2-hydroxypurine (*III*). The compound *III* was prepared also by deamination of 9-(β-D-ribofuranosyl)-2-aminopurine (*X*) with nitrous acid.

Although 2-hydroxypurine (*I*) is known for a long time[1-4], practically no attention has been paid as yet to nucleosides derived from this heterocyclic base. A synthesis of 1-(β-D-ribofuranosyl)-2-hydroxypurine (*II*) by building up the purine ring from 5-aminocytidine[5] and also a similar specific synthesis of 9-methyl-2-hydroxypurine have been described. Since 9-(β-D-ribofuranosyl)-2-hydroxypurine (*III*) is an inosine isomer, there can be little doubt that it will be biologically interesting.

Three synthetic approaches can be devised for the preparation of *III*: ribosylation of 2-hydroxypurine (*I*), deamination of 9-(β-D-ribofuranosyl)-2-aminopurine, and finally, building up the purine ring from the N^4-ribosyl derivative of 5-aminocytosine. Because the last-mentioned compound is not easily accessible and may undergo anomerisation, our present study concerns only the first two methods.

2-Hydroxypurine (*I*) was prepared by electrochemical reduction of guanine or xanthine[1,2], deamination of 2-aminopurine with nitrous acid[4] or by cyclisation of 5-aminocytosine with formic acid[3]. We have chosen the last method which has been slightly modified. The starting compound was cytosine (*IV*), obtained in high yield and purity by ammonolysis of the easily accessible[6] 4-methoxy-2-pyrimidone. Cytosine was nitrated with fuming nitric acid to give 5-nitrocytosine (*V*) which was catalytically hydrogenated to 5-aminocytosine (*VI*) according to the already described procedure[5]. Condensation of the compound *VI* with ethyl orthoformate gave smoothly 2-hydroxypurine (*I*).

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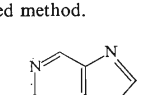
Ribosylation of the compound *I* is *a priori* complicated by the presence of two NH groups and a carbamide grouping which can undergo substitution; thus, theoretically, we can expect formation of the 2-O-, N(1)-, N(3)- and N(9)-ribosyl derivatives. Therefore, we studied three modifications of the ribosylation reaction: acid-catalysed fusion of the base *I* with 1,2,3,5-tetra-O-acetyl-D-ribofuranose, and reaction of a sugar halogenose with mercuric or sodium salt of the base *I* in a nonpolar and polar solvent.

Fusion of the compound *I* with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose and catalytic amount of sulfuric acid *in vacuo*, followed by methanolysis of the mixture and separation on cellulose, afforded, in addition to the starting compound *I*, a single product whose electrophoretic mobility in borate buffer indicated a ribonucleoside structure. However, its UV absorption spectrum (λ_{max} = 320 nm at pH 2, 7 and 12) showed unequivocally that this product was the already described 1-ribosyl derivative *II*. As shown by comparison with the authentic material (*vide supra*), the reaction mixture does not contain the 9-isomer *III* and thus under the given conditions the ribosylation leads specifically to the 1-isomer *II*. In this connection we can mention that hypoxanthine does not react under these conditions at all[7].

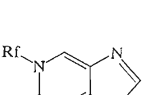
For the second synthetic alternative we needed mercuric salt of the base *I*, which was prepared from the disodium salt of *I* and equimolar amount of mercuric acetate. Reaction of this salt with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[8] in toluene in the presence of mercuric bromide[9], followed by chromatography of the reaction mixture, afforded as principal product a tribenzoyl derivative. Its ^{1}H-NMR spectrum exhibited, in addition to the expected signals of the sugar component, a signal at 8·54 ppm. Methanolysis of this compound *VII* afforded a product which on electrophoresis in borate buffer behaved as a ribonucleoside. Its UV spectrum (λ_{max} = 308 nm) was characteristic for N(9)-substituted derivatives of the base *I* (it corresponded to the published[5] data for 9-methyl-2-hydroxypurine). The compound was chromatographically and electrophoretically homogeneous and differed from the N(1)-isomer *II*, being thus in all probability the N(9)-isomer *III*.

The third alternative of the ribosylation reaction consists in the reaction of monosodium salt of the compound *I* with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide[10] in acetonitrile. This reaction can lead directly to an N- or O-ribosyl derivative which in the presence of traces of acid can undergo migration to give the N(1)- or N(3)-ribosyl derivative (*cf.* ref.[11]). In order to ensure a maximum possible conversion to the desired N(9)-isomer *III*, after the reaction had ended, the reaction mixture was heated with mercuric bromide in toluene. This reagent is known to bring about the thermodynamically controlled N(3)→N(9) migration[11] in purine nucleosides, particularly in the presence of an acid or excess halogenose[11]. In our case the addition of mercuric bromide had no effect on the composition of the reaction mixture, containing the compound *VII* as the only relevant reaction product. It follows that either the sodium salt of the compound *I* is directly ribosylated to the N(9)-isomer, or the N(3)-isomer (contingently formed from the 2-O-ribosyl derivative) rearranges

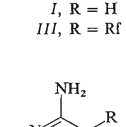
spontaneously to the compound *VII*. The N(1)-isomer of *VII* was not observed in the reaction mixture. As described for similar cases[12], the reaction was accompanied by considerable amount of elimination of the sugar halogenose, leading to 2-benzoyloxy-4-benzoyloxymethylfuran (*VIII*). Structure of the ribosylation product was rigorously proved again by methanolysis and isolation of the free nucleoside which was identical in all respects with the compound *III*, prepared by the previously mentioned method.



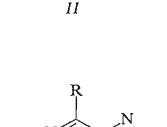
I, R = H
III, R = Rf



II

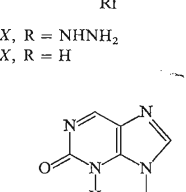


IV, R = H
V, R = NO_2
VI, R = NH_2



IX, R = $NHNH_2$
X, R = H

VIII



VII, R = Bz
III, R = H

In formulae *I* – *VIII*, Bz = benzoyl group,
Rf = β-D-ribofuranosyl residue.

The second approach, used for the preparation of *III* in this study, consists in deamination of 9-(β-D-ribofuranosyl)-2-aminopurine (*X*). This compound was obtained

either by the described procedure[13] (hydrogenation of the 2-amino-6-chloropurine derivative) or by a new synthesis, consisting in cleavage of the 2-amino-6-hydrazinopurine derivative *IX* with silver oxide. This reaction which was used by us in the pyrimidine nucleosides series[14] and later by others also for cleavage of 8-hydrazinoadenine derivatives[15], is equally facile with 6-hydrazinopurine derivatives[16] and it thus appears to be of general applicability. The nucleoside *X* reacted smoothly with nitrous acid in aqueous solution to give a compound identical with the product *III* obtained by ribosylation (*vide supra*) of 2-hydroxypurine (*I*). Since the structure of the compound *X* (and also configuration of the nucleoside bond) is firmly established (migration of the sugar moiety or anomerisation being improbable under the given conditions), the deamination product of *X* proves unequivocally the structure of the derivative *III*.

9-(β-D-Ribofuranosyl)-2-hydroxypurine exhibits, besides its typical UV absorption maximum, a characteristic blue fluorescence and has a weakly acidic character, indicated by its electrophoretic mobility in a weakly alkaline medium. This fact is in agreement with the presence of the carbamide grouping in the pyrimidine part, as well as with the value of pK_a 9·28, observed for 9-methyl-2-hydroxypurine[5].

According to preliminary results of the antibacterial activity test on synthetic medium with glucose, the compound *III* does not measurably inhibit the growth of *E. coli* in concentrations up to 1 mg/ml of medium.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Paper chromatography was performed on paper Whatman No 1 in the systems S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), S2, 1-butanol–acetic acid–water (5 : 2 : 3), thin layer chromatography on Silufol UV_{235} plates (Kavalier, Czechoslovakia) in the systems S3, chloroform, and S4, chloroform–ethanol (1 : 40). Preparative chromatography on silica gel was carried out on loose layers (30 × 16 × 0·3 cm) of silica gel, containing a fluorescent indicator (30—60 mesh, Service Laboratories of this Institute), or on a column of silica gel (150 g) according to Pitra (30—60 mesh). Paper electrophoresis was performed on a paper Whatman No 3 MM in the systems E1, 0·1M triethylammonium hydrogen carbonate, pH 7·5; E2, 0·05M triethylammonium borate, pH 7·5; E3, 1M acetic acid, at 20 V/cm for 1 h. Spots were detected in the UV light (Chromatolight). Chromatography on cellulose (Macherey–Nagel, microcrystalline cellulose) was carried out on an 80 × 3 cm column in 70% aqueous 2-propanol, elution rate 20 ml/h, the course being followed by continuous monitoring the UV absorbance on a Uvicord instrument (Uppsala, Sweden).

^{1}H-NMR spectra were taken in deuteriochloroform on a Varian 100 instrument (chemical shifts in ppm, coupling constants in Hz). UV spectra were measured in aqueous solutions on a Specord spectrometer (Zeiss, Jena, GDR).

Cytosine (*IV*)

A mixture of 4-methoxy-2-pyrimidinone[6] (58·5 g; 0·464 mol) and 30% methanolic ammonia (300 ml) was heated with stirring in an autoclave for 8 h at 100°C. After cooling, the product was filtered, washed with ethanol, ether and dried *in vacuo*, yielding 43·3 g (84%) of cytosine which was chromatographically (S1, S2) as well as electrophoretically (E3) homogeneous.

5-Nitrocytosine (*V*)

Cytosine (30 g; 0·27 mol) was added portionwise with stirring and cooling (0°C) to anhydrous nitric acid (250 ml) in the course of 1 h. After standing overnight at room temperature in a stoppered flask, the mixture was poured on ice (2 kg) under stirring and made alkaline with conc. aqueous sodium hydroxide (pH 8). The product was filtered, washed with water till the filtrate was neutral, dissolved in hot water (500 ml; pH adjusted to 10 with sodium hydroxide) and the solution filtered while hot. The filtrate was neutralised with acetic acid and allowed to stand in a refrigerator overnight. After filtration, the crystalline product was washed with ice-cold water, ethanol, ether, and dried *in vacuo*; yield 22·5 g (53·5%) of *V*, identical with an authentic material[17] (R_F 0·44 in S1). UV spectrum (pH 2): λ_{max} 254 nm (ε_{max} = 7600), λ_{max} 312 nm (ε_{max} = 8500), λ_{min} = 253, 272 nm.

5-Aminocytosine (*VI*) (*cf.*[5])

Compound *V* (22·5 g; 0·145 mol) in water (1 l) was hydrogenated over 10% Pd/C (2 g; Merck, West Germany) at normal pressure and room temperature till the hydrogen consumption ceased (105% theory), the mixture was filtered through Celite which was then washed with hot water (500 ml) and the filtrate was taken down *in vacuo*. Crystallisation of the residue from water afforded 15·1 g (83%) of chromatographically pure *VI* (R_F 0·30 in S1), decomposing above 200°C. For $C_4H_6N_4O$ (126·1) calculated: 38·09% C, 4·80% H, 44·43% N; found: 38·23% C, 5·05% H, 46·03% N. UV spectrum (pH 2): λ_{max} 305, λ_{min} 261 nm. E_{Cyt} = 0·78 in E3.

2-Hydroxypurine (*I*)

A mixture of the compound *VI* (7·6 g; 60 mmol), dimethylformamide (50 ml), ethyl orthoformate (50 ml) and 6M hydrogen chloride in dimethylformamide (20 ml; 120 mmol) was stirred in a stoppered flask for 2 days at room temperature, made alkaline with triethylamine and taken down at 40°C/13 Pa. Crystallisation of the residue from water afforded 5·9 g (64%) of the product. For the monohydrate $C_5H_6N_4O_2$ (154·1) calculated: 38·95% C, 3·92% H, 36·35% N; found: 38·81% C, 3·78% H, 36·12% N. Mass spectrum: M^+ 136. R_F 0·38 (S1), 0·39 (S2). E_{Up} 0·35 (E1), E_{Urd} 0·72 (E2). UV spectrum (pH 2): λ_{max} 314 nm (ε_{max} 5050); λ_{max} 308 nm (ε_{max} 6000).

Reaction of Compound *I* with 1,2,3,5-Tetra-O-acetyl-β-D-ribofuranose

Compound *I* (1·6 g; 5 mmol) and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose[18] (0·7 g, 5 mmol) were homogenised at 160°C, conc. sulfuric acid (60 µl) was added and the mixture was heated under stirring at 1·5 kPa for 15 min. After cooling, the melt was extracted with boiling ethanol (50 ml), filtered while hot, the solid on the filter washed with hot ethanol (20 ml), the filtrate taken down *in vacuo* and the residue allowed to stand overnight with 30% methanolic ammonia (50 ml) at room temperature. The mixture was taken down *in vacuo* and the residue chromatographed on a column (80 × 4 cm) of DEAE cellulose (Cellex D, HCO_3^- cycle). Elution with water (3 ml/min) afforded a UV-absorbing fraction which was evaporated *in vacuo* and the residue was chroma-

tographed on a cellulose column in 70% 2-propanol (*vide supra*). The fraction, containing the UV-absorbing product, was taken down and the residue precipitated from methanol (2 ml) with ether (100 ml). The product was filtered and dried *in vacuo*; yield 0·12 g (9%) of *II*; R_F 0·50 (S1); E_{Up} 0·19 (E1), E_{Urd} 1·23 (E2). UV spectrum (pH 2, 7, 12): λ_{max} 320 nm, λ_{min} 280 nm (ref.[5] reports for 1-methyl-2-hydroxypurine λ_{max} 320 nm).

9-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-2-hydroxypurine (*VII*)

a) A solution of mercuric acetate (6·4 g; 20 mmol) in hot methanol (100 ml) was added to a stirred hot solution of the compound *I* (3·8 g; 20 mmol) in methanol (200 ml) and 1M sodium methoxide in methanol (40 ml). The mixture was stirred till it became cold, centrifuged, washed with methanol and ether (100 ml each), dried on air and then at 13 Pa over phosphorus pentoxide for 3 days. This product was heated with mercuric bromide (6·2 g) in toluene (200 ml) under stirring. A part (100 ml) of the solvent was distilled off and to the boiling residue a solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[8] (20 mmol) in toluene (50 ml) was added dropwise with stirring. The stirred mixture was refluxed (calcium chloride protective tube) for 22 h, filtered while hot through Celite which was washed with toluene (50 ml) and the filtrate was taken down *in vacuo*. The residue was dissolved in chloroform (300 ml) and the solution washed successively (100 ml each) with 30% potassium iodide solution (twice), 10% sodium thiosulfate and water, and dried over sodium sulfate. After filtration, the solvent was evaporated *in vacuo* and the residue chromatographed on two loose layers of silica gel in the system S4. The product bands (R_F 0·27 in S4) were eluted with methanol (300 ml) and the eluate taken down *in vacuo*, yielding 4·15 g (35·7%) of compound *VII* as an amorphous foam. For $C_{31}H_{24}N_4O_8$ (580·5) calculated: 64·13% C, 4·17% H, 9·65% N; found: 64·24% C, 4·43% H, 9·80% N. ^{1}H-NMR spectrum: 4·55—5·0 (m, 3 H); 5·87 (m, 1 H); 6·04 (m, 1 H); 6·49 (m, 1 H); 8·54 (s, 1 H); 7·80 to 8·20 (m, 6 H) + 7·20—7·65 (m, 10 H) arom. protons + 1 H.

b) A solution of the monohydrate of *I* (3·0 g; 20 mmol) in methanol (50 ml) was mixed with 1M methanolic sodium methoxide (20 ml; 20 mmol), the mixture was taken down, the residue coevaporated with toluene (3 × 50 ml) and the remaining sodium salt dried *in vacuo*. A 30% solution of hydrogen bromide in acetic acid (40 ml) was added to a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (10·6 g; 20 mmol) in 1,2-dichloroethane (50 ml). After standing for 45 min at room temperature the mixture was taken down *in vacuo*. The residue was codistilled with toluene (4 × 50 ml), dissolved in acetonitrile (50 ml) and the solution added during 30 min to a stirred suspension of the above-mentioned sodium salt of *I* in acetonitrile (100 ml) under exclusion of moisture. The mixture was stirred overnight at room temperature and then refluxed for 8 h, filtered through Celite which was washed with acetonitrile (20 ml), and the filtrate was taken down *in vacuo*. The residue was codistilled with toluene (50 ml) *in vacuo* and refluxed with toluene (50 ml) and mercuric bromide (3 g) for 12 h. The mixture was worked up similarly as described under *a*), affording 2·9 g (25%) of *VII* as amorphous foam, identical with the product of the preceding preparation. The mixture further afforded 1·7 g (26·5%) of *VIII*, m.p. 73—75°C (cyclohexane). For $C_{19}H_{14}O_5$ (322·2) calculated: 70·80% C, 4·38% H; found: 71·27% C, 4·45% H, 0·0% N.

9-(β-D-Ribofuranosyl)-2-aminopurine (*X*)

a) *From* 9-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2-*amino*-6-*chloropurine*: A solution of the triacetate (prepared according to ref.[13]; 7·8 g; 18·3 mmol), sodium acetate trihydrate (8 g) and 5% Pd/C (2·0 g; Merck) in ethanol (150 ml) was hydrogenated at room temperature and atmospheric pressure overnight. The mixture was filtered through Celite which was washed with

ethanol (50 ml), the filtrate was taken down, the residue taken up in chloroform (100 ml) and the solution washed with water (2× 25 ml), dried over sodium sulfate, filtered and taken down *in vacuo*. The residue was chromatographed on a silica gel column (*vide supra*) in chloroform and the product fraction taken down *in vacuo*. The residue was dissolved in 30% methanolic ammonia (25 ml) and the solution set aside overnight at room temperature. After evaporation of the solvent the product was precipitated with ether (200 ml) from methanol (10 ml); yield 2·0 g (41%) of the compound *X*. Mass spectrum: M^+ 267 (M 267·2). R_F 0·59 (S1) (fluor.), E_{Urd} 0·70 (E2). UV spectrum (pH 2): λ_{max} 292 nm; (pH 7): λ_{max} 285 nm; (pH 12): λ_{max} 292 nm.

b) *From the compound* IX: A suspension of the compound *IX* (2·0 g; 6·7 mmol; prepared according to ref.[19]) and silver oxide (2·8 g; 12·1 mmol) in water (100 ml) was refluxed for 6 h with stirring, filtered while hot through Celite and the filtrate taken down *in vacuo*. The residue was dissolved in hot water (25 ml) and filtered through charcoal. The filtrate was again taken down in *vacuo*, the residue codistilled with ethanol (3× 25 ml), dissolved in methanol (5 ml) and precipitated with ether (100 ml), affording 1·35 g (75%) of the compound *X*, identical with the product prepared according to the procedure *a*).

9-(β-D-Ribofuranosyl)-2-hydroxypurine (*III*)

a) A solution of the compound *VII* (3·2 g; 5·5 mmol) in 0·2M methanolic sodium methoxide (80 ml) was set aside at room temperature overnight, neutralised with dry Dowex 50 X 8 (H^+ form), filtered, the ion exchange resin was washed with methanol (50 ml) and the filtrate was taken down *in vacuo*. The residue in water (50 ml) was extracted with ether (2 × 25 ml), the aqueous layer was taken down *in vacuo* and the residue chromatographed on a column of cellulose in 70% 2-propanol. Fractions, containing the product *III*, were combined, taken down, the residue dissolved in methanol (5 ml) and the product precipitated with ether (100 ml); yield 1·15 g (78%) of *III*; R_F 0·49 (S1), 0·26 (S2), E_{Up} 0·17 (E1), E_{Urd} 1·10 (E2). For $C_{10}H_{12}N_4O_5$ (268·2) calculated: 44·77% C, 4·51% H, 20·99% N; found: 44·23% C, 4·95% H, 20·70% N. UV spectrum (pH 2): λ_{max} 312 nm (ε_{max} 5000); (pH 7): λ_{max} 310 nm (ε_{max} 6700), λ_{min} 262 nm); (pH 12): λ_{max} 310 nm (ε_{max} 8400), λ_{min} 262 nm.

b) Acetic acid (1·2 ml) was added at 0°C to a solution of the compound *X* (1·2 g; 4·5 mmol) and sodium nitrite (1·2 g) in water (12 ml) and the mixture was set aside overnight at 0°C. It was then chromatographed on 10 sheets of paper Whatman No 3 MM in the system S1, the bands of the product *III* eluted with water (50 ml). The eluate was taken down and the residue was rechromatographed on a column of cellulose in 70% 2-propanol as described under *a*), affording 0·40 g (33·2%) of the compound *III*, identical with the product of the preparation *a*).

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