STEREOSPECIFIC SYNTHESIS OF β-D-RIBOPYRANOSYL NUCLEOSIDE ANALOGUES*

A.M.KRITZYN** and A.HOLÝ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received March 11th, 1975

Reaction of the sodium salt of uracil and adenine with 2-O-*p*-toluenesulfonyl-D-arabinose (*I*) afforded 1-(β-D-ribopyranosyl)uracil (*IIa*) and 9-(β-D-ribopyranosyl)adenine (*IIb*), resp. Compound *IIa* was also prepared from the sodium salt of 4-methoxy-2-pyrimidinone and the derivative *I via* demethylation of the intermediate *IIc*. Reaction of compound *I* with acetone afforded 3,4-O-isopropylidene-2-O-*p*-toluenesulfonyl-β-D-arabinofuranoside (*V*) which does not react with the sodium salts of uracil or adenine. Benzoylation of benzyl 2-O-*p*-toluenesulfonyl-β-D-arabinopyranoside (*VI*) yielded benzyl 3,4-di-O-benzoyl-2-O-*p*-toluenesulfonyl-β-D-arabinopyranoside (*VII*) which was converted by hydrogenolysis into 3,4-di-O-benzoyl-2-O-*p*-toluenesulfonyl--β-D-arabinopyranose (*VIII*). Reaction of compound *VIII* with the sodium salt of adenine and the subsequent debenzoylation afforded the pyranoside *IIb*. 1-(2,3,4-Tri-O-benzoyl-β-D-ribopyranosyl)uracil (*IX*) was prepared by reaction of the sodium salt of 4-methoxy-2-pyrimidinone with compound *VIII* followed by benzoylation and demethylation. Compound *IX* was also obtained by condensation of 2,3,4-tri-O-benzoyl-D-ribopyranosyl bromide (*XI*) with the chloromercuri salt of 4-methoxy-2-pyrimidinone and demethylation of the intermediate. Methanolysis of compound *IX* afforded 1-(β-D-ribopyranosyl)uracil (*IIa*).

In connection with investigations on analogues of nucleosides and nucleotides, attention has also been paid to the problem of a suitable stereospecific synthesis of pyrimidine and purine β-D-ribopyranosides *II*. Such nucleoside analogues occur in some nucleosidic antibiotics[1-3]. The different spatial arrangement of the sugar ring, the corresponding conformational changes of the nucleoside molecule, and especially the change of the chemical nature of the saccharidic hydroxylic functions which are equivalent in compounds of the type *II*, renders these compounds interesting from the standpoint of physicochemical properties as well as model substances in investigations on enzymatic requirements in metabolism of nucleic acids. Compounds of type *II* have been hitherto prepared by interaction of the protected halopyranose with the heterocyclic base according to the Hilbert–Johnson reaction[4] or the mercuri

* Part CLXXVIII in the series Nucleic Acid Components and Their Analogues; Part CLXXVII: This Journal *40*, 2347 (1975).

** On leave from the Institute of Molecular Biology, Academy of Science of the U.S.S.R., Moscow.

process[5] and by reaction of 1,2,3,4-tetra-O-acetyl-β-D-ribopyranose with the acylated base[6]. Both types of reactions may afford a pure β-anomer or a mixture of anomers which must be separated.

On another principle is based the following reaction which has been mentioned for the first time in the patent literature[7] and then modified in this Laboratory for other purposes[8–10]. Thus, the reaction of 2-O-(*p*-toluenesulfonyl)-D-arabinose (*I*) with the sodium salt of a pyrimidine or purine base affords a 1,2-epoxide of the *ribo* configuration as the intermediate which is stereospecifically opened by attack of the heterocyclic base anion at position 1 to afford the β-anomer of the pyranose form of the ribonucleoside *II*. The reaction is always accompanied by the formation of some ribofuranoside *III*. The potential application of this reaction has been examined in the present paper in detail.

I *II* *III*

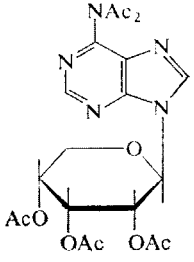
Ts = *p*-toluenesulfonyl

In formulae *II*, *III a*: B = uracil-1-yl; *b*: B = adenin-9-yl; *c*: B = 4-methoxy-2-pyrimidon-1-yl

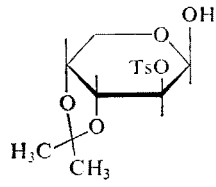
SCHEME 1

Concerning the influence of the solvent character on the pyranoside/furanoside ratio in the reaction of compound *I* with the sodium salt of adenine, both the forms *II* and *III* have been observed as reaction products in all cases. The overall yield of the reaction strongly depends on the character of the solvent (Table I) and is considerably decreased by side reactions of the intermediary epoxide in protic solvents. Thus, dimethylformamide or dimethyl sulfoxide at room temperature proved as the most advantageous solvents as shown by the synthesis of 1-(β-D-ribopyranosyl)uracil (*IIa*) and 9-(β-D-ribopyranosyl)adenine (*IIb*), see Scheme 1. Since the reaction of uracil with compound *I* may result in substitution at position 1 or 3 of the uracil nucleus, an alternative reaction was used in the present case, namely, the reaction of 4-methoxy-2-pyrimidinone with compound *I*. By this reaction, compound *IIc* was obtained as the single possible product which was then demethylated at position 4 of the heterocyclic ring by the action of anhydrous hydrogen chloride to afford compound *IIa*. This détour proved as unnecessary since (similar to the preparation[8] of ribofuranosides from the 5-O-trityl derivative of compound *I*) the N^1-isomer *IIa* is exclusively obtained from the sodium salt of uracil. Also the reaction with the sodium salt of adenine is isomer-free, compound *Id* being the exclusive product.

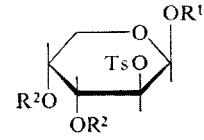
In addition to the reaction product *II*, the reaction mixture always contains the heterocyclic base and the corresponding β-D-ribofuranoside *III*. The by-products were separated by the chromatographical technique consisting in a previous chromatography on DEAE-ceilulose in the borate cycle; in the weakly alkaline medium, the ribofuranoside *III* is bound by complex formation with boric acid whereas neither compound *II* nor the heterocyclic base are retarded. The latter components may be then separated by gel-filtration on Sephadex G-10 to obtain the pure ribopyranoside *II*. Preparations on a larger scale were performed by a route based on acetylation of the reaction mixture with acetic anhydride and isolation of the pure pentaacetate *IV* by chromatography on silica gel.



IV



V



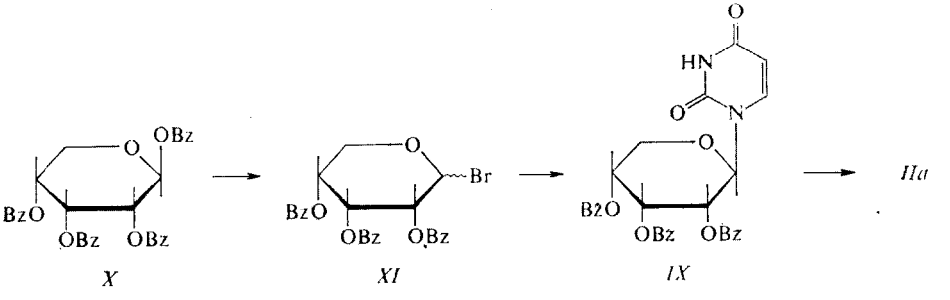
VI, $R^1 = C_6H_5CH_2$, $R^2 = H$
VII, $R^1 = C_6H_5CH_2$, $R^2 = C_6H_5CO$
VIII, $R^1 = H$, $R^2 = C_6H_5CO$

Ac = acetyl Ts = *p*-toluenesulfonyl

With the aim to limit or exclude the formation of the ribofuranoside as the by-product, the use of suitably substituted derivatives of compound *I* (excluding the formation of a five-membered ring) has been examined. One of these derivatives, namely, 3,4-O-isopropylidene-2-O-(*p*-toluenesulfonyl)-D-arabinopyranose (*V*) was prepared by acid-catalysed reaction of compound *I* with acetone. The structure of compound *V* was established by elemental analysis and ^{1}H-NMR spectrum which also suggested the β-configuration of the hemiacetal hydroxylic group in chloroform solutions. However, the attempted reaction of compound *V* with the sodium salt of adenine or 4-methoxy-2-pyrimidinone failed to give any nucleoside derivatives.

Another route was therefore used, consisting in the application of 3,4-di-O-benzoyl--2-O-(*p*-toluenesulfonyl)-D-arabinose (*VIII*). Compound *VIII* was prepared from the readily accessible[8] benzyl 2-O-(*p*-toluenesulfonyl)-β-D-arabinopyranoside (*VI*) *via* the benzoylation to the 3,4-di-O-benzoyl derivative *VII* which was characterised by elemental analysis and ^{1}H-NMR spectrum which also confirmed the unchanged pyranose ring structure and the anomeric purity. Catalytic hydrogenolysis of the dibenzoate *VII* afforded compound *VIII*. The β-configuration of the hemiacetal

hydroxylic group in compound *VIII* was established by ^{1}H-NMR spectrum (in chloroform). In contrast to the 3,4-O-isopropylidene derivative *V*, the dibenzoate *VIII* undergoes the reaction with the sodium salt of adenine. After removal of benzoyl groups, the reaction mixture contains exclusively the starting adenine and the ribopyranoside *IIb* while the isomeric ribofuranoside *IIIb* is absent as indicated by electrophoresis in a borate buffer solution. The overall yield of the product *IIb* obtained by this route from the dibenzoate *VIII* is similar to that one in the reaction with the free 2-O(*p*-toluenesulfonyl)-D-arabinose (*I*). Moreover, the reaction mixture is free of the ribofuranoside *III*.



In formulae *IX*—*XI* Bz = benzoyl.

SCHEME 2

In the analogous reaction of compound *VIII* with the sodium salt of 4-methoxy--2-pyrimidinone, the lower nucleophilicity of the anion of the base results in a decreased yield of the required product. After the condensation, benzoylation was performed by the action of benzoyl cyanide[11] and the resulting compound *IIc* was demethylated with hydrogen chloride in chloroform with the formation of the 2,3,4-tri-O-benzoate *IX*. Compound *IX* was also prepared from 1,2,3,4-tetra-O-benzoyl-β-D-ribopyranose (*X*) *via* the protected ribopyranosyl bromide *XI*. Reaction of the bromide *XI* with the chloromercuri salt of 4-methoxy-2-pyrimidinone in acetonitrile afforded the corresponding 4-methoxy derivative which was not isolated but directly converted to compound *IX* by the action of hydrogen chloride. The final methanolysis of compound *IX* yielded pure 1-(β-D-ribopyranosyl)uracil (*IIa*), see Scheme 2. When compared with this method, the arabinose derivative *VIII* affords compound *IX* in a lower yield.

In conclusion, the preparation of β-D-ribopyranosides *II* from 2-O-(*p*-toluenesulfonyl)-D-arabinose (*I*) is advantageous in that respect when a small amount of the anomerically pure product is required or when the product is sensitive towards reagents removing the protecting groups. The method is not suitable for large-scale preparations in the pyrimidine series where the classical procedures are more advantageous. On the other hand in the series of purine nucleosides, there is raison d'être

for this variant since it does not require acylation of the base and affords an isomer-free product. The starting substances are readily accessible from arabinose and less expensive than the ribose derivatives. On a large scale, the alternative using the 3,4--dibenzoyl derivative *VIII* may be recommended because of the simple purification of the final product.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and were not corrected. Unless stated otherwise, solutions were taken down on a rotatory evaporator at 40°C/15 Torr and the substances were dried over phosphorus pentoxide at 0·1 Torr.

Methods

Paper chromatography was performed on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-butanol–acetic acid–water (5 : 2 : 3). Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in solvents S_3, chloroform, and S_4, ethyl acetate. Paper electrophoresis was performed by the previously reported[12] technique on paper Whatman No 3 MM at 20 V/cm (90 min) in the buffer solution E_1, 0·1M triethylammonium borate (pH 7·5). The UV spectra were taken in aqueous solutions on a Zeiss Specord apparatus. The ^{1}H-NMR spectra were measured in hexadeuteriodimethyl sulfoxide or deuteriochloroform (hexamethyldisiloxane as internal standard) on a Varian 100 apparatus (chemical shifts in p.p.m., the coupling constants in Hz).

3,4-O-Isopropylidene-2-O-(*p*-toluenesulfonyl)-β-D-arabinopyranoside (*V*)

A suspension of 2-O-(*p*-toluenesulfonyl)-β-D-arabinose[8] (*I*; 1 g; 2·5 mmol) in acetone (50 ml) containing one drop of conc. sulfuric acid was stirred at room temperature for 12 h, treated with excess solid sodium hydrogen carbonate, and the stirring continued for 1 h. The mixture was then filtered with suction, the filtrate evaporated, and the residue dissolved in ethyl acetate (20 ml). The solution was applied to a column of silica gel (50 g) and the elution was performed with a mixture of ethyl acetate and benzene (1 : 3). Yield, 0·835 g (97%) of compound *V*, m.p. 118–119°C. For $C_{15}H_{20}O_7S$ (344·4) calculated: 52·28% C, 5·87% H, 9·32% S; found: 52·85% C, 5·95% H, 9·19% S. ^{1}H-NMR spectrum ($CDCl_3$): 3·82 (s, 3 H) and 1·23 (s, 3 H) $(CH_3)_2C$; 2·45 (s, 3 H) CH_3 (tosyl); 3·82 (d, 3 H, $J_{gem} = 14·0$) H_6; 4·05–4·30 (m, 4 H) $H_2 + H_3 + H_4 + H_6$; 5·25 (br d, 1 H, $J = 3$) H_1; 7·36 (d, 2 H) + 7·82 (d, 2 H) arom. protons.

Benzyl 3,4-Di-O-benzoyl-2-O-(*p*-toluenesulfonyl)-β-D-arabinopyranoside (*VII*)

To a solution of benzyl 2-O-(*p*-toluenesulfonyl)-β-D-arabinopyranoside[8] (*VI*; 10 g; 25 mmol) in pyridine (50 ml) there was added dropwise under cooling with ice benzoyl chloride (10·5 g; 9 ml; 75 mmol), the mixture kept at room temperature for 2 days, poured onto ice, and stirred for 2 h. The solid was collected with suction, washed with water, and dried under diminished pressure. Yield, 14·0 g (93%) of compound *VII*, m.p. 143–144°C (ethanol). For $C_{33}H_{30}O_9S$ (602·6) calculated: 65·77% C, 5·01% H, 5·31% S; found: 65·72% C, 5·15% H, 5·27% S. ^{1}H-NMR spectrum ($CDCl_3$): 2·28 (s, 3 H) CH_3; 4·20 (m, 2 H, $J_{5,4} = J_{5',4} = 2·0$, $J_{gem} = 13·5$) 2 H_5;

4·73 (m, 2 H, J_{gem} = 14·5) $CH_2C_6H_5$; 5·13 (dd, 1 H, $J_{2,1}$ = 3·4, $J_{2,3}$ = 9·8) H_2; 5·31 (d, 1 H, $J_{1,2}$ = 3·4) H_1; 5·68 (m, 1 H, $J_{4,5}$ = 1·0, $J_{4,5'}$ = 2·0, $J_{4,3}$ = 3·5) H_4; 5·78 (dd, 1 H, $J_{3,2}$ = 9·8, $J_{3,4}$ = 3·5) H_3; 6·95–8·10 (19 H) arom. protons.

3,5-Di-O-benzoyl-2-O-(*p*-toluenesulfonyl)-β-D-arabinopyranose (*VIII*)

Compound *VII* (12 g; 20 mmol) was hydrogenated in a mixture of dioxane (100 ml), glacial acetic acid (100 ml) and 40% ethanolic hydrogen chloride (4 ml) over 10% Pd/C catalyst (1 g) at room temperature and under ordinary pressure. When the hydrogen uptake was 580 ml, the catalyst was filtered off through Celite and the filtrate evaporated under diminished pressure. A mixture of the residue, water (150 ml), and conc. hydrochloric acid (1·5 ml) was then refluxed for 1 h and evaporated under diminished pressure. The residue was coevaporated with three 150 ml portions of toluene and finally chromatographed on a column of silica gel (150 g) in chloroform. The product-containing fractions were pooled, evaporated, and the residue triturated with light petroleum. Yield, 9·65 g (93%) of compound *VIII*, m.p. 60–61°C. For $C_{26}H_{24}O_9S$ (512·5) calculated: 60·92% C, 4·72% H, 6·26% S; found: 61·01% C, 5·02% H, 5·46% S.

1-(β-D-Ribopyranosyl)uracil (*IIa*)

A. To a suspension of 4-methoxy-2-pyrimidinone sodium salt[8] (7·5 mmol) in dimethylformamide (7·5 ml) there was added 2-O-(*p*-toluenesulfonyl)-D-arabinose[8] (1·95 g; 5 mmol) and the mixture stirred at room temperature for 2 days under exclusion of atmospheric moisture. The precipitate was filtered off through Celite and washed with dimethylformamide. The filtrate and washings were combined and evaporated at 40°C/0·1 Torr. The residue was dissolved in methanol (30 ml) and 55% methanolic hydrogen chloride (10 ml), the whole kept at room temperature overnight, and evaporated under diminished pressure. The residue was dissolved in water (150 ml), the uracil filtered off, and the filtrate applied to a column (150 ml) of Amberlite IR 4 B (acetate) ion exchange resin. The UV-absorbing products were eluted with water using the Uvicord apparatus. The eluate was evaporated, the residue triturated with methanol (25 ml), the mixture filtered, and the material on the filter washed with methanol. The methanolic filtrate and washings were combined and evaporated. The residue was dissolved in water (25 ml) and the solution adjusted to pH 9 with triethylamine; 2M triethylammonium borate (pH 7·5; 2·5 ml) was added and the whole applied to a column (80 × 4 cm) of DEAE-cellulose (Cellex D, high capacity) in borate cycle. The column was eluted with water to the drop of the UV absorption and then with 0·1M triethylammonium borate (pH 7·5). The fractions containing compound *IIa* and uracil (according to electrophoresis in a borate buffer solution) were pooled, evaporated, and the residue applied (in a minimum volume of water) to a column (100 × 4 cm) of Sephadex G-10. The column was eluted with water (1 ml per min), fractions containing the product *IIa* pooled, and evaporated. The residue was crystallised from ethanol to afford 0·224 g (18·5%) of compound *IIa*, m.p. 116°C; R_F values: 0·52 (S_1), 0·54 (S_2); E_1 0·20. Compound *IIIa*: R_F 0·50 and 0·41 (in S_1 and S_2, resp.); E_1 1·00.

B. To a solution of 1,2,3,4-tetra-O-benzoyl-β-D-ribopyranose[13] (*X*; 28·7 g; 52·5 mmol) in 1,2-dichloroethane (40 ml) there was added 30% hydrogen bromide in acetic acid (70 ml), the mixture kept at room temperature for 2 h under exclusion of atmospheric moisture, and evaporated. The residue was coevaporated with four 50 ml portions of toluene and the final residue of compound *XI* dissolved in acetonitrile (500 ml; distilled over phosphorus pentoxide). To this solution there was added 4-methoxy-2-pyrimidinone chloromercuri salt[14] and the whole mixture was refluxed with stirring for 10 h (calcium chloride drying tube). The mixture was then evaporated, the residue dissolved in chloroform (500 ml), the solution washed with 100 ml portions of 30% aqueous potassium iodide (two portions), water, 10% aqueous sodium thiosulfate, and water again, dried

over anhydrous magnesium sulfate, filtered, and the filtrate evaporated. The residue was applied (in chloroform) to a column of the Pitra silica gel (30—60 micron; 200 g) and the column eluted (30 ml fractions) with chloroform to afford a solid (R_F 0·10 in S_3) which was recrystallised from ethanol. Yield, 10·0 g (36%) of the tribenzoate *XI*, m.p. 218—220°C. For $C_{30}H_{24}N_2O_9$ (556·5) calculated: 64·74% C, 4·34% H, 5·03% N; found: 65·10% C, 4·18% H, 5·09% N.

A suspension of compound *IX* (5 g; 9 mmol) in 0·1M methanolic sodium methoxide (200 ml) was refluxed until the solid dissolved. The solution was stirred in a stoppered flask for 2 days at room temperature to deposit a solid which was dissolved by the addition of water. The reaction mixture was neutralised by the addition of dry Dowex 50 X 8 (H^+) ion exchange resin, filtered with suction, and the resin washed with 200 ml portions of methanol and water. The filtrate and washings were combined and evaporated under diminished pressure. The residue was diluted with water (200 ml) and extracted with three 50 ml portions of ether. The aqueous phase was evaporated under diminished pressure, the residue coevaporated with three 50 ml portions of ethanol and the final residue crystallised from ethanol. Yield of the chromatographically (S_1, S_2) and electrophoretically (E_1) homogeneous compound *IIa*, 1·77 g (80·5%), m.p. 116—118°C. For C_9H_{12} . . N_2O_6 (244·2) calculated: 44·26% C, 4·95% H, 11·47% N; found: 44·50% C, 5·04% H, 11·25% N.

C. From 15 mmol of 4-methoxy-2-pyrimidinone there was prepared the sodium salt[8], dried over phosphorus pentoxide at 0·1 Torr, mixed with a solution of compound *VIII* (12 mmol) in dimethylformamide (30 ml), the whole mixture stirred at room temperature for 24 h and then at 100°C for 8 h, and finally evaporated at 50°C/0·1 Torr. The residue was dissolved in chloroform (200 ml) and the solution washed with three 50 ml portions of water. The chloroform layer was dried over anhydrous magnesium sulfate, filtered, and the filtrate evaporated under diminished pressure. A mixture of the residue, benzoyl cyanide (3 g; 23 mmol), acetonitrile (40 ml), and triethylamine (1 ml) was stirred at room temperature for 1 h and evaporated under diminished pressure. The residue was dissolved in chloroform (100 ml), the solution washed with two 25 ml portions of water, dried over anhydrous magnesium sulfate, made up with additional chloroform to the volume of 200 ml, and saturated at 0°C with gaseous hydrogen chloride. The reaction mixture was kept at 0°C overnight and evaporated under diminished pressure. The residue was dissolved in chloroform (200 ml), the solution washed with 50 ml portions of saturated aqueous sodium hydrogen carbonate (twice) and water, dried over anhydrous magnesium sulfate, filtered, and the filtrate evaporated under diminished pressure. The residue was chromatographed on two layers (50 × 16 × 0·4 cm) of loose silica gel with fluorescent indicator in the solvent S_3. Bands of the product were eluted with methanol (200 ml) and ethyl acetate (200 ml), the eluate evaporated. and the residue crystallised from ethanol. Yield, 1·2 g (18%, referred to compound *VIII*) of the tribenzoate *IX*, identical with an authentic specimen on chromatography in the solvent S_3; m.p. 218°C, undepressed on admixture with the material prepared by procedure *B*. Deblocking of the tribenzoate *IX* with 0·1M methanolic sodium methoxide at 100°C for 2 h yielded exclusively compound *IIa* as shown by chromatography (S_1 and S_2) and electrophoresis (E_1).

9-(β-D-Ribopyranosyl)adenine (*IIb*)

A. To a solution of compound *I* (19·5 g; 50 mmol) in dimethylformamide (50 ml) there was added the sodium salt of adenine (11·8 g; 75 mmol), the mixture stirred at room temperature for 2 days under exclusion of atmospheric moisture, the precipitate filtered off through Celite, and washed with dimethylformamide. The filtrate and washings were combined and coevaporated with toluene at 40°C/0·1 Torr. The residue was stirred with pyridine (100 ml) and acetic anhydride (100 ml) at room temperature overnight, the mixture evaporated under diminished pressure, the residue diluted with methanol (75 ml) under cooling with ice, kept at room temperature for 2 h

and evaporated under diminished pressure. The residue was adsorbed (in methanol) on silica gel (75 g), the material coevaporated with ethyl acetate, and the residue applied (in ethyl acetate) to a column of silica gel (150 g) packed in ethyl acetate. The pentaacetate (R_F 0·28 and 0·45 in S_3 and S_4, resp.) was eluted with ethyl acetate, the eluate evaporated, and the residue crystallised from ethanol to afford 2·8 g (11·7%) of compound *IV*, m.p. 184–186°C. For $C_{20}H_{23}N_5O_9$ (477·4) calculated: 50·31% C, 4·86% H, 14·67% N; found: 50·12% C, 5·15% H, 14·65% N. ^{1}H-NMR spectrum ($CDCl_3$): 1·80 (s, 3 H) + 2·07 (s, 3 H) + 2·28 (s, 3 H)O—COCH$_3$; 2·35 (s, 6 H) 2 N$_6$—COCH$_3$; 4·09 (m, 2 H) 2 H$_5$; 5·28 (m, 1 H, $J_{3',4'} = 3·0$, $J_{4',5'} = 6·5$, $J_{4',5''} = 9·5$) H$_{4'}$; 5·69 (dd, 1 H, $J_{2',1'} = 10·0$, $J_{2',3'} = 3·0$) H$_{2'}$; 5·89 (t, 1 H, $J_{3',2'} = J_{3',4'} = 3·0$) H$_{3'}$; 6·13 (d, 1 H, $J_{1',2'} = 10·0$) H$_{1'}$; 8·28 (s, 1 H) H$_8$; 9·01 (s, 1 H) H$_2$.

A solution of compound *IV* (0·95 g; 2 mmol) in 30% methanolic ammonia was kept at room temperature overnight and evaporated under diminished pressure. Crystallisation of the residue from water yielded 0·45 g (80%) of compound *IIb*, homogeneous on chromatography and electrophoresis, m.p. 238–240°C. For $C_{10}H_{15}N_5O_5$ (285·3) (monohydrate) calculated: 42·09% C, 5·30% H, 24·55% N; found: 41·91% C, 5·39% H, 24·34% N. Optical rotation: $[\alpha]_D^{25} - 33·2°$ (*c* 0·5, water); reported, $[\alpha]_D^{23} - 38°$ (water). R_F values, compound *IIb*: 0·54 and 0·52 (in S_1 and S_2, resp.), E_1 0·18; compound *IIIb*: 0·57 and 0·60 (in S_1 and S_2, resp.), E_1 0·92.

B. A mixture of compound *VIII* (1·03 g; 2 mmol), the sodium salt of adenine (0·47 g; 3 mmol), and dimethylformamide (5 ml) was stirred at room temperature overnight under exclusion of atmospheric moisture, filtered off through Celite, and the material on the filter washed with dimethylformamide. The filtrate and washings were combined and evaporated at 40°C/0·1 Torr. The residue was extracted with chloroform (100 ml), the extract filtered, and the filtrate evaporated under diminished pressure. The residue was chromatographed on a layer (40 × 16 × 0·4 cm) of loose silica gel containing a fluorescent indicator (produced by Service Laboratories of this Institute) in the solvent system ethanol–ethyl acetate (5 : 95). The benzoate bands were combined and eluted with methanol (300 ml). The eluate was evaporated under diminished pressure and the residue kept at room temperature in 0·25M methanolic sodium methoxide (20 ml) overnight. The mixture was neutralised with dry Dowex 50 (H^+) ion exchange resin, filtered with suction, and the resin on the filter washed with methanol. The filtrate and washings were combined and evaporated under diminished pressure. The residue was dissolved in water (35 ml), the aqueous solution washed with four 15 ml portions of ether, and evaporated under diminished pressure. The overall content (adenine + compound *IIb*) was 8400 A_{260} (0·59 mmol) as determined spectrophotometrically. An aliquot (about 100 A_{260}) of the residue was subjected to electrophoresis in a borate buffer under standard conditions, the spots were eluted with water, and the ratio of adenine to compound *IIb* determined spectrophotometrically as 41·5 : 58·5. Yield, 0·34 mmol (17%) of compound *IIb* (referred to the total amount of the residue). The residual mixture did not contain any adenosine as shown by electrophoresis in a borate buffer solution.

Reaction of Compound *V* with Sodium Salts of Adenine and 4-Methoxy-2-pyrimidinone

A mixture of compound *V* (2 mmol), the sodium salt (3 mmol) of adenine or 4-methoxy-2-pyrimidinone, and dimethylformamide (5 ml) was stirred at room temperature for 2 days and evaporated at 40°C/0·1 Torr. The residue was refluxed in 80% aqueous acetic acid (25 ml) for 30 min, the mixture evaporated under diminished pressure, the residue coevaporated with water, and a sample of the final residue analysed by electrophoresis in a borate buffer solution and by chromatography in the solvent system S_1. The reaction mixture did not contain any product *II*, only the heterocyclic base was always present. No reaction was observed when the mixture was heated at 80°C for 5 h.

TABLE I

Effect of Solvents upon the Reaction of Adenine Sodium Salt with 2-O-(*p*-Toluenesulfonyl)-D--arabinose (*I*)

Solvent	Nucleoside yield, %	*II*/*III*
Methanol	20·0	3·0
Ethanol	23·0	4·6
Dioxane	19·0	1·7
Acetonitrile	28·0	2·5
Dimethylformamide	45·0	3·1
Dimethyl sulfoxide	43·0	6·2

Effect of Solvents upon the Reaction of Adenine Sodium Salt with Compound *I*

A mixture of compound *I* (165 μmol), adenine sodium salt (330 μmol), and the corresponding solvent (1 ml) was shaken at room temperature for 16 h under exclusion of atmospheric moisture, diluted with water (1 ml), and the aliquot (25 μl) of the mixture analysed by paper electrophoresis in the buffer solution E_1. The spots of adenine, compound *IIb*, and adenosine (*IIIb*) were eluted with 0·01M hydrochloric acid and the content determined spectrophotometrically at 260 nm (for the experimental data see Table I).

The authors wish to thank Professor Dr V. Herout, Director of this Institute, for making possible the scientific visit of A. M. K. Thanks are also due to Dr M. Masojídková for measurement and interpretation of ^{1}H-NMR *spectra, and to Mrs B. Kopecká for excellent technical assistance.*

REFERENCES

1. Ellis D. B., LePage G. A.: Can. J. Biochem. *43*, 617 (1965).
2. Watanabe K. A., Falco E. A., Fox J. J.: J. Org. Chem. *37*, 1198 (1972).
3. Watanabe K. A., Chin T. M. K., Hollenberg D. H., Fox J. J.: J. Org. Chem. *39*, 2482 (1974).
4. Onodera K., Fukumi H.: Agr. Biol. Chem. *27*, 526 (1963).
5. Naito T., Kawakami T.: Chem. Pharm. Bull. *10*, 627 (1962).
6. Furukawa Y., Honjo M.: Chem. Pharm. Bull. *16*, 1076 (1968).
7. US 2993 039 (July 18, 1961).
8. Holý A., Šorm F.: This Journal *34*, 3383 (1969).
9. Kopecký J. J., Holý A.: Radiochem. Radioanal. Lett. *7*, 361 (1971).
10. Pischel H., Holý A., Wagner G.: This Journal *37*, 3475 (1972).
11. Holý A., Souček M.: Tetrahedron Lett. *1971*, 185.
12. Markham R., Smith J. G.: Biochem. J. *52*, 552 (1952).
13. Fletcher H. G., Ness R. K.: J. Amer. Chem. Soc. *76*, 760 (1954).
14. Prystaš M.: This Journal, in press.

Translated by J. Plíml.