SYNTHESIS OF 1-β-D-ARABINOFURANOSYLCYTOSINE* **

J. BROKEŠ and J. BERÁNEK

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

Received February 7th, 1974

On treatment with acetic anhydride and acetyl chloride, 1-β-D-arabinofuranosyluracil (*IVa*) was converted to the triacetyl derivative *IVb*, the reaction of which with phosphorus pentasulfide in dioxane afforded 1-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)-4-thiouracil (*Va*). On alcoholysis, compound *Va* was converted to 1-β-D-arabinofuranosyl-4-thiouracil (*Vb*). The ammonolysis of *Va* afforded 1-β-D-arabinofuranosylcytosine (*VI*). An alternative preparation of compound *VI* was started from the acetyl derivative *IVb* which was treated with thionyl chloride under the catalysis of dimethylformamide, and then with ammonia. By reaction with phosphorus pentasulfide, the triacetyluridine *Ib* afforded the 4-thio derivative *IIa* which was alcoholysed to 4-thiouridine (*IIb*). On treatment with ammonia, compound *IIa* was converted to cytidine (*III*).

1-β-D-Arabinofuranosylcytosine (*VI*), known as Cytosar or Ara-C, probably represents in the present time the most frequently administered nucleoside analogue in the chemotherapy of cancer. In connection with the new preparation[1] of compoud *VI*, some other reactions which appeared of interest with respect to the synthesis of this antimetabolite in praxis, are object of the present paper.

The preparation of 1-β-D-arabinofuranosylcytosine (*VI*) from a naturally occurring nucleoside may be accomplished by two routes, either by modification of the aglycone portion of the molecule or by modification of the sugar moiety, *i.e.*, either by conversion of the uracil nucleoside to the cytosine one or by conversion of a cytosine ribofuranosyl derivative to an arabinofuranosyl derivative. The present paper relates to the former route, the latter one was the object of an earlier communication[1].

In the chemistry of nucleosides, there have been developed two general and convenient methods for the preparation of cytosine nucleosides starting from uracil derivatives. One[2,3] of these two methods consists in transformation of an oxo group of the protected nucleoside to a thio group by the action of phosphorus pentasulfide and the subsequent replacement of this thio group by an amino group on treatment

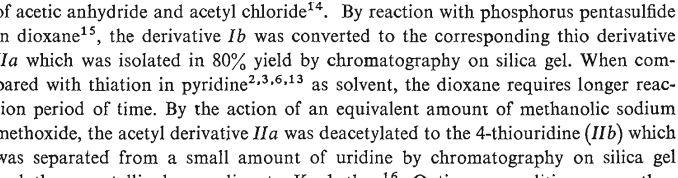
* Part CLXVII in the series Nucleic Acid Components and Their Analogues; Part CLXVI: This Journal *39*, 2115 (1974).

** Taken from the Diploma Thesis of J. Brokeš, Charles University, Prague 1973. Presented in part at the 8th International Congress of Chemotherapy, Athens, September 1973.

with ammonia. In the other method[4,5], the uracil derivative is converted to the cytosine nucleoside *via* the reactive 4-chloro derivative obtained by reaction with the Vilsmeier-Haack reagent (chloromethylenedimethylammonium chloride) and the 4-chloro atom is subsequently replaced by the amino group. In recent years, both these methods have been used in the synthesis of cytosine derivatives[6–12]. Concerning the chlorination method, the substitution at position 5 of the aglycone moiety has been observed to inhibit[10] completely the reaction or to lower markedly the reaction yield[11] (when compared with the thiation process), probably for steric reasons.

In the present work, there has been attempted to perform both the reactions by the most simplified procedures in view of the potential application for the preparation of 1-β-D-arabinofuranosylcytosine in praxis and for the sake of comparison with other known syntheses, especially with the process developed in this Laboratory[1]. The thiation procedure reported in the present paper is analogous to that of the patent literature[13].

Some reaction steps have been first examined on uridine derivatives. The triacetyluridine *Ib* was prepared from uridine in 95% yield by acetylation with a mixture of acetic anhydride and acetyl chloride[14]. By reaction with phosphorus pentasulfide in dioxane[15], the derivative *Ib* was converted to the corresponding thio derivative *IIa* which was isolated in 80% yield by chromatography on silica gel. When compared with thiation in pyridine[2,3,6,13] as solvent, the dioxane requires longer reaction period of time. By the action of an equivalent amount of methanolic sodium methoxide, the acetyl derivative *IIa* was deacetylated to the 4-thiouridine (*IIb*) which was separated from a small amount of uridine by chromatography on silica gel and then crystallised according to Kochetkov[16]. Optimum conditions were then



IIa, R^1 = Ac, R^2 = OAc, R^3 = H
IIb, R^1 = H, R^2 = OH, R^3 = H
Va, R^1 = Ac, R^2 = H, R^3 = OAc
Vb, R^1 = R^2 = H, R^3 = OH

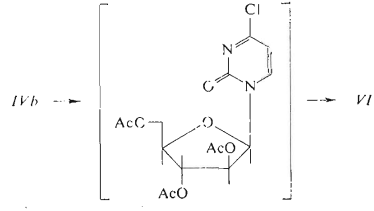
Ia, R^1 = H, R^2 = OH, R^3 = H
Ib, R^1 = Ac, R^2 = OAc, R^3 = H
IVa, R^1 = R^2 = H, R^3 = OH
IVb, R^1 = Ac, R^2 = H, R^3 = OAc

III, R^2 = OH, R^3 = H
VI, R^2 = H, R^3 = OH

examined for the conversion of the 4-thio derivatives *IIa* and *IIb* to the 4-amino compound *III*. Thus, while the reaction of 4-thiouridine (*IIb*) with liquid ammonia at room temperature for 12 days resulted in an incomplete conversion of the 4-thio group to the 4-amino group and in formation of a small amount of uridine as by-product, both the compounds *IIb* and *IIa* afforded cytidine (*III*) as the single product by reaction with methanolic ammonia at 90°C.

In the synthesis of 1-β-D-arabinofuranosylcytosine (*VI*), there was used as the starting compound 2,2'-anhydro-1-β-D-arabinofuranosyluracil, obtained from uridine by reaction with diphenyl carbonate[17,18]. By the action of an equivalent amount of 0·1M-NaOH at room temperature[7], the anhydro compound was converted to the arabinosyluracil *IVa* in an almost quantitative yield. Compound *IVa* afforded the triacetyl derivative *IVb* in 95% yield by acetylation with acetic anhydride in the presence of acetyl chloride and glacial acetic acid[14]. The derivative *IVb* was transformed to 1-β-D-arabinofuranosylcytosine (*VI*) by two routes. One route, analogous to the above conversion of uridine to cytidine, consisted in reaction of compound *IVb* with phosphorus pentasulfide in dioxane[15] with the formation of the thio derivative *Va* which was obtained in 68% yield after chromatography on silica gel. By the action of an equivalent amount of methanolic sodium methoxide, the derivative *Va* was deacetylated with the formation of 1-β-D-arabinofuranosyl-4-thiouracil (*Vb*) which was isolated in the form of the monohydrate[19]. Prior to the last step, there was examined the stability of the arabinosylcytosine *VI* towards heating with liquid ammonia at 70°C for 43 h. Under these conditions, compound *VI* was stable and did not undergo any cleavage. Reaction of the acetyl derivative *Va* with methanolic ammonia (25%) in an autoclave at 90°C for 6 h afforded 1-β-D-arabinofuranosylcytosine (*VI*) which was isolated by chromatography on a column of Dowex 50(H^+) ion exchange resin in 95% yield.

Alternatively, the acetyl derivative *IVb* was converted to the chloro derivative *VII* by reaction with thionyl chloride in chloroform in the presence of a catalytic amount of dimethylformamide[5]. The crude compound *VII* was treated with methanolic



VII

ammonia (20%) and the resulting reaction mixture chromatographed on silica gel to afford 41% of 1-β-D-arabinofuranosylcytosine (*VI*) and 11% of the starting compound *IVb*. As shown by comparison of the two routes, the thiation process gave a higher yield, *i.e.*, 60% of the arabinosylcytosine *VI* as referred to the starting triacetylarabinosyluracil *IVb*, while the yield of the chlorination process was 41%. On the other hand, the chlorination process is less laborious and less expensive than the thiation route. None of these procedures, however, is more advantageous than the earlier reported preparation of 1-β-D-arabinofuranosylcytosine (*VI*) from cytidine[1].

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Pyridine was dried over potassium hydroxide pellets. The other solvents were purified as usual and stored over molecular sieves Potassit 3 (Research Institute of Petroleum and Hydrocarbons, Bratislava, Czechoslovakia). Analytical samples were dried at 50°C/0·1 Torr for 8 h unless stated otherwise.

Methods. Descending paper chromatography was performed on paper Whatman No 1 in the solvent systems S_1, butanol–ethanol–water (40 : 11 : 19), and S_2, butanol–water (86 : 14). Thin-layer chromatography was carried out on ready-for-use Silufol UV_{254} silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_3, benzene–ethyl acetate–methanol (35 : 10 : 5), and S_4, methanol–1M ammonium acetate saturated with sodium tetraborate[1] (9 : 1). Column chromatography was performed on silica gel (particle size, 30–60 micron), previously partially deactivated by the addition of 12% water. Paper electrophoresis was carried out on a high-voltage water-cooled apparatus on paper Whatman No 3 MM in 0·05M sodium tetraborate (pH 9·5). The spots were detected under UV light (Chromatolite).

2′,3′,5′-Tri-O-acetyluridine (*Ib*)

A mixture of uridine (*Ia*; 6·1 g; 2·5 mmol), acetic acid (25 ml), acetic anhydride (10 ml), and acetyl chloride (1 ml) was stirred at room temperature until a solution was obtained (about 2 h). The solution was allowed to stand overnight and evaporated under diminished pressure. The residue was coevaporated with four 50 ml portions of 1 : 1 ethanol–toluene and finally with methanol (50 ml). Crystallisation from ethanol (20 ml) afforded 8·15 g (88%) of the triacetyl derivative *Ib*, m.p. 128–129°C, undepressed on admixture with an authentic specimen[20]. The mother liquors were processed as usual to afford an additional crop (646 mg; 7%) of compound *Ib*. UV spectrum (ethanol): λ_{max} 258 nm (log ε 3·90) and λ_{min} 228 nm (log ε 3·33). IR spectrum (chloroform): 3385 cm^{-1} (NH), 1750 cm^{-1} (CO acetate), 1719 and 1697 cm^{-1} (CO uracil), and 1636 cm^{-1} (C=C). Optical rotation: $[\alpha]_D^{20}$ +18·2° (methanol; *c* 0·50).

2′,3′,5′-Tri-O-acetyl-4-thiouridine (*IIa*)

To a refluxing suspension of phosphorus pentasulfide (2·7 g) in dioxane (30 ml) there was added a solution of the triacetyl uridine *Ib* (4·47 g; 12 mmol) in dioxane (30 ml) and the reflux continued for 7 h. The mixture was kept at +3°C overnight, poured into ice-cold water (100 ml), the whole stirred for 20 min, and extracted with three 50 ml portions of chloroform. The combined extracts were washed with two 15 ml portions of water, filtered with active charcoal, and the filtrate evaporated under diminished pressure. The chromatographically non-homogeneous

residue was purified by chromatography on a column of silica gel (500 g) with benzene (2000 ml; fractions 1—120) and 3 : 1 benzene–ethyl acetate (1500 ml; fractions 121—210) as eluants. Fractions 145—205 afforded 3·44 g (80%) of the chromatographically homogeneous nucleoside *IIa*. UV spectrum (ethanol): λ_{max} 245 and 328 nm (log ε 3·59 and 4·20); λ_{min} 224 and 275 nm (log ε 3·15 and 3·23). IR spectrum (nujol): 1749 cm^{-1} (CO acetate), 1713 cm^{-1} and sh 1693 cm^{-1} (CO thiouracil), 1617 cm^{-1} (C═C), 1156 cm^{-1} (C═S), 3220 and 3100 cm^{-1} (NH). Optical rotation: $[\alpha]_D^{20}$ +18·4° (*c* 0·67; ethanol). For $C_{15}H_{18}N_2O_5S$ (386·4) calculated: 46·63% C, 4·70 H, 7·25% N, 8·30% S; found: 46·54% C, 4·94% H, 6·87% N, 8·03% S.

4-Thiouridine (*IIb*)

To a solution of the triacetyl derivative *IIa* (1·855 g; 4·8 mmol) in methanol (200 ml), there was added 1M methanolic sodium methoxide (14·5 ml), the whole kept at room temperature for 1 h, neutralised with Dowex 50 W X 4 (H^+) ion exchange resin, and filtered with active charcoal through diatomaceous earth. The filtrate was evaporated under diminished pressure and the residue coevaporated with three 50 ml portions of 1 : 1 ethanol–benzene and one 50 ml portion of methanol. The residual product *IIb* (1·247 g; weakly contaminated with uridine) was chromatographed on a column of silica gel (200 g) with ethyl acetate (3000 ml; fractions 1—150) as eluant. Fractions 75—135 were evaporated under diminished pressure to afford 1·13 g (90%) of compound *IIb*, yellow needles, m.p. 137—139°C (ethanol–ether); reported[16], m.p. 135—138°C. UV spectrum (water): λ_{max} 247 and 330 nm (log ε 3·50 and 4·15); λ_{min} 227 and 278 nm (log ε 3·32 and 3·09). IR spectrum (nujol): 1737 cm^{-1} and 1717 cm^{-1} (CO), 1614 cm^{-1} (C═C), 1154 cm^{-1} (C═S), 3500, 3380, and 3320 cm^{-1} (OH), 3110 cm^{-1} (NH). Optical rotation: $[\alpha]_D^{20}$ +49·7° (*c* 0·67; water). For $C_9H_{12}N_2O_5S$ (260·3) calculated: 41·53% C, 4·65% H, 10·76% N, 12·32% S; found: 41·54% C, 4·75% H, 10·63% N, 12·21% S.

Cytidine (*III*)

A. To the triacetyl derivative *IIa* (50 mg; 0·13 mmol) there was added 19% methanolic ammonia (15 ml) and the whole was heated in a steel flask at 90°C for 6 h. The resulting solution was evaporated under diminished pressure, the residue dissolved in water (50 ml), the solution filtered with active charcoal, and the filtrate evaporated under diminished pressure. The residue (30 mg) was chromatographically as well as electrophoretically homogeneous and identical with an authentic sample of cytidine.

B. A mixture of 4-thiouridine (*IIb*; 130 mg; 0·5 mmol) and 19% methanolic ammonia (15 ml) was heated in a pressure vessel at 90°C for 9 h and then processed as above to afford 108 mg (93·5%) of a chromatographically as well as electrophoretically homogeneous residue. Crystallisation from 50% aqueous ethanol gave compound *III*, identical with an authentic specimen.

C. A mixture of 4-thiouridine (*IIb*; 129 mg; 0·5 mmol) and liquid ammonia (10 ml) was kept at room temperature in a pressure vessel for 12 days and evaporated under diminished pressure. The chromatographically non-homogeneous residue was dissolved in water (50 ml), the solution filtered through diatomaceous earth, and the filtrate applied to a column of Dowex 50 (H^+; 100—200 mesh) cation exchange resin (15 ml). The column was first eluted with water (30 ml), the eluate evaporated, the residue (45 mg) dissolved in boiling methanol (25 ml), the solution filtered while hot, and the filtrate evaporated under diminished pressure to afford 38 mg of a solid consisting of a mixture of uridine (*Ia*), 4-thiouridine (*IIb*), and a little cytidine (*III*) as shown by electrophoresis. The column was then eluted with 5% aqueous ammonia, the chromatographically as well as electrophoretically homogeneous eluate evaporated under diminished pressure, the residue (62 mg; 47%) dissolved in 50% aqueous methanol, the solution filtered with active

charcoal, the filtrate evaporated, and the residue crystallised from 50% aqueous ethanol to afford cytidine (*III*), identical with an authentic sample.

1-β-D-Arabinofuranosyluracil (*IVa*)

2,2'-Anhydro-1-(β-D-arabinofuranosyl)uracil[17] (2·26 g; 10 mmol) was dissolved with stirring in 0·1M-NaOH (105 ml) and the stirring continued at room temperature for 90 min. The solution was then applied to a column (50 ml) of Dowex 50 (H^+) ion exchange resin and the column eluted with water (100 ml). The eluate was evaporated under diminished pressure, the residue coevaporated with three 50 ml portions of ethanol–benzene (1 : 1) and one 50 ml portion of methanol, and dried at 50°C to afford 2·41 g (98·6%) of compound *IVa*, chromatographically as well as electrophoretically homogeneous and identical with an authentic specimen[21]. This residue was directly used without any further purification in the next step. The analytical sample, m.p. 222—223°C (methanol), as reported[21] earlier.

1-(2,3,5-Tri-O-acetyl-β-D-arabinofuranosyl)uracil (*IVb*)

A mixture of the crude arabinofuranosyluracil *IVa* (0·98 g; 4 mmol), acetic acid (10 ml), acetic anhydride (4 ml), and acetyl chloride (0·6 ml) was stirred at room temperature overnight and then at 35°C for 8 h. The solution was evaporated under diminished pressure, the residue coevaporated with five 50 ml portions of ethanol–toluene (1 : 1) and one 50 ml portion of methanol, and the final chromatographically as well as electrophoretically homogeneous residue (1·485 g) crystallised from ethanol (3 ml) to afford 1·364 g (92%) of compound *IVb*, m.p. 131—132°C; reported[20], m.p. 129—130°C. Work-up of mother liquors yielded additional 63 mg (4%) of compound *IVb*. UV spectrum (ethanol): λ_{max} 259 nm (log ε 3·91) and λ_{min} 229 nm (log ε 3·31). IR spectrum (chloroform): 3385 cm^{-1} (NH), 1749 cm^{-1} (CO acetate), 1721 and 1694 cm^{-1} (CO uracil), 1632 cm^{-1} (C=C). Optical rotation: $[\alpha]_D^{20}$ + 83·9° (methanol; *c* 0·50).

TABLE I

Chromatography (R_F values) and Paper Electrophoresis

Compound	S_1[a]	S_2[a]	S_3[b]	S_4[b]	S_5[b]	electrophoresis[c]
Ia	0·42	0·22	0·06	0·22	0·26	+ 72
Ib	0·82	0·79	0·48	0·71	—	+ 14
IIa	0·88	0·86	0·84	0·73	—	+ 54
IIb	0·60	0·43	0·20	0·20	0·62	+121
III	0·29	0·11	0·00	0·16	—	+ 60
IVa	0·45	0·27	0·07	0·62	0·38	+ 17
IVb	0·79	0·76	0·52	0·72	—	+ 21
Va	0·90	0·87	0·85	0·71	0·95	+ 58
Vb	0·67	0·53	0·20	0·61	0·68	—
VI	0·32	0·13	0·00	0·52	0·00	— 11

[a] Paper Whatman No 1; [b] silica gel; [c] mobilities in mm, 1 500 Volt/2 h, paper length 57 cm.

1-(2,3,5-Tri-O-acetyl-β-D-arabinofuranosyl)-4-thiouracil (*Va*)

To a refluxing suspension of phosphorus pentasulfide (1·2 g) in dioxane (15 ml) there was added a solution of the triacetyl derivative *IVb* (1·9 g; 5·1 mmol), the mixture refluxed for 8 h, kept at +3°C overnight, poured into ice-cold water (50 ml), and the whole extracted with three 30 ml portions of chloroform. The combined extracts were washed with two 10 ml portions of water, dried over anhydrous magnesium sulfate, filtered with active charcoal through diatomaceous earth, and the filtrate evaporated under diminished pressure. The residue (1·87 g) was chromatographed on a column of silica gel (350 g) and the column washed with benzene (2000 ml; fractions 1—110) and then with a 4 : 1 mixture of benzene and ethyl acetate (2500 ml; fractions 111—250). Fractions 175—240 were evaporated under diminished pressure and the residue triturated with benzene (20 ml) to afford 1·63 g (68%) of compound *Va*, m.p. 82—88°C. UV spectrum (ethanol): λ_{max} 249 and 329 nm (log ε 3·58 and 4·23), λ_{min} 224 and 275 nm (log ε 3·12 and 3·14). IR spectrum (KBr): 1757 cm^{-1} (CO acetate), 1731 cm^{-1} (CO thiouracil), 1613 cm^{-1} (C═C), 1161 cm^{-1} (C═S), 3200 and 3110 cm^{-1} (NH). Optical rotation: $[\alpha]_D^{20}$ +120·0° (ethanol; *c* 0·67). For $C_{15}H_{18}N_2O_8S.C_6H_6$ (464·5) calculated: 50·81% C, 4·94% H, 6·59% N, 7·53% S; found: 50·81% C, 4·95% H, 6·72% N, 7·58% S. A sample (200 mg) was recrystallised from 2-propanol with a drop of benzene to afford 155 mg of the same solvate, m.p. 82—88°C.

1-β-D-Arabinofuranosyl-4-thiouracil (*Vb*)

A solution of the triacetyl derivative *Va* (0·50 g; 1·3 mmol) in 0·04M methanolic sodium methoxide (104 ml) was kept at room temperature for 1 h and neutralised by the addition of Dowex 50 X 4 (H^+) cation exchange resin. The neutral mixture was filtered with active charcoal through diatomaceous earth and the filtrate evaporated under diminished pressure. The residue was co-evaporated with three 50 ml portions of 1 : 1 ethanol–benzene and on 50 ml portion of methanol. The final chromatographically homogeneous residue (0·327 g; 97%) was crystallised from 96% ethanol to afford compound *Va* in the form of a monohydrate, yellow needles, m.p. 85—87°C. UV spectrum (water): λ_{max} 248 and 333 nm (log ε 3·47 and 4·14) and λ_{min} 228 and 278 nm (log ε 3·33 and 3·07). IR spectrum (nujol): 1696 cm^{-1} (CO), 1619 cm^{-1} (C═C), 1159 cm^{-1} (C═S). Optical rotation: $[\alpha]_D^{20}$ +190·3° (*c* 0·67; water). For $C_9H_{12}N_2O_5S.H_2O$ (278·3) calculated: 38·84% C, 5·07% H, 10·07% N, 11·52% S; found: 38·95% C, 5·06% H, 10·35% N, 11·42% S.

1-β-D-Arabinofuranosylcytosine (*VI*)

A. A solution of the triacetyl derivative *Va* (520 mg; 1·35 mmol) in 25% methanolic ammonia (30 ml) was heated at 90—100°C in a pressure vessel for 6 h, cooled down, and evaporated. The residue was coevaporated with three 50 ml portions of methanol and dissolved in water (30 ml). The aqueous solution was applied to a column (50 ml) of Dowex 50 (H^+) cation exchange resin and column washed with water (300 ml). Compound *VI* was eluted with 5% aqueous ammonia (80 ml), the eluate evaporated under diminished pressure, the residue dissolved in methanol (50 ml), the solution filtered with active charcoal through diatomaceous earth, and the filtrate evaporated under diminished pressure to afford 312 mg (95%) of a chromatographically homogeneous residue which was recrystallised from a mixture (4 ml) ethanol–water (1 : 1). Yield, 225 mg (69%) of the nucleoside *VI*, m.p. 214·5—216°C, identical with an authentic specimen[1]. Work-up of mother liquors yielded additional 58 mg (18%) of compound *VI*, m.p. 214—215·5°C. Reported[22], m.p. 212—213·5°C.

B. To a solution of the triacetyl derivative *IVb* (926 mg; 2·5 mmol) in chloroform (20 ml) there was added thionyl chloride (2 ml) and dimethylformamide (0·1 ml), the whole refluxed

for 7 h, kept at room temperature overnight, and evaporated under diminished pressure and exclusion of atmospheric moisture. The residue was dried at 50°C/0·1 Torr for 2 h and dissolved in chloroform (20 ml). The solution was added dropwise with cooling and stirring to a 3% solution of ammonia in chloroform (30 ml). The mixture was filtered with the addition of diatomaceous earth, and the material on the filter washed with three 15 ml portions of chloroform and two 15 ml portions of ethanol. The filtrate and washings were combined and evaporated under diminished pressure. The residue was coevaporated with two 50 ml portions of methanol and the final residue dissolved in 20% methanolic ammonia (40 ml). The solution was kept in a stoppered vessel for 48 h at room temperature and then evaporated under diminished pressure. The residue was coevaporated with three 50 ml portions of methanol and finally dissolved (974 mg) in methanol. The solution was chromatographed on a column of silica gel (50 g) with the use of 4 : 1 chloroform–methanol as eluant to afford 71 mg (11·6%) of compound *IVa* and 252 mg (41·4%) of compound *VI* from the corresponding chromatographic fractions. Compound *VI* was in every respect identical with an authentic specimen[1] and with the product from paragraph *A*.

Stability. Crystals of compound *VI* (54 mg; 0·22 mmol) were heated in a pressure vessel with liquid ammonia (10 ml) at 70°C for 43 h and excess ammonia was then evaporated under diminished pressure. The residue (chromatographically as well as electrophoretically homogeneous and identical with the starting material) was dissolved in water, the solution filtered, the filtrate evaporated, and the residual solid (56 mg) crystallised from a little water by the addition of ethanol. Yield, 36 mg (67%) of compound *VI*, m.p. 210—215°C. As shown by chromatography and electrophoresis, the mother liquors contained an additional crop of compound *VI* as the single UV-absorbing component.

1-β-D-*Arabinofuranosylcytosine hydrochloride.* To a solution of compound *VI* (159 mg; 0·66 mmol) in water (5 ml) there was added 2M-HCl (0·35 ml), the solution evaporated under diminished pressure, and the residue coevaporated with three 5 ml portions of ethanol–toluene (1 : 1). Crystallisation of the final residue from methanol (2 ml) afforded 81 mg of 1-β-D-arabinofuranosylcytosine hydrochloride, m.p. 190—194°C; reported[13], m.p. 186—188°C. Optical rotation: $[\alpha]_D^{25}$ +130·6° (*c* 0·33; water); reported[13], $[\alpha]_D^{23}$ +129° (*c* 1·41; water). The product was chromatographically and electrophoretically identical with the authentic hydrochloride[1]. Work-up of mother liquors afforded an additional crop (40 mg) of the hydrochloride. UV spectrum: λ_{max} 213 and 281 nm (log ε 4·19 and 4·33) and λ_{min} 242 nm (log ε 3·31) in 0·01M-HCl; λ_{inax} 214 and 272 nm (log ε 4·03 and 3·99) and λ_{min} 250 nm (log ε 3·77) in 0·01M-NaOH. For $C_9H_{13}N_3O_5$. .HCl (279·7) calculated: 38·68% C, 5·05% H, 15·05% N, 12·68% Cl; found: 38·41% C, 4·83% H, 14·89% N, 12·92% Cl.

The authors wish to thank Mr P. Formánek for measurement of UV and IR spectra and Dr P. Fiedler for interpretation. The analyses were kindly performed in the Analytical Department (Dr J. Horáček, Head) of this Institute by Mrs V. Rusová, Mrs E. Šipová, and Mr V. Štěrba. The excellent technical assistance of Mrs J. Hlaváčková is gratefully acknowledged.

REFERENCES

1. Beránek J., Delia T. J.: Czechoslovak Patent Application (1971). The abstract was presented at the 8th International Congress of Chemotherapy, Athens, 1973 (to be published).
2. Fox J. J., Wempen I., Hampton A., Doerr I. L.: J. Am. Chem. Soc. *80*, 1669 (1958).
3. Fox J. J., Van Praag D., Wempen I., Doerr I. L., Cheong L., Knoll J. E., Eidinoff M. L., Bendich A., Brown G. B.: J. Am. Chem. Soc. *81*, 178 (1959).
4. Žemlička J., Šorm F.: This Journal *30*, 1880 (1965).

5. Žemlička J., Šorm F.: This Journal *30*, 2052 (1965).
6. Beránek J., Šorm F.: This Journal *28*, 469 (1963).
7. Farkaš J., Beránek J., Šorm F.: This Journal *31*, 4002 (1966).
8. Černeckij V., Chládek S., Šorm F., Smrt J.: This Journal *27*, 87 (1962).
9. Pliml J., Šorm F.: This Journal *28*, 546 (1963).
10. Prystaš M., Šorm F.: This Journal *28*, 2598 (1963).
11. Bobek M., Farkaš J., Šorm F.: This Journal *32*, 3581 (1967).
12. Prystaš M., Šorm F.: This Journal *34*, 1104 (1969)
13. Hunter J. H.: US-Pat. 3 116 282; Chem. Abstr. *61*, 4467 (1964).
14. Beránek J., Pitha J.: This Journal *29*, 625 (1964).
15. Klein R. S., Wempen I., Watanabe K. A., Fox J. J.: J. Org. Chem. *35*, 2330 (1970).
16. Kochetkov N. K., Budowsky E. I., Shibaev V. N., Yeliseeva G. I., Grachev M. A., Demushkin V. P.: Tetrahedron *19*, 1207 (1963).
17. Hampton A., Nichol A. W.: Biochemistry *5*, 2076 (1966).
18. Beránek J.: Czechoslovak Patent Application (1971).
19. Saenger W.: J. Am. Chem. Soc. *94*, 621 (1972).
20. Brown D. M.. Todd A. R., Varadarajan S.: J. Chem. Soc. *1956*, 2388.
21. Beránek J., Šorm F.: This Journal *33*, 913 (1968).
22. Walvick E. R., Roberts W. K., Dekker C. A.: Proc. Chem. Soc. (London) *1959*, 84.

Translated by J. Pliml.