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

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One-step synthesis of the cytostatically active 1-β-D-arabinofuranosylcytosine (yield, 31·9%; with respect to the recovered cytidine, the yield was 47·5%) was effected from cytidine by the action of diphenyl carbonate in dimethylformamide at 120°C in the presence of sodium hydrogen carbonate or one equivalent of water. 1-β-D-Arabinofuranosylcytosine was separated from the unreacted cytidine by chromatography on an anion exchange resin in the borate cycle. Chromatographic data of the above compounds, their uracil counterparts, and acetyl derivatives were tabulated.

1-β-D-*arabino*-Pentofuranosylcytosine is clinically administered as a cytostatically active substance particularly against acute myeloblastic forms of leukemia[2]. Considerable attention has been paid to the synthesis and biological activity assays and a number of reviews has been published (see for example refs[3-5] and citations therein quoted). The first synthesis[6] of arabinosylcytosine and cyclocytidine in 1959 was followed by numerous further preparations until the present time[7]. One group comprises syntheses in which the sugar component is attached to the base by the nucleosidation reaction[8,9] or the cytosine base is synthesised directly on the sugar component[10-13]. Another approach to arabinosylcytosine could make use of general methods for the preparation of cytosine nucleosides from uracil derivatives *via* the 4-thio[15] and 4-chloro[17] intermediates (both the reactions were also developed in the purine nucleoside series)[14,16], the 4-alkoxy derivatives[18] or derivatives in which position 4 is activated by silylation[19]; all these intermediates are converted to the required product on treatment with ammonia. The methods using the thio[15] and chloro[17] intermediates were used some time ago in this Laboratory in the preparation of 1-β-D-arabinofuranosylcytosine[20] for purposes of comparison with the patent literature[21] and the method applied to the present work[22,23]. Analogous preparations were performed from 5'-O-trityluridine *via* the 2,4-dithio derivative[24] and from

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triacylarabinofuranosyl uracil *via* the 4-chloro derivative obtained by reaction with phosphorus oxychloride[25,26]. Further routes to arabinosylcytosine consist in modification of the sugar moiety, *i.e.*, conversion of ribonucleosides to 2,2′-anhydro nucleosides and the subsequent hydrolysis to *arabino*-nucleosides. These methods make use of 2′-alkyl(aryl)sulfonyl esters[27], nitric acid esters[28] or active derivatives of phosphoric acid for the direct conversion of cytidine to 2*R*-(2α,3β,3aβ,9aβ)-2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-6*H*-furo[2′,3′ : 4,5]oxazolo[3,2-*a*]pyrimidine-2-methanol.* Nucleotide derivatives are obtained by reaction of polyphosphoric acid[6,29] with cytidine (the subsequent enzymatic dephosphorylation affords cyclocytidine from which 1-β-D-arabinofuranosylcytosine results by hydrolysis) and by syntheses starting from phosphoric acid esters of cytidine[30–34]. Cyclocytidine also results from reactions with partly hydrolysed phosphorus oxychloride[35], fresh phosphorus oxychloride[34,36] or thionyl chloride[36–39] in dimethylformamide (the Vilsmeier–Haack reagent[37]). The latter preparation of cyclonucleosides was developed in both the pyrimidine[7] and the purine series[40]. The acyl derivatives of cyclocytidine are obtained by reaction of cytidine with acyl halides[41,42].

Furthermore, two general methods were devised in the nucleoside field for a direct conversion of unsubstituted *ribo*-nucleosides to 2,2′-anhydro nucleosides (O^2,2′-cyclonucleosides) and the subsequent cleavage to *arabino*-derivatives, namely, reaction with thiocarbonyldiimidazole[43–45] and reaction with diphenyl carbonate[46]. However, none of these methods has been applied to cytidine. The reaction of cytidine with diphenyl carbonate was therefore examined in detail[22] to detect an unexpected course. Later on, the reaction of cytidine with diphenyl carbonate was reported in a short communication[47] and a patent application[48].

Under conditions analogous to those applied to uridine[46], *i.e.*, when heated with diphenyl carbonate in dimethylformamide at 150°C for 15–30 minutes in the presence of sodium hydrogen carbonate, cytidine does not afford any cyclonucleoside. Instead of cyclocytidine, another substance was isolated from the reaction mixture and identified as 1-β-D-arabinofuranosylcytosine. Concerning the temperature, no reaction takes place below 100°C. However, when the reaction is performed on a larger scale (by one or two order of magnitude), the use of a lower temperature (120°C) and the correspondingly longer reaction time appears advisable from the standpoint of yields and work-up of the reaction mixture. The reaction may be effected even in the absence of sodium hydrogen carbonate when one equivalent of water is used. The presence of alkali does not appear indispensable, the basicity of cytidine being sufficient for the required reaction. It may be assumed that the mechanism of the

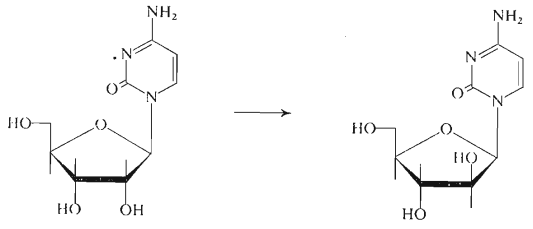
* The nomenclature recommended by Chemical Abstracts ((Chem. Abstr. Index Guide *76*, 211 (1972)) is used instead of the earlier designations 2,2′-anhydro-1-β-D-arabinofuranosylcytosine or O^2,2′-cyclocytidine. For the sake of brevity, the term "cyclocytidine" appears in the further text.

present reaction is analogous to that of other pyrimidine nucleosides[46]. Most probably the primarily formed 2′,3′-O-carbonyl derivative is transformed to cyclocytidine which is hydrolysed by water present in the reaction medium with the formation of 1-β-D-arabinofuranosylcytosine.

Attempts to improve the yield by the use of a more active carbonate such as di(*p*-nitrophenyl) carbonate or by the use of a greater excess of diphenyl carbonate did not meet with success. Under strictly anhydrous conditions and with the use of 2,5 – 3 equivalents of diphenyl carbonate, an insoluble substance resulted which was not identified.

The attempted preparation of cyclocytidine from cytidine hydrochloride instead of the free nucleoside failed since a cleavage of the nucleoside bond occurred and only cytosine was isolated from the reaction mixture. The same cleavage of the nucleoside bond with the formation of cytosine was observed when cytidine hydrochloride was heated in dimethylformamide in the absence of diphenyl carbonate.

On the basis of the above observations, a procedure was developed for the preparation of 1-β-D-arabinofuranosylcytosine (*II*) affording a 47·5% yield of the required substance, with respect to the recovered cytidine (*I*). The reaction was performed in dimethylformamide at 120°C for 6 h in the presence of one equivalent of water. The resulting arabinosylcytosine was separated from the unreacted cytidine by chromatography on a strongly basic anion exchange resin in the borate cycle; nucleosides with the vicinal *cis*-diol system are detained on the resin.



I *II*

Furthermore, arabinosylcytosine was separated from uracil derivatives – (minor by-products of the reaction) – by chromatography on a strongly acidic cation exchange resin in the H^+ cycle, eluted with dilute aqueous ammonia and isolated partly as the free nucleoside, partly as the hydrochloride. Cytidine was liberated from the borate column with dilute aqueous acetic acid and recovered from the eluate (after removal of boric acid) by chromatography on a strongly basic anion exchange resin in 33·3% yield. For the chromatography see Table I.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Ultraviolet spectra were measured on a CF-4 apparatus (Optica Milano). Optical rotation was measured on an automatic Bendix Ericson ETL-NPL Type 143A polarimeter. Analytical samples were dried at 0·5 Torr. Solutions were taken down on a rotatory evaporator at 20—40°C/20 Torr. Ascending thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, benzene–ethyl acetate–methanol (35 : 10 : 5); S_2, ethyl acetate-methanol (1 : 1); S_3, ethyl acetate–5% solution of ammonia in methanol (6 : 4); S_4, ethyl acetate–methanol (9 : 1); and S_5, methanol–1M ammonium acetate in water saturated with sodium tetraborate (9 : 1). Descending paper chromatography was carried out on Whatman paper No 1 in the solvent systems S_6, butanol–ethyl acetate–water (40 : 11 : 19); and S_7, butanol–water (86 : 14).

Chromatographic data of the cytosine and uracil nucleosides and their acetyl derivatives are shown in Table I.

1-(β-D-*arabino*-Pentofuranosyl)-4-aminopyrimidin-2(1*H*)-one (*II*)

A stirred mixture of cytidine (*I*; 600 g; 2·56 mol), diphenyl carbonate[49,50] (560 g; 1·19 equivalent), dimethylformamide (750 ml), and water (45 ml; 2·5 mol) was heated at 120°C. Additional diphenyl carbonate (three 40 g portions; total 0·25 equivalent) were introduced after 1·5, 2·5, and 3·5 h of heating. After 6 h, the mixture was cooled down and poured with stirring into ether (2·5 litre). The precipitate was collected, dried, and dissolved in 60% aqueous methanol

TABLE I

Thin-Layer and Paper Chromatography (for conditions see Experimental)

Compound	Thin-layer chromatography					Paper chromatography	
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
2′,3′,5′-Tri-O-acetyl-1-β-D-arabinosyluracil	0·52	0·90	0·84	0·90	0·75	0·75	0·79
1-β-D-Arabinosyluracil	0·07	0·90	0·26	0·33	0·66	0·39	0·19
2′,3′,5′-Tri-O-acetyluridine	0·44	0·90	0·86	0·86	0·74	0·77	0·82
Uridine	0·06	0·76	0·18	0·25	0·22	0·31	0·13
2′,3′,5′-Tri-O-acetyl-1-β-D-arabinosylcytosine	0·07	0·74	0·83	0·10	0·60	0·69	0·81
2′,3′,5′-Tri-O-acetyl-N^4-acetyl-1-β-D-arabinosylcytosine	0·34	0·86	0·90	0·61	0·69	0·79	0·90
1-β-D-Arabinosylcytosine	0	0·45	0·13	0	0·52	0·25	0·10
2′,3′,5′-Tri-O-acetylcytidine	0·05	0·74	0·68	0·09	0·60	0·71	0·75
2′, 3′,5′-Tri-O-acetyl-N^4-acetylcytidine	0·28	0·90	0·85	0·59	0·69	0·80	0·90
N^4-Acetylcytidine	0·08	0·80	0·26	0·15	0·30	0·41	0·18
Cytidine	0	0·41	0·09	0	0·14	0·21	0·08

(5 litre). The solution was filtered with active charcoal, the filtrate evaporated, the residue dissolved in water (2·5 litre) and the solution applied to a column of Zerolite FF ion exchanger (14—52 mesh; 9 kg) in the borate cycle (the column in the original OH^- form was prewashed with an aqueous solution of 5 kg of sodium tetraborate and then with water until the pH value of the effluent was about 7·5). Elution of the column with water was checked by thin-layer chromatography in the solvent system S_5. Fraction 1 (about 4·6 litre containing mainly compound *II*) was collected until the brown colour began to turn to yellow. Fraction 2 (about 105 litre) was collected as long as the effluent contained the UV-absorbing compound *II* and then evaporated. The residue of fraction 2 was repeatedly coevaporated with twelve one-litre portions of methanol and the residue crystallised from methanol to afford (after work-up of mother liquors) total 104 g of compound *II* (in the form of the free nucleoside), m.p. 205—216°C, which was recrystallised from 50% aqueous ethanol. Yield, 85·5 g of compound *II*, m.p. 215—217°C; $[\alpha]_D^{25}$ +155·2° (*c* 0·7; water). UV spectrum (water): λ_{max} 275 nm (log ε 3·90) and λ_{min} 254 nm (log ε 3·73). Physical data are in accordance with those reported[20,25,29,35,36].

Hydrochloride. Mother liquors from crystallisation of the free arabinoside *II* were combined with fraction 1 from the borate column and the mixture was evaporated. The residue was dissolved in water (800 ml), the solution applied to a column of Dowex 50X2 ion exchange resin (H^+ cycle; 50—100 mesh; 1·5 kg), and the column washed with water (about 8 litre) in order to remove the uracil derivatives. Compound *II* was then liberated by elution with 4% aqueous ammonia (about 15 litre) and the effluent evaporated. The residue containing compound *II* as the single UV-absorbing substance, was dissolved in methanol (500 ml), the solution adjusted to pH 2—3 (as determined in a sample diluted with water) with 30% methanolic hydrogen chloride, and evaporated. Crystallisation of the residue from 50% aqueous ethanol and work-up of mother liquors yielded total 135 g of the hydrochloride of compound *II*. Recrystallisation from the same solvent yielded 121·1 g of 1-β-D-arabinofuranosylcytosine hydrochloride (*II* . HCl), m.p. 188 to 193°C; $[\alpha]_D^{25}$ +131·0° (*c* 0·7; water). UV spectrum (water): λ_{max} 276 nm (log ε 4·00), λ_{min} 246 nm (log ε 3·59). The data are in accordance with those reported[20,21].

The overall yield of compound *II* was 31·9% (*i.e.*, 47·5% with respect to the recovered cytidine). This value corresponds to 190·85 g of the free nucleoside *II* or to 219·2 g of the hydrochloride.

Isolation of the unreacted cytidine. The borate column was eluted with 5% aqueous acetic acid (about 30 litre). The eluate containing cytidine as the single UV-absorbing substance, boric acid, and acetic acid was concentrated to the volume of 5 litre and the boric acid was filtered off. The filtrate was concentrated again to the volume of 1 000 ml and then 500 ml, and the boric acid was repeatedly filtered off. The final filtrate was applied to a column of Dowex 50 W X 2 ion exchange resin (H^+ cycle; 50—100 mesh; 1 kg) and the column was washed with water. Cytidine was liberated from the column by elution with 5% aqueous ammonia (about 5 litre), the effluent evaporated, the residue dissolved in 30% aqueous methanol (1 000 ml), and the solution applied to a column of Dowex 2X4 ion exchange resin (OH^- cycle; 1 kg). The column was washed with 30% aqueous methanol and then eluted with 0·1M aqueous ammomium hydrogen carbonate[29]. The cytidine-containing fractions were combined, evaporated, and the residue was coevaporated with water to remove ammonium hydrogen carbonate. The final residue was crystallised from 50% aqueous ethanol. Recovery, 198·45 g (33·3%) of cytidine (*I*).

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