SELECTIVE O-ACYLATION. PREPARATION OF 1-(2,3,5-TRI-O-ACETYL-β-D-*arabino*-PENTOFURANOSYL)CYTOSINE HYDROCHLORIDE* **

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2′,3′,5′-Tri-O-acetylcytidine hydrochloride (*IIIb*) is selectively obtained in 91% yield from cytidine (*Ia*) by reaction with acetyl chloride in acetic acid at room temperature; under analogous conditions, arabinosylcytosine hydrochloride (*IIb*) affords the tri-O-acetyl derivative hydrochloride *IVb* in 80% yield. The hydrochloride *IVb* is proposed as the water-soluble "depot" derivative of the cytostatically active arabinosylcytosine.

The acetylation of pyrimidine nucleosides by the action of acetic anhydride in the presence of acetyl chloride or with acetyl chloride alone (the so called acidic acetylation) has been applied to the preparation of numerous acetyl derivatives of the ribo[2–4] arabino[3–6], and deoxy[7,8] series. In the case of purine nucleosides, this method resulted in a cleavage of the nucleoside bond[8] with the formation of an acetylated halogenose. With pyrimidine nucleosides bearing the amino group in the aglycon[9] or sugar[10] moiety, the method has selectively furnished the O-acetyl derivatives.

The selective N- and O-acylation has been object of several papers[11–18]. Martinez and coworkers[11] prepared tri-O-acetylarabinofuranosylcytosine by reaction of arabinocytosine hydrochloride with a mixture of acetic acid and trifluoroacetic anhydride. Montgomery and Thomas[12] prepared the tri-O-acyl derivatives of arabinofuranosylcytosine by N-deacylation of the tetraacyl derivative by the action of picric acid in refluxing methanol or on treatment with hydrazine in a mixture of pyridine and acetic acid. The selective N-deacetylation in refluxing methanol has been applied to derivatives of 6-azacytidine (*cf.*[2]). In the preparation of 5′-O-esters, Gish and coworkers[13] protected the amino group of cytosine derivatives by protonation. The selective N-acylation of cytosine nucleosides with acetic anhydride in refluxing methanol has been developed by Fox and coworkers[14–16].

* Part XIII in the series Analogues of Nucleosides; Part XII: see ref.[8]

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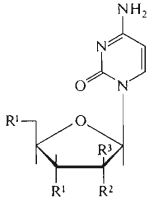
The pharmacological value of the cytostatic agent 1-β-D-arabinofuranosylcytosine is lowered by deamination during the clinical application. For this reason, numerous derivatives of arabinosylcytosine have been prepared such as N^4-substituted derivatives that should be less sensitive towards deamination[16], a series of "depot" derivatives such as the peracyl derivatives[11,12], tri-O-acyl derivatives[11,12], 5'-O-esters[13], 2'-O-esters, and 3'-O-esters[19], and cyclocytidine[20].

On the basis of the earlier experience with the preparation of 6-azauridine acyl derivatives, particularly 6-azauridine tri-O-acetyl derivative as the depot and considerably less toxic derivative of 6-azauridine[21], the preparation of tri-O-acetylcytosine hydrochloride (*IVb*) has been now attempted with the hope to obtain a depot and water-soluble derivative of the cytostatic agent arabinosylcytosine. Despite some negative allusions in the literature[11,12], compound *IVb* was obtained in a high yield with the use of the acidic acetylation[1,9].



I, $R^1 = R^2 = OH$, $R^3 = H$
II, $R^1 = R^3 = OH$, $R^2 = H$
III, $R^1 = R^2 = OCOCH_3$, $R^3 = H$
IV, $R^1 = R^3 = OCOCH_3$, $R^2 = H$

a free bases
b hydrochlorides

Acetylation of cytosine nucleosides by the action of acetyl chloride in acetic acid afforded selectively the corresponding O-acetyl derivatives. The same results were obtained with the use of a free nucleoside or nucleoside hydrochloride as the starting substance. From cytidine (*Ia*), the tri-O-acetyl derivative hydrochloride *IIIb* is formed in 91% yield (in order to increase the solubility of the product, the reaction mixture was diluted with chloroform). The triacetate hydrochloride *IVb* is obtained in 72% yield under analogous conditions from arabinosylcytosine hydrochloride[22,23] (*IIb*). When the preparation was performed on a larger scale and the mother liquors were worked up, the total yield was 80%. Spectral and analytical data of products *IIb* and *IVb* were in accordance with their structure. The UV spectra correspond to earlier measurements[2,11,12,16] and confirm that a selective O-acetylation took place, free of any substitution on the amino group. The UV spectra of compounds *IIb* and *IVb* did not exhibit any bathochromic shifts characteristic of cytosine N-acetyl derivatives.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Boetius). The UV spectra were measured on a CF-4 apparatus (Optica Milano). The IR spectra were recorded on a UR-20 apparatus (Carl Zeiss, Jena). The ^{1}H-NMR spectra were measured on a Varian HA-100 apparatus (chemical shifts are shown in δ units and expressed in p.p.m.; the coupling constants are given in Hz). Optical rotation was measured on a 141 MC Perkin-Elmer polarimeter. Analytical samples were dried at 0·5 Torr. Solutions were taken down on a rotatory evaporator at 40°C/20 Torr. Thin-layer chromatography was performed on ready-for-use Silufol^R (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in 6 : 4 (v/v) ethyl acetate- 5% methanolic ammonia.

1-(2,3,5-Tri-O-acetyl-β-D-*ribo*-pentofuranosyl)-4-aminopyrimidin-2(1*H*)-one Hydrochloride (*IIIb*)

A mixture of cytidine (*Ia*; 486 mg; 2 mmol), acetic acid (3 ml), and acetyl chloride (0·6 ml) was stirred at room temperature for 10 min, diluted with chloroform (6 ml), and the stirring continued for 40 h. The mixture was then evaporated, the residue coevaporated with ethanol and then 1 : 1 chloroform-methanol, crystallised from chloroform–methanol, and recrystallised from 2-propanol–methanol. Yield, 738 mg (91%) of the hydrochloride *IIIb*, m.p. 142—148°C; $[\alpha]_D^{25}$ +37·2° (*c* 0·4; ethanol). UV spectrum (water): plateau 230—246 nm (log ε 3·67), λ_{max} 271 nm (log ε 3·84). IR spectrum (nujol): 3015 v br (NH), 3095 (CH), 1739 (C=O), 1684 (C=O, cytosine), and 1622 cm^{-1} (C=C). The ^{1}H-NMR spectrum (deuteriochloroform with hexadeuteriodimethyl sulfoxide, trimethylsilane as internal standard): 6·41 (d, 1 H, H_5, $J_{5,6}$ = 8·0), 7·93 (d, 1 H, H_6, $J_{6,5}$ = 8·0), 9·17 (broad, 1 H, NH), 5·94 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 4·0), 5·48 (dd, 1 H, $H_{2'}$, $J_{2',1'}$ = 4·0, $J_{2',3'}$ = 6·0), 5·35 (m, 1 H, $H_{3'}$), 4·35 (m, 3 H, $H_{4'}$ + $H_{5'}$ + $H_{5''}$), 2·10 (s, 9 H, $OCOCH_3$). R_F value, 0·68 (cytidine *Ia*, 0·09; tetraacetylcytidine, 0·85). For $C_{15}H_{20}ClN_3O_8$ (405·8) calculated: 44·40% C, 4·97% H, 10·35% N, 8·74% Cl; found: 44·78% C, 5·14% H, 10·08% N 8·54% Cl.

1-(2,3,5-Tri-O-acetyl-β-D-*arabino*-pentofuranosyl)-4-aminopyrimidin-2(1*H*)-one Hydrochloride (*IVb*)

A suspension of arabinosylcytosine hydrochloride[22,23] (*IIb*; 200 mg; 0·72 mmol), acetic acid (2 ml), and acetyl chloride (1 ml) was stirred at room temperature for 3 h, diluted with chloroform (1 ml), stirred for additional 18 h, and evaporated. The residue was coevaporated with several portions of ethanol and then 1 : 1 ethanol–chloroform, and crystallised from a mixture of 2-propanol and ethanol. Yield, 240 mg (72%) of the hydrochloride *IVb*, m.p. 171—174°C; $[\alpha]_D^{25}$ +96·9° (*c* 0·7; ethanol). UV spectrum (water): λ_{max} 271 nm (log ε 3·88), λ_{min} 243 nm (log ε 3·70). IR spectrum (nujol): 3310, 3115 and 1677 (NH), 2720 and 1545 (N^+H), 1756 and 1742 (C=O, acetyl), and 1716 cm^{-1} (C=O, cytosine). The ^{1}H-NMR spectrum (deuteriochloroform with hexadeuteriodimethyl sulfoxide, trimethylsilane as internal standard): 6·48 (d, 1 H, H_5, $J_{5,6}$ = 7·5), 7·77 (d, 1 H, H_6, $J_{6,5}$ = 7·5), 9·28 (broad, 1 H, NH), 6·23 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 4·0), 5·40 (dd, 1 H, $H_{2'}$, $J_{2',1'}$ = 4·0, $J_{2',3'}$ = 1·5), 5·11 (dd, 1 H, $H_{3'}$, $J_{3',2'}$ = 1·5, $J_{3',4'}$ = 4·0), 4·15—4·50 (m, 3 H, $H_{4'}$ + $H_{5'}$ + $H_{5''}$), 2·06 (s, 3 H, $OCOCH_3$), 2·12 (s, 3 H, $OCOCH_3$), and 2·15 (s, 3 H, $OCOCH_3$). R_F value, 0·83 (arabinosylcytosine *IIa*, 0·13; tetraacetylarabinosylcytosine, 0·90). For $C_{15}H_{20}ClN_3O_8$ (405·8) calculated: 44·40% C, 4·97% H, 10·35% N, 8·74% Cl; found: 44·30% C, 4·83% H, 10·13% N, 8·62% Cl.

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REFERENCES

1. Beránek J., Delia T. J.: in the book: *Progress in Chemotherapy* (K. G. Daikos, Ed.), Vol. 3, p. 832. Hellenic Society for Chemotherapy, Athens 1974.
2. Beránek J., Píťha J.: This Journal *29*, 625 (1964).
3. Brokeš J., Beránek J.: This Journal *39*, 3100 (1974).
4. Drašar P., Hein L., Beránek J.: This Journal *41*, 2110 (1976).
5. Brokeš J., Beránek J.: This Journal *40*, 3071 (1975).
6. Farkaš J., Beránek J., Šorm F.: This Journal *31*, 4002 (1966).
7. Hein L., Drašar P., Beránek J.: Nucl. Acids Res. *3*, 1125 (1976).
8. Hřebabecký H., Beránek J.: Nucl. Acids Res. *3*, 1387 (1976).
9. Beránek J., Drašar P.: Czech. 164 080 (1973).
10. Brokeš J., Beránek J.: This Journal *40*, 3061 (1975).
11. Martinez A. P., Lee W. W., Goodman L.: J. Med. Chem. *9*, 268 (1966).
12. Montgomery J. A., Thomas H. J.: J. Med. Chem. *15*, 116 (1972).
13. Gish D. T., Kelly R. C., Camiener G. W., Wechter W. J.: J. Med. Chem. *14*, 1159 (1971).
14. Watanabe K. A., Fox J. J.: Angew. Chem. *78*, 589 (1966).
15. Otter B. A., Fox J. J. in the book: *Synthetic Procedures in Nucleic Acid Chemistry* (W. W. Zorbach, R. S. Tipson, Eds), p. 285. Interscience, New York 1968.
16. Wempen I., Miller N., Falco E. A., Fox J. J.: J. Med. Chem. *11*, 144 (1968).
17. Sasaki T., Mizuno Y.: Chem. Pharm. Bull. *15*, 894 (1967).
18. Bleaney R. C., Jones A. S., Walker R. T.: Tetrahedron *31*, 2423 (1975).
19. Warner D. T., Neil G. L., Taylor A. J., Wechter W. J.: J. Med. Chem. *15*, 790 (1972).
20. Kikugawa K., Ichino M.: J. Org. Chem. *37*, 284 (1972).
21. Grafnetterová J., Beránek J., König J., Šmahel O., Šorm F.: Neoplasma *3*, 241 (1966).
22. Beránek J., Delia T. J.: Czech. 164 401 (1971).
23. Beránek J., Delia T. J., Drašar P. in the book: *Nucleic Acid Chemistry*, Vol. 1 (L. B. Townsend, Ed.). Wiley-Interscience, in press.

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