

## Pseudouridine Transformations. Formation of 2'- and 3'-Deoxypseudouridines *via* Halogen Intermediates using $\alpha$ -Acetoxyisobutyryl Chloride

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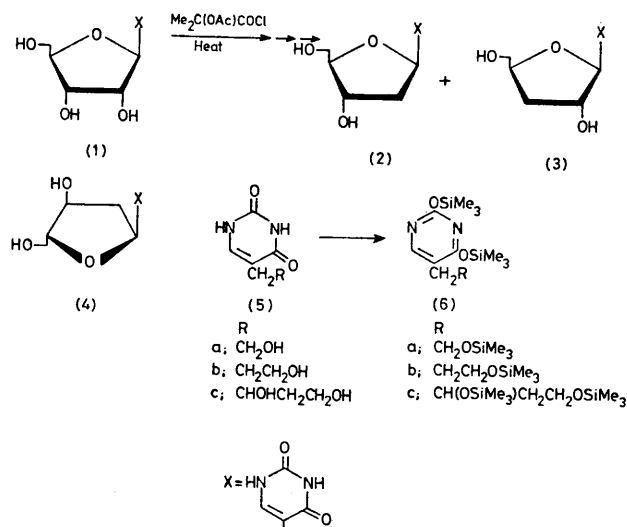
**Summary** Treatment of pseudouridine (**1**) with  $\alpha$ -acetoxyisobutyryl chloride (neat or in acetonitrile solution) at elevated temperatures followed by hydrogenolysis of the resulting mixture of chloro-sugar intermediates and deblocking gave the 2'- and 3'-deoxypseudouridines (**2**) and (**3**) (*ca.* 90%) in a ratio of *ca.* 55:45 with no evidence for the previously suggested  $\alpha$ -anomer (**4**) of 2'-deoxypseudouridine.

PSEUDOURIDINE (5- $\beta$ -D-ribofuranosyluracil) was the first naturally occurring C-nucleoside (C-glycosyl heterocycle) to be identified and studied.<sup>1</sup> The chemistry of pseudouridine ( $\psi$ ) and other C-nucleosides as well as biochemical aspects have been reviewed extensively.<sup>2</sup> The enzyme-mediated synthesis of 2'-deoxypseudouridine-5'-phosphate was reported in 1972<sup>3</sup>,<sup>4</sup> and this product was noted as an inhibitor of thymidylate synthetase.<sup>4</sup> As suggested by the reaction conditions of Michelson and Cohn,<sup>5</sup> cyclonucleoside formation is significantly more difficult with  $\psi$  than with uridine. Indeed, the easy O-2  $\rightarrow$  C-2' uridine cyclonucleoside closure employing diphenyl carbonate<sup>6</sup> is unsuccessful (O-4  $\rightarrow$  C-2') with  $\psi$ . The Mattocks-Moffatt<sup>7</sup> reagent ( $\alpha$ -acetoxyisobutyryl chloride) gives the O-2  $\rightarrow$  C-2' cyclonucleoside and/or 2'-halogeno-2'-deoxy-ribo product with uridine.<sup>7</sup> Our results with  $\psi$  show a different ultimate course and are at variance with recently published work.<sup>8-10</sup>

Treatment of  $\psi$  (**1**) with  $\alpha$ -acetoxyisobutyryl chloride (neat) at 130 °C gave a mixture of chloro-sugar intermediates. Hydrogenolysis using tri-n-butyltin hydride and deblocking gave two major products whose <sup>1</sup>H n.m.r. and electron impact mass spectra were compatible with their assignment as 2'-deoxypseudouridine (2'-d $\psi$ ) (**2**) and 3'-deoxypseudouridine (3'-d $\psi$ ) (**3**). Two groups have very recently communicated syntheses of 2'-d $\psi$  (**2**). Brown and his co-workers<sup>11</sup> employed condensation of a lithio pyrimidine and a protected aldehyde deoxy sugar to give 2'-d $\psi$  (**2**) and its  $\alpha$ -anomer, 5-(2-deoxy- $\alpha$ -D-erythro-pentofuranosyl)uracil (**4**). Watanabe *et al.* treated  $\psi$  (**1**) with  $\alpha$ -acetoxyisobutyryl chloride in hot acetonitrile and obtained a mixture containing variously blocked cyclonucleosides and 2'-chloro-2'-deoxy derivatives.<sup>8-10</sup> Hydrogenolysis of the mixture and deblocking resulted in isolation of two compounds which were assigned the  $\beta$  and  $\alpha$  2'-deoxypseudouridine structures, (**2**) and (**4**), respectively.<sup>9</sup> We have repeated the reaction of  $\psi$  and  $\alpha$ -acetoxyisobutyryl chloride in hot acetonitrile (20 h) and have again obtained (**2**) and (**3**) (*ca.* 55:45) in *ca.* 90% combined yield after hydrogenolysis and deblocking.

Our data for the structure of  $\beta$ -2'-d $\psi$  (**2**) are in agreement with the two previously noted studies.<sup>9,11</sup> However, the structure of the second deoxynucleoside is now demonstrated to be 3'-d $\psi$  (**3**) and we have observed no

evidence of any  $\alpha$ -anomer. The <sup>1</sup>H n.m.r. spectrum [(CD<sub>3</sub>)<sub>2</sub>SO] of (**3**) has a doublet of doublets at  $\delta$  4.47 for the anomeric proton (H-1') with apparent coupling constants of *ca.* 1 and 2.4 Hz. The *ca.* 1 Hz splitting results from long-range coupling with H-6 of the uracil base. The  $J_{1'-2'}$  = 2.4 Hz coupling is consistent with values observed with 3'-deoxy- $\beta$ -D-erythro-pentofuranosyl nucleosides derived from adenosine,<sup>12</sup> guanosine,<sup>†</sup> inosine,<sup>13</sup> tubercidin,<sup>14</sup> and formycin.<sup>14</sup> The 3'-methylene protons of (**3**) give rise to a relatively narrow multiplet whereas the 2'-methylene protons of the  $\alpha$ -anomer (**4**) were reported by Brown<sup>11</sup> to exhibit a distinctly separated pair of multiplet resonances. The H-4' resonance of the 3'-deoxy isomer is shifted downfield by *ca.* 0.4 p.p.m. at 100 MHz relative to that of the 2'-deoxy isomer as discussed previously.<sup>14</sup>



This trend is also apparent in reported data of Watanabe *et al.*<sup>9,10</sup> As well, the u.v. c.d. spectra of  $\psi$  (**1**), 2'-d $\psi$  (**2**), and (**3**) all have parallel negative long wavelength and positive shorter wavelength Cotton effect envelopes. The long wavelength transition amplitudes are closely similar for  $\psi$  (**1**) and (**3**), and somewhat reduced for 2'-d $\psi$  (**2**). These Cotton effects are compatible with the  $\beta$ -3'-d $\psi$  structure of (**3**), but would not be expected for an  $\alpha$ -anomer (whose c.d. spectrum should exhibit a long wavelength transition of reversed sign).<sup>15</sup>

Chemical degradation conclusively demonstrated the positional *vs.* stereo isomerism. The C-1'-O-4' bond of  $\psi$  was hydrogenolysed (rhodium catalyst<sup>1</sup>) and the ribityl product was treated with sodium metaperiodate followed

† R. Mengel and W. Muhs, unpublished data.

by sodium borohydride. The resulting 5-(2-hydroxyethyl)-uracil (**5a**) was examined by t.l.c. and was subjected to trimethylsilylation and mass spectrometric evaluation [ $m/e$  372·1718, calc. for (**6a**)  $M^+$  ( $C_{15}H_{32}N_2O_3Si_3$ ): 372·1721]. The identical reaction sequence was applied to 2'-d $\psi$  (**2**) and compound (**3**). Mass spectra were compatible with formation of 5-(3-hydroxypropyl)uracil (**5b**) [ $m/e$  386·1876, calc. for (**6b**)  $M^+$  ( $C_{16}H_{34}N_2O_3Si_3$ ): 386·1877] and 5-(2,4-dihydroxybutyl)uracil (**5c**) [ $m/e$  488·2384, calc. for (**6c**)  $M^+$  ( $C_{20}H_{44}N_2O_4Si_4$ ): 488·2378], respectively. T.l.c. indicated that (**5b**) and (**5c**) were exclusive products of the treatment of (**2**) and (**3**) with periodate-borohydride. When (**5c**) was again subjected to the oxidation-reduction sequence, no change was detected. This eliminates the possibility of prior incomplete periodate cleavage and affirms the 1,3-diol nature of (**5c**).

Thus, in contrast with the parallel reaction of uridine,<sup>7</sup>  $\psi$  is converted by  $\alpha$ -acetoxyisobutyryl chloride into a

mixture of 2'- and 3'-chlorodeoxynucleoside intermediates. Our full paper will describe reversible equilibrium mechanisms that rationalize the observed results in the published transformations of  $\psi$  based on our isolation of O-4  $\rightarrow$  C-2' (cyclonucleoside) and C-2'-C-3' (epoxide) anhydro intermediates.

Biological testing inferences<sup>9,10</sup> based on the assignment of the  $\alpha$ -anomeric structure (**4**) to 3'-deoxypseudouridine (**3**) and the products derived therefrom *via* Fox and Watanabe's heterocyclic interconversions must now be reassessed.

We acknowledge generous support from the National Cancer Institute of Canada, the National Research Council of Canada, and The University of Alberta. We thank Dr. J. R. Lake for preliminary experiments.

(Received, 17th March 1978; Com. 287.)

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