Tetrahedron Letters No. 37, pp 3911 - 3914, 1972. Pergamon Press. Printed in Great Britain.

NUCLEOSIDES LXXVII. ROTATIONAL ISOMERISM OF CERTAIN 3-β-D-GLUCOPYRANOSYL-6-METHYLURACILS [1]

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(Received in USA 17 July 1972; received in UK for publication 14 August 1972)

Recently, Yamaoka et al. [2] reported that the condensation of 6-methyluracil with tetra-O-acetyl-β-D-glucopyranosyl bromide or with tri-O-benzoyl-D-ribofuranosyl chloride by the nitromethane-$Hg(CN)_2$ method [3] afforded the 3-glycosylated pyrimidines (1 and 2a respectively). On the basis of two pairs of doublets observed by them in the nmr spectrum of the de-acylated glucosyl derivative (3), they concluded that compounds 1 and 3 were anomeric mixtures. Further, since the dialdehydes produced by periodate oxidation of 3 and 4 exhibited similar ORD curves, they claimed that the 3-ribofuranosyl nucleosides (2a and 4) were also anomeric mixtures.

We had previously reported [4] the synthesis of 2a in crystalline form by the same procedure and we assigned the 3-β-D-ribofuranosyl structure by conversion of 2a to the known [5] crystalline triacetate 2b. Indeed, we exploited compound 2a for conversion to the thiono derivative (5) and, thence, after desulfurization, to the tribenzoate of 4-methyl-1-β-D-ribofuranosyl-2-pyrimidinone (6). Compound 6 was identical with the product obtained by condensation of 4-methyl-2-pyrimidinone and tri-O-benzoyl-D-ribofuranosyl chloride in nitromethane in the presence of $Hg(CN)_2$. We had found no evidence in the synthesis of 2a or in its conversion to 5 and 6 to suggest that any of these compounds (2a, 5, 6) were anomeric mixtures.

The nmr data given by Yamaoka et al. [2] for 3-D-glucopyranosyluracil (3) do not support their claim that 3 is an anomeric mixture. They reported that 3 showed (in D_2O) $J_{1',2'}$ values of 9.33 and 9.67 Hz at δ = 5.76 and 5.65 respectively. We have repeated their synthesis of 3 and obtained values of $J_{1',2'}$ similar to those they reported. Obviously, neither splitting (of > 9 Hz) would support an α-isomer where a gauche relationship should obtain, therefore the assertion [2] that compounds 1 and 3 (and, indeed, 2 and 4) are anomeric mixtures is incorrect.

The possibility that 3 contains O-glycoside contamination is excluded since we find that treatment of 1 with methanolic hydrogen chloride gives the free nucleoside (3). O-Glycosides

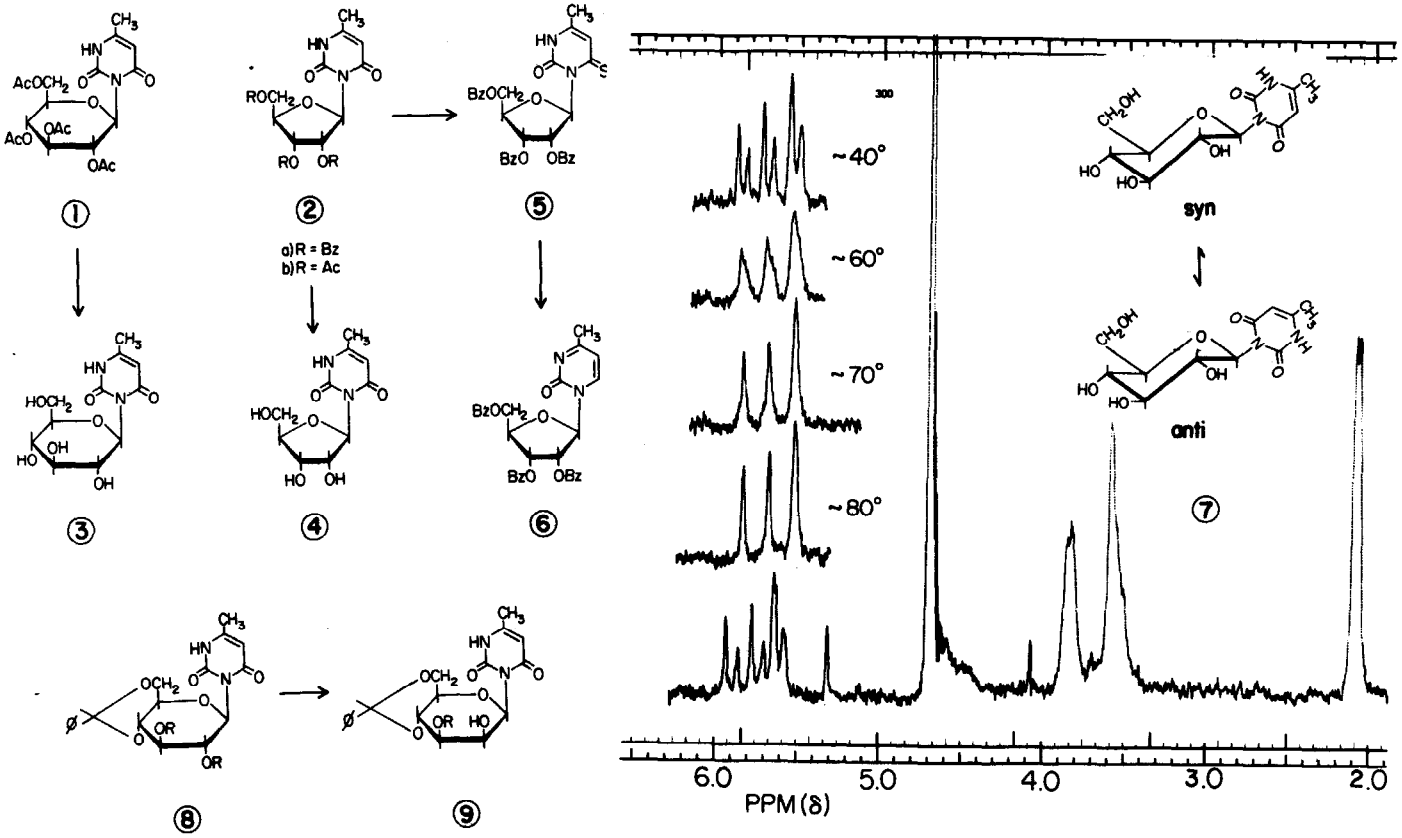

CHART 1

are solvolyzed under much milder conditions [6]. Any significant contamination of 1-4 with N-1-glycosylated derivatives is also excluded since the uv spectra of these compounds are wholly consistent with N-3 substitution [7]. We therefore investigated the possibility that 3-glucopyranosyl-6-methyluracil (3) is the beta anomer and that it exists in solution (e.g. D_2O) as a mixture of two rotational isomers (7) (syn and anti) due to restricted rotation about the glycosyl bond.

The nmr spectrum of 3-glucopyranosyl-6-methyluracil (3) in D_2O at $\sim 40^{\circ}$ gave a multiplet at $\delta \sim 5.75$ which integrated for 2 protons. The 6-methyl signal appeared at $\delta = 2.17$ as a distinct narrow doublet with a spacing of 0.7 Hz (due apparently to a long range interaction with H-5). Addition of one drop of NaOD to the sample further resolved the multiplet (Chart 1). Of these the higher field signals are easily assigned to H-5 on the basis of the small splittings caused by long range coupling with the 6-methyl protons. The remaining two doublets are therefore assigned to H-1' (anomeric signals). On heating the sample to $\sim 80^{\circ}$, all the signals at $\delta \sim 5.75$ collapsed with the formation of a doublet for the anomeric proton ($J_{1',2'} = 9.6$ Hz) and a narrow signal for H-5. On cooling this sample to $\sim 40^{\circ}$, the original spectrum gradually re-appeared (Chart 1).

These nmr data for 3 are consistent only with a β-D-nucleoside in the expected C1 conformation since any change in the sugar ring conformation away from C1 would result in a much smaller coupling for the anomeric signals. The additional fact that at higher temperature the pairs of anomeric signals coalesce into a doublet can be explained only by the existence of 3 as a mixture of two rotational isomers (7) of the beta-D-configuration in solution at lower temperature. At $\sim 80^{\circ}$, the energy barrier for the conversion of one rotational isomer into the other is overcome with resultant free rotation. The fact that addition of NaOD to the sample in D_2O helped to resolve the anomeric signals is fully consistent with the anisotropic effect of the aglycon monoanion acting differently on each of the rotational isomers (7).

The report by Yamaoka et al. [2] must therefore be re-interpreted in light of rotational isomerism. For example, they claimed that their "anomeric mixture" 3 was converted into an anomeric mixture of 4',6'-benzylidene-2',3'-di-O-mesyl derivatives (8, R = mesyl) and that these, upon treatment with base, afforded a mixture of the 2,2'- and 2,3'-anhydro derivatives of the β-D-manno and the α-D-allo configuration respectively. On hydrolysis of this mixture with NaOH, they report the isolation of pure, crystalline 3-(4,6-O-benzylidene-3-O-mesyl-β-D-

mannosyl)-6-methyluracil (9).

A rational explanation of this reaction sequence is that 8 (R = mesyl) exists as two rotamers in solution (syn and anti) analogous to 7 and that the reaction of these rotamers led to 9. We have therefore examined compounds 8 (R = H or mesyl) and have found that, indeed, they do exist (in DMSO) as a mixture of rotamers. Thus 8 (R = H) shows 2 signals for H-5 at δ = 5.48 and 5.40 and two anomeric doublets at δ = 5.73 and 5.62 ($J_{1',2'}$ = 9.0 Hz each). With 8 (R = mesyl), the H-5 signals appeared at δ = 5.50 and 5.44 and the anomeric signals occurred as a pair of doublets at δ = 6.34 and 6.23 ($J_{1',2'}$ = 9.0 Hz each). Upon heating the nmr tubes containing compounds 8, the H-5 signals collapsed to a sharp singlet while the pair of anomeric doublets merged into a sharp doublet. Upon cooling these nmr tubes, each of their original spectra reappeared.

To our knowledge, these observations are the first examples of the discrete existence of two rotamers of a pyrimidine nucleoside in solution [8] The existence of rotamers in 3 and 8 (R = H or mesyl) is obviously due in large measure to restricted rotation imposed by both carbonyl groups which flank the glycosyl linkage at N-3. The fact that a 1:1 mixture of these rotamers is not observed (the actual proportions are about 3:2) is readily explained by the differences in electronegativity of the 2- and 4-carbonyl groups which may favor to greater or lesser degree one rotamer over the other. Though rotamers have been observed with nucleosides 3 and 8 (R = H or mesyl) we were unable to see this phenomenon with the tetraacetate 1, and 8 (R = acetyl) because the chemical shifts of H-1' and H-2' of these compounds were almost identical in the solvents employed.

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1. This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA 08748).

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8. During the preparation of this manuscript, we were informed by Dr. W. Pfleiderer, Konstanz, Germany, that a similar phenomenon has been observed in his laboratory with certain β-D-glycopyranosylpteridines.