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5'-O-Benzoyl-2'-keto-3'-deoxy-α-uridine: First Unambiguous Synthesis, Crystal Structure and its use to Establish the Anomerisation of 2'-Ketouridines under Basic Conditions.

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Abstract: 5'-O-benzoyl-3'-deoxy-2'-keto-α-uridine was characterised unambiguously with the help of crystal structure analysis and provided ^{1}H-NMR data to differentiate it from its β-anomer. We have also established, with the help of the above ^{1}H-NMR data that the earlier reported procedures (ref. 4,5) of synthesising 2'-ketouridine produced a mixture of anomers.

INTRODUCTION

During the course of our studies on the reactions of sulphonylated pyrimidine nucleosides with secondary amines we observed[1-3] that 2',3'-di-O-mesyl-5'-O-trityl-*lyxo*-uridine **1a** produced[3] an anomeric mixture of 1-(2,3-dideoxy-2-N-dialkylamino-5-O-trityl-D-*glycero*-pent-2-eno-furanosyl) uracils. The structure of the α-enamine, such as **2α** was proved unambiguously with the help of crystal structure analysis. The β-enamine, such as **2β** was, however, hydrolysed and the structure of the keto- compound was established[3] by comparing its spectral data with those of the authentic 5'-O-benzoyl-2'-keto-β-uridine **3β**.

A perusal of the literature revealed that Sasaki and his co-workers synthesised 5'-O-benzoyl-2'-ketouridine by heating a mixture of 1-(5-O-benzoyl-3-O-mesyl-2-O-tosyl-β-D-*lyxo*-furanosyl) uracil[4] **1b** or 1-(2,5-di-O-benzoyl-3-O-mesyl-β-D-*lyxo*-furanosyl) uracil[5] **1c** and sodium benzoate in DMF at 120°C and 115°C respectively and reported that the compound they isolated was **3β**. However, a comparison of the spectra and melting points of the ketouridines reported by them and isolated in our laboratory had led us to think that the structure of the 2'-ketouridine reported earlier might not be the β-anomer. Our doubt about the identity of the product was further compounded by two other recent reports. Minamoto and his co-workers reported[6] the synthesis of 5'-O-benzoyl-2'-ketouridines, where 3'-O-tosyl-5'-O-benzoyl-*lyxo*uridine **1d** was treated with sodium benzoate, lithium benzoate, potassium *tert*-butoxide and sodium hydride under various conditions and anomeric mixture was isolated in each case. The second report[7] was on the synthesis of (1'R, 3'S and R, 5'S)-4'-Oxo-2'-oxabicyclo [3.1.0] hexan-3'-yl pyrimidines and purines from β-ribonucleosides; in all reported cases the products were formed as anomeric mixtures.

This paper is dedicated to Prof. Amalendu Banerjee on occasion of his 65th birthday.

RESULTS AND DISCUSSION

Synthesis of 5'-O-benzoyl-2'-keto-α-uridine **3α**. The title compound was synthesised by hydrolysing the corresponding α-enamine **2α** under strongly acidic conditions. Concurrent detritylation also occurred and the resulting keto compound was isolated as the 5'-O-benzoyl derivative. To establish that the above reaction conditions did not promote anomerisation, the known 5'-O-trityl-3'-deoxy-2'-keto-β-uridine **4** (ref. 8b) was detritylated and benzoylated under similar conditions to produce anomerically pure[3] **3β**. Compound **3α** was crystallised from methanol and the compound was subjected to crystal structure analysis to establish the structure unambiguously (**fig. 1**). The ^{1}H-NMR spectrum of **3α** was distinctly different from that of **3β** not only in terms of the chemical shift values of the respective protons but also the splitting pattern of the 3'-methylene protons which appeared around 2.97-2.63 ppm. It is evident, therefore, that on the basis of the ^{1}H-NMR spectra alone, it is possible to distingush between α and β ketouridines.

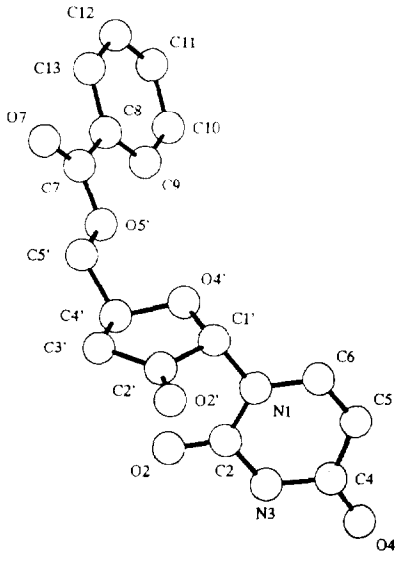


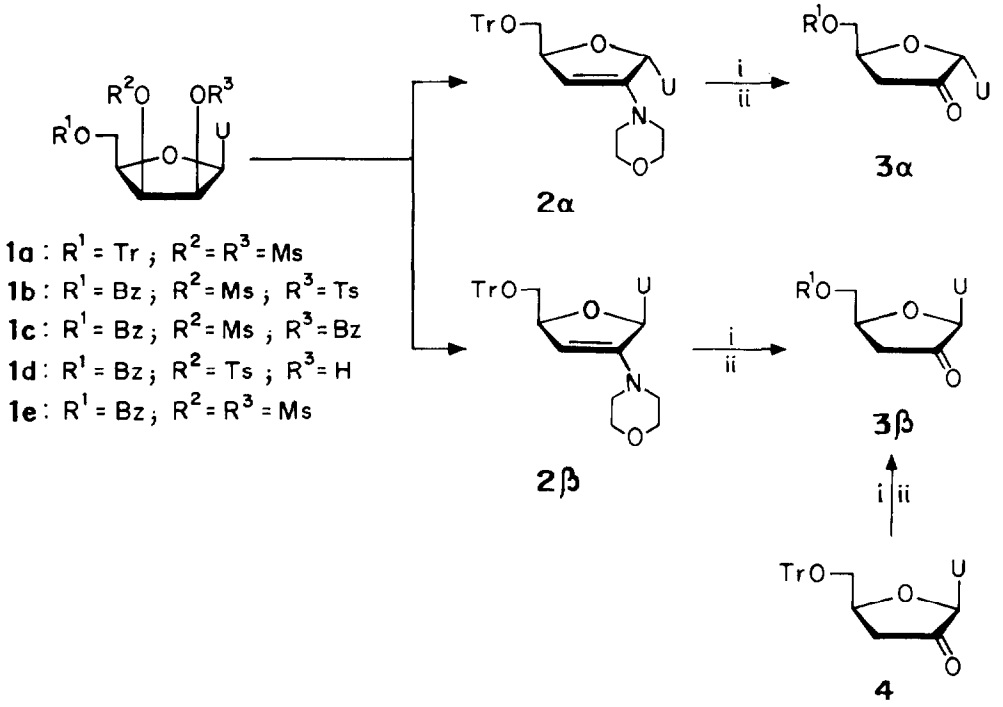
Fig. 1: PLUTO[11] Plot of the Molecule **3α** from its Crystal Structure

Reinvestigation on Sasaki's reactions: We repeated two selected but very important reactions which were reported[4,5] by Sasaki and his co-workers. Compound **1b** was heated[4] with sodium benzoate in DMF at 120°C. The reaction produced a mixture of **3α** and **3β** in roughly 1:1 ratio which was evident from the ^{1}H-NMR spectrum of the purified mixture. Almost similar result was obtained when compound **1e** was subjected to similar reaction conditions. To establish the anomerisation reaction of the 2'-ketouridine beyond doubt, the authentic keto compound **3β** was heated with sodium benzoate in DMF at 120°C for 1h; the product obtained was indeed a mixture of **3α** and **3β**.

Sasaki and his co-workers deprotected the 5'-O-benzoyl-2'-ketouridine using triethylamine in methanol at elevated temperature[5]. We subjected the authentic compound **3β** to the same reaction conditions. The deprotected products were rebenzoylated using the conditions mentioned earlier. The isolated mixture was again shown to be a 1:1 mixture of **3α** and **3β**. This experiment led us to believe that the deprotected 2'-ketouridine was indeed a mixture of anomers.

Comparison: A comparison of the selected ^{1}H-NMR data and melting points (**Table 1**) of 2'-ketouridines obtained from various sources revealed some interesting information.

Scheme-1



Scheme-2

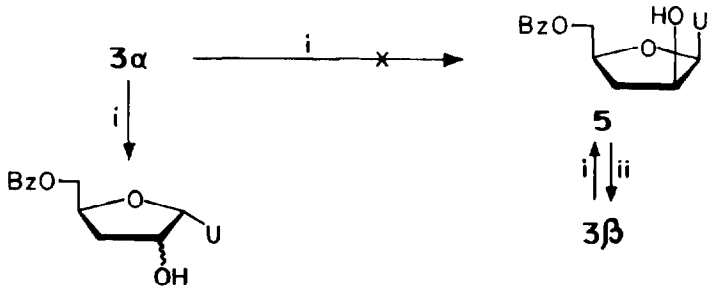


Table 1

2'-Ketouridines	H-1'	H-4'	H-3',3''
Iwasaki α-keto? (mpt 170.5-174°C)[9]	5.58	4.82	2.79 (d, J=8Hz)[9]
Sasaki β-keto? (mpt 195°C)[4]	5.60	5.00	2.80 (t)[10]
Pathak β-keto **3β** (mpt 191°C)	5.51	4.75	2.75 (d, J=7.9Hz)[3]
Pathak α-keto **3α**(mpt 195°C)	5.59	4.97	2.81 (m)

The data taken together, indicate that our α-ketouridine is closer to Sasaki's β-ketouridine (Sasaki βketo? in **Table 1**). Melting points of both the anomers synthesised by us are very close and the melting point of the mixture was 181°C. However, we assumed that Sasaki's 2'-ketouridine showing a "triplet" at 2.8 ppm (60 MHz) was infact a simplified version of the "8 lines" splitting pattern shown by our **3α** at 2.81 ppm (200 MHz) and did the following experiments. The first experiment was to reduce our **3α** with sodium borohydride which produced an inseparable mixture of two products epimeric at C-2' centers; none of the products was identical with 1-(5-O-benzoyl-3-deoxy-β-D-ara-furanosyl)uracil[5] **5** (mixed ^{1}H-NMR) whereas the single product obtained from the sodium borohydride reduction of our **3β** (generated from the enamine **2β**) was identical with compound **5** (mixed ^{1}H-NMR). On the other hand, the single 2'-ketouridine generated by the DMSO-acetic anhydride oxidation of **5** was identical to our **3β** compound. These experiments provide additional proof in support of the correctness of the structure of our compounds **3α** and **3β** .

In conclusion, we have characterised 5'-O-benzoyl-3'-deoxy-2'-keto-α-uridine **3α** unambiguously for the first time with the help of crystal structure analysis and provided ^{1}H-NMR data to differentiate it from its β-anomer. We have also established, with the help of the above ^{1}H-NMR data that Sasaki and his co-workers' procedure[4,5] of synthesising 2'-ketouridine did produce a mixture of anomers. Our observation raises doubt about the correctness of the anomeric configuration of the 5'-O-benzoyl-3'-deoxy-2'-ketouridine reported by Sasaki and his co-workers and it could possibly be concluded on the basis of ^{1}H-NMR data and melting points that the product they reported was 5'-O-Benzoyl-3'-deoxy-2'-keto-α-uridine; moreover, the free 2'-ketouridine[5] was definitely a mixture of anomers.

EXPERIMENTAL

Melting points were uncorrected. Uridine was purchased from Pharma Waldhof GmbH, Germany and used as received. Thin Layer Chromatography was performed on Merk precoated 60 F_{254} plates. Compounds were visualised on TLC plate under UV light. Column chromatographic separations were done using silica gel (Silica gel 60, 230-400 mesh, E. Merck) or basic alumina (Brockmann Grade I for Chromatography, S.D. Fine Chem. Ltd., India). ^{1}H-NMR (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on Bruker ACF200 NMR spectrometer (δ scale) using TMS or solvent chloroform-d as internal standards. Mass spectra were recorded on Finnigan MAT 1020B GC/MS.

5'-O-Benzoyl-3'-deoxy-2'-keto-α-uridine 3α from enamine 2α: A solution of compound **2α** (1mmol) and conc. HCl (3mmol) in THF/H_2O (6ml, 5:1) was heated under reflux for 12 h. After completion of the reaction, solvents were evaporated under reduced pressure. The oily residue was coevaporated with pyridine and redissolved in the same solvent (6ml). The solution was cooled at 0°C and benzoyl chloride in pyridine was added to it . After the addition, the reaction mixture was stirred at the same temperature for 2h. The reaction mixture was poured into saturated sodium bicarbonate solution and was extracted with ethyl acetate. Organic layer was dried over sodium sulphate and evaporated under reduced pressure. The oily residue was purified on silica gel column. Yield: 67 %, m.p. 195°C. ^{1}H-NMR (DMSO-d_6): δ 11.62 (bs, H) N-H; 7.99-7.46 (m, 6H)

benzoyl, H-6 ; 5.65 (d, 7.9 Hz 1H) H-5; 5.59 (s, 1H) H-1'; 4.97 (m, 1H) H-4'; 4.54-4.35 (m, 2H) H-5', 5''; 2.97-2.63 (m, 2H) H-3', 3''. ^{13}C-NMR (DMSO-d_6): δ 206.5, C-2'; 166.0, benzoyl keto; 163.8, C-4; 151.2, C-2; 145.6, C-6; 133.9, 129.7, 129.2, phenyl; 102.3, C-5; 86.67, C-1'; 76.9, C-4'; 66.85, C-5'; 37.6, C-3'. MS (EI): m/z 330 (M^+, 10%); 219 (M^+-uracil, 15%); 208 (M^+-BzOH, 25%); 195 (M^+-$BzOCH_2$, 5%).

Reactions of 1-(5-O-benzoyl-3-O-mesyl-2-O-tosyl-β-D-lyxo-furanosyl) uracil 1b with sodium benzoate. A mixture of compound **1b** (1mmol) and sodium benzoate (4mmol) in DMF was stirred at 120°C. After 3 hours the reaction mixture was poured into water (20ml) and extracted with ethylacetate (3X50ml). The ethylacetate solution was dried over anhydrous sodium sulphate and evaporated to oily material under reduced pressure. The residue thus obtained was purified on silica gel column to produce a mixture of compounds **3α** and **3β** in ratio ca. 1:1. Yield: 50%.

Reactions of 1-(5-O-benzoyl-2,3-O-dimesyl-β-D-lyxo-furanosyl) uracil 1e with sodium benzoate. A mixture of compound **1e** (1mmol) and sodium benzoate (4mmol) in DMF was stirred at 110°C. After 2h the reaction mixture was poured into water (20ml) and extracted with ethylacetate (3X50ml). The ethylacetate solution was dried over anhydrous sodium sulphate and evaporated to oily material under reduced pressure. The residue thus obtained was purified on silica gel column chromatography to give compounds **3α** and **3β** in ratio ca. 1:1.Yield: 53%.

Anomerisation reactions of 5'-O-benzoyl-3'-deoxy-2'-keto-β-uridine 3β with sodium benzoate. Compound **3β** (0.3mmol) was dissolved in a mixture of DMF (10ml) and sodium benzoate (1mmol). The solution was heated at 120°C for 1h. The reaction mixture was poured into the saturated sodium bicarbonate solution and was extracted with ethyl acetate. Ethyl acetate solution was dried over sodium sulphate and evaporated under reduced pressure. The oily residue was purified on a silica gel column. Yield: 53 %.

Anomerisation reactions of 5'-O-benzoyl-3'-deoxy-2'-keto-β-uridine 3β with triethylamine. Compound **3β** (0.3mmol) was dissolved in a mixture of methanol (10ml) and triethylamine (1ml). The solution was heated under reflux gently. After 1h an additional amount of triethylamine (1ml) was added and the mixture was left at room temperature overnight. The reaction mixture was evaporated to oily material and dissolved in pyridine (10ml). The reaction mixture was cooled to 0°C and benzoyl chloride in pyridine was added to it . After the addition, the reaction mixture was stirred at same temperature for 2h. The reaction mixture was poured into the saturated sodium bicarbonate solution and was extracted with ethyl acetate. Organic layer was dried over sodium sulphate and evaporated under reduced pressure. The oily residue was purified on a small silica gel column. Yield: 20 %.

Oxidation of 1-(5-O-benzoyl-3-deoxy-β-D-ara-furanosyl)uracil 5 with DMSO-acetic anhydride. Acetic anhydride (1ml) was added to a solution of compound **5** (1mmol) in a mixture of DMSO (9ml) and dry benzene (2ml), and the mixture was heated at 60°C for 4 hours; after cooling, ethyl acetate (50ml) was added and reaction mixture was neutralised by shaking with 5% sodium bicarbonate solution. The organic layer was then washed with water (3x10ml), dried over sodium sulphate and evaporated to oily material under reduced pressure. The residue thus obtained was purified on silica gel column chromatography to give compound **3β**. Yield: 33%.

Sodium borohydride reduction of 5'-O-benzoyl-3'-deoxy-2'-keto-β-uridine 3β. Compound **3β** (0.5 mmol) was dissolved in ethanol (5ml) and sodium borohydride (2.5 mmol) was added. After stirring at room temperature for 1.5 hours solvents were removed under reduced pressure. The residue was partitioned between chloroform (50ml) and water. The chloroform layer was dried over sodium sulphate and evaporated to dryness. The residue thus obtained was purified on silica gel column chromatography to give compound **5**. Yield: 63%; m.p. 165°C (lit. 163-165°C). ^{1}H-NMR (DMSO-d_6): δ 7.86-7.81 (m, 2H) & 7.45-7.19 (m, 4H) benzoyl, H-6 ; 5.78 (d, 3.8 Hz 1H) H-1'; 5.34 (d, 8 Hz, 1H) H-5; 5.02 (d, 4.1 Hz, 1H) OH-2';4.46-4.16 (m, 4H) H-2',4',5',5''; 2.31-2.18

(m, 1H) & 1.82-1.7 (m, 1H) H-3', 3''. ^{13}C-NMR (DMSO-d_6): δ 166.53, benzoyl keto; 164.31, C-4; 151.3, C-2; 143.0, C-6; 134.19, 130.3, 130.0, 129.5, phenyl; 100.7, C-5; 87.14, C-1'; 75.5/69.8, C-2'/ C-4'; 66.9, C-5'; 35.6, C-3'.

Sodium borohydride reduction of 5'-O-benzoyl-3'-deoxy-2'-keto-α-uridine 3α. Compound 3α (0.5 mmol) was dissolved in ethanol (5ml) and sodium borohydride (2.5 mmol) was added. After stirring at room temperature for 1.5 hours, solvents were removed under reduced pressure. The residue was partitioned between chloroform (50ml) and water. The chloroform layer was dried over sodium sulphate and evaporated to dryness. The residue thus obtained was purified on silica gel column chromatography to give an inseparable mixture of two products epimeric at C-2' centers. Yield: 60%. ^{1}H-NMR (DMSO-d_6): δ 7.81-7.75 (m, 4H) & 7.34-7.04 (m, 8H) 2x(benzoyl, H-6) ; 5.83 (d, 3.1 Hz, 1H) & 5.51 (d, 2.8 Hz, 1H) 2xH-1'; 5.36 (m, 2H) 2xH-5; 4.6-4.08 (m, 8H) 2x(H-2',4',5',5''); 2.3-1.6 (m, 2H) 2x(H-3', 3'').

Crystal structure determination of 5'-O-benzoyl-3'-deoxy-2'-keto-α-uridine 3α. The purified anomer was crystallised from methanol. Molecular formula $C_{16}H_{14}N_2O_6$; Formula weight 330.29. Unit cell was determined and X-ray data upto $2\theta_{max}$=47° were collected on an EnrafNonius CAD4 p.c. controlled diffractometer.using zirconium filtered MoKα (λ=0.71069 Å) radiation. Crystals were monoclinic in space group C 2 with unit cell parameters a=20.3374 (30), b=5.8139 (6), c=13.3621 (24) Å; β=105.97 (2)°; V=1519.0(4) (Å)^3, Z=4; D_c=1.44gm cm^{-3}; Structure was solved by direct methods using SHELXS86[12] and refined using SHELXL93[13]. Nonhydrogen atoms were treated anisotropic and hydrogens isotropic. The positions of hydrogens were determined from ideal geometry. All parameters including positions and temperature factors of hydrogens were refined. Convensional R factors at the end of the refinement was 0.0365 for 734 observed reflections (I>2σ(I)) and the goodness of fit s=0.776. Maximum and minimum electron densities in a final difference Fourier map were 0.14 and -0.20 eÅ^{-3} respectively[14].

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