Carbohydrate Research, 27 (1973) 455–459

Note

Reaction of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl) uracil with excess thionyl chloride Formation of bis[1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl) uracil] 5′-sulfite

PREM C SRIVASTAVA AND ROBERT J ROUSSEAU*

ICN Nucleic Acid Research Institute, Irvine, California 92664 (U S A)

(Received September 9th, 1972, accepted with revisions October 30th, 1972)

Recently the 9-(5-chloro-5-deoxy-β-D-ribofuranosyl) analogs of adenine and hypoxanthine have been employed in the synthesis of dinucleoside phosphates[1] These chloro compounds were conveniently prepared by the action of excess thionyl chloride on 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine and 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)hypoxanthine The subject of this report deals with reaction of the pyrimidine nucleoside 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-uracil (**1**) with excess thionyl chloride

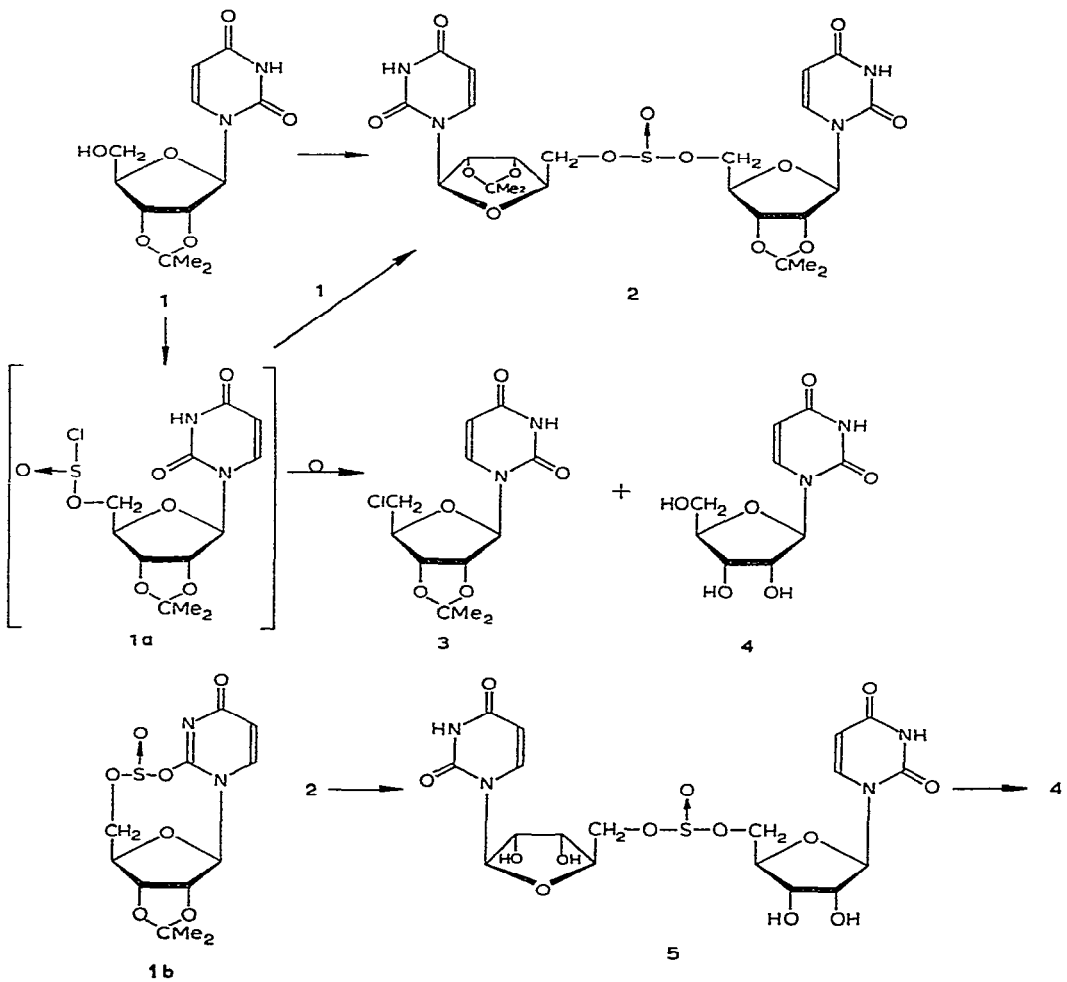
When **1** was treated with excess thionyl chloride for 16 h at room temperature, three products were isolated bis[1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil] 5′-sulfite (**2**) in 28% yield, 1-(5-chloro-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil (**3**) in 4 5% yield, and 1-β-D-ribofuranosyluracil (**4**) in 50% yield

Formation of **2** was unexpected, as the purine nucleoside derivatives previously mentioned undergo facile chlorination with excess thionyl chloride at room temperature[1] A search of the literature pertinent to this subject revealed that certain alcohols (ethanol[2], amyl alcohol[2], fatty alcohols[3]) are not easily chlorinated by excess thionyl chloride but are converted into the respective bis(sulfites) Others (methanol[2], secondary, and tertiary alcohols[3]) undergo facile chlorination with excess thionyl chloride and the bis(sulfites) can be obtained only if a limiting quantity is employed[2] A recent example was reported by May and Kaiser[4], where a limiting quantity of thionyl chloride was used to suppress chlorination in the synthesis of bis(*p*-nitrophenyl)sulfite

The differences in product formation with thionyl chloride between the purine nucleosides 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine or 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)hypoxanthine and the pyrimidine nucleoside **1** are presumably due to the properties of the respective nucleoside bases One difference is that 9-β-D-ribofuranosyladenine ($pK_a = 3\ 5$)[5] and 9-β-D-ribofuranosylhypoxanthine ($pK_a = 1\ 2$)[5] are more readily protonated than 1-β-D-ribofuranosyluracil (pK_a of

*To whom inquiries should be directed

uracil = −3 38)[6] As Darzens[7] found that excess thionyl chloride in the presence of tertiary base facilitated formation of chlorides, it was thought that the more basic nature of adenosine and inosine could be responsible for enhanced formation of the chloro derivative When this postulate was tested by addition of an equimolar quantity of 9-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine[8] to the reaction mixture of thionyl chloride and **1**, the ratio of products **2**, **3**, and **4** was unaltered Treatment of **1** with excess thionyl chloride and pyridine afforded an intractable mixture with no apparent formation of **2**, **3**, or **4**



A second difference in the properties of these nucleoside bases is that certain uracil nucleosides readily form anhydronucleosides through the 2-oxygen atom of the uracil ring The possibility of formation of the intramolecular sulfite intermediate **1b** cannot be ruled out, however, no evidence of this type of compound was found

Reaction of the intermediate chlorosulfite **1a** with **1** can explain formation of the bis(sulfite) **2**, whereas intramolecular rearrangement* of **1a** can afford the chloro compound **3** When the bis(sulfite) **2** was treated with excess thionyl chloride in which 2 equivalents of hydrogen chloride gas had been dissolved, no chloro compound could be detected This suggested that the bis(sulfite) **2** does not act as an intermediate in the formation of compound **3** When **1** was treated with ten times the amount of thionyl chloride used in the previous experiments, the amount of **2** isolated was decreased slightly, to 22 6% The yield of the chloro compound **3** under these conditions remained unchanged

The isopropylidene moieties of compound **2** were readily removed by the action of 88% formic acid to afford bis(1-β-D-ribofuranosyluracil) 5'-sulfite (**5**) This reaction proceeded without detectable cleavage of the glycosyl or sulfite bonds Treatment of **2** with 0 1M sodium hydroxide at 100° caused scission of the sulfite bonds and formation of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil (**1**)

It was concluded from this study that the reaction of excess thionyl chloride with primary hydroxyl groups of nucleosides may not provide the desired chloro compounds as major products, but instead yield bis(sulfites)

EXPERIMENTAL

General — Physical properties of these compounds were determined with the following instruments Thomas–Hoover apparatus (m p uncorrected); Cary 51 u v spectrometer (u v spectra), Hitachi Perkin–Elmer R20A High Resolution n m r spectrometer (n m r sodium 4,4-dimethyl-4-silapentane-1-sulfonate) Elemental analyses were performed by Galbraith Laboratories, Inc , Knoxville, Tenn Chromatography was conducted on Cellulose F t l c plates (Brinkman) and compounds were detected with a Minerolite (UVS 11) short-wavelength lamp Thionyl chloride utilized in this study was freshly distilled from linseed oil

Bis[1-(2,3-O-isopropylidene-β-D-ribofuranosyl)uracil] 5'-sulfite (**2**) — 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)uracil[12] (**1**, 2 84 g, 10 mmole) was dissolved in thionyl chloride (10 ml) and kept for 16 h at ~25° with exclusion of moisture Excess thionyl chloride was removed under diminished pressure below 25° and the remaining traces were removed by evaporation of benzene from the residue The latter was taken up in dichloromethane (25 ml), cooled, and an ice-cold mixture of triethylamine (2 ml) and ethanol (5 ml) was added. This well shaken mixture was kept for 10 min at 0° and then evaporated *in vacuo* to dryness The residue was again taken up in dichloromethane (25 ml) and washed with water (15 ml, 3 times) The aqueous layer (*A*) contained 1-β-D-ribofuranosyluracil (**4**) and was utilized in the isolation of **4**

The organic layer was dried ($MgSO_4$) filtered, and evaporated *in vacuo* to dryness The residue was fractionally crystallized from an excess (~100 ml) of boiling water to yield 875 mg (28 3%) of **2**, m p 195° (softened ~130°), u v , λ_{max}^{pH1} 258 5 nm

*For a discussion of the mechanism of reaction of thionyl chloride with alcohols, see Ref 9

(ε 19,730), λ_{max}^{pH11} 259 nm (ε 14,950), R_F (butanol saturated with water) 0 82, (4 1 5 butanol–acetic acid–water) 0 89, n m r (Me_2SO-d_6) δ 11 5 (s, 2, 1-NH), 7 7 (d, 2, $J_{5,6}$ 8 Hz, 6-CH), 5 85 (s, 2, H-1′), 5 7 (d, 2, 5-CH), 5 1 (d, 2, $J_{2',3'}$ 5 Hz, H-2′), 4 8 (d, 2, H-3′), 4 2 (s, 6, H-4′, H-5′)

Anal Calc. for $C_{24}H_{32}N_4O_{13}S$ C, 46 74, H, 5 23, N, 9 08, S, 5 19 Found C, 46 94, H, 4 80, N, 8 94, S, 4 86

*1-(5-Chloro-5-deoxy-2,3-*O*-isopropylidene-β-*D*-ribofuranosyl)uracil* (**3**) — The mother liquor from the filtration of **2** was concentrated to low volume (~15 ml), cooled, and the resultant pale-yellow crystals were filtered and recrystallized from water to yield 136 mg (4 5%) of **3**, m p 167° (reported[10] 175–177°, also[11] 180–181°), R_F (4 1 5 butanol–acetic acid–water) 0 85

Anal Calc for $C_{12}H_{15}ClN_2O_5$ C, 47 61, H, 4 99, Cl, 11 71, N, 9 25 Found C, 47 48, H, 5 04, Cl, 11 90, N, 9 13

*1-β-*D*-Ribofuranosyluracil* (**4**) — The filtrate from the crystallization of **3** was combined with the aqueous extract (*A*) and evaporated *in vacuo* to dryness The residue was crystallized from methanol containing a trace of water to afford 1 2 g (50%) of **4**, m p 162–163° This product was identical with authentic 1-β-D-ribofuranosyluracil by mixed m p and by R_F values in several solvent systems

Formation of compounds **2**, **3**, *and* **4** *with tenfold dilution of thionyl chloride* — Compound **1** (2 84 g, 10 mmole) was dissolved in thionyl chloride (100 ml) and the reaction mixture was kept for 16 h at ~25° The solution was treated according to the previous procedures to yield 700 mg (22 6%) of **2**, 130 mg (4 5%) of **3**, and 500 mg of uridine Compound **1** was detected in the reaction mixture by t l c, but was not isolated

*Bis(1-β-*D*-ribofuranosyluracil) 5′-sulfite* (**5**) — Bis[1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil] 5′-sulfite (**2**, 154 mg, 0 25 mmole) was dissolved in 88% formic acid (1 0 ml) and kept for 5 h at ~25° in a stoppered flask The formic acid was removed under diminished pressure, water (2 ml) was added to the residue, and the mixture was again evaporated *in vacuo* to dryness This process was repeated twice, and the residue obtained was triturated with acetone, filtered, and dried to afford 125 mg (93%) of **5** Recrystallized from a mixture of water and acetone, the product had m p 200°, R_F (butanol saturated with water) 0 05, (4 1 5 butanol–acetic acid–water) 0 23, u v λ_{max}^{pH1} 259 nm (ε 17820), λ_{max}^{pH11} 260 nm (ε 14486)

Anal Calc for $C_{18}H_{22}N_4O_{13}S$ C, 40 45, H, 4 14, N, 10.48, S, 5 99. Found C, 40 23, H, 3 90; N, 10 24, S, 5 75

Alkaline hydrolysis of **2** *formation of 1-(2,3-*O*-isopropylidene-β-*D*-ribofuranosyl)-uracil* (**1**) — To 0.1M sodium hydroxide (10 ml) was added **2** (154 mg, 0 25 mmole) and the solution was refluxed for 3 h The reaction mixture was evaporated *in vacuo* to a volume of 2 ml and then the pH of the solution was adjusted to 6 by the dropwise addition of dilute acetic acid while the temperature of the solution was maintained at 0–5°. The solution was kept for 1 h at 0° and then the solid (100 mg) that separated was filtered off and dried An additional 15 mg of **1** could be obtained by concentration of the filtrate, total yield of **1** 110 mg (81%), m p and m m p. 161–162°, having the

same R_F values as an authentic sample[12] of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil (**1**)

REFERENCES

1 P C SRIVASTAVA, K L NAGPAL, AND M M DHAR, *Experientia*, 25 (1969) 356
2 L CARIUS, *Ann Chem*, 111 (1859) 93
3 R. H CLARK AND H R L STREIGHT, *Trans Roy Soc Canada*, Section III (1929) 77
4 S W MAY AND E T KAISER, *J Amer Chem Soc*, 91 (1969) 6491
5 H A SOBER (ed), *Handbook of Biochemistry, Selected Data for Molecular Biology*, Chemical Rubber Co, Cleveland, Ohio, 1968, p G21 and G35
6 R SHAPIRO AND M DANZIG, *Biochemistry*, 11 (1972) 23
7 M G DARZENS, *C R Acad Sci Paris*, 1314 (1911) 1601
8 G HUBER, *Chem Ber*, 89 (1956) 2853
9 J MARSH, *Advanced Organic Chemistry Reactions, Mechanisms, and Structure*, McGraw-Hill Book Co, New York, 1968 p 268
10 R F DODS AND J S ROTH, *J Org Chem*, 34 (1969) 1627
11 J P H VERHEYDEN AND J G MOFFATT, *J Org Chem*, 37 (1972) 2289
12 P A LEVENE AND R S TIPSON, *J Biol Chem*, 106 (1934) 113