Carbohydrate Research, 43 (1975) 208–212

Note

The action of alkali on keto-nucleosides: the formation of saccharinic acid nucleosides during the alkaline degradation of 7-(6-deoxy-3,4-*O*-isopropylidene-*β*-L-*lyxo*-hexopyranosylulose)theophylline

THÉRÈSE HALMOS* AND KOSTAS ANTONAKIS

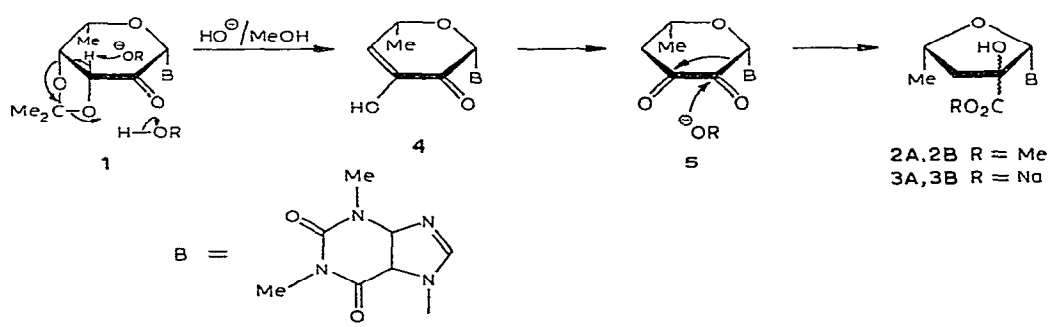
Institut de Recherches Scientifiques sur le Cancer du C.N.R.S., 94 800–Villejuif (France)

(Received December 10th, 1974; accepted for publication, February 17th, 1975)

The use of ketohexosylpurines as synthetic intermediates is based on their stability, particularly in alkaline media. Unlike ketopentosylpyrimidines, which are instantaneously decomposed[1] in alkaline media, ketohexosylpurines react slowly and often without concomitant glycosidic cleavage; some nucleophilic additions have been recently reported[2]. Moreover, the growth inhibitory activity exhibited by hexosylulose-purines[3] prompted us to investigate their stability in relation to avoiding the formation of toxic degradation products.

We now report on the action of alkali on 7-(6-deoxy-3,4-*O*-isopropylidene-*β*-L-*lyxo*-hexopyranosylulose)theophylline (**1**) recently reported as a synthetic intermediate[4] and cell-growth inhibitor[3].

The protected keto-nucleoside **1**, obtained[4] by direct oxidation using the Pfitzner–Moffatt system[5], was treated with 0.01–2M methanolic sodium hydroxide at room temperature. When the reaction was monitored by t.l.c., two products (**2A** and **2B**) were revealed together with a minor spot having the same R_F value as theophylline; **2A** and **2B** were gradually converted into **3**. The rate of these reactions was directly proportional to alkali concentration.



*Attaché de Recherche à l'I.N.S.E.R.M.

It was subsequently shown that **2A** and **2B** were 7-(3,5-dideoxy-2-*C*-methoxycarbonyl-β-L-*erythro*-pentofuranosyl)theophylline and its *threo* isomer, and **3A** and **3B** the sodium salts of the corresponding 2′-*C*-carboxy derivatives.

The degradation products did not reduce alkaline silver nitrate and reacted very slowly with 30% sulphuric acid at 100°, giving yellowish spots which fluoresced at 360 nm.

During the reaction of **1**, the u.v. absorbance at 272.5 nm decreased slightly during the first hour and a broad absorption at 300–400 nm appeared. Thereafter, this peak declined, and after 2 h, there was no absorbance above 300 nm. Simultaneously, after 1 h, the absorbance at 272 nm started to increase and reached a final value after ~12 h. The absorption curves showed an isosbestic point at ~260 nm, indicating an ionization equilibrium.

In comparison with protected pyrimidine ketonucleosides, the cleavage of the *N*-glycosidic bond in **1** was not a major reaction pathway. Thus, in 0.1M sodium hydroxide, during the first 10 min, only 10% of the theophylline was liberated and this increased to 20% after 2 h.

Treatment of methyl 4-alkoxyribopyranosiduloses[6,7] with alkali results in elimination of the 4-alkoxy group, giving an α,β-unsaturated ketoglycoside. The same type of reaction was postulated by Cook and Moffatt[1] for protected pyrimidine ketonucleosides. Furthermore, it was proposed[8] that an intermediate in saccharinic acid formation would be an α-dicarbonyl compound, which undergoes a benzylic acid type of rearrangement. Such intermediates have been isolated[9].

The products formed when **1** was treated with 0.1M sodium hydroxide did not give a red, complex nickel salt after treatment with hydroxylamine, characteristic of α-dicarbonyl compounds. The reaction mixture contained at least three products, each having an intact glycosidic bond, and these were subsequently isolated. When the reaction was interrupted after 1 hour, **2A** and **2B** could be isolated, and, after 7 h, **2A**, **2B**, and **3** were present in approximately equal proportions, and **3** was fractionated chromatographically into the isomers **3A** and **3B**.

Elemental analysis, and i.r. and n.m.r. spectroscopic data (Table I) indicated **2A**/**2B** and **3A**/**3B** to be pairs of isomers, namely, the methyl esters and sodium salts of 7-(3,5-dideoxy-2-*C*-carboxy-β-L-*erythro*-pentofuranosyl)theophylline and the *threo* isomer.

The esters **2A** and **2B** reacted with alkaline hydroxylamine, producing hydroxamic acids, which formed red iron-complexes with ferric chloride, and **3A** and **3B** could be esterified with methanol, giving **2A** and **2B**, respectively. In aqueous sodium hydroxide, **2A** and **2B** yielded **3A** and **3B**, respectively.

The configurational assignment at C-2′ was based on the observation by Hruska *et al.*[11] that, in a flexible furanose system, the chemical shift for H-1′ is influenced by the differential shielding effects of HO-2′. Thus, H-1′ is more shielded by a *cis* than by a *trans* HO-2′. Thus, in the pair of isomers **2A**/**2B**, H-1′ is shielded more in **2A**. Therefore HO-2′ and H-1′ are *cis*, *i.e.*, **2A** is the *erythro* and **2B** the *threo* isomer. This assignment is consistent with the chemical-shift differences of the ester

TABLE I

N.M.R. DATA FOR SACCHARINIC ACID NUCLEOSIDES

Compound	*Chemical shifts (δ p.p.m.)*[a,b]							
	H-1'	*H-3'a*	*H-3'b*	*H-4'*	*H-5'*	*H-8*	N-*Me*	O-*Me*
2A	6.39	2.17	2.82	4.98	1.50	7.87	3.41 3.60	3.40
2B	6.77	2.39	2.39	4.71	1.45	7.87	3.33 3.53	3.94
3A	6.41	1.98	2.87	4.93[d]	1.48	8.10	3.42 3.60	
3B	6.90	2.35	2.35	4.70[d]	1.41	8.17	3.37 3.58	
	J values (Hz)							
	3'a,3'b	*3'a,4'*	*3'b,4'*	*4',5'*				
2A	13	9.5	5.5	6.0				
2B		7.6[c]	7.6[c]	6.0				
3A	13.2	7.6	6.2	6.2				
3B		9.5	5.0	6.0				

[a]Internal tetramethylsilane. [b]Solvents: $CDCl_3$ for **2A,B**; CD_3OD for **3A,B**. [c]$J \approx 1/2(J_{3'a,4'} + J_{3'b,4'})$. [d]Signal partially obscured by the HDO resonance.

methyl groups; greater deshielding occurs in the *threo* isomer, because of the proximity of the COOMe group to the C=O group at position 6 in the nitrogenous base (Table I).

The mechanism of the formation of the branched-chain pentofuranosylpurines may be postulated as follows.

The attack by base on H-3' in **1** gives the conjugated keto-nucleoside **4** *via* the acetal, as postulated for 2',3'-*O*-benzylideneuridine[10]. Isomerisation of the enediol **4** into an α-dicarbonyl intermediate **5** is followed by a benzilic acid rearrangement, leading to the saccharinic acid nucleosides **2A** and **2B**.

The esters **2A** and **2B** were weakly active against KB tumour cells at 0.7 mg/ml, whereas keto-nucleoside **1** was more active at the same dose[3]. It is noteworthy that the *erythro* isomer **2A** showed the greatest inhibitory activity, particularly on the second day.

EXPERIMENTAL

General methods. — U.v. spectra were measured with a Jobin–Yvon multi M VI spectrophotometer. I.r. spectra were determined for potassium bromide pellets by use of a Perkin–Elmer Model 137 spectrometer. N.m.r. spectra were recorded with a Varian T-60 instrument, and decoupling was effected with a Varian T-6059 spin

decoupler, using the frequency-sweep mode. Optical rotations were determined with a Roussel–Jouan "Quick" polarimeter. Melting points are uncorrected.

T.l.c. was performed on silica gel HF (Merck), using *A* chloroform–methanol (9:1), *B* ethyl acetate–methanol (9:1), and *C* ethyl acetate–methanol (8:2). Nucleoside spots were detected by visual examination under u.v. light.

*Alkaline treatment of 7-(6-deoxy-3,4-O-isopropylidene-β-*L*-lyxo-hexopyranosyl-ulose)theophylline*[4] (**1**). — A solution of 200 mg of **1** in methanol (22.5 ml) and 0.2M methanolic sodium hydroxide (22.5 ml) was stored under anhydrous conditions, and monitored by t.l.c. (solvent *A*) and u.v. spectroscopy. The following R_F values were observed: **2A** 0.75, **2B** 0.65, **3A,B** 0.05. After 7 h, the reaction was stopped by neutralization with Dowex-50W-X (H^+) resin, the mixture was filtered and concentrated *in vacuo*, and the residue was subjected to p.l.c. on six plates (20 × 20 cm), using three consecutive developments with solvent *A*; the running distances were 5, 10, and 15 cm, respectively. Elution of the products in the two faster-moving, well-separated bands with methanol gave 7-(3,5-dideoxy-2-*C*-methoxycarbonyl-β-L-*erythro*-pentofuranosyl)theophylline (**2A**, 42 mg) and the *threo* isomer **2B** (38 mg).

Isomer **2A** had m.p. 119–122°, $[\alpha]_D^{20}$ +40° (*c* 0.1, methanol), λ_{max}^{MeOH} 275 nm (ε 7500), ν_{max}^{KBr} 1750 (ester C=O) and 1440 cm^{-1} (COOMe).

Anal. Calc. for $C_{14}H_{18}N_4O_6$: C, 49.7; H, 5.33; N, 16.56. Found: C, 49.9; H, 5.33; N, 16.1.

Isomer **2B** had m.p. 139–142°, $[\alpha]_D^{20}$ +110° (*c* 0.1, methanol), λ_{max}^{MeOH} 273 nm (ε 8400), ν_{max}^{KBr} 1740 (ester C=O) and 1430 cm^{-1} (COOMe).

Anal. Found: C, 49.9; H, 5.6; N, 16.2.

The n.m.r. data for **2A** and **2B** are given in Table I.

The product in the slower-moving band was eluted with methanol, the solution was concentrated, and the residue was chromatographed on four plates (20 × 20 cm) using four consecutive developments with solvent *B* and four with solvent *C*, which cleanly separated two very close bands. The product in the slower band was eluted with methanol to give the sodium salts of 7-(3,5-dideoxy-2-*C*-carboxy-β-L-*erythro*-pentofuranosyl)theophylline (**3A**, 25 mg) and the *threo* isomer **3B** (26 mg).

Isomer **3A** decomposed at 210°, and had $[\alpha]_D^{20}$ +90° (*c* 0.1, methanol), λ_{max}^{MeOH} 275 nm (ε 6560), ν_{max}^{KBr} 1610 cm^{-1} (COO^-).

Anal. Calc. for $C_{13}H_{15}NaN_4O_6 \cdot 2H_2O$: C, 39.3; H, 4.97; N, 14.63. Found: C, 39.4; H, 4.83; N, 13.4.

Isomer **3B** decomposed at 252°, and had $[\alpha]_D^{20}$ +30° (*c* 0.1, methanol), λ_{max}^{MeOH} 273 nm (ε 6640), ν_{max}^{KBr} 1620 cm^{-1} (COO^-).

Anal. Found: C, 38.9; H, 4.50; N, 13.8.

The n.m.r. data for **3A** and **3B** are given in Table I.

Esterification of **3A** *and* **3B**. — To a solution of **3A** (or **3B**) (12 mg) in methanol (1 ml) was added 10 mg of Dowex-50 W-X (H^+) resin, and the mixture was heated under reflux with vigorous stirring for 2 h. The filtered mixture was concentrated, and the residue was purified by t.l.c. (solvent *A*). The crystalline material was identified as **2A** (or **2B**) by m.p., and i.r. and n.m.r. spectroscopy.

REFERENCES

1 A. F. Cook and J. G. Moffatt, *J. Amer. Chem. Soc.*, 89 (1967) 2697; U. Brodbeck and J. G. Moffatt, *J. Org. Chem.*, 35 (1970) 3552.
2 F. Leclercq, M. Bessodes, J. Jumelet, and K. Antonakis, *J. Carbohyd. Nucleosides Nucleotides*, 1 (1974) 349.
3 K. Antonakis and I. Chouroulinkov, *Compt. Rend.*, 273D (1972) 2099; *Biochem. Pharmacol.*, 23 (1974) 2095.
4 K. Antonakis, *Carbohyd. Res.*, 24 (1972) 229.
5 K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, 85 (1963) 3027; 87 (1965) 5670.
6 O. Theander, *Tappi*, 48 (1965) 105.
7 L. Kenne and S. Svensson, *Acta Chem. Scand.*, 26 (1972) 2144.
8 J. C. Sowden, *Advan. Carbohyd. Chem.*, 12 (1957) 36.
9 E. F. L. J. Anet, *J. Amer. Chem. Soc.*, 82 (1960) 1502; R. L. Whistler and J. N. BeMiller, *ibid.*, 82 (1960) 3705; H. P. Humphries and O. Theander, *Acta Chem. Scand.*, 25 (1971) 883.
10 G. H. Jones, J. P. H. Verheyden, and J. G. Moffatt, *Int. Congr. Pure Appl. Chem., XXI, Prague*, 1967, Abstract N-26.
11 F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 92 (1970) 4088.