Studies on 7-(3,5-Dideoxy-2-*C*-methoxycarbonyl-β-L-pentofuranosyl)-theophyllines. Configuration at the Branch Point and Biological Activity

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The structures of the recently prepared ' saccharinic acid ' nucleosides have been established by chemical and spectroscopic means. Reduction of 7-[3,5-dideoxy-2-*C*-methoxycarbonyl-β-L-*erythro*-(and *threo*-)pentofuranosyl]theophylline (1A and 1B) with sodium borohydride gave 7-[3,5-dideoxy-2-*C*-hydroxymethyl-β-L-*erythro*-(and *threo*-)pentofuranosyl]theophylline (2A and B). Selective tosylation of (2A and B) produced the 2'-tosyloxymethyl derivatives (3A and B). On heating in dioxan (3A) gave the cyclonucleoside (4A), indicating the *erythro*-structure of (2A) and consequently the *threo*-structure of (2B). The *erythro*-isomer showed significant growth inhibitory activity against KB cells, whereas the *threo*-hydroxymethyl derivative was inactive under the same conditions.

We have recently [1] reported the isolation and the characterization of the ' saccharinic acid ' nucleosides formed during the alkaline degradation of a protected ketohexosylpurine. The configurations of 7-(3,5-dideoxy-2-*C*-methoxycarbonyl-β-L-*erythro*-pentofuranosyl)theophylline (1A) and its *threo*-isomer (1B) were assigned on the basis only of n.m.r. spectroscopic data. We now report a study of the structure of these new branched-chain sugar nucleosides and the direct proof of the configuration at the branch point. This structure determination was particularly important in order to evaluate comparatively the biological activity of both isomers.

Under the usual experimental conditions carboxylic esters are resistant to reduction with sodium borohydride. However, there are some examples (steroidal esters [2] and methyl esters of uronic acid glycosides [3]) where such reduction occurs. By use of a large excess of sodium borohydride at elevated temperature, esters of heterocyclic, aromatic, and aliphatic acids can be reduced, although the reduction is much slower than that for aldehydes and ketones.[4]

When sodium borohydride was added to a methanolic solution of (1A or B), reduction occurred at room temperature and the reaction was complete within 40 min in the case of (1A) and 80 min in the case of (1B). The hydroxymethyl derivatives (2A and B) were isolated and purified by chromatography and crystallization. The structures of (2A and B) were established by the lack of ester carbonyl bands in the i.r. spectra and by the appearance of a new CH_2 signal and the lack of the ester methyl signal in the n.m.r. spectra.

Valuable structural information has been obtained by observation of the cleavage of a hydrogen bond between

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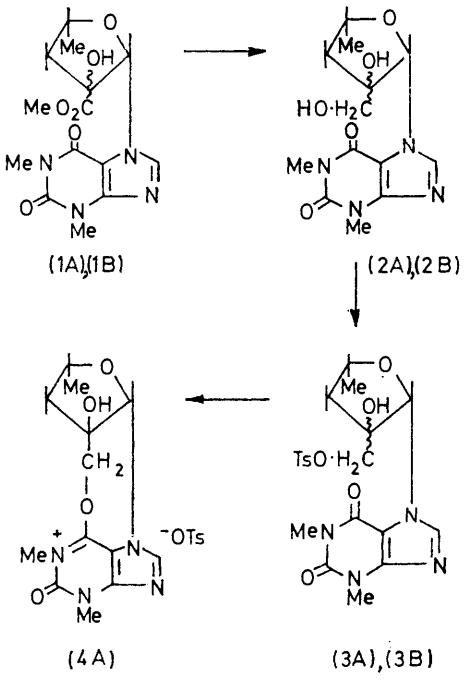
[2] H. Heyman and L. F. Fieser, *J. Amer. Chem. Soc.*, 1951, **73**, 5252.

[3] M. L. Wolfrom and K. Anno, *J. Amer. Chem. Soc.*, 1952, **74**, 5583.

[4] M. S. Brown and H. Rapoport, *J. Org. Chem.*, 1963, **28**, 3261.

a sugar hydroxy-group and the 2-carbonyl group of cytosine or uracil in alkaline medium,[5,6] which is reflected by changes in u.v. absorption.[5] However, molecular models of (2A and B) showed that both should be capable



of forming a hydrogen bond between the 6-oxo-group of the purine and either the CH_2OH branch or the 2-OH of the sugar, and their u.v. spectra would therefore not be expected to exhibit marked differences in the high pH range, where ionisation of sugar OH occurs.

As far as the stereochemistry at C-2′ is concerned, n.m.r. analysis of coupling patterns did not give any information. However, the coupling constants yielded an indication of the possible ring puckerings existing in (2A and B), and thus of whether cyclonucleoside formation could be used as unequivocal proof of structure. Calculations based on measured $J_{3'a,4'}$ and $J_{3'b,4'}$ values for structures (2A and B) (see Experimental section) with appropriate dihedral angles indicated that for compound (2B) there are four possible conformations. Of these, models show that only the twist conformation $^2T_3$ is likely. In the case of compound (2A) it is concluded that a rapid equilibrium exists between the ^2E and $^2T_3$ conformations. For these conformations, a *cis*-relationship between the purine base and a 2′-tosyloxymethyl group is necessary if cyclonucleoside formation is to occur.

Selective tosylation of compounds (2A and B) gave the monotosylates (3A and B). These derivatives had the same coupling constants as the parent compounds. When (3A) was heated in dioxan, cyclisation occurred to give (4A). From (3B) no cyclonucleoside was formed. Compound (4A) was characterized by its n.m.r. and u.v. spectra. The u.v. spectrum ($A_{270}/A_{250} = 1.40$) showed differences from that of (3A) ($A_{270}/A_{250} = 2.63$), reflecting changes in electron distribution of the purine system, consistent with cyclonucleoside formation. The n.m.r. spectrum of (4A) showed downfield shifts of N-1 and N-3 methyl signals indicating quaternization. The N-1 methyl shift (−0.18 p.p.m.) was greater than that of the N-3 methyl (−0.07 p.p.m.), consistent with the observation [7] that in xanthines the latest dissociating proton was that at N-1. This signifies that N-1 is less electronegative, and therefore more liable to be quaternized. On the basis of these results, it seems likely that (2A) has the *erythro*- and (2B) the *threo*-configuration. Furthermore, this indicates that the initial configurational assignment of the precursors (1A and B) was correct.

Biological assays were performed [8] in relation to the growth inhibitory activity exhibited by 7-(6-deoxy-3,4-*O*-isopropylidene-β-L-*lyxo*-hexopyranosulosyl)theophylline. Only the *erythro*-isomer (2A) inhibited KB cancerous cell growth at 0.7 mg ml^{-1}; the methyl ester (1A) of the parent ' saccharinic acid ' exhibited a lower inhibitory activity at the same dose. No inhibitory activity was observed for the sodium salt of the same 2′-*C*-carboxy-derivative.

EXPERIMENTAL

U.v. spectra were measured with a Varian UV-VIS M 635 spectrophotometer. I.r. spectra were determined for potassium bromide pellets by use of a Perkin-Elmer 137 spectrometer. N.m.r. spectra were recorded with a Varian T-60 instrument; decoupling was effected with a Varian T-6059 spin decoupler, operating in the frequency-sweep mode. Optical rotations were determined with a Roussel-Jouan Quick polarimeter. T.l.c. was performed on silica gel 60 PF (Merck) in (*A*) chloroform–methanol (9 : 1) or (*B*) water-saturated ethyl acetate.

7-(3,5-*Dideoxy*-2-C-*hydroxymethyl*-β-L-erythro-*pentofuranosyl*)*theophylline* (2A) *and its* threo-*Isomer* (2B).—A solution of the ester (1A) (50 mg) in methanol (1.5 ml) was reduced with sodium borohydride (10 mg). The reaction was followed by t.l.c. After 40 min, the mixture was diluted with water (1 ml), acidified with Dowex 50 W-X (H^+) resin in order to destroy the excess of sodium borohydride, and neutralized with Amberlite IR 45 (OH^-) resin to remove borate ions. The filtered solution was concentrated *in vacuo*, and the residue was purified by preparative t.l.c. [solvent (*B*)] giving the crystalline *alcohol* (2A), m.p. 144—146°, $[\alpha]_D^{20}$ +68° (*c* 0.1 in MeOH) R_F (*A*) 0.297, R_F (*B*) 0.256, $\lambda_{max.}$ (MeOH) 275 nm (ε 8 000), δ ($CDCl_3$) 7.85 (1 H, s, H-8), 6.31 (1 H, s, H-1′), 4.70 (1 H, m, H-4′), 3.62 (3 H, s, 3-Me), 3.43 (3 H, s, 1-Me), *ca.* 3.34 (2 H, branch CH_2, partially overlapped with N·CH_3), 2.47 (1 H, q, $J_{3'a,4}$ 6.4, $J_{3'a,3'b}$ 13.1 Hz, H-3′$_a$), 1.95 (1 H, q, $J_{3'b,4'}$ 7.1 Hz, H-3′$_b$), and 1.50 (3 H, d, $J_{4',5'}$ 6.1 Hz, H-5′) (Found: C, 50.5; H, 5.85; N, 17.5. $C_{13}H_{18}N_4O_5$ requires C, 50.3; H, 5.8; N, 18.0%).

The threo-*isomer* (2B) (20 mg) was obtained in the same manner, except that the reaction was stopped after 80 min, and for chromatographic purification solvent (*A*) was used; m.p. 205° (decomp.; water lost at 86—89°), $[\alpha]_D^{20}$ +80° (*c*

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0.1 in methanol), R_F (*A*) 0.275, R_F (*B*) 0.308, $\lambda_{max.}$ (MeOH) 275 nm (ε 7 900), δ ($CDCl_3$) 7.95 (1 H s, H-8), 6.50 (1 H, s, H-1′), 4.82 (1 H, m, H-4′), 3.90 (1 H, d, J_{gem} 13 Hz) and 3.59 (1 H, d) (2′-CH_2), 3.58 (3 H, s, 3-Me), 3.38 (3 H, s, 1-Me), 2.25 (1 H, q, $J_{3'a,4'}$ 5.0, $J_{3'a,3b'}$ 13.2 Hz, H-3′a), 1.83 (1 H, q, $J_{3'b,4'}$ 9.5 Hz, H-3′b), and 1.37 (3 H, d, $J_{4',5'}$ 6.1 Hz, H-5′) (Found: C, 47.6; H, 6.2; N, 16.5. $C_{13}H_{18}N_4O_5$, H_2O requires C, 47.6; H, 6.1; N, 17.0%).

7-[3,5-*Dideoxy*-2-C-(p-*tolylsulphonyloxymethyl*)-β-L-erythro-*pentofuranosyl*]*theophylline* (3A) *and its* threo-*Isomer* (3B). *Cyclisation of the Former.*—A solution of the alcohol (2A) (3.8 mg, 1.22 × 10^{-2} mol) in dry pyridine (0.15 ml) was cooled to 0 °C and toluene-*p*-sulphonyl chloride (5.3 mg, 2.78 × 10^{-5} mol) was added. The mixture was stirred at 0 °C for 2 h, then stored at 4 °C for 18 h. The excess of sulphonyl chloride was decomposed with water (1 drop) at 0 °C. The mixture was poured into ice–water (2 ml) and extracted with chloroform (4 × 1 ml). The extracts were washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated to dryness *in vacuo*. The *tosylation product* was separated by t.l.c. [solvent (*A*)]; m.p. 168—169°, $[\alpha]_D^{20}$ +30° (c 0.1 in methanol), R_F (*A*) 0.792, R_F (*B*) 0.606, $\lambda_{max.}$ (MeOH) 273 nm (ε 6 800; A_{270}/A_{250} 2.63), δ ($CDCl_3$) 8.0 (1 H, s, H-8), 7.51 (4 H, AA′BB′ system, aromatic H of tosyl), 6.30 (1 H, s, H-1′), 4.81 (1 H, m, H-4′), 4.02 (1 H, d, J_{gem} 16 Hz) and 3.87 (1 H, d), (2′-CH_2), 3.70 (3 H, s, 3-Me), 3.40 (3 H, s, 1-Me), 2.66 (1 H, q, $J_{3'a,4'}$ 6.4, $J_{3'a,3'b}$ 13 Hz, H-3′a), 2.22 (1 H, q, $J_{3'b,4'}$ 7.1 Hz, H-3′b), 2.51 (3 H, s, tosyl Me), and 1.50 (3 H, d, $J_{4',5'}$ 6.0 Hz, H-5′).

A solution of the tosylate (3A) (2.6 mg) in dioxan (0.8 ml) was heated at 100 °C. The reaction was followed by t.l.c. [solvent (*B*)]. After 2.5 h *ca.* 80% of (3A) had reacted. The *O*(6)3′-methylenepentofuranosyl theophyllinium tosylate (4A) was separated by t.l.c. with solvent (*B*), giving a glass, $\lambda_{max.}$ (MeOH) 271 nm (A_{270}/A_{250} 1.40), δ ($CDCl_3$) 7.95 (1 H, s, H-8), 7.50 (4 H, AA′BB′ system, aromatic H of tosyl), 6.29 (1 H, s, H-1′), 3.77 (3 H, s, 3-Me), 3.58 (3 H, s, 1-Me), 2.50 (3 H, s, tosyl Me), and 1.48 (3 H, d, H-5′).

Tosylation of the alcohol (2B) (7.5 mg, 2.28 × 10^{-5} mol) with toluene-*p*-sulphonyl chloride (9.53 mg, 5 × 10^{-5} mol) in dry pyridine (0.3 ml) as described for (2A), and purification by t.l.c. [solvent (*A*)] gave the crystalline tosylate (3B), m.p. 144—146°, R_F (*A*) 0.632, R_F (*B*) 0.556, $[\alpha]_D^{20}$ +97° (*c* 0.1 in methanol), $\lambda_{max.}$ (MeOH) 273.5 nm (ε 7 700), δ ($CDCl_3$) 7.90 (1 H, s, H-8), 7.62 (4 H, AA′BB′ system, aromatic H of tosyl), 6.38 (1 H, s, H-1′), 4.86 (1 H, m, H-4′), 4.28 (2 H, s, 2′-CH_2), 3.64 (3 H, s, 3-Me), 3.40 (3 H, s, 1-Me), 2.50 (3 H, s, tosyl Me), 2.37 (1 H, q, $J_{3'a,4'}$ 5.1, $J_{3'a,3'b}$ 13.2 Hz, H-3′a), 1.96 (1 H, q, $J_{3'b,4'}$ 9.5 Hz, H-3′b), and 1.38 (3 H, d, $J_{4',5'}$, 6.1 Hz, H-5′).

A solution of (3B) (4.6 mg) in dioxan (1.4 ml) was heated at 100 °C for 13 h. Only unchanged (3B) was detected by chromatography and u.v. and n.m.r. spectra.

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