4'-Oxo-nucleosides. Synthesis and Properties of 6-Chloro-9-(6-deoxy-α-L-*lyxo*-hexopyranos-4-ulosyl)purine and the Corresponding 7-Glycosyltheophylline

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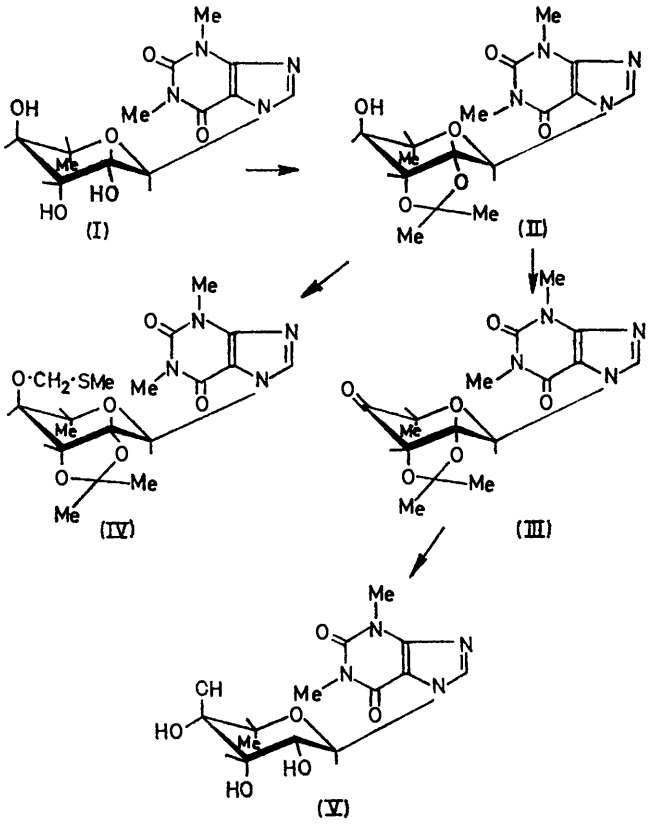
The first synthesis of 7- and 9-linked 4'-oxo-deoxynucleosides has been accomplished by direct oxidation of sterically hindered 6-deoxyhexosylpurines by the Pfitzner–Moffatt system; use of the dimethyl sulphoxide–acetic anhydride system led only to a 4'-methylthiomethyl ether. The intermediate 7-(α-L-rhamnopyranosyl)theophylline and 6-chloro-9-(α-L-rhamnopyranosyl)purine were obtained by direct acid-catalysed fusion of theophylline and 6-chloropurine with 1,2,3,4-tetra-*O*-acetyl-α-L-rhamnose. A conformational inversion taking place on acetal formation from the 6-chloropurine nucleoside was revealed by n.m.r. spectral analysis. The behaviour in acidic and alkaline media of the 4'-oxohexosyl-purines has been studied. The oxo-nucleosides showed growth inhibitory activity against KB cells, whereas the parent nucleosides were inactive.

THE natural occurrence of nucleosides containing amino-sugars, keto-sugars, and branched-chain sugars has created considerable interest in their synthesis. The biological importance of such compounds has been emphasized by the discovery of the antitumour and antibiotic activity of a variety of 'modified' hexosyl nucleosides [1] especially those containing 4-aminohexoses.

We have recently reported the synthesis of a hexosulosylpurine [2] and of a deoxyhexosulosylpurine,[3,4] and we have shown that these oxo-nucleosides can be used as synthetic intermediates to many 'modified' nucleosides possessing biological interest. Additional interest in oxo-nucleosides has resulted from the recent discovery of the biological activity [5] of 7-(β-L-fucos-2-ulosyl)theophylline [3] and of 7-(3-*O*-acetyl-4-deoxy-β-L-fucos-3-en-2-ulosyl)theophylline.[6]

We now describe the synthesis of the first 4'-oxo-nucleosides as a step towards the synthesis of 4'-amino- and 4'-branched chain nucleosides. The 6'-deoxy-4'-oxo-nucleosides (III) and (VIII) were obtained in over 60% yield by direct oxidation of 7-(6-deoxy-2,3-isopropylidene-α-L-mannosyl)theophylline (II) [7] and 6-chloro-9-(6-deoxy-2,3-*O*-isopropylidene-α-L-mannosyl)-purine (VII) with the Pfitzner–Moffatt reagent; [8] the procedure was modified as described for the hexos-2-ulosylpurines.[2,3] The reaction was found to be much more rapid than those of the foregoing hexosylpurines.

The required 7- and 9-(α-L-rhamnosyl)purines, (I) and (VI), were obtained by acid-catalysed fusion of theophylline and 6-chloropurine with 1,2,3,4-tetra-*O*-acetyl-α-L-rhamnose. These compounds have been previously prepared [9,10] by the heavy metal salt procedure. This method did not appear to be suitable to us because of the difficulty of removing the contaminating heavy metal (which is an inhibitor in biological systems) from the final nucleoside. The n.m.r. spectrum of (I) in [$^2H_6$]dimethyl sulphoxide showed a large coupling constant for H-1' and H-2' (8 Hz), indicating that these protons were *trans*-diaxial. This is compatible with the assigned



α-configuration in the *C*1 conformation, which is confirmed by the negative rotation sign, by the small coupling constants for the other protons (indicating that

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[3] K. Antonakis and M. J. Arvor, *Compt. rend.*, 1971, **272***C*, 1928; K. Antonakis, *Carbohydrate Res.*, 1972, **24**, 229.

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[6] K. Antonakis and M. J. Arvor-Egron, *Carbohydrate Res.*, 1973, **27**, 468.

[7] K. Antonakis and J. Herscovici, *Compt. rend.*, 1972, **274***C*, 2099.

[8] K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1963, **85**, 3027; 1965, **87**, 5670.

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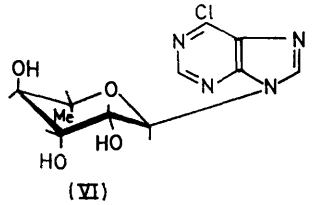
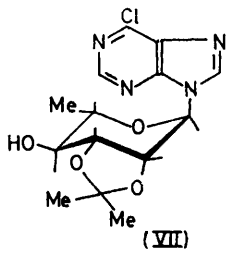
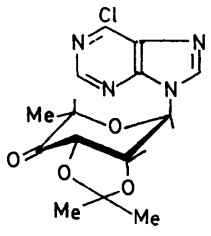
[10] R. B. Baker, K. Hewson, H. J. Thomas, and J. A. Johnson, *J. Org. Chem.*, 1957, **22**, 954.

H-2′ and H-3′ are axial–equatorial and H-3′ and H-4′ as well as H-4′ and H-5′ equatorial–equatorial), and by the multiplicity of the signals (H-3′ and H-4′, triplets, H-2′ quartet, and H-5′ octet).


(VI)


(VII)


(VIII)

In a similar way the configuration of (VI) was established by the value of $J_{1',2'}$ (8 Hz), indicative of a *trans*-diaxial relationship and consistent with α-L-configuration in the *C*1 conformation. The H-5′ and H-6′ signals were well separated and resolved. The 2′-proton signal was separated from the OH envelope by addition of [$^2H_4$]-methanol (33%) to the solution. The peak at δ 5·1 was then displaced to δ 4·7 ($J_{1',2'}$ 8, $J_{2',3'}$ 3 Hz).

Acetonation of 7-(α-L-rhamnopyranosyl)theophylline (I) [9] and 6-chloro-9-(α-L-rhamnopyranosyl)purine (VI) [10] gave the 2′,3′-*O*-isopropylidene derivatives (II) and (VII). In contrast to the theophylline derivative (II), which exhibited coupling constants similar to those of (I), the 6-chloropurine derivative (VII) exhibited a small value of $J_{1',2'}$ (4 Hz) and large values of $J_{3',4'}$ (7 Hz) and $J_{4',5'}$ (7 Hz). This is compatible with the α-configuration in the 1*C* conformation. Onodera and his co-workers [11] have also reported unexpected values for the coupling constants of 9-α-L-rhamnopyranosyladenine, and an axial orientation of the aglycone has been postulated for *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside.[11]

Oxidation of compounds (II) and (VII) with dimethyl sulphoxide and dicyclohexylcarbodi-imide in the presence of dichloroacetic acid for 10 min at room temperature afforded the 4′-oxo-nucleosides (III) and (VIII), which were isolated in pure form by distillation followed by chromatography on a silica gel column. No unchanged material was detected by chromatographic methods in either case.

Compound (III) had an i.r. spectrum similar to that of (II) except for a carbonyl band at 1755 cm^{-1}. The n.m.r. spectrum showed clearly the presence of the 4′-oxo-group. Whereas in the alcohol (II) the H-5′ signal was an octet at δ 4 ($J_{4',5'}$ and $J_{5',6'}$) the H-5′ signal of the oxo-nucleoside (III) appeared as a quartet at δ 4·44, confirming the absence of a proton at C-4′. Spin-decoupling confirmed the assignment of this quartet to H-5′.

The oxo-nucleoside (III) is easily hydrated, giving the pure *gem*-diol characterized by an OH band in the i.r. spectrum in the absence of the C=O band. The u.v. absorption maximum [$\lambda_{max.}$ (H_2O) 274 nm] was identical with those reported for 7-hexosyltheophyllines.[3,11]

Structure (VIII) was established from the n.m.r. spectrum, which was very similar to that of (VII), and the 9-position of glucosidation was assigned from a comparison of the u.v. absorption data for (VII) with those of 9-(α-L-rhamnosyl)adenine [10] and 6-chloro-9-(β-L-fucosyl)-purine.[4]

Treatment of (II) with dimethyl sulphoxide–acetic anhydride did not yield the expected ketone (III), but afforded the 4′-methylthiomethyl ether (IV) in 90% yield without detectable accumulation of other products. A similar thioether nucleoside derivative has been recently reported [12] as the main product of the oxidation of 7-(4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranosyl)-theophylline,[2] and a number of carbohydrate methylthiomethyl ethers have been isolated.[13] The n.m.r. spectrum in deuteriochloroform revealed two sharp singlets at δ 2·5 and 4·9, characteristic of the *S*-methyl group.[12] The methylthiomethyl ether (IV) is presumably formed after the elimination of acetate from the acetoxysulphonium (A) by reaction of the resulting sulphonium ylide (B) with an alcohol.[12]

$$\underset{(A)}{Me_2\overset{+}{S}OAc} \longrightarrow \underset{(B)}{CH_2{=}\overset{+}{S}Me} \xrightarrow[+AcOH]{R_4CHOH} R_2CH{\cdot}O{\cdot}CH_2{\cdot}SMe$$

In alkaline medium, the 6-chloropurine oxo-nucleoside (VIII) was less stable than the corresponding theophylline derivative (III). In methanolic 0·1N-sodium hydroxide compound (VIII) was cleaved after 30 min, whereas the theophylline (III) was unaffected; under similar conditions the parent nucleosides (II) and (VII) are stable. The stability of the theophylline derivative (III) may be explained by the establishment of a hydrogen bond between the 4′-*gem*-diol system [(III) has been isolated as the hydrate] and the 2-oxo-group of the theophylline (V). The assigned *C*1 conformation would facilitate this, whereas it could not occur with the 6-chloropurine derivative whatever the conformation.

On treatment of the nucleoside (III) with 2N-hydrochloric acid at room temperature, complete deacetalization was achieved after 1 h, with no concomitant glycosidic cleavage. The 4′-oxo-nucleoside (V) was thus obtained, as a pure hydrate. Attempts to achieve a similar deacetalization of (VIII) were unsuccessful; this oxo-nucleoside was less stable than (III) under acidic conditions.

Compounds (V) and (VIII) showed growth inhibitory

[11] K. Onodera, S. Hirano, and F. Masuda, *Carbohydrate Res.*, 1968, **7**, 27.

[12] K. Antonakis and F. Leclercq, *Bull. Soc. chim. France*, 1971, 4309.

[13] N. A. Hughes, *Carbohydrate Res.*, 1967, **5**, 149; J. L. Godman and D. Horton, *ibid.*, 1968, **6**, 229; G. J. F. Chittenden, *ibid.*, 1970, **15**, 101.

activity against KB cells at 0·17 mg ml^{-1} whereas the parent nucleosides (II) and (VII) were inactive at 0·7 mg ml^{-1}; the method for studying this effect was reported recently.[5]

EXPERIMENTAL

Solutions were evaporated at 40° under diminished pressure. Optical rotations were measured with a Roussel–Jouan 'Quick' polarimeter. I.r. spectra were determined with a Jobin–Yvon MVI spectrometer. N.m.r. spectra were recorded with a Varian T-60 instrument. I.r. spectra were obtained on a Perkin-Elmer 137 spectrometer. T.l.c. was performed on 0·25 mm layers of Merck silica gel HF with ethyl acetate; the products were detected by u.v. absorption or by spraying with 30% sulphuric acid and heating at 105°. Elemental analyses were obtained from the Laboratoire de Microanalyse du C.N.R.S.

7-(6-*Deoxy*-α-L-manno-*hexopyranosyl*)*theophylline* (I).—7-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)theophylline [9] (4·52 mg, 1 mmol), obtained by acid-catalysed fusion,[2,3] was kept at 0° for 1 h in methanol saturated with ammonia. The solution was evaporated *in vacuo* and the residue crystallized from ethanol; yield 250 mg, m.p. 167° (lit.,[9] 190°); $[\alpha]_D^{20}$ −90° (*c* 0·1 in H_2O) (lit.,[9] −90°); $\lambda_{max.}$ 274 nm (ε 8850); δ [$(CD_3)_2SO$] 6 (d, $J_{1',2'}$ 8 Hz), 4·8 (q, $J_{2',3'}$ 2 Hz), and 1·1 (octet, $J_{5',6'}$ 7 Hz).

7-(6-*Deoxy*-2,3-O-*isopropylidene*-α-L-manno-*hexopyranosyl*)*theophylline* (II).—7-(α-L-Rhamnopyranosyl)theophylline (I) (1 g) was shaken with acetone (100 ml). After dissolution, concentrated sulphuric acid (0·2 ml) was added and the mixture was left at room temperature during 1 h. The solution was neutralized with N-sodium hydroxide and filtered. The filtrate was evaporated under reduced pressure and the *isopropylidene nucleoside* (II) crystallized from ethanol–pentane; yield 900 mg, m.p. 153°; $[\alpha]_D^{20}$ −60° (*c* 0·1 in MeOH); $\lambda_{max.}$ 274 nm (ε 10,300); R_F 0·3; δ ($CDCl_3$) 5·8 (d, $J_{1',2'}$ 7 Hz) and 4 (octet, $J_{5',6'}$ 6 Hz) (Found: C, 52·4; H, 5·9; N, 15·6. $C_{16}H_{22}N_4O_6$ requires C, 52·4; H, 6·0; N, 15·3%).

7-(6-*Deoxy*-2,3-O-*isopropylidene*-α-L-lyxo-*hexopyranos*-4-*ulosyl*)*theophylline* (III).—To a solution of (II) (500 mg, 1·36 mmol) in dimethyl sulphoxide (10 ml), dicyclohexylcarbodi-imide (DCC) (1·4 g, 6·8 mmol), benzene (10 ml), and dichloroacetic acid (0·15 ml) were added and the mixture was kept for 5 min at room temperature. After removal of the excess of DCC and dicyclohexylurea by high vacuum distillation at 40°, the crude material was dissolved in ethyl acetate and applied to a silica gel (Merck 0·05—0·2 mm) column (diam. 2 cm) packed in ethyl acetate. Elution with ethyl acetate (500 ml) and evaporation gave a syrup. The *oxo-nucleoside* (III) crystallized as the hydrate from benzene–cyclohexane; yield 450 mg (90%), m.p. 72—76°; $[\alpha]_D^{20}$ −56° (*c* 0·1 in MeOH); $\lambda_{max.}$ (MeOH) 274 nm (ε 11,700); R_F 0·51, and after heating at 100° $\nu_{max.}$ (KBr) 1755 cm^{-1} (C=O); δ ($CDCl_3$) 6·1 (d, $J_{1',2'}$ 7 Hz) and 4·44 (q, $J_{5',6'}$ 7 Hz) (Found: C, 49·45; H, 5·5; N, 14·4. $C_{16}H_{20}N_4O_6,H_2O$ requires C, 50·2; H, 5·7; N, 14·6%).

7-(6-*Deoxy*-α-L-lyxo-*hexopyranos*-4-*ylosyl*)*theophylline* (V).—The isopropylidene nucleoside (III) (360 mg, 1 mmol) was dissolved in methanol (1 ml), 2N-hydrochloric acid (9 ml) was added, and the solution was kept for 1 h at room temperature. The crystalline material was filtered off; recrystallization from ethanol gave pure *oxo-nucleoside* (V) as the hydrate, m.p. 140—141°; $[\alpha]_D^{20}$ −55° (*c* 0·1 in Me_2SO); $\lambda_{max.}$ (Me_2SO–H_2O) 274 nm (ε 7000); $\nu_{max.}$ (KBr) 3600 cm^{-1} (OH-*gem*); R_F 0·1; δ [$(CD_3)_2SO$] 6·2 (d, $J_{1',2'}$ 4 Hz) (Found: C, 45·05; H, 5·25; N, 16·15. $C_{13}H_{16}N_4O_6,H_2O$ requires C, 45·6; H, 5·25; N, 16·4%).

7-(6-*Deoxy*-2,3-O-*isopropylidene*-4-O-*methylthiomethyl*-α-L-manno-*hexopyranosyl*)*theophylline* (IV).—A solution of 7-(6-deoxy-2,3-*O*-isopropylidene-α-L-*manno*-hexopyranosyl)-theophylline (II) (500 mg, 1·36 mmol) in dimethyl sulphoxide (6 ml) and acetic anhydride (4 ml) was kept at room temperature for 48 h. Evaporation gave a syrup which was dissolved in ethyl acetate and chromatographed on silica gel in ethyl acetate (3 ml fractions). Tubes 100—130 gave a syrup which crystallized from ethanol to give the *thioether* (IV), m.p. 146°; δ ($CDCl_3$) 2·5 and 4·9 (s, SMe) (Found: C, 50·55; H, 6·45; N, 12·8; S, 7·65. $C_{18}H_{26}N_4O_6S$ requires C, 50·5; H, 6·1; N, 13·1; S, 7·5%).

6-*Chloro*-9-(6-*deoxy*-α-L-manno-*hexopyranosyl*)*purine* (VI).—6-Chloro-9-(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnopyranosyl)-purine [11] (408 mg, 1 mmol), obtained by fusion procedures [2,3] in the presence of boron trifluoride, was kept at 0° for 1 h in methanol saturated with ammonia. The solution was evaporated to a syrup which readily crystallized from ethanol. The *nucleoside* (VI) (300 mg) had m.p. 172°; $[\alpha]_D^{20}$ −60° (*c* 0·1 in MeOH); R_F 0·15; δ [$(CD_3)_2SO$] 6 (d, $J_{1',2'}$ 8 Hz), 4·6 (t, $J_{3',4'}$ 4, $J_{2',3'}$ 2·5 Hz), 4 (t, $J_{3',4'}$ 4, $J_{4',5'}$ 4 Hz), and 3·5 (q, $J_{4',5'}$ 4, $J_{5',6'}$ 7 Hz); δ [$(CD_3)_2SO$–CD_3OD] 5·1 (q, $J_{2',3'}$ 2·5, $J_{1',2'}$ 8 Hz) (Found: C, 44·2; H, 4·65; N, 18·95. $C_{11}H_{13}ClN_4O_4$ requires C, 44·0; H, 4·4; N, 18·7%).

6-*Chloro*-9-(6-*deoxy*-2′,3′-O-*isopropylidene*-α-L-lyxo-*hexopyranos*-4′-*ulosyl*)*purine* (VIII).—Selective acetalization of (VII) during 2 h, as described for (I), gave the isopropylidene nucleoside (VII) as a semi-crystalline material; t.l.c. R_F 0·7; $\lambda_{max.}$ 264 nm (ε 9200); δ [$(CD_3)_2SO$] 6 (d, $J_{1',2'}$ 4 Hz), 5·4 (d, $J_{2',3'}$ 4 Hz), 4·8 (t, $J_{3',4'}$ 6·5 Hz), 4 (t, $J_{4',5'}$ 6·5 Hz), and 3·5 (m, $J_{5',6'}$ 6 Hz).

To a solution of (VII) (500 mg, 1·5 mmol) in dimethyl sulphoxide (8 ml), dicyclohexylcarbodi-imide (1·6 g, 8·5 mmol) in benzene (10 ml) and dichloroacetic acid (0·15 ml) were added. The solution was set aside for 10 min, then diluted with ethyl acetate, and a solution of oxalic acid (0·6 ml) was added. Dicyclohexylurea (DCU) was filtered off after 30 min and the filtrate was washed with water and evaporated to a syrup. This was dissolved in acetone; the solution was filtered to remove DCU and evaporated. Crystallization from isopropyl alcohol gave the pure *oxo-nucleoside* (VII) (100 mg), m.p. 134—138°; $[\alpha]_D^{20}$ −45° (*c* 0·1 in MeOH); R_F 0·78; $\lambda_{max.}$ (MeOH) 265 nm (ε 8900); δ [$(CD_3)_2SO$] 6·2 (d, $J_{1',2'}$ 2 Hz) (H-2′ and H-3′ superimposed at δ 5·2—5·4) and 4·3 (q, $J_{5',6'}$ 6 Hz) (Found: C, 49·9; H, 4·3; N, 16·6. $C_{14}H_{15}ClN_4O_4$ requires C, 49·7; H, 4·4; N, 16·6%).

Action of Alkali on the Oxo-nucleosides (III) *and* (VIII).—The nucleosides (III) and (VIII) (10^{-2} mmol) were each dissolved in methanol (0·09 ml) and the solutions were diluted with methanolic N-sodium hydroxide (0·01 ml). The reaction was followed by t.l.c.; free base was detected by u.v. absorption and free sugar by spraying with 30% sulphuric acid and heating at 105°. In ethyl acetate the R_F values of the bases are different from those of starting oxo-nucleosides.

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