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Note

Reaction of the D-ribose moiety of adenosine and AMP with periodate and 5,5-dimethylcyclohexane-1,3-dione (dimedone)

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The *cis*-glycol group of ribonucleosides and ribonucleotides can be oxidized specifically by periodate to form dialdehydes (**1** → **2**) which subsequently polymerise to oligo- or poly-ribo-oxynucleosides and -ribo-oxynucleotides[1]

On investigating the reaction of the dialdehyde **2** (R = H), derived from adenosine, with dimedone, a single product was isolated, the combustion analysis of which was consistent with structure **4** (R = H) The mass spectrum of **4** (R = H) exhibits a molecular ion at *m/e* 527 and therefore provides further evidence for the presence of two dimedone residues Furthermore, in the n m r spectrum of **4** (R = H), the two acidic protons (H-2″) of the dimedone residues could be identified by D_2O exchange The splitting of the signals for the methyl and methylene protons of the

TABLE I

ELECTROPHORESIS, CHROMATOGRAPHY, AND U V SPECTROSCOPY OF **2–4**

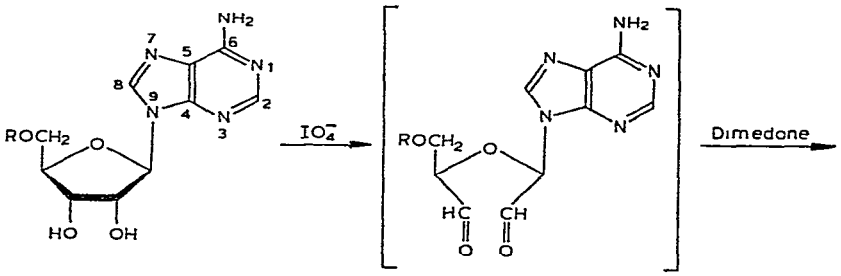
Compound	*Electrophoretic mobilities*[a]		*T l c* R_F[b]	*U v spectra*	
	A	B		λ_{max} (*nm*)	$\lambda_{shoulder}$ (*nm*)[c]
2 R = $(HO)_2PO$	0 37 (+)	1 0 (+)	0 85	258	—
2 R = H	—	0 28 (−)	0 74	258	—
Dimedone	0 59 (+)	0 7 (+)	0 55	280	—
3 R = $(HO)_2PO$	0 28 (+)	—	0 60	265	286 (weak)
4 R = $(HO)_2PO$	1 0 (+)	—	0 22	264 5	290
3 R = H	—	0 0	0 30	265	286 (weak)
4 R = H	0 27 (−)	0 24 (+)	0 15	264 5	290

[a] *A*, Cellulose thin-layer plates (Merck, Darmstadt, GFR), 0 1M acetate buffer (pH 5 0), 600 V, 31→51 mamp, *B*, silica gel thin-layer plates (Woelm, Eschwege, GFR), 0 1M Sørensen citrate buffer (pH 6 5), 600 V, 29 mamp (apparatus from Desaga, Heidelberg, GFR) [b] Silica gel (as above), 0 25M LiCl [c] 0 1M Phosphate buffer (pH 7 0)

dimedone moieties may be based on interactions with the purine ring, and on the fact that C-3′ and C-5′ are additional asymmetric centers and that, therefore, stereoisomers can be formed From these results, together with chromatography, electrophoresis, and u v data (Table I), we suggest the structure **4** From u v and electrophoresis data, **3** or structures generated from **3** by ring closure are proposed as intermediates

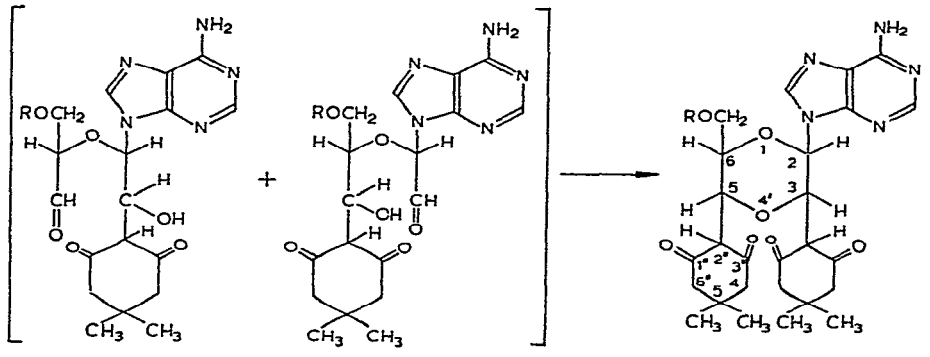
The reaction product of **2** [R = $(HO)_2PO$], derived from adenosine monophosphate (AMP), and dimedone is probably **4** [R = $(HO)_2PO$], since dephosphorylation by alkaline phosphatase yields **4** (R = H)

Glitz and Sigman[2] treated the dialdehyde **2**, derived from AMP, as well as several periodate-oxidized RNA species, with [^{14}C]-labelled dimedone under slightly different conditions From the incorporation of radioactivity and u v data, they concluded that a 1 1 stoichiometry of the dialdehyde to dimedone existed in these compounds



1 R = H
1 R = $(HO)_2PO$

2 R = H
2 R = $(HO)_2PO$



3

4 R = H
4 R = $(HO)_2PO$

EXPERIMENTAL

Adenosine, AMP, and dimedone were commercial products of analytical grade Alkaline phosphatase from *E coli* (E C 3 1 3 1) was a product of Boehringer, Mannheim N m r. spectra were measured at 24° on a Varian HA-100 spectrometer, using methyl sulphoxide-d_6 as solvent and tetramethylsilane as internal standard Mass spectra were obtained on a Varian-MAT CH-4 spectrometer I r spectra were measured in KBr pellets, using a Perkin–Elmer Infracord spectrometer Zeiss PMQ II and UNICAM SP 1800 spectrometers were used for measuring the u v spectra

Preparation and characterization of 6-amino-9-[3′,5′-bis(5″,5″-dimethylcyclohexane-1″,3″-dione-2″-yl)-6′-hydroxymethyl-1′,4′-dioxan-2′-yl]purine (**4**, *R = H*) — Adenosine (10 mmol) was treated with 10 mmol of $NaIO_4$ in 100 ml of water at 23° in the dark for 1 h Traces of periodate then were reduced[3] with butane-2,3-diol A solution of dimedone (20 mmol) in 600 ml of water was filtered and added to the foregoing solution, and the mixture was stored at 23° overnight The product was collected, recrystallized from aqueous methanol, and dried at 80° over P_2O_5 *in vacuo* to give **4** (4 5 g, 81%; R = H), m p 184 5° (Found C, 57 4, H, 6 56, N, 12 97 $C_{26}H_{33}N_5O_7$ H_2O calc C, 57 3, H, 6 46, N, 12 83%)

On boiling a solution of **4** (R = H) in ethanol, the anhydrous compound was obtained, m p beginning at 190° (Found C, 59 02, H, 6 50, N, 13 45 $C_{26}H_{33}N_5O_7$ calc C, 59 1; H, 6 32, N, 13 29%)

At pH 3, **4** (R = H) has λ_{max} 260 nm (ε 25,800), pH 7, λ_{max} 264 nm (ε 24,500), shoulder at 290 nm, pH 10 5, λ_{max} 265 nm (ε 26,500), shoulder at 290 nm, ν_{max} 1715 (weak, ketone of a six-membered ring) and 1620 cm^{-1} (strong, 1,3-diketone in the enolic form) Mass spectrum *m/e* 527 (6 5%) $M^+ - H_2O$, 509 (21 5) $527 - H_2O$, 491 (20) $509 - H_2O$, 473 (9) $491 - H_2O$, 136 (100) BH^+, 135 (100) B^+, 392 (13) 527 − B, 374 (95) 509 − B, 356 (93) 481 − B, 338 (92) 473 − B, 479 (53) $509 - CH_2O$, 344 (73) 479 − B. N m r data δ 8 2 (*s*, 1 H, H-8), 8 18 (*s*, 1 H, H-2), 7 23 (*s*, 2 H, NH_2), 6 76 (*s*, 1 H, H-2′), 4 18 (*d*, *J* 4 5 Hz, after D_2O exchange *s*, 1 H, H-3′), 3 5 (*s*, 2 H, H-5′,6′), 3 34 (*s*, 2 H, CH_2-6′), 1 03 (*s*) and 0 87 (*d*) (12 H, 4 Me-5″), 2 3 (*m*, 8 H, 4 CH_2-4″,6″), 5 75 (*d*, *J* 4 5 Hz, after D_2O exchange *s*, 2 H, CH-acid-2″) Sometimes, the sugar protons exhibited coupling constants of 1–2 Hz, but in most cases, no splitting of the signals could be detected

Synthesis of 6-amino-9-[3′,5′-bis(5″,5″-dimethylcyclohexane-1″,3″-dione-2″-yl)-6′-phosphoryloxymethyl-1′,4′-dioxan-2′-yl]purine [**4**, R = $(HO)_2PO$] and further preparations of **4** (R = H) were carried out in 10mM solution by treating **1** with 1 equiv of $NaIO_4$ for 30 min at 4° in the dark and subsequently with 2 equiv of dimedone in 15mM Teorell–Stenhagen buffer, either at pH 4 9 or 6 9 or 8 8, for 12 h at 4° Analyses were carried out as described above

Ester cleavage with alkaline phosphatase — A reaction mixture (50 μl) of 0 5 μmol of **2** [R = $(HO)_2PO$] and 1 μmol of dimedone were incubated with 10 μl of alkaline phosphatase solution (= 1 μg of enzyme) at pH 8 8 and 37° for 40 min The reaction products were worked up and analyzed as described above

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