Reactions Between Ribonucleoside Derivatives and Formaldehyde in Ethanol Solution

By PETER K BRIDSON, JOSEF JIRICNY, OZNUR KEMAL, and COLIN B REESE*
(*Department of Chemistry, King's College, Strand, London* WC2R 2LS)

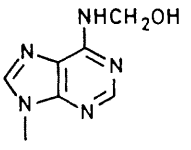
Summary 2′,3′-*O*-Isopropylidene-adenosine, -cytidine, and -guanosine, (**2a**), (**3a**), and (**4a**), respectively react with formaldehyde in ethanol solution to give the corresponding *N*-ethoxymethyl derivatives, (**2b**), (**3b**), and (**4b**), respectively in good yields

THE reaction between nucleic acids and formaldehyde has been much investigated [1–3] The fact that the base residues in double-stranded regions are virtually resistant to attack by formaldehyde has been used as a probe of secondary structure in nucleic acids [1–3] Formaldehyde has also been used to stabilize single-stranded polynucleotides [1,2] Studies involving the reaction between formaldehyde and nucleic acids have generally been carried out in aqueous solution and the principal products, which are believed to result from *N*-hydroxymethylation of the base residues [as in the modified adenine residue (**1**)], are relatively unstable [1–3] It is not surprising, therefore, that the products obtained at the nucleoside level, that is in the reactions between formaldehyde and adenosine, cytidine and guanosine derivatives in aqueous solution, have not been fully characterized [2]

TABLE Reaction between 2′,3′-*O*-isopropylidene ribonucleosides and formaldehyde in ethanol.

Substrate	Mol equiv of formaldehyde	Reaction time[a]/h	Product[b] (% Yield)	m p /°C	λ_{max}[c]/nm (ϵ_{max})
(**2a**)	5·9	5	(**2b**) (90)	134—135	263 (17,600)
(**3a**)	5·8	7	(**3b**) (72)	125—126	272 (9,250), 247 (9,000)
(**4a**)	6 7	48	(**4b**) (>95)	*ca* 260 (decomp)	255 (14,800)

[a] In ethanol, under reflux [b] Satisfactory microanalytical and n m r spectroscopic (^{1}H and ^{13}C) data were obtained for products (**2b**—**4b**) [c] In 95% ethanol

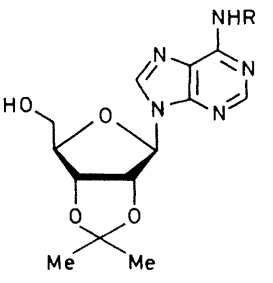


(1)

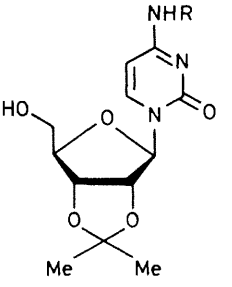
We now report that when 2′,3′-*O*-isopropylidene-adenosine, -cytidine and -guanosine,[4] (**2a**), (**3a**), and (**4a**), are heated, under reflux, with an excess of aqueous formaldehyde in ethanol solution, they are converted into the corresponding *N*-ethoxymethyl derivatives (**2b**), (**3b**), and (**4b**), respectively The latter three products may all be isolated† as analytically pure crystalline solids in good yields (Table) In a typical reaction (Table), 2′,3′-*O*-isopropylideneadenosine (**2a**, 3·25 mmol) and aqueous formaldehyde (*ca* 40% w/v, *ca* 19 mmol) are heated, under reflux, in ethanol (25 ml) solution After 5 h, the products are evaporated under reduced pressure and chromatographed to give 2′,3′-*O*-isopropylidene-6-*N*-ethoxymethyladenosine (**2b**) [^{1}H n m r ($CDCl_3$) δ 1·21 (3H, t, *J* 7·0 Hz), 1·38 (3H, s), 1·65 (3H, s), 3 71 (2H, q, *J* 7·0 Hz), 3 91 (2H, m), 4·54 (1H, m), 5·18 (4H, m), 5·90 (1H, d, *J* 4·4 Hz), 6·53 (1H, dd, *J* 3 2, 7·6 Hz), 7 58 (1H, m), 7·93 (1H, s), and 8·39 (1H, s)] in 90% isolated yield (Table) The reaction of nucleoside base residues with formaldehyde and ethanol may be compared to their related reactions with formaldehyde and thiols recently reported by us[5] and by other workers [6]

The stabilities of the above *N*-ethoxymethyl derivatives (**2b**—**4b**) were measured in 0 05 M-hydrogen chloride and in 0 05 M-sodium hydroxide in dioxan-water (1 : 1 v/v) at 20 °C In the acidic medium, the half-times of hydrolysis of (**2b**), (**3b**), and (**4b**) were found to be *ca* 1·5, 120, and 20 min, respectively In the alkaline medium, (**2b**) and (**4b**) were *ca* 50% decomposed after 10 and 15 h, respectively and (**3b**) was *ca* 5% decomposed after 45 h ‡

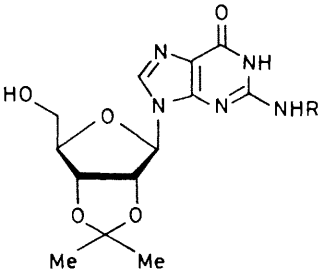
The present results suggest that the base residues of single-stranded regions of nucleic acids could be modified by



(2)



(3)



(4)

a, R = H b; R = CH_2OEt

treatment with formaldehyde in a suitable alcoholic solvent Furthermore, such modified base residues would be expected to be more stable (except in strongly acidic media) than the corresponding putative *N*-hydroxymethylated residues introduced[1–3] by treatment of the same substrates with formaldehyde in aqueous solution

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† 2′,3′-*O*-Isopropylidene derivatives (**2a**), (**3a**), and (**4a**), rather than the corresponding unprotected ribonucleosides, were used as substrates in this study only to facilitate the isolation of the products

‡ Preliminary results suggest that the *N*-ethoxymethyl derivatives (**2b**—**4b**) are converted back into the 2′,3′-*O*-isopropylidene nucleosides (**2a**—**4a**) by alkaline hydrolysis but that the corresponding *N*-hydroxymethyl derivatives are formed under the above conditions of acidic hydrolysis

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