SYNTHESIS OF RIBONUCLEOSIDE 5′-O-HYDROXYMETHANEPHOSPHONATES

Preliminary communication

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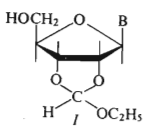
Within the series of nucleotide analogues, various derivatives containing phosphorus–carbon linkage have been already reported in the literature[1–5]. In this preliminary communication we wish to describe a synthesis and some properties of a new class of compounds bearing a hydroxylic function attached to the alkyl group of the alkanephosphonate residue, namely the nucleoside 5′-O-hydroxymethanephosphonates *II*.

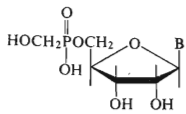
In contrast to alkanephosphonates[1–3], the title compounds are not accessible by a simple condensation of hydroxymethanephosphonic acid[6] with protected nucleoside derivatives in the presence of usual activating agents such as N,N′-dicyclohexylcarbodiimide or 2,4,6-triisopropylbenzenesulfonyl chloride in pyridine. In this particular case, the activation of hydroxymethanephosphonic acid by 1,1′-carbonyldiimidazole[7] *via* phosphonic acid imidazolide affords satisfactory results.

Thus, anhydrous hydroxymethanephosphonic acid (3 mmol) was treated with 1,1′-carbonyldiimidazole (6 mmol) in anhydrous dimethylformamide (7 ml) for 2 h at room temperature and, afterwards, 2′,3′-O-ethoxymethyleneuridine (*Ia*) (2 mmol) (*cf.*[8]) was added to the reaction mixture. After stirring for 24 hours at room temperature, the solvent was removed *in vacuo* and the residue treated with 50% aqueous acetic for 30 min at 50°C. After evaporation to dryness, compound *IIa* was isolated on a DEAE-cellulose column or by preparative paper chromatography; Whatman No 1, 2-propanol–aqueous ammonia–water (7 : 1 : 2), R_F 0·44; ethanol–1M ammonium acetate (5 : 2), R_F 0·56. Paper electrophoresis (pH 7·5): 0·56, referred to uridylic acid. In this way, compound *IIa* was obtained in 40—50% yield. Ammonium salt: $C_{10}H_{18}N_3O_9P$ (355·3): calculated: 11·82% N, 8·73% P; found: 12·30% N, 9·05% P. The cytidine derivative *IIb* was obtained similarly. Compounds *II* are stable at 50°C for 6 h in 50% aqueous acetic acid or dilute (1 : 1) ammonia.

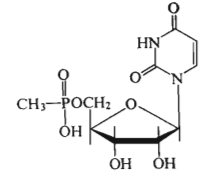
The ester linkage of *II* is resistant to the action of alkaline phosphatase *E. coli*, intestinal alkaline phosphatase, or snake venom phosphodiesterase (0·05M-Tris-HCl, pH 9, 6 h at 37°C).



I



II



III

In formulae *I*, *II* *a*, B = uracil; *b*, B = cytosine.

Derivatives *II* are good substrates for snake venom 5'-nucleotidase (*Crotalus adamanteus*) in contrast to *III* (synthetized from *Ia*, *cf.* ref.[3]) which is a poor substrate (10 μmol substrate and 30 μg enzyme protein in 150 μl 0·05M-Tris-HCl, pH 9, 5 h at 37°C; in brackets, percentage of splitting): UMP (46%), *IIa* (36%), *III* (8·5%). The enzymatic hydrolysis affords nucleoside and hydroxymethanephosphonic acid. The different behaviour of 5'-nucleotidase towards *II* and *III* might be explained rather in terms of the absence of a hydrophilic function in *III* than by a steric effect of the methyl group attached to the phosphorus atom.

The reactivity of secondary hydroxylic function of nucleoside sugar moieties towards the activated phosphonate derivative is much lower than that of the primary hydroxylic group. Consequently, unprotected nucleosides provide almost pure 5'-isomeric compounds *II* under the above conditions.

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