Tetrahedron Letters No. 25, pp 2595 - 2596, 1972. Pergamon Press. Printed in Great Britain.

PREPARATION OF 8-CHLOROADENOSINE AND ITS PHOSPHATE ESTERS

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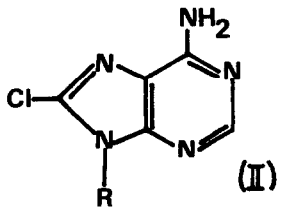
(Received in UK 8 May 1972; accepted for publication 18 May 1972)

Since the discovery that double stranded polyribonucleotides can induce the formation of interferon,[1] there has been considerable interest in the synthesis of modified polynucleotides.[2-4] Purines undergo electrophilic attack at the 8-position and the synthesis of 8-bromo ADP[5] and 8-bromo GDP[6] has been achieved by direct bromination of the nucleoside diphosphates. However, the introduction of a bulky group in the 8-position of the purine causes the nucleotides to adopt the syn-conformation[7] and 8-bromo GDP does not act as a substrate for polynucleotide phosphorylase.[8] There are no reports in the literature of the preparation of 8-chloropurine nucleosides.

We wish to report the chlorination of adenosine by tetrabutylammonium iodotetrachloride (I)[9] to give 8-chloroadenosine (II).

$(n\text{-}C_4H_9)_4NICl_4$

(I)



(II)

where R = β-D-ribofuranosyl

Using (I) we have prepared 8-chloro AMP and 8-chloro ADP, and have polymerised the latter using polynucleotide phosphorylase bound to an insoluble support.

To a solution of adenosine (1.03 g) in dry DMF (3.0 ml) was added a solution of (I) (3.05 g) in dry DMF (3.0 ml), and the reaction mixture stirred in a well stoppered flask for 24 hours. The solution was poured into water (100 ml) and the aqueous phase extracted with chloroform (10 x 25 ml). The aqueous phase was then applied to a Dowex 50 column (2 x 40 cm, H^+ form) and the column eluted with aqueous ammonium hydroxide. The ammonium hydroxide was removed from the eluate which contained (II) by repeated evaporation in vacuo, the residue was crystallised from methanol/ethyl acetate to give 8-chloroadenosine (0.44 g, 38%); λ_{max} 0.1 N HCl 262 nm, ε 13,900, 0.1 N NaOH 263 nm, ε 11,700).

The mass spectrum of 8-chloroadenosine confirms its structure. There was no peak corresponding to the molecular ion, the base peak occurred at 169.0155 and there was another intense peak

at 171.0121 (27%). These peaks correspond to $C_5H_4N_5{}^{35}Cl$ and $C_5H_4N_5{}^{37}Cl$ respectively and arise from one of the principal fragmentation routes of nucleosides[10] which involves the breaking of the sugar-purine bond to give a fragment corresponding to B+H. Loss of HCN from these two ions gives peaks at 142 (46.2%) and 144 (15.5%) and there are metastable peaks at 119.3 and 121.2 corresponding to these decompositions. The position of attachment of chlorine as C_8 is confirmed by a peak at m/e 80 corresponding to B+H - HCN - ClCN. The ribose residue appears as a peak at 133.

The ^1H n.m.r. 100 MHz spectrum (D_2O) showed a signal at 1.91 τ (1 Hs) due to H_2, the signal due to H_8 which appears at 1.69 τ in adenosine was absent, confirming the position of substitution in the purine ring. The 2', 3' and 4' protons of the sugar residue appear as a complex multiplex together with the signal due to HDO in the region 5 - 6 τ, the 1' proton appears as a doublet (J = 7 c/s) at 3.92 τ and the 5' protons as a poorly resolved doublet (J = 1 c/s) at 6.11 τ.

8-Chloro AMP (30 mg) was prepared in 23% yield from AMP using a similar chlorination procedure, the product being isolated by ion exchange chromatography on a Dowex 1 column (2 x 30 cm, $HCOO^-$ form). Dephosphorylation of 8-chloro AMP with Crotalus adamanteus venom[2] followed by thin layer chromatography showed that only 8-chloroadenosine was present.

^{14}C-Labelled 8-chloro ADP (112 mg, 25%) was prepared from ADP as above, the product being isolated by chromatography on DEAE cellulose (1 x 20 cm, HCO_3^- form) with elution by triethylammonium bicarbonate and was converted into the sodium salt using a Dowex 50 column. Dephosphorylation of this sodium salt with Crotalus adamanteus venom followed by thin layer chromatography again showed that only 8-chloroadenosine was present.

The attempted polymerisation of 8-chloro ADP with soluble polynucleotide phosphorylase[11] was unsuccessful. However, when polynucleotide phosphorylase which had been insolubilised by attachment to cellulose[12] was used, polymerisation occurred to the extent of 20% after 30 minutes. Further studies on this polymerisation reaction are in progress.

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