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STUDIES ON THE ISOPROPYLATION OF ADENOSINE-5'-MONOPHOSPHATE

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The isopropylation of 5'-AMP **1** under strongly alkaline conditions using isopropyliodide gave 3'-isopropyl 5'-AMP **2** as the major product, the minor alkylation product from this reaction has been isolated in 6.2% yield and characterised as the 5'-AMP-isopropylester **3**. Isopropylation of 5'-AMP using DMSO and potassium carbonate gave N^1-isopropyl-5'-AMP **4** in 18.7% and N^1-isopropyl-5'-AMP isopropylester **5** in 28.9% yield, while the same reaction using potassium tert-butoxide and DMSO gave the N^6-isopropyl-5'-AMP **6**. The structure of the product has been assigned using ultraviolet spectroscopy proton NMR and mass spectrometry. In some isopropylated nucleotides described in this paper, it is possible to get useful structural information using a combination of FAB and E.1. mass spectrometery.

Key words: 5'-AMP, Isopropylation, NMR.

INTRODUCTION

Among the alkylated nucleic acid components the methylated nucleosides are of interest due to their wide spread occurrence in RNA which is extensively methylated in the base as well as sugar moieties [1]. Various methylating agents are also found to be cytotoxic due to possible alkylation of DNA bases [2]. Significant increase in 1-methylhypoxanthine and 7-methylguanine in the urine of patients suffering from leukaemia has been reported [3]. Homopolynucleotides containing 2'-methoxy and ethoxy groups have been synthesised and their interaction with complementary polynucleotides has also been reported [4-6]. Such 2'-alkoxy homopolynucleotides have shown interesting biological properties such as they act as selective viral replication inhibitors [7]. Recently synthesis of 2'-methoxyoligonucleotides of defined sequence has also been carried out and their use as probes in RNA hybridization has been reported [8].

The synthesis of various 2'-alkoxylated nucleotides has been the subject of our previous communication [10] in which it was reported that alkylation of 5'-AMP with alkylhalides under strongly alkaline conditions gave predominantly sugar alkylated 5'-AMP derivatives. The major products obtained in the case of methyl, ethyl, *n*-propyl iodides were the 2'-alkoxylated nucleotides but the isopropyliodide gave the 3'-alkoxylated isomer which was attributed to steric factors. The reaction of isopropyliodide with 5'-AMP has been re-investigated again under strongly alkaline conditions in order to investigate the structure of the minor products formed during this reaction. Furthermore it was also of interest to investigate this reaction using DMSO as the solvent and anhydrous potassium carbonate as the acid scavenger and also by using DMSO and potassium *tert*-butoxide. The results obtained by isopropylation using above noted conditions are being presented in this publication. Such isopropylated nucleotides may possess interesting biological properties.

EXPERIMENTAL

Adenosine-5'-monophosphate was purchased from BDH Laboratories, England. Paper chromatography was carried out on Whatman No. 1 paper in a descending manner using all glass apparatus. For preparative paper chromatography whatman 3MM paper was used. The solvent systems used were A. Isopropanol: Ammonium hydroxide: Water (7:1:2), B. Isopropanol: Ammonium hydroxide: 0.1M Boric acid (7:1:2), C. Ethanol: IM Ammonium acetate (7:3).

Paper electrophoresis was done on Whatman 3MM paper strips 43.5 x 8.8cms, at 400 volts and 18 amperes for two hours using phosphate buffer 0.05M, pH 8.5.

^{1}H-NMR spectra were determined on Bruker AM 300 NMR spectrometer, for FAB & E.I. mass measurements Varian MAT 112 and MAT 312 spectrometer connected to MAT 188 data system and PDP 11/34 computer system was used.

Isopropylation of 5'-AMP using aqueous base and dioxane. 5'-AMP (500mg) was dissolved in IN sodium hydroxide (6 ml) and dioxane (2.5 ml). Isopropyliodide (1.5 ml) was added and the reaction mixture was stirred at 70° for 3 hours. Paper chromatography on Whatman No. 1 using solvent A showed the formation of three products excluding the unreacted starting material. The crude reaction mixture was loaded on Whatman 3MM sheets and devel-

oped by using solvent system A. The bands were cut, eluted with distilled water (20 ml each) and after low temperature concentration were obtained as amorphous solids. The product corresponding to fastest mobility, Rf 0.73 in solvent A was *Adenosine-5'-monophosphate isopropylester* **3** and was obtained as an amorphous solid (29mg; 6.25%). U.V. λ max 261nm, pH 1 and λ max 260nm, pH 12; paper electrophoretic mobility in phosphate buffer pH 8.5, 3.3cm, (AMP 6.5 cm). ^{1}H-NMR 1.15 (Isopropyl, m, 6H), 6.03 (H-1',d, 1H, J = 2.5'Hz), δ 8.21 (H-2,s, 1H) and δ 8.14 (H-8,s, 1H). Mass E.1. m/z 135 (base), 136 (base + 1).

The band corresponding to Rf 0.61 in solvent A was a non-nucleotide material and that corresponding to Rf 0.46 in solvent A was identical with 3'-0-isopropyl AMP 2 in its NMR, U.V. spectral characteristics and electrophoretic mobility [10].

Isopropylation of 5'-AMP in DMSO using potassium carbonate. 5'-AMP (100 mg) was dissolved in DMSO (5 ml) and anhydrous potassium carbonate (350 mg) was added. After addition of isopropyliodide (0.3 ml) the mixture was stirred at ambient temperature for 3 hours. Additional quantities of isopropyliodide (0.15 ml)) and potassium carbonate (300 mg) were added and stirring was continued for further 6 hours. The crude reaction mixture on paper chromatography showed the formation of three new products and the unreacted starting material. The reaction mixture was concentrated under vacuum at 37° and separated on two 3MM Whatman chromatography sheets using solvent A. The bands were eluted with distilled water and concentrated below 40° to get pure compounds.

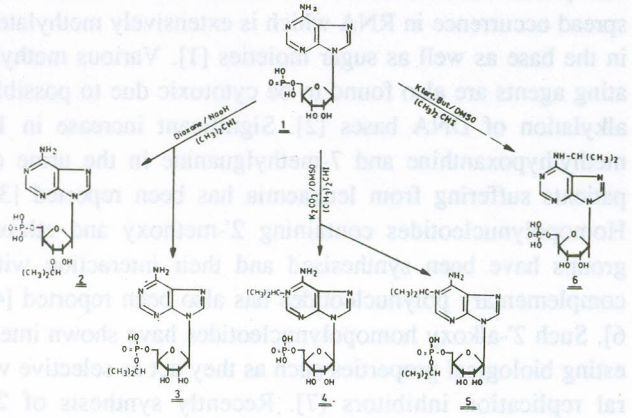
The slower moving product, Rf 0.48 in solvent A was obtained as an amorphous solid (21 mg: 18.7%) and was characterised as *N^1-isopropyl-5'-AMP* **4**. U.V. λ max 256nm, pH 1 and λ max 257nm, pH 12; paper electrophoretic mobility in phosphate buffer pH 8.5, 7.3cm (AMP, 7.3cm). ^{1}H NMR (D_20), δ 1.06 (Isopropyl, m, 6H), δ 6.15 (H-1', d, ^{1}H, J = 2. 5Hz), δ 8.41 (H-2,s, ^{1}H) and δ 8.20 (H-8,s, ^{1}H). FAB Mass-M^+ 390, M^+ Na 412, M^+ K 428. E.1. Mass m/z 178 (Base + isopropyl), m/z 135 (Base + 1) and m/z 108, 81 and 54.

The product corresponding to faster moving spot Rf 0.81 in solvent A was isolated as amorphous solid (36mg; 28.9%) and was identified as *N^1-isopropyl-5'-AMP isopropylester* **5** U.V. λ max 256nm, pH 1, λ max 257nm, pH 12; paper electrophoretic mobility in phosphate buffer pH 8.5 4.0cm (AMP 7.8cm) ^{1}H-NMR (D_20), δ 1.15 (isopropyl,m, 14H), δ 6.03 (H-1'd, 1H, J = 2.5Hz), δ 8.21 (H-2,s, 1H), δ 8.14 (H-8,s, ^{1}H). M.S., E.1. M^+, m/z 432, m/z 416 (M^+-CH_3), m/z 177 (Base + isopropyl) and m/z 136 (Base + 1).

Isopropylation of 5'-AMP in DMSO using potassium tert-butoxide. 5'-AMP (100 mg) was dissolved in dry DMSO (2.5 ml) and potassium *tert*-butoxide (880mg in 2.5ml DMSO) was added and the mixture was stirred at room temperature for 1 hour. After addition of isopropyliodide (0.3 ml), the mixture was stirred further for 1 hour. The crude reaction mixture was separated by preparative paper chromatography in solvent a and separated bands were eluted with distilled water (20 ml). The major product corresponding to Rf 0.48 in solvent A was isolated as an amorphous powder, (34mg, 30.3%), and was characterised as *N^6-isopropyl-5'-AMP* **6** U.V. λ max 264nm pH 1 and 267nm pH 12. Paper electrophoretic mobility 6.5cm (AMP = 6.5 cm). ^{1}H-NMR (D_20); δ 1.28 (Isopropyl,m, 6H), δ 6.08 (H-1',d, 1H, J = 2. 5Hz), δ 8.4 (H-2,s, ^{1}H) and δ 8.2 (H-8, s, 1H).

M.S. FAB m/z 428 (M^+. K).

M.S. E.1. m/z 177 (Base + isopropy1),
m/z 162 (Base-CH_3), m/z 135 (B + H) and
m/z 108, 81 and 54.



DISCUSSION

Except for the methylation of 5'-AMP using dimethylsulphate and methylmethane sulphonate [11, 12] not much information on the direct alkylation of nucleotides is reported in the literature. A reinvestigation of the isopropylation under strongly alkaline conditions gave the already reported [10] 3'-isopropoxy 5'-AMP **2**, the structure of which has already been assigned in our previous publication. From the reaction mixture a second product with faster mobility, Rf 0.73 in solvent A, Table 1, was also isolated in 6.2% yield and has now been assigned as the isopropylester of 5'-AMP **3**. U.V. spectrum of the product did not show any change at acid or alkaline pH as compared to 5'-AMP and therefore substitution at the base moiety can be excluded. The paper electrophoretic results, Table 1, of this

Table 1. Paper chromatographic and paper electrophoretic data for isopropylated-5'-AMP derivatives.

Compound	Rf Values			Paper electrophoresis (migration in cm.)
	Solvent A	Solvent B	Solvent C	
5'-AMP **1**	0.06	0.19	0.33	7.5
5'-AMP isopropylester **3**	0.73	0.79	0.83	3.8
N^1-isopropyl-5'-AMP **4**	0.48	0.50	0.67	7.5
N^1-isopropyl-5'-AMP isopropylester **5**	0.81	0.80	0.82	3.9
N^6-isopropyl-5'-AMP **6**	0.48	0.38	0.71	7.5

product confirmed the assigned structure as the mobility of **3** was found to be half of that of 5'-AMP at pH 8.5 in phosphate buffer indicating it as the mono-anion. The ^{1}H-NMR spectrum of **3** exhibited a multiplet at δ 1.15 integrating for 6 protons due to isopropyl group and singlets for H-8 and H-2 protons of adenine at δ 8.14 and δ 8.21 respectively. The anomeric proton of ribose was found at δ 6.03 as doublet with a small coupling constant, $J_{1,2}$ 2.5 Hz. The E.1. mass spectrum exhibited characteristic base peak at m/e 135 and base plus peak at m/e 136. The other ions arising from the fragmentation of the base were at m/e 108, m/e 81 and m/e 54.

Among the purine nucleosides adenosine has been reported to give 1-methyladenosine using methyliodide in dimethylacetamide while methylation of guanosine and deoxyguanosine using methyliodide in N, N-dimethylformamide and dimethylsulphoxide respectively gave substitution at the 7-position of guanine moiety of the nucleoside [11]. It was therefore of interest to study the isopropylation of 5'-AMPusing the alkylhalide in DMSO as solvent and anhydrous potassium carbonate as the acid scavenger. The reaction was carried out at room temperature for 3 hours and after addition of a further quantity of the alkylhalide and potassium carbonate, for 6 hours. The crude reaction mixture on paper chromatography in solvent A, B & C showed the formation of three new products out of which only two products, exhibiting Rf 0.48 and 0.81 in solvent A, Table 1, possessed a typical nucleotide spectrum. The products were isolated by preparative paper chromatography as amorphous solids in 18.7 and 28.9% yields. The slower moving product of Rf 0.48 in solvent A has been assigned the structure of N^1-isopropylated-5'-AMP **4** on the basis of typical ultraviolet spectral shifts and also by ^{1}H. NMR spectrum and FAB and E.1. mass spectroscopy. The U.V. spectrum of **4** is shown in Table 2, the N^1-isopropyl AMP showed a characteristic shift in the U.V. Spectrum at λ max 256nm at pH 1 and λ max 257nm at pH 12 which is

Table 2. U.V. Spectra of isopropylated 5'-AMP derivatives.

Compound	λ max (nm)		λ min (nm)	
	pH-1	pH-12	pH-1	pH-12
5'AMP **1**	259	260	230	228
5'-AMP isopropylester **2**	259	260	230	230
N^1-isopropyl-5'-AMP **3**	256	257	230	229
N^1-isopropyl-5'-AMP isopropyleste **4**	256	257	230	228
N^6-isopropyl-5'-AMP **5**	264	267	233	234

consistent with a substitution at N^1 of the base [11]. The ^{1}H-NMR spectrum in D_2O exhibited the protons for isopropylgroup as a multiplet at δ 1.06 integrating for 6 protons, the ribose anomeric proton at δ 6.15 as a sharp doublet, $J_{1,2}$ 2.5Hz integrating for single proton and the H-8 and H-2 protons of the base as singlets at δ 8.20 and δ 8.4 respectively integrating for a single proton in each case.

In spite of their polarity, nucleosides are generally volatile enough to be analysed by mass spectrometery [12] but nucleotides are not ameable to this type of analysis unless they are made volatile by derivatization. The trimethylsilyl derivatives have been subjected to mass spectroscopy and useful structural information has been published [13] which can be utilized for structural diagnosis of nucleotides. For the structural diagnosis of N^1-isopropyl-5'-AMP **4** we have used a combination of FAB and E.1. mass spectroscopic data to get usefull structural information. The FAB mass spectrum of **4** showed a M^+ at m/z 390 and additionally M^+. Na and M^+.K signals at m/z 412 and m/z 428 confirming the molecular weight while the E.1 gave fragments at m/z 178 for base plus isopropylgroup, m/z 135 for base and the characteristic fragments derived from base at m/z 108, 81 and 54 due to loss of three HCN fragments from the base successively.

The second product isolated from the reaction mixture possessed faster Rf value of 0.81 in solvent A and was obtained in 28.9% yield. It was characterised as the N^1-isopropyl-5'-AMP isopropylester **5** using spectroscopic techniques. The U.V. spectrum of **4** resembled the N^1-isopropyl-5'-AMP spectrum at pH 1 and pH 12 (Table 2) while its electrophoretic mobility was found to be half of that of 5'-AMP indicating substitution at the phosphate group. The ^{1}H-NMR spectrum exhibited a multiplet at δ 1.15 integrating for 15 protons representing two isopropylgroups, the ribose anomeric proton at δ 6.03 as a sharp doublet integrating for a single proton and the base protons for H-2 and H-8 at δ 8.21 and δ 8.14 as singlets integrating for single protons. The E.1. mass spectrum gave molecular ion at m/z

432, M^+CH_3 signal at m/z 416, the base plus isopropyl signal m/z 178 and the usual fragments obtained by decomposition of the base. It is possible that the diminution of charge by substitution of the phosphate group and the presence of a second isopropylgroup at the base renders this compound volatile enough to get meaningful structural information from electron impact mass spectrometery.

Another alkylation procedure which is often used in sugar alkylations [14] and has also been applied to nucleosides [15] involves the reaction of alkylhalide and sodium hydride in DMSO or DMF. It is also reported that similar alkylations can be carried out in sugars by using potassium tert-butoxide in DMSO and this reagent15 is as efficient as that prepared from sodium hydride. The isopropylation of 5'-AMP was carried out using the alkylhalide and potassium tert-butoxide in DMSO. The major product obtained in 30% yield was assigned the structure as N^6-isopropyl-5'-AMP **6** on the basis of spectroscopic evidence. This compound exhibited Rf 0.48 in solvent A and its electrophoretic mobility was equal to that of 5'-AMP indicating an unsubstituted phosphate group. The U.V. spectrum showed hyper chromic shifts in acidic and alkaline solutions which were identical with those due to exocyclic amino group substitution [11]. The ^{1}H-NMR spectrum in D_20 exhibited a multiplet at δ 1.28 integrating for 6 protons, the ribose anomeric proton at δ 6.08 as a sharp doublet, integrating for single proton and the base protons at δ 8.4 and δ 8.2 for H-8 and H-2 respectively integrating for a single proton. The FAB mass showed M^+K at m/z 428 and E.1. mass gave the expected fragments at m/z 177 for base plus isopropylgroup, m/z 162 for base minus methyl, m/z 135 for base and at m/z 108, 81 and 54 indicating successive loss of three HCN molecules from the base. It therefore appears that by varying the reaction conditions the isopropylation can be carried out at different reactive centres of the 5'-adenosine monophosphate.

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