Alkylation of Adenosine-5'-Monophosphate with Alkyl Halides under Strongly Alkaline Conditions

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Summary: Alkylation of adenosine-5'-monophosphate (5'-AMP) with alkyl halides under strongly alkaline conditions has been investigated. Reaction with methyl-,ethyl-, normal and iso-propyl iodides using a mixture of 1N NaOH and dioxane gave predominantly sugar substituted derivatives. The structure of major products, with special reference to orientation of alkyl groups at the ribose moiety, has been established through U.V. and NMR spectroscopy, and through paper chromatography.

Introduction

Occurrance of 2'-O-methylated nucleosides in the messenger as well as transfer RNA isolated from diverse type of organisms is well established [1,2]. Although the precise biological role of these modifications is still speculative, much work has been reported on the synthesis and properties of individual 2'-O-methylated nucleosides, nucleotides and polynucleotides. Such modified 2'-O-methylated polynucleotides have exhibited interesting biological properties, thus, for example, single stranded poly-2'-O-methyl-cytidylic acid is a specific template for viral directed RNA dependent DNA polymerase [3]. Similarly poly 2'-O-methyl adenylic acid has been reported to be an inhibitor of leukaemia virus replication in mouse embryo cells [4]. Synthesis and chemical properties of 2'-O-ethylnucleosides and polynucleotides have also been reported in the literature [5].

In view of the very interesting biological properties of these 2'-O-methylated polymers, especially with reference to their clinical potential, the need was felt to extend the range of alkyl groups at the 2'-O-position of ribose in order to prepare novel alkylated polymers for biological evaluation.

Various methods are reported in the literature for methylation of nucleosides which yield 2'-O-methylated products. Diazomethane in dimethoxyethylene-glycol ether has been widely used for methylation of unprotected nucleosides [6,7], but the use has been limited due to difficult accessibility of higher diazoalkanes. The reaction of dialkyl-sulphates in strongly alkaline medium has also been reported [8] to yield a mixture of 2'-O-,3'-O, and 5'-O-alkyl nucleosides. The method is again limited due to difficulty in getting higher dialkyl sulphates and the formation of complicated mixtures of various isomers, leading to diminution in yields of the required 2'-O-methylated products. The reaction of methyl and ethyl iodides with nucleoside-3,5-cyclic phosphates under strongly alkaline conditions has also been reported [9], but the difficult and unsatisfactory hydrolysis of the cyclic phosphate to

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the 2'-O-alkylated nucleotides makes the method less attractive in its application.

Since the alkyl iodides are readily and cheaply available, and the reports that under strongly alkaline conditions the alkylation at the heterocyclic amino group is minimised [8,9] while the hydroxyls of ribose are easily alkylated, it was decided to study the reaction of adenosine-5'-monophosphate directly with different alkyl iodides under alkaline conditions. The present studies describe the results obtained by such a reaction using methyl-, ethyl-, n-propyl- and iso-propyl iodides with adenosine-5'-monophosphate. The present approach has provided a method of direct alkylation of 5'-AMP, under conditions which lead to the formation of sugar alkylated products as major compounds and has led to the synthesis of hitherto unknown 2'-O-n-propyl and 3'-O-isopropyladenosine 5'-monophosphates. The 2'-O-alkylated nucleotides can be converted to the respective diphosphates and finally to polynucleotides, by enzymatic polymerisation, thus considerably shortening the synthetic route.

Results and Discussion

5'-AMP reacted readily with methyl and ethyl iodides at room temperature using 1N NaOH and dioxane as the solvent. The crude reaction mixture on paper chromatography exhibited the formation of products possesing higher mobility as compared to the starting material in all the solvents used, (Table-1). No reaction was observed in the case of normal and isopropyl iodides with 5'-AMP at ambient temperatures, therefore the reaction was carried out at 60-70°C when the crude reaction mixture on paper chromatography showed the formation of alkylation products (Fig. 1). The major and minor products were separated by paper chromatography using 3 MM-

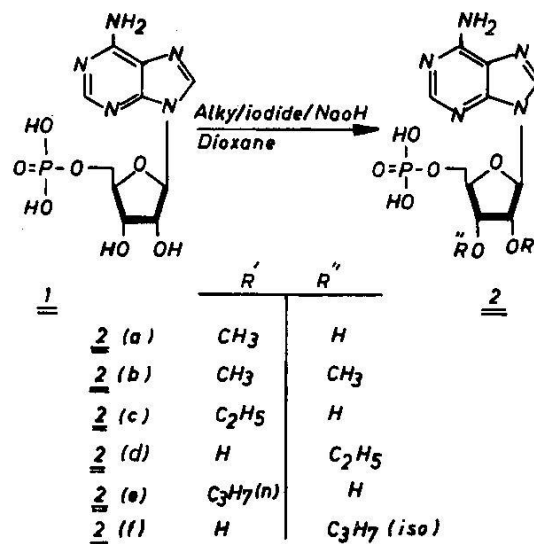|	R'	R''
2 (a)	CH_3	H
2 (b)	CH_3	CH_3
2 (c)	C_2H_5	H
2 (d)	H	C_2H_5
2 (e)	$C_3H_7(n)$	H
2 (f)	H	C_3H_7 (iso)

FIG. 1

chromatography paper in solvent A and pure products were eluted from paper strips by distilled water. Evaporation at low temperature (below 40°C) and reduced pressure gave pure compounds which were then used for structural investigations. The yield of 2'-O-alkylated products ranged from 60% for 2'-O-methyl and 70% for 2'-O-ethyl, while yields of 2'-O-normal and 3'-O-isopropyl adenosine nucleotides were lower, 21 and 25% respectively, possibly due to the formation of other side products.

Previous work has shown that under strongly alkaline conditions the alkylation at the exocyclic amino group of the base moiety is not favoured. In our reactions a similar pattern is followed as is evidenced by ultraviolet and proton NMR spectroscopy. The ultraviolet spectra of adenosines are sensitive to alkylations at the base moiety [10] and any substitution at the base will show shifts in the absorption maximum. The U.V. spectra of alkylated derivatives as shown in Table-2 hardly show any shift in the absorption maxima thereby excluding

Table-1: Paper chromatography of alkylated adenosine-5'-monophos-
phates

S.No	Compound	Rf.Values		
		Solvent A	Solvent B	Solvent C
1.	5'-AMP (1)	0.08	0.31	0.42
2.	2'-O-methyl-AMP (2a)	0.16	0.45	0.54
3.	2',3'-di-O-methyl-AMP (2b)	0.23	0.53	0.62
4.	2'-O-ethyl-AMP (2c)	0.20	0.54	0.61
5.	3'-O-ethyl-AMP (2d)	0.38	0.63	0.74
6.	2'-O-n-propyl-AMP (2e)	0.29	0.57	0.69
7.	3'-O-isopropyl-AMP (2f)	0.25	0.54	0.66

Table-2: U.V.spectra of 2'-O-alkyladenosine-5'-monophosphates

S.No.	Compound	λ_{max} (nm)		λ_{min} (nm)	
		pH.1	pH.12	pH.1	pH.12
1.	2'-O-methyl-AMP (2a)	260	260	230	230
2.	2'-3,-O-dimethyl-AMP (2b)	260	260	230	230
3.	2'-O-ethyl-AMP (2c)	259	260	231	230
4.	3'-O-ethyl-AMP (2d)	259	260	232	230
5.	2'-O-n-propyl-AMP (2e)	260	260	231	231
6.	3'-O-isopropyl-AMP (2f)	259	260	230	230

the possibility of base alkylation. This finding is further confirmed by the proton NMR spectroscopy, where $O-CH_3$ and $N-CH_3$ groups can be very easily distinguished due to difference in their chemical shifts. The evidence presented above, therefore, confirmed that alkylation has taken place in the sugar moiety of the nucleotides.

The possibility of esterification of phosphate group has been excluded on the basis of paper electrophoresis at pH 8.5 where no difference was observed between Rf. of 5'-AMP and the substituted products. Furthermore potentiometric titrations showed that the phosphate group was unsubstituted as it showed the presence of both primary and secondary dissociations in all the products being reported in this publication. The possibility that the dialkyl derivatives may have alkyl groups at the ribose as well as at the phosphate moieties can be excluded on the basis of evidence derived from potentiometric and electrophoretic studies of these derivatives. In the literature Griffin and Resse [11] have reported the methylation of 5'-AMP using dimethyl sulphate at pH 7.2 and have isolated phosphate methylated products in considerable amounts. In the present studies, under our conditions, the major and minor products isolated by us do not show any evidence of phosphate methylation, though the presence of other minor products in the reaction mixture possessing alkylation at phosphate group cannot be completely excluded.

NMR spectroscopy has been of special value in structure elucidation of nucleosides and nucleotides. The presence of 5'-phosphate group can be easily established by the shielding effect of the group on the 5'-methylene hydrogens and interaction with the H-8 of purine ring. An examination of Table-3 indicates the expected shifts of the 5' -methylene protons as well as H-8 of

the purine ring. These values conform with those reported in the literature [9].

The site of alkylation at the sugar hydroxyl groups of ribose can be conveniently shown with the help of proton NMR spectroscopy. It has already been established [7,12] that substitution at the 2'-OH group shifts the position of anomeric H-1' proton to higher values while 3'-O-substitution shifts the position of the H-1' resonance to lower values as compared to the unsubstituted nucleoside or nucleotide. An examination of Table-3 indicates that the major product, in the case of methylation is 2'-O-methyl-AMP as the signal for H-1' is shifted to higher value, and further more $O-CH_3$ resonance is exhibited at δ 3.47 intergrating for three protons. The structure of the minor product can be established as 2'-3'-di-O-methyl-AMP due to the position of the anomeric proton and the presence of two signals for two methoxy groups at δ 3.42 and δ 3.57, each signal integrating for 3 protons, similar values are reported in the literature for these two methoxyl protons [8].

In the case of ethylation, NMR evidence indicated that the major product is 2'-O-ethyl-AMP while the minor product is 3'-O-ethyl AMP furthermore the O-ethyl protons are at the expected positions, δ 1.10 (CH_3,3H) and δ 3.67 (CH_2,2H). The minor alkylation product obtained by using n-propyl iodide has been characterised as 2'-O-n-propyl-AMP by displacement of H-1' proton to higher values, the n-propyl group exhibiting resonance at δ 0.75 (CH_3,3H), δ 1.52 (CH_2,2H) and δ 3.0 (CH_2,2H). The major product obtained by reaction of AMP with isopropyl iodide is interesting and has been assigned the structure 3'-O-isopropyl AMP on the basis of NMR spectroscopy. The predomimnt synthesis of the 3'-isopropyl isomer in contrast to the 2'-

Table-3: N.M.R.Spectra* (in D_2O) of alkylated adenosine-5'-monophosphates

Compound	H-2	H-8	H-1'	H-5',5"	O-alkyl groups		
Adenosine	8.25	8.07	6.05	3.90			
5'-AMP (1)	8.60	8.39	6.18	4.18			
2'-O-CH$_3$AMP (2a)	8.60	8.14	6.20	4.02	3.47		
2'-,3'-di-O-methyl-AMP (2b)	8.65	8.20	6.17	4.02	3.42	3.57	
2'-O-ethyl-AMP (2c)	8.62	8.22	6.20	4.03	3.67	1.10	
3'-O-ethyl-AMP (2d)	8.65	8.22	6.17	4.02	3.65	1.15	
2'-O-n-propyl-AMP (2e)	8.65	8.25	6.20	4.04	3.00	1.52	0.75
3'-O-isopropyl-AMP (2f)	8.60	8.22	6.15	4.05	3.82	1.10	

*Chemical shifts are denoted as δ values.

O-isomers as in methyl, ethyl and n-propyl AMP derivatives is not presently clear. It is quite plausible that the steric hindrance offered to the bulkier isopropyl group at the 2'-O-position, by the heterocyclic ring does not favour 2'-O-substitution, whereas the 3'-O-hydroxyl group is free to undergo this reaction. Further work to elaborate these findings is in progress.

Experimental

5'-AMP was purchased from E.Merck, Darmstadt. Paper chromatography was performed in all glass apparatus, in a descending manner using whatman No.1 or 3 MM paper. The solvent systems used were A, isopropanol: ammonium hydroxide: 0.1 M boric acid (7:1:2); B, n-propanol: ammonium hydroxide: water (55:10:36) and C, ethanol: 1M ammonium acetate pH 5.0 (7:3). The U.V. spectra were determined on a spectromom 204 spectrometer. NMR spectra were determined on a 100 MHZ, Jeol PMX 60 spectrophotometer.

General alkylation procedure

5'-AMP (400 mg) was suspended in dioxane (2.0 ml) and 1N NaOH solution (4.8 ml) was added. The mixture was stirred magnetically and alkyl iodide was added. The stirring was carried out at room temperature or at 60-70° for 3 hours depending upon the alkyl halide. The pH throughout the reaction remained on the alkaline side. The reaction mixture was then concentrated to 2 ml under vaccum below 40°C and was loaded on 4 sheets of 3 MM whatman chromatography paper the chromatography was done in solvent A. The bands of major and minor products were cut and eluted with distilled water (25 ml). The eluate was concentrated in vaccum below 40° to afford the respective alkylation products.

2'-O-Methyl-AMP (2a)

5'-AMP (400 mg) was reacted with methyl iodide (1.2 ml) in 1N NaOH (4.8 ml) and dioxane (2.0 ml) at room

temperature. The major product (Rf. 0.16, solvent A) was eluted and repeatedly evaporated with methanol (3x20 ml) till constant weight was obtained to afford 2'-O-methyl-AMP (2a), (242 mg, 68%) as an amorphous powder.

2',3',-Di-O-methyl-AMP (2b)

The minor band after the isolation of 2'-O-methyl-AMP (Rf.0.23, solvent A) was eluted and similarly worked up to yield the dimethylated AMP (2b) (80 mg, 20%) as an amorphous powder.

2'-O-Ethyl-AMP (2c)

5'-AMP (400 mg) was reacted with ethyl iodide as described above at 70° for 3 hours. Work up of the reaction mixture in the usual manner afforded the major product (Rf. 0.20, solvent A) as amorphous solid (319 mg. 79%).

3'O-Ethyl-AMP (2d)

The minor band after separation of 2'-O-ethyl AMP which had Rf. (0.38, solvent A) was worked up as described above to afford 3'-O-ethy-AMP (2d) (47 mg, 12%).

2'-O-Propyl-AMP (2e)

5'-AMP (400 mg) was reacted with n-propyl iodide (1.2 ml) at 70°C. Work up of the reaction mixture by usual procedure gave 2'-O-n-propyl AMP (2e) as a major product (89 mg 22.2%), Rf.(0.29, solvent A)

3'-O-Isopropyl-AMP (2f)

Treatment of 5'-AMP (400 mg) with isopropyliodide at 70° and following the usual work up procedure gave 3'-O-isopropyl-AMP (2f) as the major product (100 mg, 26%), Rf. (0.25, solvent A).

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