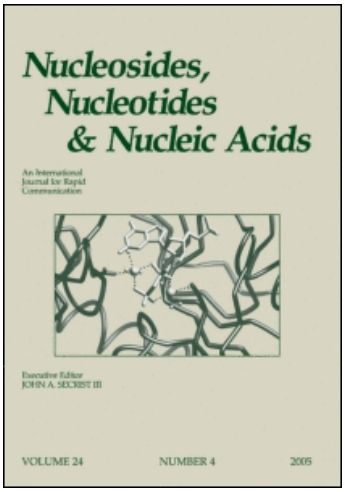

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^{13}C NMR SPECTRA OF SOME RIBOSE ALKYLATED ADENOSINE- 5'- MONOPHOSPHATE DERIVATIVES

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ABSTRACT: The ^{13}C NMR spectra of 2'-O-methyl-2, 2', 3'-di-O-methyl-3, 2'-O-ethyl-4, and 3'-O-ethyl-5'-AMP-5 derivatives were measured in D_2O and are compared with those of non-alkylated 5'-AMP. The assignments for the ribose as well as base carbons have been made using DEPT experiments. The O-alkylation of ribose hydroxyls by methyl or ethyl groups resulted in pronounced downfield shifts of the signals for the substituted carbons, while the signals for adjacent carbons are shifted upfield, but to a much smaller extent. This behaviour is consistent with that reported for other model compounds. The pronounced downfield shift of the quaternary C-5 carbon of the base, in the protonated form of 5'-AMP as compared to its sodium salt in the non-protonated form, can be readily explained by protonation of the N-1 position of the adenine base.

INTRODUCTION: The structural modifications which the nucleic acids undergo on exposure to various carcinogens and mutagens may affect the replication and transcription of genetic material.[1] The elucidation of the structure of such modified nucleotides and their biological consequences may throw some light on the mechanism of chemical carcinogenesis and mutagenesis. The modern spectroscopic methods like NMR spectroscopy or mass spectrometry are now being increasingly applied to determine the structure of modified nucleic acids and their components. During our work on the synthesis of a number of 2'-O- and 3'-O-alkylated derivatives of 5'-AMP,[2] a number of such nucleotides were readily available and therefore it was of interest to study their ^{13}C NMR spectra. Such studies will lead to the understanding of the effect of ribose alkylations on the ^{13}C NMR spectral characteristics of

5'-AMP derivatives and also provide a method for distinguishing between a pair of 2'-O- and 3'-O-alkyl derivatives of the nucleotides. Furthermore, except for 5'-AMP, data for ^{13}C NMR of these compounds are not reported in the literature. ^{13}C NMR spectroscopy has found wide application in the structural elucidation of a variety of organic compounds. In the carbohydrate area extensive data are available about the ^{13}C NMR of methyl-O-alkyl pentofuranosides. These data indicate that O-alkylation of a hydroxyl group in the pentofuranosides is characterized by a downfield shift of the signal for the alkoxy substituted carbon while the adjacent carbons are shifted, in most cases, upfield but to a smaller extent.[3] In the case of uridine and inosine the alkylation sites on the ribose moiety have been unequivocally assigned by using ^{13}C NMR spectroscopy, and this has lead to an efficient method for the differentiation of 2'-O-, 3'-O- and 5'-O-alkylated nucleosides.[4] A variety of nucleosides and their derivatives have been examined by ^{13}C spectroscopy and assignments for the base as well as sugar carbons have been reported in the literature.[5,6,7]

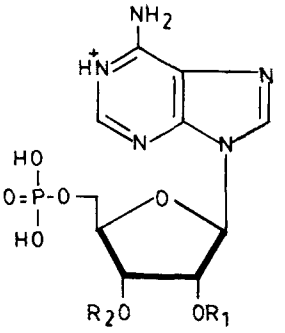
DISCUSSION: Previously the site of substituents in a pair of 2'-O- and 3'-O-alkyl nucleosides and nucleotides has been determined by the use of ^{1}H NMR spectroscopy.[8,9] These studies have established that the signal for the anomeric proton is shifted downfield on 2'-O-alkylation and upfield on 3'-O-substitution, as compared to the unsubstituted nucleoside or nucleotide. Many applications of this method for determining the site of alkylation are found in the literature.[10] However, these data reveal that the shift of anomeric protons are very small and vary in the range of 0.05 to 0.19 ppm depending on the concentration of the sample hence difficulty in assignment of signals may arise. ^{13}C shifts are generally of a larger magnitude and assignments for ribose alkylations would therefore be expected to be unambiguous.

The ^{13}C spectrum of the disodium salt of 5'-AMP (Table-1) corresponds closely with that reported in the literature[11,12] for the free adenylic acid and assignments of various ^{13}C signals have been made by comparison with the reported values. The values of C-2 and C-4 are reported to be 153.3 and 149.3 ppm, respectively,[11] but since in the present study the assignments are based upon DEPT experiments whereby the methylene, methine and quaternary carbons are unambiguously assigned and C-2 being a methine carbon resonates at 147.99 while C-4 a quaternary carbon resonates at 151.04 ppm. It therefore appears that the values for C-2 and C-4 cited in the

TABLE-1: ^{13}C Chemical shifts (ppm) in the NMR Spectra of ribose alkylated 5'-AMP derivatives in D_2O

5'-AMP Derivative	Base carbons					Ribose carbons					
	C-2	C-4	C-5	C-6	C-8	C-1'	C-2'	C-3'	C-4'	C-5'	C-O-alkyl
5'-AMP (Disodium salt)	147.99	151.04	121.23	153.08	144.87	90.63	77.36	73.01	86.92	66.97	-
5'-AMP (Free Acid) 1	152.76	151.17	160.00	157.12	143.86	90.82	77.44	73.05	86.96	67.09	-
2'-O-methyl-5'-AMP 2	155.67	151.75	164.74	158.40	143.05	88.06	86.00	72.07	87.96	66.12	60.76
2',3'-Di-O-methyl-5'-AMP 3	155.72	151.85	164.91	158.47	143.06	88.14	86.08	85.17	86.41	66.59	60.76 60.16
2'-O-ethyl-5'-AMP 4	155.68	151.50	164.75	158.43	143.09	88.26	84.41	72.30	87.96	66.10	69.95 17.02
3'-O-ethyl-5'-AMP 5	155.60	151.77	164.73	158.35	143.00	88.94	79.82	83.65	86.46	66.49	69.48 17.00

* The status of each carbon was confirmed by DEPT experiments.



	R_1	R_2
1.	H	H
2.	CH_3	H
3.	CH_3	CH_3
4.	C_2H_5	H
5.	H	C_2H_5

FIG. 1

literature[11] may require to be corrected. The spectrum of 5'-AMP (Table-1, Fig-1) in the protonated form as well as the sodium salt exhibits signals for two methine carbons C-2 and C-8 and three quaternary carbons C-4, C-5 and C-6 of the adenine base; while the ribose portion of the spectrum exhibits signals for four methine carbons C-1', C-2', C-3' and C-4' and one methylene carbon C-5'.

When the ^{13}C spectrum of 5'-AMP was measured in the free acid form, prepared by passing an aqueous solution of sodium salt on a column of strong cation exchange resin, Amberlite IR - 120, H^+ form, the signals for C-5 and C-6 quaternary carbons were shifted downfield, as compared to those in the disodium salt (Table-1), while the ribose carbons were unaffected. Jones and Robins[13] have reported that in adenosine the nitrogen at position 1 is the most basic and accepts a proton readily. Similar conclusions were also obtained by Windmueller and Kaplan[14] and Tsuboi and coworkers[15] after infrared studies of adenosine. It is therefore reasonable to assume that on neutralization with strong acidic resin the N-1 protonated AMP is obtained. The downfield shift of C-5 from 121 ppm to 160 ppm can therefore be attributed to a charge redistribution in the adenine ring resulting in the reduction of electron density at C-5. The possibility of protonation at N-7 has been excluded on the basis of U.V. spectral measurements; the spectra of the protonated derivatives similar to the sodium salt of 5'-AMP at pH 1; protonation at N-7 would have resulted into a considerably changed U.V. spectrum.[16] A comparison of ^{13}C NMR spectra of 5'-AMP as the sodium salt and as the free acid in the protonated form clearly supports protonation at N-1 and consequent downfield shift of C-5 in AMP and also in all the alkylated derivatives 2 - 5; as expected the C-6 carbon also exhibits a downfield shift but to a smaller extent.

TABLE-2: Displacement* of ^{13}C signals (ppm) in ribose alkylated 5'-AMP derivatives (in D_2O) relative to the corresponding carbon atoms in the unsubstituted 5'-AMP in the protonated form.

5'-AMP Derivative	C-1'	C-2'	C-3'	C-4'	C-5'
2'-O-methyl-5'-AMP 2	-2.76	+8.56	- 0.98	+1.02	-0.87
2',3'-O-Dimethyl-5'-AMP 3	-2.68	+8.64	+12.12	-0.55	+0.50
2'-ethyl-5'-AMP 4	-2.56	+6.97	- 0.75	+1.00	-0.99
3'-O-ethyl-5'-AMP 5	-1.88	+2.38	+10.60	-0.50	-0.60

* In signal displacement (+) represents downfield shifts and (-) represents upfield shifts.

The ^{13}C spectra of the ribose part of 5'-AMP show expected shift of the substituted carbons, after alkylation. In the case of 2'-O-methyl 2 and 2'-O-ethyl 4 derivative of 5'-AMP the 2'-carbon is shifted downfield to the extend of 8.56 and 6.97 ppm, respectively, as shown in Table-2. The adjacent carbon atoms i.e. C-1' and C-3' are shifted upfield, but to a smaller extent, which is consistent with the alkylation pattern in methylpentofuranosides[3] as well as with the alkylation pattern of uridine and inosine.[4] As would be expected the 2', 3'-di-O-methyl-5'-AMP 3 exhibits downfield shift of 2' and 3'-carbons to the extent of 8.64 and 12.12 ppm, respectively, while the adjacent C-1' and C-4' carbons are shifted upfield to the extent of 2.94 and 0.51 ppm, respectively. Similarly the 3'-O-ethyl 5'-AMP, 5 exhibits a downfield shift of 3'-carbon to the extent of 10.68 ppm. From this work it is apparent that ^{13}C spectroscopy can be used for distinguishing between the 2' and 3'-O-alkyl substitutions in nucleotides.

EXPERIMENTAL: The synthesis and complete characterization of alkyl derivatives of 5'-AMP is described in reference 2. ^{13}C spectra were determined on a Bruker AM-300 NMR spectrometer as 10 mg/ml solution in D_2O using DSS as the internal standard. The free acids were obtained by passing an aqueous solution of the disodium salt of the nucleotides through a cation exchanger, Amberlite IR 120 (H^+ form). The acidic eluate was collected, evaporated to dryness under vacuum at 37°C and thoroughly dried under vacuum before measurement of spectra.

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