Pak. j. sci. ind. res., vol. 38, nos. 9-10, September - October 1995

NEGATIVE ION FAB MASS SPECTROMETRY OF SOME RIBOSE ALKYLATED CYTIDINE 5'-MONOPHOSPHATE DERIVATIVES

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(Received September 21, 1991; revised June 14, 1995)

Characteristic fragment ions obtained in FAB mass spectrometry of ribose alkylated cytidine 5'-monophosphate (5'-CMP) derivatives in the negative mode are described. All the compounds examined exhibited either molecular ion M or quasimolecular ion M-H, the fragment ions can be used to characterise these nucleotides.

Key words: FAB mass spectrometry, Ribose alkylation, Cytidine-5'-monophosphate.

Introduction

Mass spectroscopy of nucleosides and modified nucleosides has been very useful in determination of the structure of these compounds [1,2], however, nucleotides due to their high polarity and low volatility are generally not amenable to the electron impact mass spectrometry. Mass spectrometry of more volatile trimethylsilyl derivatives of nucleotides has been successfully used for structural correlations and principal fragmentation patterns for these derivatives have been elaborated [3]. The development of many soft ionisation techniques, such as field desorption [4], californium-252-plasma desorption [5], secondary ion MS [6], pulse laser induced desorption [7], atmospheric pressure ionisation MS [8] and pyrolysis electron impact as well as chemical ionisation MS [9] have been successfully used in nucleoside and nucleotide area.

Fast atom bombardment (FAB) spectrometry is a milder desorption ionisation technique and has proved useful in the study of free [10] and protected nucleosides and nucleotides as well as in studies of the adducts of nucleosides with chemical carcinogens [11,12] and adducts of metal nucleotide complexes [13].

FAB mass spectrometry requires a stream of fast atoms of argon or xenon to be bombarded on to a solution of a sample in a liquid matrix such as glycerol. The solution volatilises and ionises generating positive or negative ions which can be detected by usual techniques. It has also been reported that the use of FAB - MS in the negative ion mode reduces interference from positive counter ions and allows rapid sequence determination of simple di- and trinucleotides [14].

A number of new ribose - alkylated nucleotides were available from our work in these Laboratories and it was desirable to study their FAB mass spectra specially in the negative ion mode, the result of such a study on the derivatives of 5'-CMP are being presented in this publication. A literature survey has indicated that biological activities of such compounds like inhibiting activities of the enzymes involved in nucleic acid metabolism are not reported.

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Experimental

5'-CMP disodium salt was purchased from BDH (England). The purity of all the compounds used in this study was checked by paper chromatography in three solvents: (i) Iso-propanol:NH_4OH:H_2O (7:1:2), (ii) Ethanol: 1 M ammonium acetat pH 7.0 (7:3; v/v) and (iii) n-Propanol: ammonium hydroxide:water (5.5:1:3.5;v/v). All the compounds showed single homogeneous spots. The synthesis of the ribose alkylated 5'-CMP derivatives is described as in reference [15].

FAB mass spectra in the negative mode were recorded on a Finnigal MAT - 312 mass spectrometer connected to a PDP 11/34 computer system using glycerol as the matrix.

Results and Discussion

Some significant ions observed in the FAB (negative) mass spectra of ribose alkylated 5'-CMP derivatives are shown in Table 1. The parent nucleotide, 5'CMP (Scheme 1) as disodium salt exhibited the molecular ion $[M]^-$ at *m/z* 367 which corresponded with its correct molecular formula (Table 1) while the loss of two sodium atoms from the molecule exhibited ions at *m/z* 344 and *m/z* 322 due to the ions $[M - Na]^-$ and $[M+H-2Na]^-$ respectively. A peak at *m/z* 459 was observed due to the clustering of molecular ion with glycerol producing the ion $[M+glycerol]^-$, while the ion *m/z* 436 was due to the ion $[M-Na+glycerol]^-$ and that at *m/z* 414 due to the ion $[M+H-Na_2+glycerol]^-$.

The compound 2'-O-methyl 5'-CMP (**2**) ($R_1 = CH_3$; $R_2=R_3=R_4=H$) exhibited the quasimolecular ion $[M-H]^-$ at *m/z* 336 corresponding to the molecular formula $C_{10}H_{15}N_3O_8P$,

TABLE 1. SOME SIGNIFICANT IONS OBSERVED IN THE FAB (NEGATIVE) MASS SPECTRA OF RIBOSE ALKYLATED 5'-CMP DERIVATIVES.

Compound	[M]⁻ / [MH]⁻	[M-Alkyl]⁻	M+H-Na [M-Na]⁻	[M]/[M-H+glycerol]⁻	[M-Na+glycerol]⁻	Molecular Formula
5'-CMP 2Na **1**	367(52)	-	344	459(18)	436(26)	$C_9H_{14}N_3O_8P_2Na_2$
2'-O-Methyl 5'CMP **2**	366(15)	322	-	428(5)	-	$C_{10}H_{16}N_3O_8P$
3'-O-Methyl 5'CMP 2Na **3**	380(7)	367	-	471(6)	-	$C_{10}H_{14}N_3O_8P_2Na_2$
2',3'-Di-O-Methyl 5'-CMP **4**	350(12)	325(6)	-	442(19)	-	$C_{11}H_{18}N_3O_8P$
2'-O-Ethyl 5'-CMP Na **5**	394(25)	367(22)	-	459(8)	-	$C_{11}H_{16}N_3O_8P_2Na$
3'-O-Ethyl 5'-CMP **6**	350(22)	-	-	442(8)	-	$C_{11}H_{18}N_3O_8P$
2',3'-Di-O-Ethyl 5'-CMP **7**	379(100)	-	-	470(47)	-	$C_{13}H_{22}N_3O_8P$
2'-O-n-Propyl 5'-CMP Na **8**	387(18)	343(18)	365(35)	479(8)	-	$C_{12}H_{19}N_3O_8P_2Na$
2',3'-Di-O-n-Propyl 5'-CMP Na **9**	429(30)	386(87)	407(31)	521(10)	478(25)	$C_{15}H_{25}N_3O_8P_2Na$
N³,2'-Di-O-n-Propyl 5'-CMP Na **10**	429(12)	321(9)	407(20)	521(10)	499(29)	$C_{15}H_{25}N_3O_8P_2Na$
3'-O-iso-Propyl 5'-CMP 2Na **11**	409(30)	387(48)	365(58)	501(7)	593(5)	$C_{12}H_{18}N_3O_8P_2Na_2$

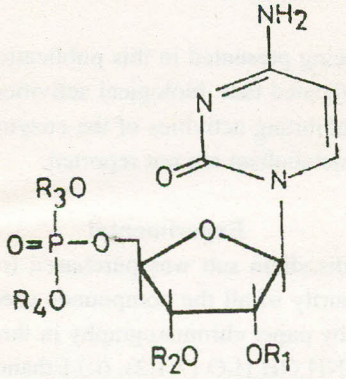

Fig. 1. Cytidine-5-monophosphate (R_1=R_2=H, R_3=R_4=Na)

the peak at *m/z* 322 was due to the loss of alkyl group giving rise to the ion $[M+H=CH_3]^-$. The glycerol solvated ion [M-H+glycerol]⁻ was exhibited at *m/z* 428. The compound 3'-O-methyl 5'-CMP monosodium salt (**3**) (R_1=R_3=H; R_2=CH_3; R_4=Na) exhibited the quasimolecular ion [M-H]⁻ at *m/z* 380 corresponding the molecular formula of the compound. The ion [M-H-alkyl]⁻ appeared at *m/z* 367 while the glycerol-solvated quasimolecular ion [M-2H+glycerol] appeared at *m/z* 471. The compound 2', 3'-di-O-methyl 5'-CMP, (**4**) R_1=R_2=CH_3; R_3=R_4=H) exhibited [M-H]⁻ at *m/z* 351 which corresponded to its molecular formula (Table 1).The ion [M-H+glycerol]⁻ at *m/z* 242 appeared due to the addition of glycerol.

The compound 2'-O-ethyl 5-CMP disodium salt, (**5**) R_1=C_2H_5; R_2=H; R_3=R_4=Na) exhibited the molecular ion [M-H]⁻ at *m/z* 394 which corresponded with the molecular formula, the loss of the ethyl group results into ion [M-H-ethyl]⁻ at *m/z* 367 and the ion at *m/z* 459 was due to the fragment [M-ethyl+glycerol]⁻. The 3'-O-ethyl 5'-CMP, (**6**) (R_1=R_3=R_4=H; R_2=C_2H_5) exhibited [M-H]⁻ at *m/z* a 350 and the ion at *m/z* 442 appeared due to the addition of glycerol i.e. [M-H+glycerol]⁻. The compound 2', 3'-di-O-ethyl 5'-CMP (**7**) (R_1=R_2=C_2H_5; R_3=R_4=H) exhibited the molecular ion [M-H]⁻ at *m/z* 379 and loss of the alkyl groups resulted into the formation of the ion [M+2H-2 ethyl]⁻ to give a peak at *m/z* 325. The addition of glycerol resulted into the formation of the ion [M-2H+glycerol]⁻ at *m/z* 470.

The compound 2'-O-n-propyl 5'-CMP monosodium salt (**8**) (R_1=n-propyl; R_2=R_3=H; R_4=Na) exhibited the molecular ion [M]⁻ at *m/z* 387, loss of alkyl group gave the ion which exhibited the [M-H-n-propyl] peak at *m/z* 343. Loss of sodium resulted into the formation of ion [M+H-Na]⁻ at *m/z* 365 and glycerol adduct ion [M+glycerol]⁻ appeared at *m/z* 479.

The compound 2',3'-di-O-n-propyl 5'-CMP monosodium salt (**9**) (R_1= R_2= n-propyl; R_3 = H; R_4 = Na) showed the molecular ion [M]⁻ at *m/z* 429. Successive loss of two n-propyl groups produced ions [M-n-propyl]⁻ and [M-2 × n-propyl]⁻ at *m/z* 386 and 342 respectively. The glycerol solvated ion [M+glycerol]⁻ appeared at *m/z* 521. The compound N³, 2'-O-di-n-propyl 5'-CMP monosodium salt (**10**) also exhibited the molecular ion [M]⁻ at *m/z* 429, the peak at *m/z* 321 resulted due to the ion [M- 2 × n-propyl+H-Na]⁻. The glycerol solvated ion [M+glycerol]⁻ was present at *m/z* 521.

The compound 3'-O-isopropyl 5'-CMP disodium salt (**11**) (R_1=H; R_2=isopropyl; R_3=R_4=Na) exhibited the molecular ion [M]⁻ at *m/z* 409 corresponding to correct molecular formula, other expected diagnostic fragments were also present.

The results of the present studies indicate that FAB mass spectrometry specially in the negative ion mode is a useful technique for characterization of nucleotide derivatives. Fragments below *m/z* 200 were not recorded due to complexities of matrix bound ions, therefore, base and ribose-derived ions could not be characterized.

In all our ribose alkylated 5'-CMP derivatives the molecular ion [M]⁻ or quasimolecular ion [M-H]⁻ could be invariably characterized. The ions arising due to loss of alkyl groups, sodium ions and those due to glycerol solvated molecular ions can be easily recognised. The application of high resolution mass spectrometry will provide exact molecular

formula of these nucleotides for their accurate and unambiguous characterization.

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