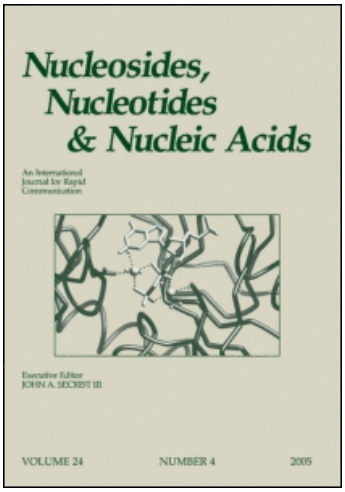

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Differentiation of Isomeric Purine and Pyrimidine Mononucleotides by Fast Atom Bombardment Tandem Mass Spectrometry

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To cite this Article Walton, Terence J. , Ghosh, Dipankar , Newton, Russell P. , Brenton, A. Gareth and Harris, Frank M.(1990) 'Differentiation of Isomeric Purine and Pyrimidine Mononucleotides by Fast Atom Bombardment Tandem Mass Spectrometry', Nucleosides, Nucleotides and Nucleic Acids, 9: 7, 967 — 983
To link to this Article: DOI: 10.1080/07328319008045212
URL: http://dx.doi.org/10.1080/07328319008045212

NUCLEOSIDES & NUCLEOTIDES, 9(7), 967-983 (1990)

DIFFERENTIATION OF ISOMERIC PURINE AND PYRIMIDINE MONONUCLEOTIDES BY FAST ATOM BOMBARDMENT TANDEM MASS SPECTROMETRY

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Abstract: The use of positive ion fast atom bombardment mass-analysed ion kinetic energy (FAB/MIKE) spectroscopy to differentiate the 2',3'- and 5'-monophosphate isomers of adenosine, guanosine and cytidine is described.

Early attempts to differentiate isomeric mononucleotides by mass spectrometry employing electron impact ionization, which required derivatization of free nucleotides to yield sufficiently volatile compounds, met with only limited success, allowing the 5'-mononucleotides to be distinguished from their 2'- and 3'-isomers, but not allowing the latter two isomers to be distinguished (1,2). Developments in 'soft' ionization techniques have more recently significantly increased the sensitivity of the mass spectrometric analysis of such nucleotide derivatives (3). However a major advantage of 'soft' ionization procedures such as fast atom bombardment-mass spectrometry (FAB-MS) is that it permits analysis of many thermally labile and polar molecules without derivatization, and this has already been used to advantage in nucleotide studies (4-7). In addition tandem mass spectrometry procedures involving collisionally activated dissociation/mass analysed ion kinetic energy spectroscopy (CAD/MIKES) of ions generated by fast atom bombardment have been developed. Such techniques offer a number of advantages in analysis of tissue extracts (8), of

which two are of particular value. Selection of an appropriate characteristic ion in the FAB mass spectrum for subsequent CAD analysis constitutes an effective separation procedure and often allows subsequent analysis of the compound of interest free from interference by either other components of the extract. Secondly for many different types of biochemically significant molecules CAD/MIKES has been shown to have the capacity to distinguish isomeric structures (6,9). This is of particular value in the nucleotide field, where positive ion FAB in conjunction with CAD/ MIKES has been used to distinguish isomeric 2',3'- and 3',5'-cyclic nucleotides and thus to establish unambiguously the natural occurrence of a number of 3',5'-cyclic nucleotides in animal and plant tissue (6, 10-12). In the case of non-cyclic nucleotides, it has been established that 3'- and 5'-isomers of purine and pyrimidine ribomononucleotides and deoxyribomononucleotides can generally be distinguished by CAD of the anion $(M-H)^-$ generated by negative ion FAB. In these CAD spectra, the relative intensity of the peak representing loss of the protonated heterocyclic base moiety from the parent anion was observed to be generally, though not invariably, characteristic for each isomer (13). In contrast, the CAD spectra obtained from the quasimolecular ion $(MH)^+$ generated by positive ion FAB of nucleotides show prominent peaks representing ions containing the heterocyclic base moiety which arise by fragmentation of the ribose ring system. These fragmentations, termed S_1 and S_2 (SCHEME 1), were of considerable diagnostic value in assigning structures of nucleosides substituted in the ribose moiety (14), as is the case in the isomeric nucleoside monophosphates. Here we describe the application of positive ion fast atom bombardment combined with CAD/MIKES to the analysis of the 2'-, 3'- and 5'-monophosphates of adenosine, guanosine and cytidine and present data which allows differentiation of each nucleotide isomer.

MATERIALS AND METHODS

Free acid (2'-, 3'- and 5'-AMP and 2'- and 3'-CMP), monosodium salt (3'-GMP) and disodium salt (5'-CMP, and 2'- and 5'-GMP) forms of nucleotides were obtained from the Sigma Chemical Co. (Poole, U.K.) with the exception of 3'GMP (monosodium salt) which was from Boehringer Mannheim (Lewes, U.K.).

Positive FAB mass spectra were obtained on a VG ZAB-2F mass spectrometer under conditions previously specified (6,10,11,15). Solutions of nucleotides in the range 5 $\mu g/\mu l$ to 25 $\mu g/\mu l$ were made up in glycerol:water (1:1 v:v) and 3 μl placed on the FAB target. Under the operating conditions employed sample lifetime varied from 90 sec to 5 minutes. CAD spectra were generated by using N_2 as collision gas in the second field-free region gas cell at a pressure of 800 μPa (6 $\mu Torr$) indicated on an external ion gauge.. MIKE spectra were obtained by selecting a specific protonated ion and scanning the electric sector under data system control. For scans over small voltage ranges sample was replenished when necessary and up to six sweeps accumulated.

RESULTS AND DISCUSSION

General Features of Positive Ion FAB Spectra

FAB mass spectra were determined on isomeric purine and pyrimidine nucleotides at the level of the free acid, monosodium salt or disodium salt. The spectra in all cases contained in addition to the matrix derived ions (m/z 93 $[GroH]^+$, m/z 115 $[GroNa]^+$, m/z 185 $[Gro_2H]^+$, m/z 207 $[Gro_2Na]^+$, m/z 277 $[Gro_3H]^+$, m/z 299 $[Gro_3Na]^+$, and m/z 369 $[Gro_4H]^+$) relatively few prominent nucleotide derived peaks. As expected in the case of nucleotides analysed as the free acid, the ion $[BH_2]^+$, derived from the heterocyclic base moiety of each nucleotide (m/z 136 adenosine nucleotides, m/z 112 cytidine-2'- and cytidine 3'-phosphate) was generally intense, whilst the most intense sample-related peak at high mass corresponded to the protonated molecular ion $[MH]^+$ (m/z 348 for each adenine nucleotide, m/z 324 for 2'- and 3'-CMP) which was accompanied by its abundant glycerol adduct ion $[MGroH]^+$ (m/z 440, each adenine nucleotide; m/z 416 2'- and 3'-CMP). In the spectra of nucleotides determined at the level of their sodium salts, the peak corresponding to the sodium adduct ion $[BHNa]^+$ (m/z 174 2'-, 3'- and 5'-guanosine monophosphate, m/z 134, 5'-cytidine monophosphate were generally significantly stronger than the corresponding protonated base peak, $[BH_2]^+$, whilst in the high mass region peaks to sodium containing species dominated the spectra (m/z 408 and m/z 430, disodium 2'- and 5'-guanosine monophosphate $[MNa_2]^+$ and $[MNa_3]^+$ respectively; m/z 368,

monosodium 3'-guanosine monophosphate; m/z 346, 368 and 390 disodium 5'-cytidine-phosphate). In the spectrum derived from each nucleotide sodium salt there was in addition a peak corresponding to the protonated molecular ion $[MH]^+$ of the free acid form [m/z 364 guanosine nucleotides; m/z 324 cytidine nucleotides]. As expected, the relative intensities of the characteristic ion peaks in the FAB mass spectra alone were insufficient to differentiate between each isomeric form. In order to assess the value of tandem mass spectrometry in differentiating the isomeric nucleoside monophosphates, the protonated molecular ion of the free acid ($[MH]^+$) generated by the FAB ionization process was selected with the magnetic sector and subjected to CAD/MIKES analysis. It seemed probable that this generally abundant ion (m/z 348 adenosine monophosophate isomers, m/z 364 guanosine monophosphate isomers and m/z 324 cytidine monophosphate isomers) would represent the ion of lowest relative molecular mass whose CAD process might reflect the influence of the phosphate group esterified in each set of isomers at C-2', C-3' or C-5' of the ribose ring, and in which effects of sodium and glycerol on the CAD process were precluded.

CAD/MIKES ANALYSIS

Isomeric Adenosine Nucleotides

For each adenosine nucleotide, as exemplified by 2'-AMP (FIG. 1a), the largest peak in the MIKE spectrum obtained by CAD of the ion at m/z 348 generated by FAB ionization occurred at m/z 136 and is assigned to the ion formed by cleavage of the glycoside bond with hydrogen transfer and charge retention on the heterocyclic system (I, SCHEME 1), which was also the major fragmentation process during CAD/MIKES analysis of adenosine-3',5'-cyclic monophosphate (5).

This very intense ion was accompanied by a series of prominent ions (FIG. 1a) the masses of which, in the range m/z 160-265, were consistent with the presence of all or part of the ribotide moiety in their structure, suggesting that this series, including the S_1 and S_2 fragment ions (13), would be of particular value in distinguishing the isomeric nucleotides. The MIKE spectra over the corresponding voltage

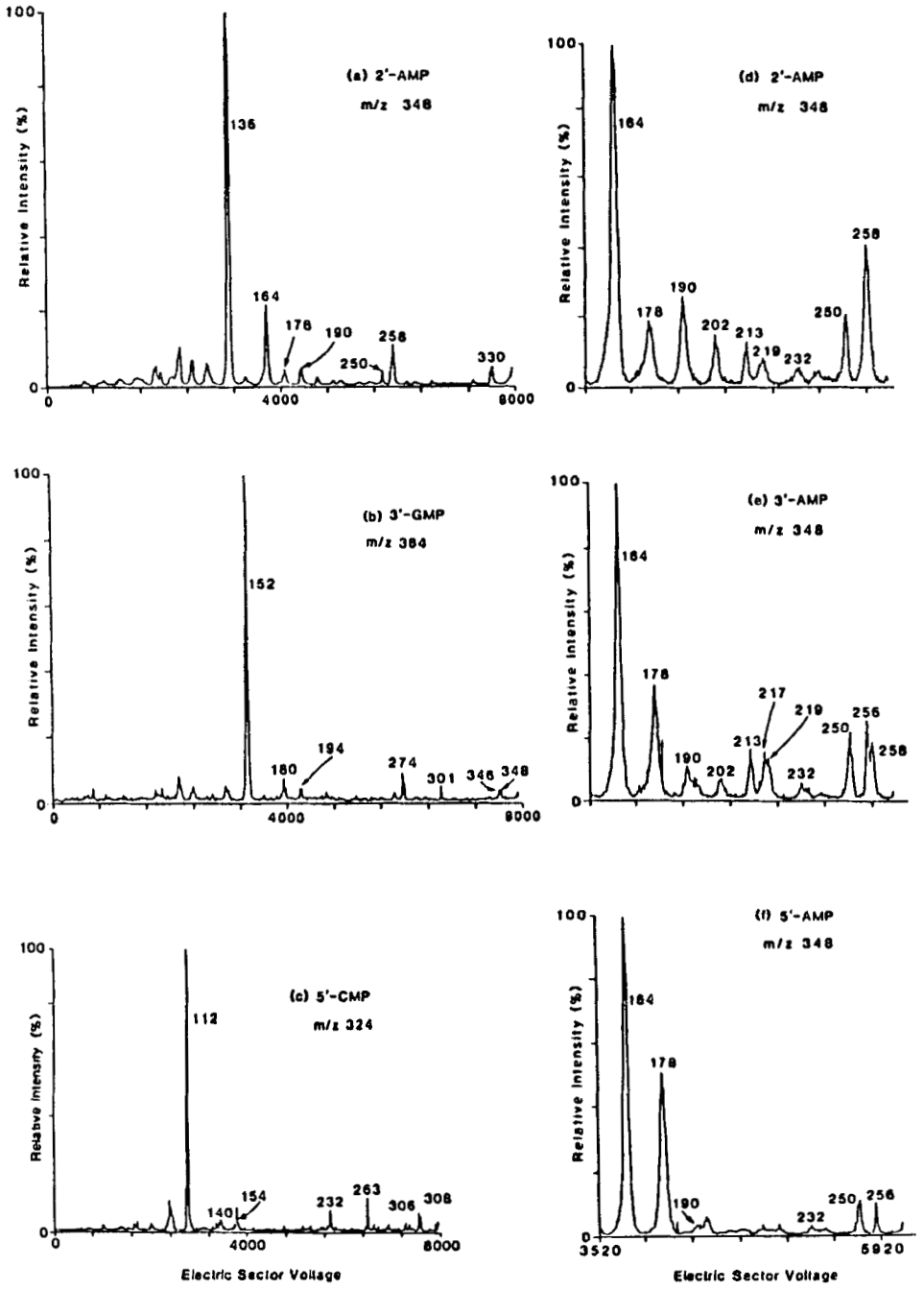
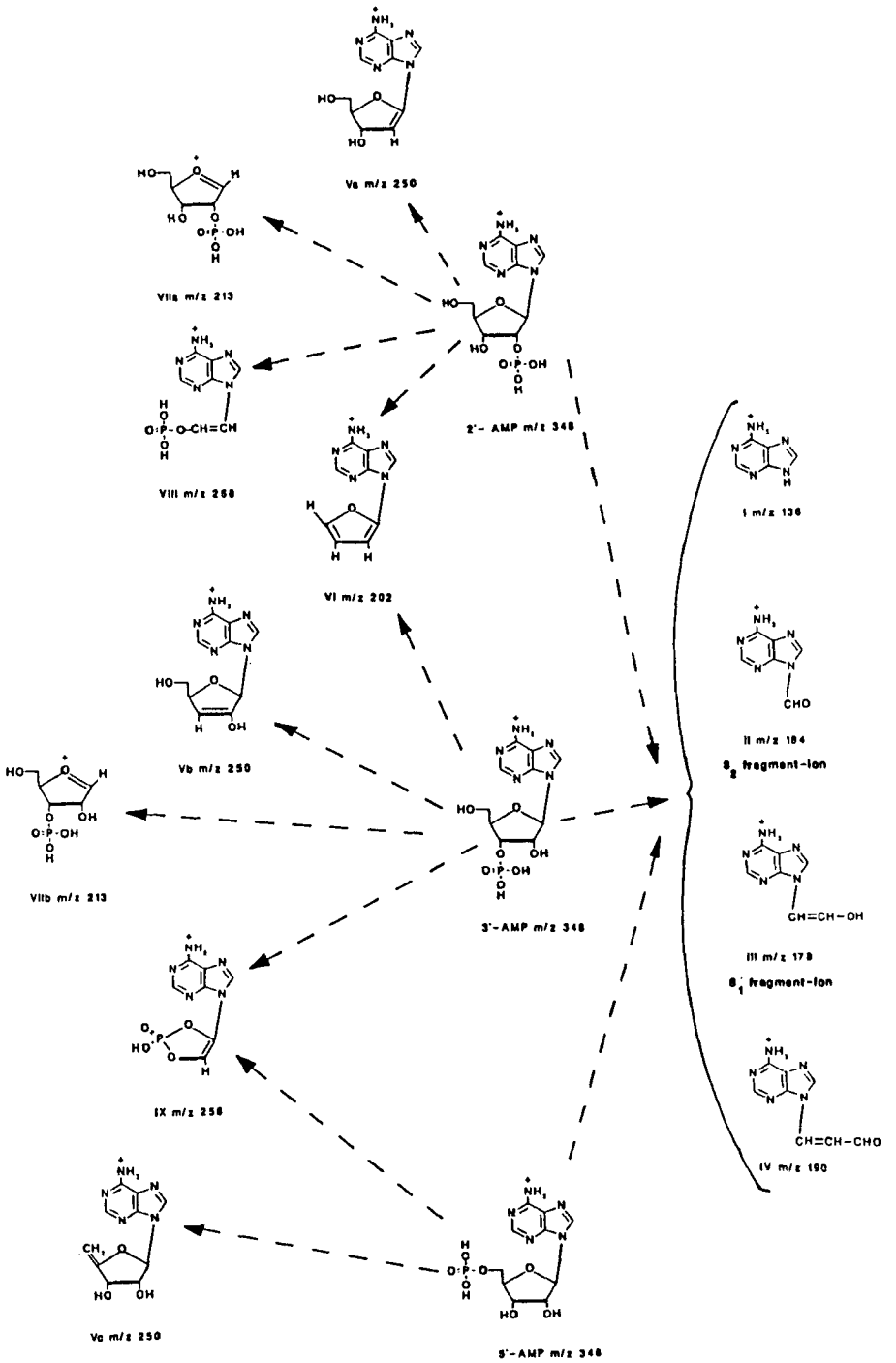


FIG. 1. MIKE spectra of the protonated molecular ion of (a) & (d) adenosine 2-monophosphate (m/z 348), (b) guanosine 3'-monophosphate (m/z 364), (c) cytidine 5'-monophosphate (m/z 324), (e) adenosine 3'-monophosphate (m/z 348) and (f) adenosine 5'-monophosphate (m/z 348). The m/z values of major peaks are indicated.



SCHEME 1 Fragmentation of adenosine mononucleotides.

range were therefore redetermined (FIG. 1d-f), the sample being replenished as necessary. For each isomer the S_2 ion at m/z 164, formed by cleavage of the ribotide ring, gave rise to the largest peak in this region of the spectrum (II, SCHEME 1). The peak due to the S_1 fragment ion derived by cleavage of the ribotide ring (III, SCHEME 1) at m/z 178 was significantly larger in the spectra of 3'-AMP and 5' AMP than in that of 2'-AMP, which is consistent with the location of the phosphate ester group at C-2' of the ribose system, an analogous difference again having been observed in the spectra of 3',5'-cyclic AMP and 2',3'-cyclic AMP (5). The spectrum of each isomer contained a prominent peak at m/z 250, consistent with a fragment arising by elimination of phosphoric acid from the parent ion to yield the isomeric structures (Va (2'-AMP), Vb (3'-AMP) and Vc (5'-AMP) shown in SCHEME 1. The abundance of this ion relative to the ion at m/z 164 is significantly lower in the spectrum of 5'-AMP than in those of the 2'- and 3'-isomers, and this is consistent with the probable relative stabilities of the proposed structures for the isomeric ions at m/z 250 yielded by each nucleotide; in the case of 5'-AMP this contains an exocyclic double bond system (structure Vc, SCHEME 1) whilst those arising from the 2'- and 3'-isomers contain an endocyclic double bond (Va and Vb, respectively, SCHEME 1). The spectra of 2' AMP and 3' AMP are further characterized by the presence of five prominent peaks at m/z 190, m/z 202, m/z 213, m/z 219 and m/z 258 (FIG. 1d and 1e) which are essentially absent from the spectrum of 5'-AMP (FIG. 1f), thus allowing the 5'-isomer to be readily distinguished from either its 2'- or 3'-isomer. The spectrum of 3'-AMP may be differentiated from that of 2' AMP by the presence in the former of a prominent peak at m/z 256, assigned to ion structure IX, SCHEME 1, which is not observed in the spectrum of 2'-AMP (FIG. 1d and 1e). Further to this point of absolute difference, differentiation of the 2'- and 3'-isomers is facilitated by consideration of the relative intensities of the four key ions. In the spectrum of 3'-AMP the pattern in the relative intensity of the ions at m/z 178, m/z 190, m/z 202 and m/z 258 (5.8:1.8:1.0:3.3) is quite distinct from that of the same ions in the spectrum of 2'-AMP (1.3:1.9:1.0:2.8). In this series the ions at m/z 178 and m/z 258 show particularly marked differences in their relative abundancies which can be rationalized in terms of the isomeric ribotide ring structures.

Thus, in the spectrum of 3'-AMP (FIG. 1e), and also 5'-AMP (FIG. 1d), the relatively high abundance of the S_1 ion at m/z 178 is consistent with the assignment of the structure III (SCHEME 1) retaining the free hydroxyl group from C-2' of the ribose ring, whilst this ion is of significantly lower abundance in the spectrum of the isomer containing phosphate substitution at C-2'. Conversely in 2'-AMP the ion at m/z 258, assigned structure VIII (SCHEME 1) which contains the C-2'-phosphate ester group, is the most abundant ion in this series, but was totally absent from the spectrum of 5'-AMP (FIG. 1f). The presence of a significant but relatively weak peak at m/z 258 in the spectrum of 3'-AMP (FIG. 1e) suggests that some migration of the 3'-phosphate ester to the adjacent C-2' hydroxyl of the ribotide system occurs.

Isomeric Guanosine Nucleotides

The general features of the CAD/MIKE spectrum obtained from the ion at m/z 364 generated by FAB ionization of 3'-guanosine monophosphate (FIG. 1b) was typical of each guanosine nucleotide. The peak at m/z 152, corresponding to the protonated guanine base (I, SCHEME 2) was extremely large (FIG. 2a-c); differences in the pattern of the minor peaks in the mass range m/z 160-290 were, however, apparent in the spectra of the three isomers and this range was reexamined. The spectra determined by narrow range scanning (FIG. 2a-c) contained a series of peaks due to fragment ions arising by cleavage of the ribotide ring, analogous to those observed in the spectra of the adenosine nucleotide isomers, in particular the prominent S_2 fragment-ion peak at m/z 180 (II, SCHEME 2) and the S_1 fragment-ion peak at m/z 194 (III, SCHEME 2). In the case of the 2'-isomer, the S_1 fragment ion at m/z 194 was of significantly lower abundance relative to that at m/z 180 (FIG. 2a-c) consistent with the location of the phosphate group at C-2' of the ribose system whilst the corresponding S_1 fragment ion containing the C-2' phosphate group at m/z 274 (IV, SCHEME 2) was of significantly higher relative abundance in the spectrum of 2'-GMP than in the spectra of 3'- and 5'-GMP (data not shown) in a similar pattern to that seen for the adenosine nucleotide isomers. Also, in an analogous manner to the adenosine nucleotides, the ion arising by elimination of phosphoric acid from the guanosine nucleotides at m/z 266 was significantly more

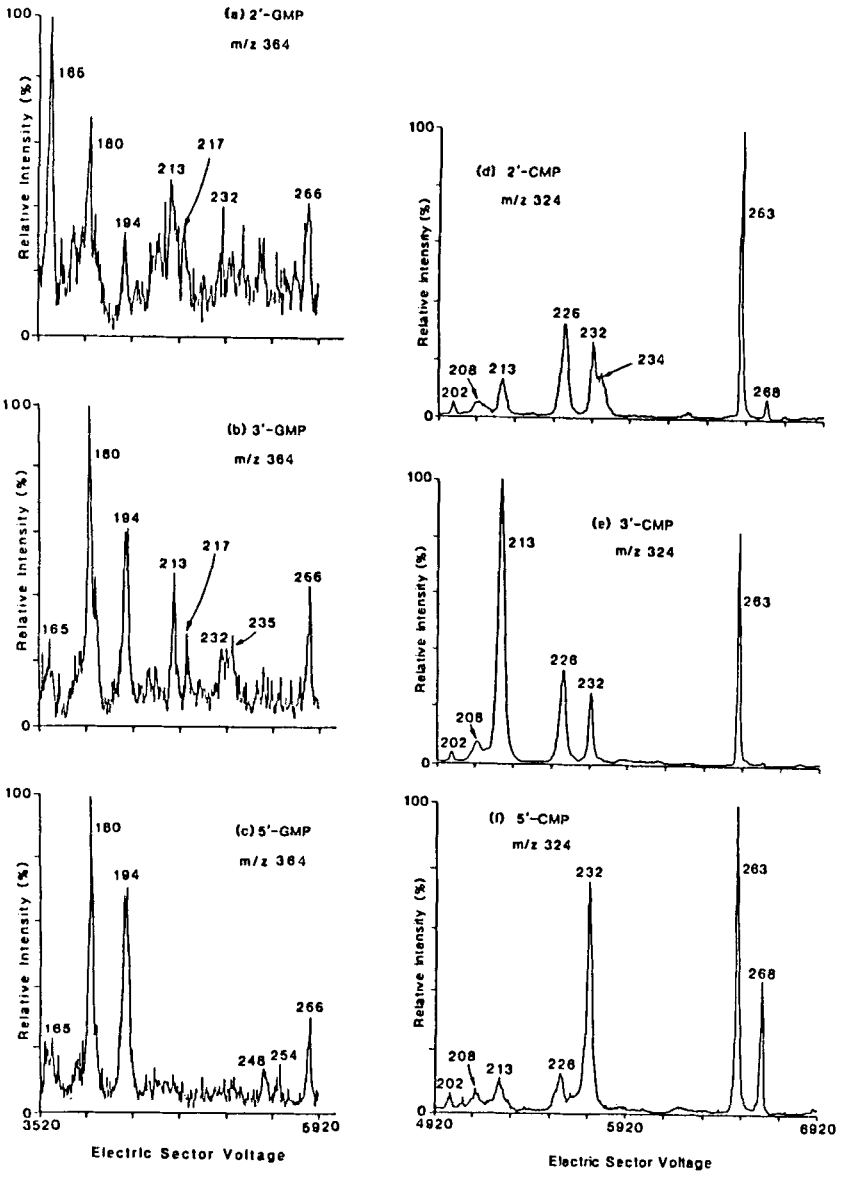
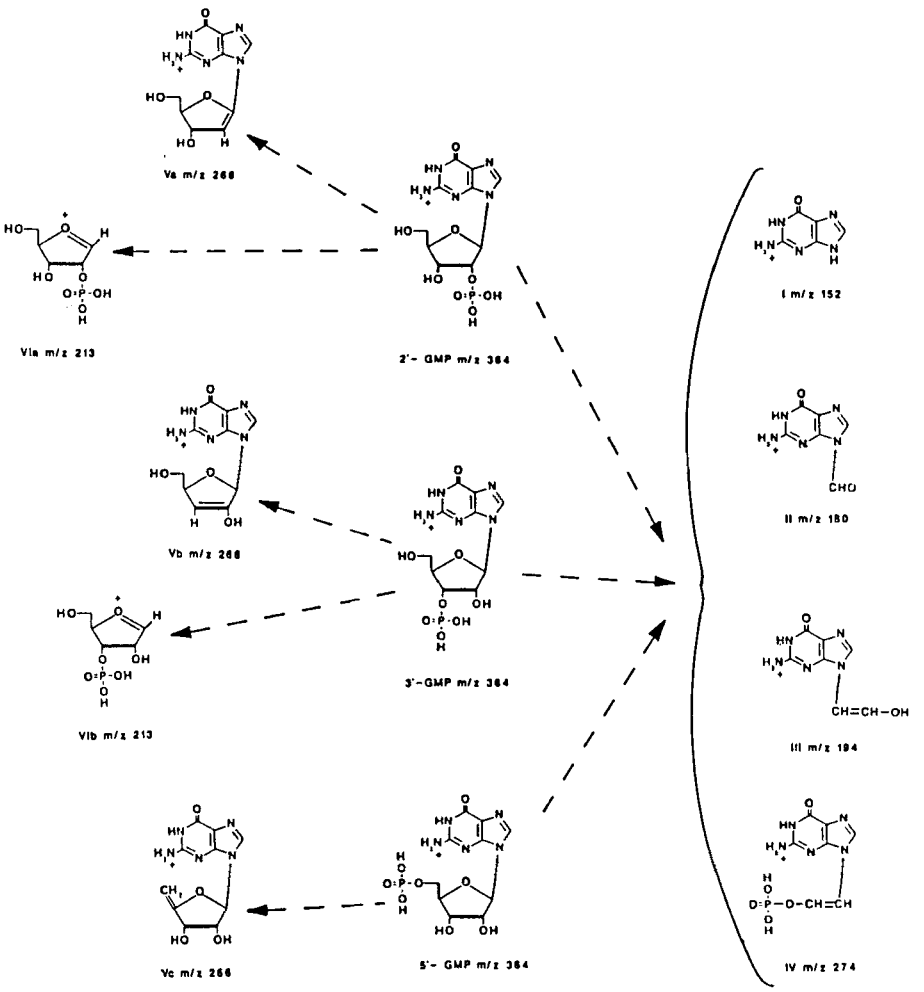


FIG. 2. MIKE spectra of the protonated molecular ion (m/z 364) of (a) guanosine 2'-, (b) guanosine 3'-, and (c) guanosine 5'-monophosphate and of the protonated molecular ion (m/z 324) of (d) cytidine 2'-, (e) cytidine 3'-, and (f) cytidine 5'-monophosphate. The m/z value of major peaks are indicated.



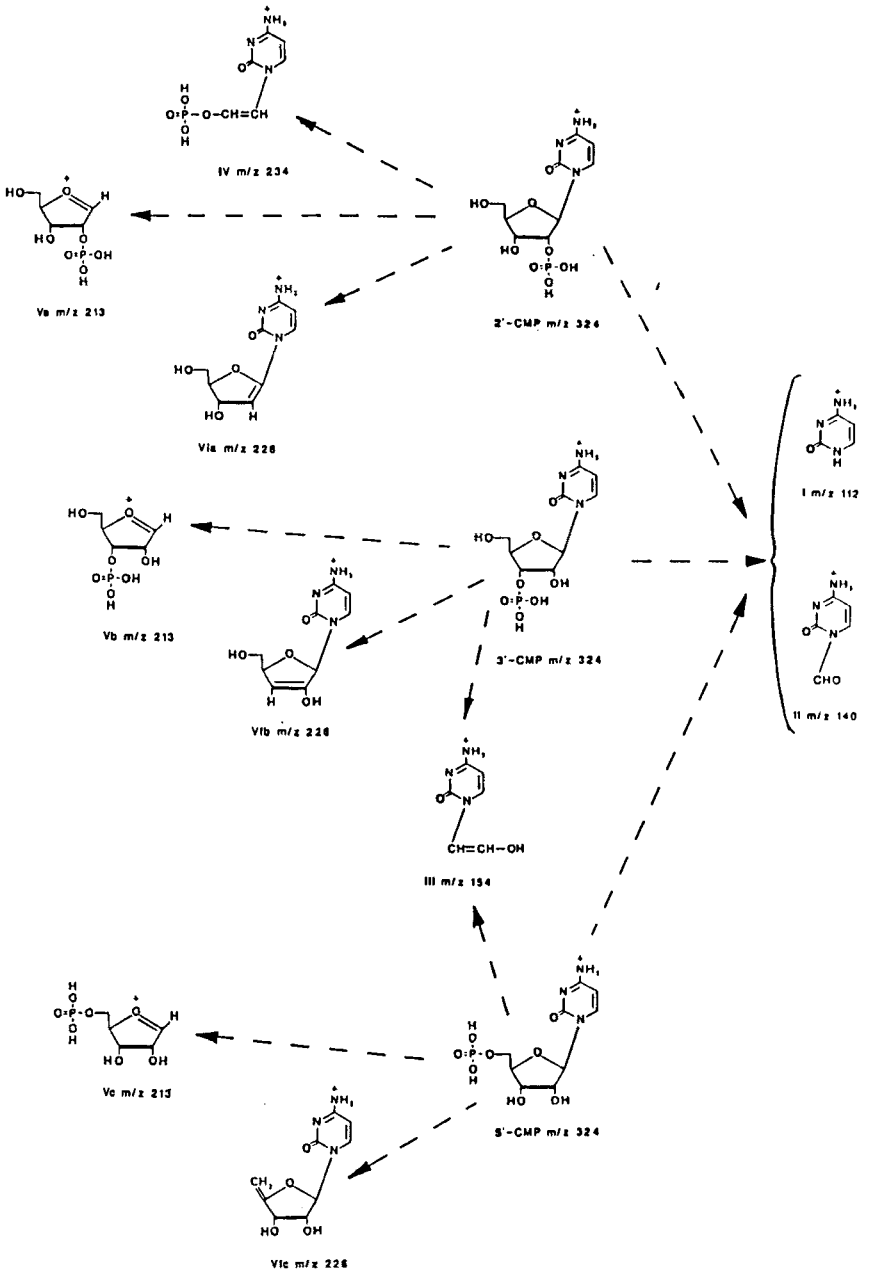
SCHEME 2 Fragmentation of guanosine mononucleotides.

abundant, relative to m/z 180, in the case of the spectra of the 2'- and 3'-isomers (Va and Vb, SCHEME 2) than in that of the 5'-isomer (Vc, SCHEME 2). The spectrum of 2'-GMP was readily distinguished from those of the 3'- and 5'-isomers by comparison of the relative intensities of the ions at m/z 165, m/z 180 and m/z 194. In the case of 2'-GMP, the peak at m/z 165 is the largest in this series (FIG. 2a) but is very small in the spectra of the 3'- and 5'-GMP (FIG. 2b and 2c), thus enabling 2'-GMP to be distinguished from either 3'- or 5'-GMP. The latter two isomers may however be readily distinguished by the presence of a series of peaks at m/z 213, m/z 217, m/z 232 and m/z 235, which are

prominent in the spectrum of 3'-GMP (FIG. 2b) but are essentially absent from the spectrum of 5'-GMP (FIG. 2c). The most prominent peak in this sequence in 3'-GMP occurred at m/z 213, and a significant isobaric peak was also present in the spectrum of 2' GMP (FIG. 2b and 2a, respectively). Prominent peaks at m/z 213 are also present in the spectra of 2'- and 3'-AMP (FIG. 1d and 1e), whilst in the case of 5'-AMP (FIG. 1f), this peak was also very small, supporting the structures proposed for these fragment ions (VIIa and VIIb, SCHEME 1 and VIa and VIb, SCHEME 2) and their origin from the common ribotide structures. These observations suggest that an abundant m/z 213 ion may be generally characteristic of 2'- and 3'-purine nucleotides.

Isomeric Cytidine Nucleotides

In the CAD/MIKE spectra obtained from the protonated molecular ion at m/z 324 generated by FAB ionization of each isomeric cytidine mononucleotide, represented by the spectrum of 5'-cytidine monophosphate (FIG. 1c), the most intense peak occurred at m/z 112, corresponding to the fragment ion arising by cleavage of the glycosidic band with hydrogen transfer and charge retention on the pyrimidine base (I, SCHEME 3). This peak was accompanied by significant peaks at m/z 140 in the case of each isomer, arising by S_2-cleavage of the ribotide ring system (structure II, SCHEME 3). The spectra of 3'- and 5'-CMP also contained a significant S_1 fragment-ion peak at m/z 154, assigned structure III, SCHEME 3, whilst this peak was essentially absent from the spectrum of 2'-CMP. This analogy to the fragmentation processes of the 2'-substituted purine nucleotides indicates that a weak fragment at $[B+42]^+$ is generally characteristic of nucleoside 2'-monophosphates. Additional points of difference between the spectra of these isomeric pyrimidine nucleotides were apparent in the mass range m/z 200 - m/z 270, and this region was reexamined by narrow range scanning. In this region a very strong peak at m/z 263, to which the intensity of other peaks could be related, was a common feature of each spectrum (FIG. 2d-f); since no analogous ion was observed in either set of purine nucleotide isomers, it is probable that this ion arises by cleavage of the pyrimidine ring. The spectrum of 2'-CMP in this region was readily distinguished by the presence of a prominent peak at m/z 234 (FIG. 2d)



SCHEME 3 Fragmentation of cytidine mononucleotides.

which was absent from the spectra of 3'- and 5'-CMP (FIG. 2e and 2f). The ion at m/z 234 may be assigned structure IV, SCHEME 3 corresponding to the S_1 fragment ion and containing the vinylic phosphate group, and is thus analogous to fragment VIII, SCHEME 1, which is observed as a

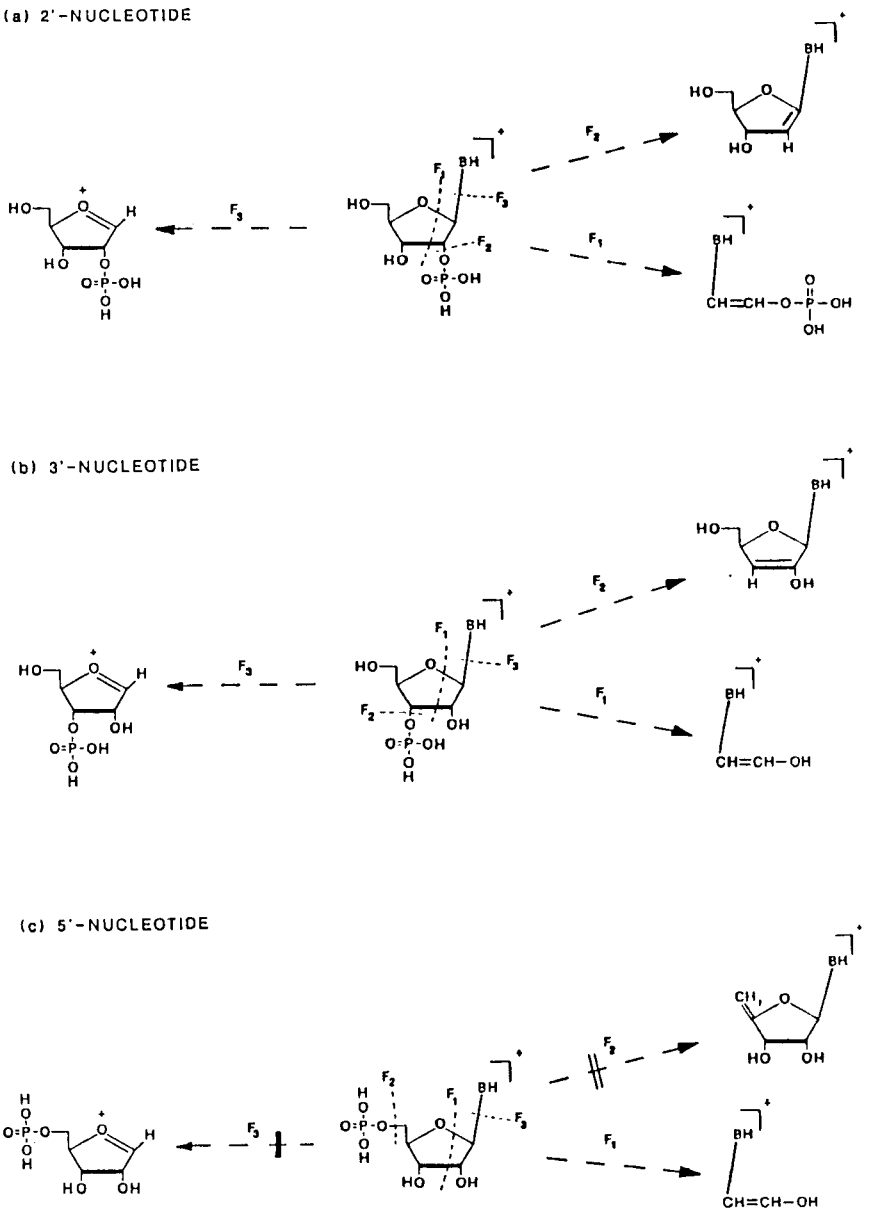
strong ion at m/z 258 in the spectrum of 2'-AMP (FIG. 1d). Thus the presence of the ion at m/z 234, in conjunction with the absence of a peak at m/z 154 allows the MIKES/CAD spectrum of 2'-CMP to be readily distinguished from those of either 3'- or 5'-CMP. The spectrum of the 3'-isomer may be clearly distinguished from that of the 5'-isomer by comparison of the abundance of the peaks at m/z 213, m/z 226, m/z 232 and m/z 268 relative to the abundance of the peak at m/z 263 (FIG. 2e and 2f). In the case of 3'-CMP the ion at m/z 213, arising by cleavage of the glycosidic linkage with charge retention on the ribotide system (Vb, SCHEME 3), was the most abundant ion in this region of the spectrum and was of greater relative intensity than the ion at m/z 263 (FIG. 2e) whereas the fragment ion at m/z 213 gave rise to a relatively weak feature in the spectrum of 5'-CMP (FIG. 2f). This pattern is thus consistent with that observed for the adenosine and guanosine nucleotide isomers, and indicates that the relative intensity of the ion at m/z 213 may be used generally to differentiate between isomeric 3'- and 5'-nucleotides. This region of the spectrum of 5'-CMP was further characterized by the relatively high abundance of the ions at m/z 268 and m/z 232 (FIG. 2f), which although present in the spectrum of 3'-CMP were of much lower intensity relative to the ion at m/z 263 (FIG. 2e). As had been the case for the purine nucleotides, the ion arising by elimination of phosphoric acid from the cytidine nucleotides (m/z 226, structures VIa-c, SCHEME 3) was significantly more prominent in the case of the 2'- and 3'-isomers (FIG. 2d and 2e) than for the 5-isomer (FIG. 2f).

The observations described above indicate that differentiation of the mononucleotide 2'-, 3'- and 5'-phosphate isomers can generally be based on the relative abundance of ions in the CAD/MIKES spectrum arising by the three fragmentations designated F_1 F_2 and F_3 (SCHEME 4).

In distinguishing 2'-mononucleotides from 3'- and 5'-mononucleotides, the relative intensities of the ions arising by the F_1 fragmentation process is diagnostic. In the case of the 2'-isomer, the peak corresponding to this fragment ion, $[BH-CH=CH-O-P-O_3H_2]^+$, occurs 80 mass units higher than the corresponding ion $[BH-CH=CH-O-H]^+$ yielded by the 3'- and 5'-isomers. It should be noted however that peaks

corresponding to the 2'-isomer are seen in the spectra of the 3'-isomer, and ***vice versa***, possibly arising from migration of the phosphate group to the adjacent hydroxyl function during analysis. Nevertheless, in the spectra of the 2'-isomer the peak corresponding to the phosphate containing F_1 fragment ion is of much greater relative abundance than the peak corresponding to $[BH-CH=CH-OH]^+$, whereas in 3'- and 5'-isomers, the ion corresponding to $[BH-CH=CH-O-P-O_3H_2]^+$ is either a much weaker feature or absent altogether. In the identification of 5'-nucleotides, the relative intensity of the F_2 fragment ion (SCHEME 4), corresponding to loss of phosphoric acid from the parent mononucleotide $[MH-98]^+$ is a useful indicator, as this ion is generally of lower relative abundance in the spectra of 5'-nucleotides than in the spectra of the 2'- and 3'-isomers. Further the CAD/MIKE spectra derived from 5'-nucleotides are also characterized by an absent (5'-AMP and 5'-GMP) or very weak (5-CMP) peak at m/z 213, formed from the ribotide system by process F_3 (SCHEME 4), an analogous situation to that previously described for CAD analysis of the (M-H) anion of 5'-mononucleotides previously described (13), whereas the spectra of 2'- and 3'-isomers of each contain significant peaks at m/z 213.

Currently nucleotide profiles are produced from hplc analysis, and due to the difficulty of separating 2'-, 3'- and 5'-nucleoside monophosphates, there is generally little or no attempt at establishing their isomeric composition. The tandem mass spectrometry procedure described above will enable more precise characterization of the nucleoside monophosphate isomer content in total nucleotidce fractions extracted from tissue, and direct, unequivocal identification of the monophosphate isomers of adenosine, guanosine and cytidine which have undergone prior separation by hplc. This will be of particular value not only in establishing detailed changes in tissue nucleotides, which are useful indices of metabolic state (16), but also in studies of cyclic nucleotide phosphodiesterases, such as the multifunctional phosphodiesterases found in mammals which are capable of hydrolysing both 2',3'- and 3',5'-cyclic nucleotide substrates to 2'-, 3'- and 5'-nucleotide products (17).



SCHEME 4 Fragmentations which characterize 2',3' - and 5' - isomers of nucleoside monophosphates (B = purine or pyrimidine base).

ACKNOWLEDGEMENT

We gratefully acknowledge financial support from The Wellcome Trust and the University College of Swansea.

REFERENCES

1. Lawson, A.M., Stillwell, R.N., Tacker, M.M., Tsuboyama, K. and McCloskey, J.A. (1971) J. Amer. Chem. Soc. 93, 1014-1023.

2. Budzikiewicz, H. and Feistner, G. (1978) Biomedical and Environmental Mass Spectrometry 5, 512-513.

3. Weng, Q-M., Hammargren, W.M., Slowikowski, D., Schram, K.H., Borysko, K.Z., Wotring, L.L. and Townsend, L.B. (1989) Anal. Biochem. 178, 102-6.

4. Eagles, J., Javanaud, C. and Self, R. (1984) Biomedical and Environmental Mass Spectrometry 11, 41-6.

5. Schulten, H-R and Schiebel, H.M. (1985) Fresenius Z. Anal. Chem. 321, 531-537.

6. Kingston, E.E., Beynon, J.H., Newton, R.P. and Liehr, J.G. (1985) Biomedical and Environmental Mass Spectrometry 12, 525-534.

7. Sakurai, T., Matsuo, T., Kusai, A. and Nojima, K. (1989) Rapid Comm. Mass Spectrom. 7, 212-6.

8. McLafferty, F.W. (Ed.) 'Tandem Mass Spectrometry', John Wiley, 1983.

9. Walton, T.J., Mullins, C.J., Newton, R.P., Brenton, A.G. and Beynon, J.H. (1988) Biomedical and Environmental Mass Spectrometry 16, 289-298.

10. Newton, R.P., Kingston, E.E., Hakeem, N.A., Salih, S.G., Beynon, J.H. and Moyse, C.D. (1986) Biochem. J. 236, 431-439.

11. Newton, R.P., Hakeem, N.A., Salvage, B.J., Wassenaar, G. and Kingston, E.E. (1988) Rapid Comm. Mass Spectrom. 2, 118-126.

12. Newton, R.P., Chiatante, D., Ghosh, D., Brenton, A.G., Walton, T.J., Harris, F.M. and Brown, E.G. (1989) Phytochemistry 28, 2243-2254.

13. Cerny, R.L., Gross, M.L. and Grotjamn, L. (1986) Anal. Biochem. 156, 424-435.

14. Crow, F.W., Tomer, K.B., Gross, M.L., McCloskey, J.A. and Bergstrom, D.E. (1984) Anal. Biochem. 139, 243-62.

15. Kingston, E.E., Beynon, J.H. and Newton, R.P. (1984) Biomedical and Environmental Mass Spectrometry 11, 367-374.

16. Donovan, M.P., Newton, R.P. and Brown, E.G. (1987) Biochem. Soc. Trans. 15, 676-677.

17. Helfman, D.M., Katoh, N. and Kuo, J.F. (1984) Adv. Cyclic Nucleotide Res. 16, 403-416.

Received April 30, 1990.