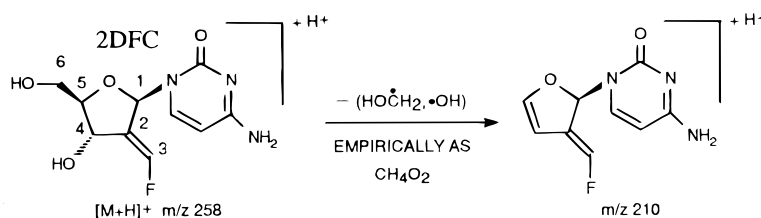


A Rare Mass Spectrometric Neutral Loss of 48 u

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Fast atom bombardment and thermospray mass spectrometry results are consistent with the 2-deoxyfluoromethine derivative of the nucleoside (cytidine) shown below and designated 2DFC. However, a mass loss of 48 u from $[M + H]^+$ (m/z 258) was observed forming an abundant fragment ion at m/z 210. The loss seemed peculiar since a 48 u neutral is more often associated with H_3CSH or H_3SiOH , which are not possible neutrals to be eliminated from 2DFC. Accurate mass measurement of m/z 210 indicated the 48 u neutral lost had the molecular formula CH_4O_2 . Constant neutral loss (48 u) linked scan experiments showed the fragmentation (depicted below) is concerted within the microsecond time frame of the mass spectrometer.



Other structurally homologous nucleosides, such as cytidine, do not exhibit this prominent fragmentation. Computerized molecular modeling was used to propose why the marked elimination occurs with 2DFC. © 1997 by John Wiley & Sons, Ltd.

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KEYWORDS: 48 u neutral loss; CH_4O_2 neutral loss; nucleoside; cytidine; linked scans; computer modeling; mass spectrometry

INTRODUCTION

The CH_4O_2 neutral loss is not reported in the open literature, nor is it described upon particular review of manuscripts covering the mass spectrometry of nucleosides. Other nucleosides, such as cytidine or deoxycytidine where C-2 is an sp^3 hybrid containing 'H and 'OH or two 'H, respectively, do not exhibit this prominent neutral loss. The reported abundant fragment ions, using a variety of ionizations (fast atom bombardment (FAB) including high-energy collision-induced dissociation (CID),^{1–3} electron ionization including direct exposure,^{4–10} chemical ionization including direct exposure,^{11–13} field desorption,¹⁴ field ionization¹⁵ and laser desorption^{16–18}), concentrate on those of the pyrimidine base, independent loss of H_2O , 'OH, $H_2C=O$ and 'CH₂OH from the pentose ring and cleavage of the pentose ring. The papers do not report the loss of 48 u, nor do mass spectral figures in the papers give noticeably abundant peaks 48 u less than the intact molecular mass. The 48 u CH_4O_2 is not reported in either the table of common neutral fragments by McLafferty^{19a} or the examples of other rearrangement reactions^{19b} focusing on eliminations ejecting small stable radicals or

neutral molecules. This paper describes an abundant fragmentation involving an unusual neutral loss not previously addressed in the literature.

EXPERIMENTAL

FAB spectra were recorded on a Kratos Concept S sector mass spectrometer of Nier–Johnson geometry operating at ion acceleration 8 kV, resolving power 2000 and a source temperature of 80°C. The 2-deoxyfluoromethine derivative of the nucleoside cytidine (2DFC) was dissolved in a matrix of glycerol and bombarded by a beam of xenon atoms produced by a saddle field gun operating at 7 kV. Accurate mass calibrant ions of polyethylene glycols 400 and 600 (~1:2)²⁰ were collected just prior to the sample in the same run while the magnet was sweeping at 5 s per decade. Linked scan mass spectra were recorded after CID of the primary ion beam with helium target gas introduced in the first field-free region. Linked scan ion signals were multi-channel accumulated after adjusting the collision gas to attenuate by half the primary ion beam of the $[M + H]^+$ (m/z 258) ion signal.

Thermospray data was collected on a Hewlett-Packard Model 5989A mass spectrometer. Approximately 2 mg of 2DFC were dissolved in ~1 ml of CH_3OH-H_2O (1:1) from which 20 μ l were directly

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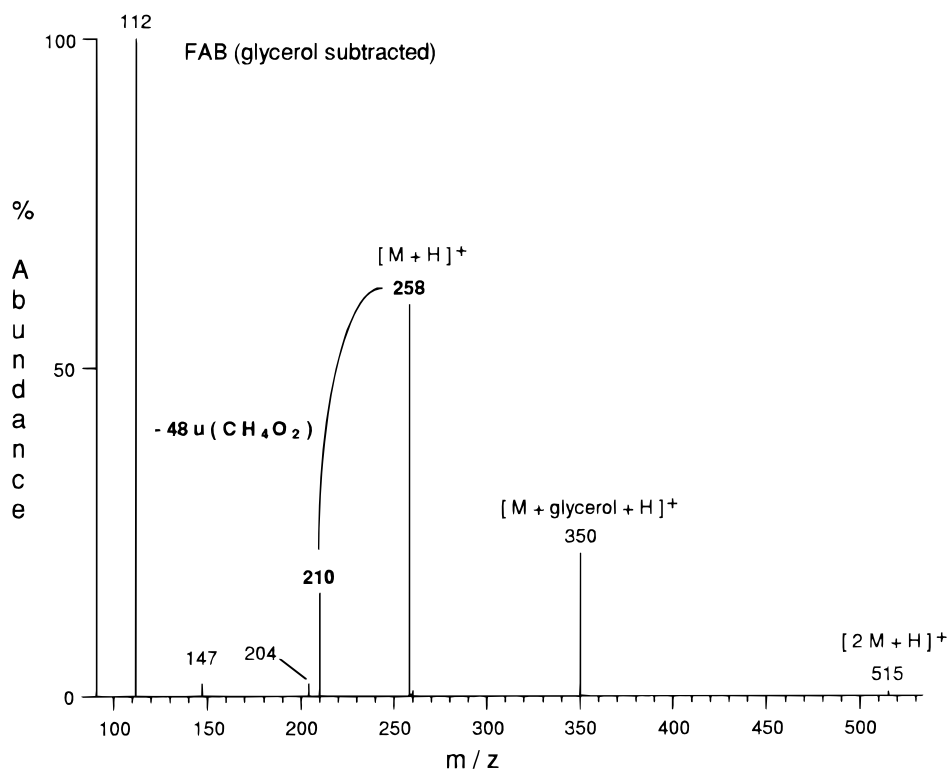


Figure 1. Fast atom bombardment mass spectrum of 2DFC (glycerol matrix subtracted).

infused into the thermospray ion source via a 1.0 ml min^{-1} carrier eluent of $\text{CH}_3\text{OH}-\text{H}_2\text{O}$ (1:1) made 0.05 M in NH_4OAc . The quadrupole mass analyzer temperature was 100°C , the source temperature 224°C and the thermospray tip temperature $\sim 245^\circ\text{C}$ during infusion of the sample. Thermospray ionization was performed with the filament on (100 eV) and the fragmenter set to 100 V.

RESULTS AND DISCUSSION

The FAB (glycerol subtracted) and thermospray mass spectra are presented in Figs 1 and 3 and interpretive mass to ion structure correlations are shown in Figs 2 and 4, respectively. FAB accurate mass measurement results (Fig. 2) corroborate the molecular formulas of the ion structures. Both FAB and thermospray ionizations resulted in abundant fragment ions at m/z 210 corresponding to the CH_4O_2 neutral loss. Additionally, a FAB CID product ion linked scan mass spectrum of $[M + H]^+$ (m/z 258) also revealed an abundant m/z 210 ion signal due to CH_4O_2 neutral loss.

To illustrate the loss of CH_2OH and OH as a concerted process, FAB CID 48 u constant neutral loss (CNL) product ion and precursor ion mass spectra were recorded. An abundant m/z 210 product ion signal corroborated the $[M + H]^+ - 48 u (\text{CH}_4\text{O}_2)$ fragmentation. In addition, and as would be expected, product ion signals at m/z 190 and 99 also denoted CNL of 48 u since these fragment ions also contain the pentose moiety. They correspond to m/z 238 ($[M + H]^+ - \text{HF}$) $- 48 u \rightarrow m/z$ 190, and m/z 147 (pentose fragment ion (Fig. 2)) $- 48 u \rightarrow m/z$ 99. By

corollary, recording the CID precursor ions having CNL of 48 u revealed the signals at m/z 258 ($[M + H]^+$), 238 ($[M + H]^+ - \text{HF}$) and 147 (the pentose fragment ion).

The planar sp^2 hybridization of C-2 in 2DFC may vector the CH_4O_2 neutral loss by decreasing the torsion angle between hydrogens on C-4 and C-5, making them more planar. C-4 and C-5 may be promoting their σ electrons bonded with CH_2OH and OH to a new π bond. This would render CH_2OH and OH eager leaving groups, giving $[M + H]^+$ a propensity for simultaneous loss of these moieties, resulting in abundant formation of m/z 210 fragment ions. Computer modeling of 2DFC supports this proposal.²¹ Comparative energy-minimized Dreiding structures of 2DFC and deoxycytidine are shown in Fig. 5. The sp^2 hybridization of C-2 in 2DFC decreases the torsion angle between adjacent hydrogens on C-4 and C-5 by $\sim 28^\circ$, making them more planar, relative to the same C-4 and C-5 hydrogens of deoxycytidine where C-2 is an sp^3 $-\text{CH}_2-$.

CONCLUSION

An abundant 48 u (CH_4O_2) neutral loss has been demonstrated which has not been addressed previously in the literature. The preferred elimination is proposed to arise through induced planar character on two sp^3 carbons, adjacent to each other, vectored by an sp^2 carbon α to them in a pentose ring. The preferred elimination may prove useful in determining evidence for similar sp^2 modifications in the pentose ring of endogenous nucleosides using 48 u CNL experiments.

FAST ATOM BOMBARDMENT

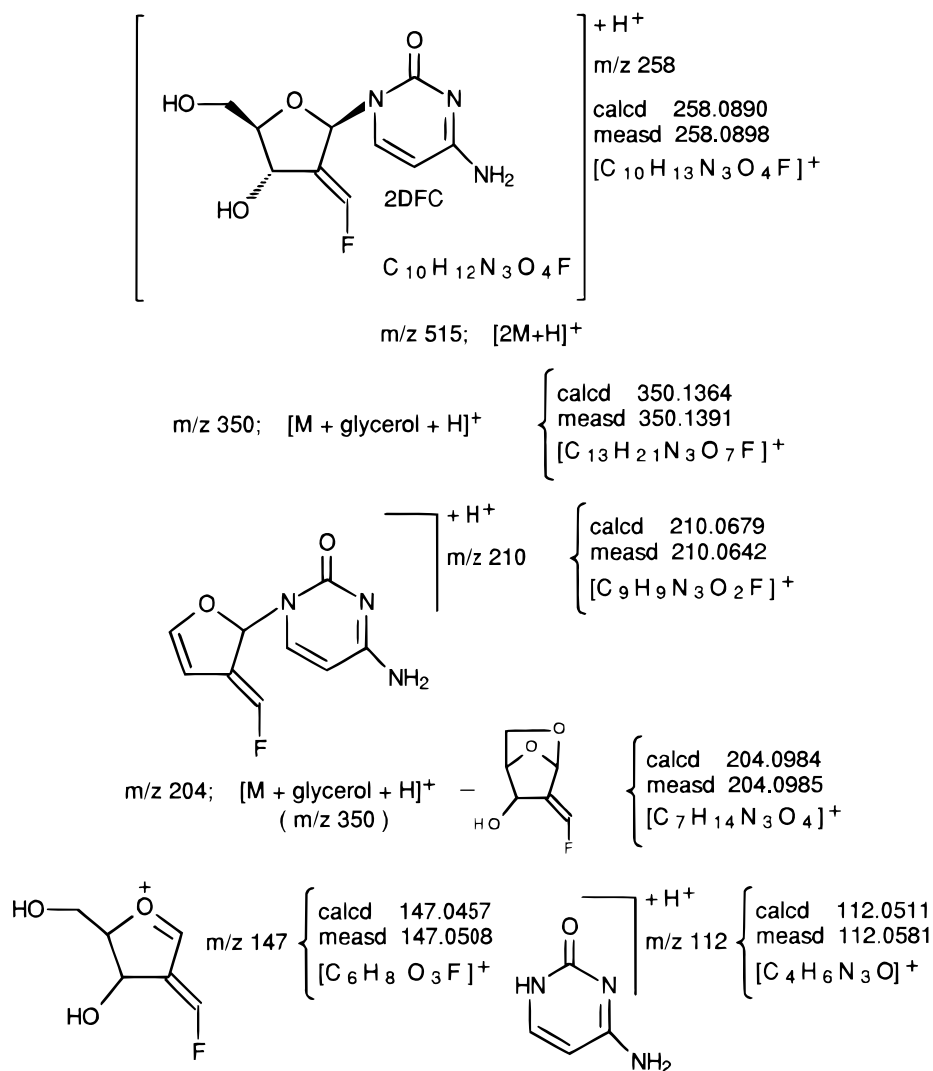


Figure 2. Mass to ion structure correlations for the FAB mass spectrum of 2DFC (Fig. 1).

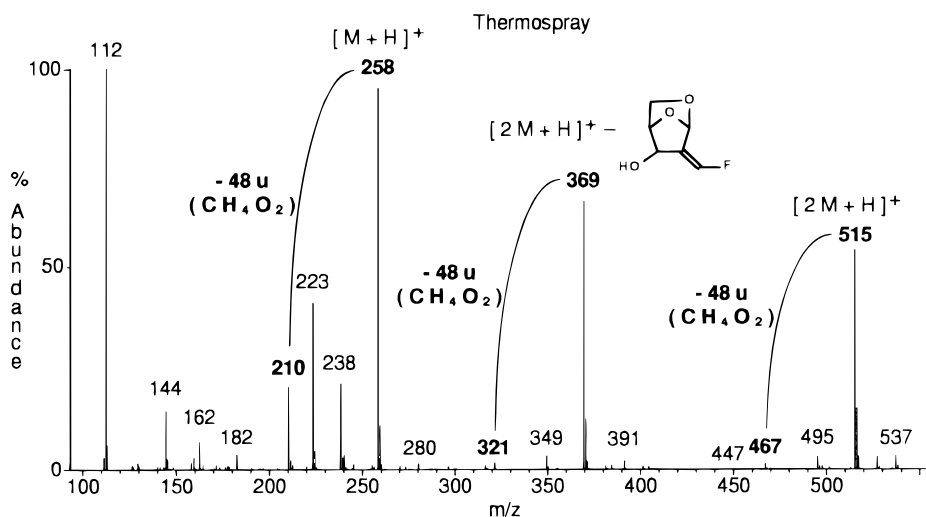


Figure 3. Thermospray mass spectrum of 2DFC.

THERMOSPRAY

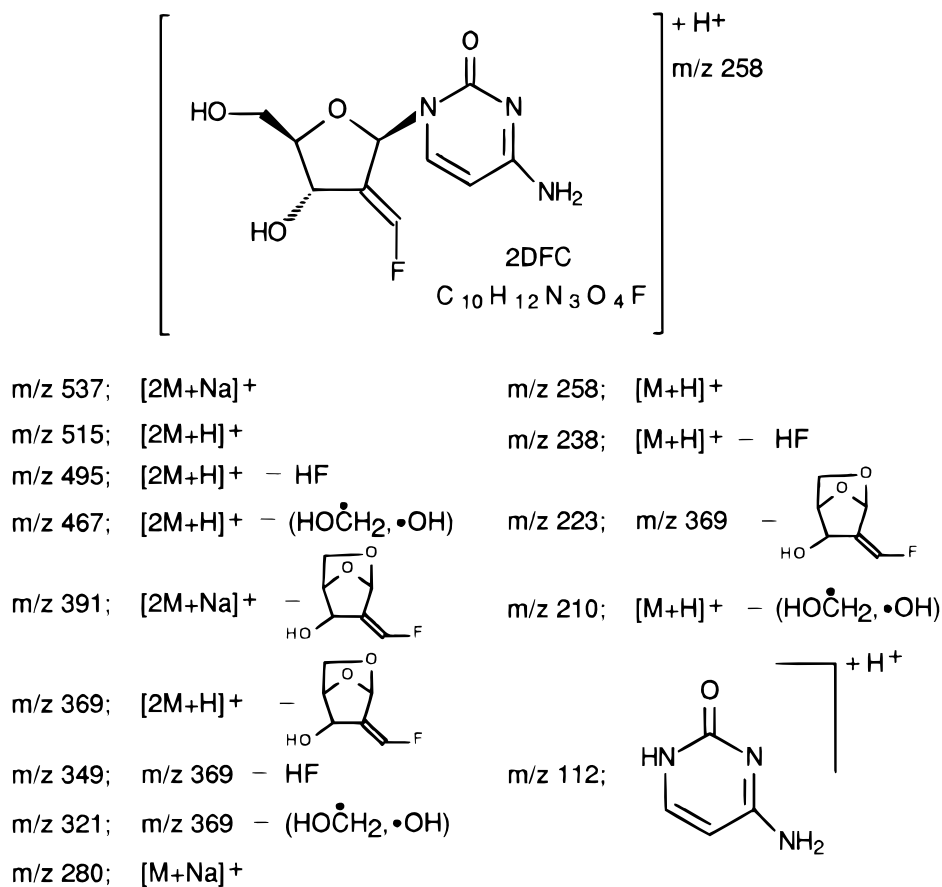


Figure 4. Mass to ion structure correlations for the thermospray mass spectrum of 2DFC (Fig. 3).

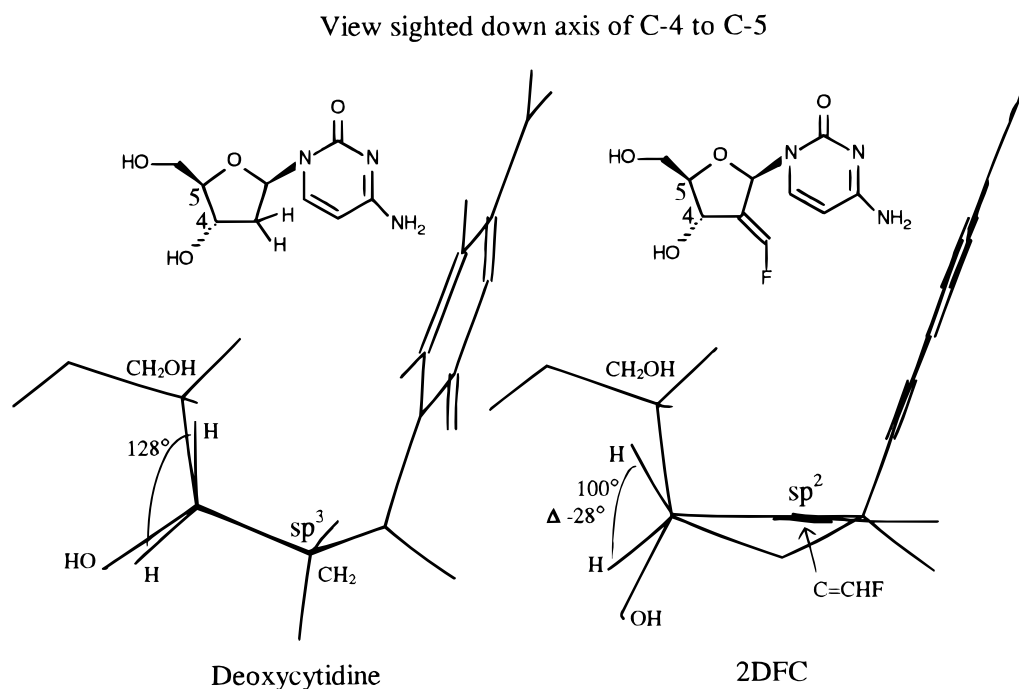


Figure 5. Energy-minimized Dreiding structures showing the hydrogens of 2DFC twisted to more planar configuration relative to deoxycytidine.

REFERENCES

1. F. W. Crow, K. B. Tomer, M. L. Gross, J. A. McCloskey and D. E. Bergstrom, *Anal. Biochem.* **139**, 243 (1984).
2. T. Sakurai, T. Matsuo, A. Kusai and K. Nojima, *Rapid Commun. Mass Spectrom.* **3**, 212 (1989).
3. D. L. Slowikowski and K. H. Schram, *Nucleosides Nucleotides* **4**, 347 (1985).
4. J. A. McCloskey, in *Basic Principles in Nucleic Acid Chemistry*, edited by P. O. P. Ts'o, Vol. 1, p. 209. Academic Press, New York (1974).
5. BenchTop/PBM Computerized Library, *Wiley Registry of Mass Spectral Data*, Vers. 2.0. Palisade, Newfield, NY (1991).
6. J. G. Liehr, D. L. von Minden, S. E. Hattox and J. A. McCloskey, *Biomed. Mass Spectrom.* **1**, 281 (1974).
7. S. M. Hecht, A. S. Leonard and J. Nelson, *Anal. Biochem.* **38**, 230 (1970).
8. L. K. Liu, W. S. Li and C. Y. Su, *Proc. Natl. Sci. Counc. Repub. China, Part A* **10**, 359 (1986).
9. K. Tsujimoto, H. Nomura and M. Ohashi, *Shitsuryo Benseki* **33**, 255 (1985).
10. L. Alder, G. R. Kim, R. Grimm, C. D. Pein and D. Cech, *Z. Chem.* **26**, 136 (1986).
11. M. S. Wilson and J. A. McCloskey, *J. Am. Chem. Soc.* **97**, 3436 (1975).
12. R. R. Weller, J. A. Mayernik and C. S. Giam, *Biomed. Environ. Mass Spectrom.* **15**, 529 (1988).
13. E. L. Esmans, E. J. Freyne, J. H. Vanbroeckhoven and F. C. Alderweireldt, *Biomed. Mass Spectrom.* **7**, 377 (1980).
14. N. B. Zolotoi, G. V. Karpov and V. E. Skurat, *Khim. Fiz.* 575 (1982).
15. P. Brown, G. R. Pettit and R. K. Robins, *Org. Mass Spectrom.* **2**, 521 (1969).
16. R. J. Perchalski, R. A. Yost and B. J. Wilder, *Anal. Chem.* **55**, 2002 (1983).
17. L. Li, D. M. Lubman, *J. Mass Spectrom. Ion Processes* **88**, 197 (1989).
18. L. Van Vaeck, P. Van Espen, F. Adams, R. Gijbels, W. Lauwers and E. Esmans, *Biomed. Environ. Mass Spectrom.* **18**, 581 (1988).
19. F. W. McLafferty, in *Interpretation of Mass Spectra*, edited by N. J. Turro, 3rd edn, pp. (a) 282 and (b) 174 University Science Books, Mill Valley, CA, (1980).
20. L. J. Goad, M. C. Prescott and M. E. Rose, *Org. Mass Spectrom.* **19**, 101 (1984).
21. Alchemy II Molecular Modeling Software. Tripos Associates, St. Louis, MO (1988).