MASS SPECTROMETRY OF ELECTROPHORE-LABELED NUCLEOSIDES

PENTAFLUOROBENZYL AND CINNAMOYL DERIVATIVES

THOMAS M. TRAINOR

Department of Chemistry and Barnett Institute of Chemical Analysis, Northeastern University, Boston, MA 02115 (U.S.A.)

ROGER W. GIESE

Barnett Institute of Chemical Analysis and College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115 (U.S.A.)

and

PAUL VOUROS*

Department of Chemistry and Barnett Institute of Chemical Analysis, Northeastern University, Boston, MA 02115 (U.S.A.)

SUMMARY

Three structurally similar deoxynucleosides (thymidine, O^4-ethylthymidine, and 2′-deoxyuridine) were studied by mass spectrometry as pentafluorobenzyl, cinnamyl, or mixed derivatives. The purpose of the work was to define the usefulness of such derivatives for structural elucidation of deoxynucleosides. The compounds were ionized in three ways: electron capture negative ion, positive ion chemical ionization, and electron impact. For each of the derivatives examined, the combined spectra were well suited for structural elucidation purposes.

INTRODUCTION

Mass spectrometry (MS) has been shown to be particularly useful in the characterization of DNA and RNA structures through analysis of the components nucleobases, nucleosides, and nucleotides[1–5]. The study of the non-volatile nucleosides has been accomplished by a variety of desorption ionization techniques, including fast atom bombardment[6], field desorption[7], [252]Cf desorption[8], and secondary ion MS[9,10]. These ionization techniques permit the analysis of highly polar, thermally labile compounds directly, without the need for chemical derivatization. However, relatively large quantities of sample are normally required to carry out such experiments, typically in the microgram scale.

We have been interested in developing analytical methodology for the trace level detection of chemically modified nucleobases and nucleosides in physiological samples. This requires detection limits not normally associated with the aforementioned desorption MS techniques. Electron capture negative ion mass spectrometry (ECNIMS) has been shown to provide particularly low detection limits,

approaching the femtogram (10^{-15} g) level, for compounds amenable to electron capture[11]. For compounds not inherently electrophoric, the preparation of a suitable derivative is necessary to permit efficient ionization under ECNIMS conditions[11–14]. Numerous volatile derivatives of the naturally occurring and modified nucleosides have been proposed for analysis by both gas chromatography (GC)[15] and MS[16]. These include derivatives based on acetyl[17], permethyl[18], alkylsilyl[19], or trifluoro-acetyl[20,21] chemistry. However, very little has appeared on the preparation of electrophoric derivatives suitable for ECNIMS. Smith *et al.*[22] have described the preparation of trifluoroacetyl and nitrobenzyl nucleoside derivatives. The negative ion mass spectra observed, obtained under low pressure electron impact (EI) conditions, consisted primarily of fragment ions related to the introduced electrophore, and not the original nucleoside.

Recent studies conducted in our laboratories concerning the development of volatile, chemically stable, and highly electrophoric derivatives of nucleosides[23,24] and nucleobases[25,26] for analysis by GC with electron capture detection (ECD) and GC–MS have provided several new approaches to nucleoside derivatization method-ology. For the nucleosides, two promising derivatives are those obtained by treatment with pentafluorobenzyl bromide[23] and cinnamoyl chloride[24]. We wish to report here the methane negative ion and positive ion chemical ionization mass spectra of several derivatives of the nucleoside thymidine, and two modified nucleosides 3-methyl-thymidine and 5-hydroxymethyl-2'-deoxyuridine. Presented in Table I are the structures of the seven compounds prepared for this study. Three different derivatives of thymidine 1–3, were prepared incorporating pentafluorobenzyl or cinnamoyl

TABLE I

STRUCTURES OF ELECTROPHORE-LABELED NUCLEOSIDES

CIN = COCH=CHC$_6$H$_5$; PFB = CH$_2$C$_6$F$_5$.
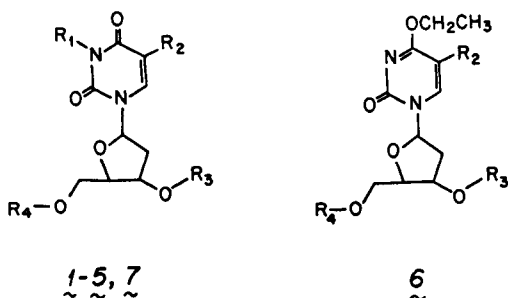
Compound	MW	R_1	R_2	R_3	R_4
1	502	H	CH$_3$	CIN	CIN
2	682	PFB	CH$_3$	CIN	CIN
3	782	PFB	CH$_3$	PFB	PFB
4	516	CH$_3$	CH$_3$	CIN	CIN
5	616	CH$_3$	CH$_3$	PFB	PFB
6	630	—	CH$_3$	PFB	PFB
7	978	PFB	CH$_2$OPFB	PFB	PFB

electrophores. Two derivatives of the modified nucleoside 3-methylthymidine, 4 and 5, were synthesized by introduction of either electrophore to the sugar hydroxyl sites. In addition, the pentafluorobenzyl derivatives of the modified nucleosides O^4-ethyl-thymidine (6) and 5-hydroxymethyl-2'-deoxyuridine (7) have been prepared. As reported previously[23,24] these derivatives have been found to be quite stable chemically, particularly in comparison to the popular silyl adducts. Moreover, the high sensitivity exhibited by these compounds by GC–ECD[23,24] suggests possible utility for the related technique of ECNIMS.

EXPERIMENTAL

The preparation of these derivatives has been described previously[23,24]. All mass spectra were obtained by placing approximately 100 ng of each compound in the glass tip of a direct insertion probe of the Finnigan 4021B mass spectrometer. Ion source temperature was set at 200°C and the probe was heated balistically to 300°C. For the chemical ionization experiments, methane served as reagent gas at a source pressure of 0.30 Torr.

RESULTS

Electron capture negative ion mass spectrometry

The spectra obtained for compounds 1–7 by ECNIMS are summarized in Table II. The behavior of the derivatives in terms of fragmentation is clearly dependent on

TABLE II

METHANE ECNIMS OF ELECTROPHORE-LABELLED NUCLEOSIDES

Values in parentheses are percentages relative abundance.

Ion	m/z						
	1	2	3	4	5	6	7
M $^{\tau}$	502	682	782	516	616	630	978
	(100)	(100)	(0.5)	(100)	(0)	(0)	(0)
(M − 181)⁻	—	501	601	—	435	449	797
		(0)	(5)		(8)	(35)	(8)
b⁻	125	125	305	139	139	153	501
	(17)	(12)	(5)	(8)	(1)	(2)	(9)
Other	147*	—	762	—	178**	178**	178**
	(16)		(2)		(100)	(100)	(100)
			178				196***
			(100)				(80)

   * $C_6H_5CH = CHCOO^-$
  ** $CH_2C_6F_4O^-$.
 *** $CHOC_6F_5^{\tau}$

the choice of added electrophore. The cinnamoyl esters 1, 2, and 4 are distinguished by the production of the molecular anion, $M^{\overline{\cdot}}$, as the base peak. Fragmentation in the spectra of these derivatives is limited; a portion of the ion current is carried by ions formed as a result of cleavage of the nitrogen–carbon glycosidic bond to give the base anion, b^-. Also formed is the complementary anion $C_6H_5CH=CHCOO^-$, m/z 147, by retention of the charge on the electrophore moiety. The high relative abundance of the radical anion $M^{\overline{\cdot}}$, created by the initial electron capture process, is most likely due to the stabilization of the charge by one or both of the cinnamoyl groups.

In contrast to the spectra of the cinnamoyl compounds, those of the derivatives which contain only the pentafluorobenzyl group, 3, 5, 6, and 7, show virtually no molecular anion, $M^{\overline{\cdot}}$. This absence of $M^{\overline{\cdot}}$, due to ionization via a dissociative electron capture process, is a common feature of ECNIMS of the pentafluorobenzyl derivatives of a variety of functionalities[12]. The base peak for 5, 6, and 7 is found to be an ion at m/z 178. A probable composition for this ion is $CH_2C_6F_4O^-$, produced via either an inter-molecular nucleophilic oxygen–fluorine exchange or an intra-molecular rearrangement. Bimolecular exchange reactions have been reported previously for halogenated aromatics under methane chemical ionization conditions and may occur due to traces of oxygen in the ion source[27]. Nevertheless, except for that of 2 (see below), the spectra of the pentafluorobenzyl derivatives studied here exhibit the fragment $(M-181)^-$. This loss of the pentafluorobenzyl radical is quite common[12,28,29] and provides an ion suitable for selected ion monitoring studies for trace level analyses. It is important to note that for compound 2, in which both the pentafluorobenzyl and cinnamoyl electrophores are present, the ionization is dominated by the cinnamoyl groups, yielding only $M^{\overline{\cdot}}$ (100%) and b^- (12%) ions. No signal corresponding to $(M-181)^-$ or m/z 178 was observed. Given these favorable properties of cinnamoyl-derivatized nucleosides for detection, it is unfortunate that recent work in our laboratories has revealed that 4 is a difficult solute to handle by GC.

Methane positive ion chemical ionization

The analysis of nucleosides by positive ion chemical ionization (PICI) mass spectrometry has been accomplished using both the free nucleosides[30] and the trimethylsilyl derivatives[3,31]. The PICI mass spectra of compounds 1–7 are summarized in Table III. The behavior of both the cinnamoyl and pentafluorobenzyl derivatives is quite similar to that documented previously for the corresponding trimethylsilyl derivatives[3]. In general, the mass spectra provide both molecular weight confirmation via the MH^+ ions and details of structural features through the production of several significant fragment ions. The major fragmentation paths for these derivatives are outlined in Scheme 1. Ions indicative of the nucleobase, $(b+2H)^+$ and $(b+30)^+$, the sugar, S^+ and $(S-OR)^+$, and the intact nucleoside, $(M-OR)^+$ and $(M-OR-OR)^+$, are readily discernible and should aid in the structure elucidation of modified nucleosides. With the exception of compound 1, the MH^+ ion is a prominent feature for all the derivatives studied. This ion, when also considered in conjunction with the $M^{\overline{\cdot}}$ or $(M-181)^-$ ions in the ECNI mass spectra, serves to establish the molecular weight of the derivative. Chemical modifications at the sugar or base portions of the nucleoside may be ascertained by location of the appropriate fragment ions. In particular, the pair of ions $(b+2H)^+$ and $(b+30)^+$, separated by 28 a.m.u., are clearly featured for all the compounds studied.
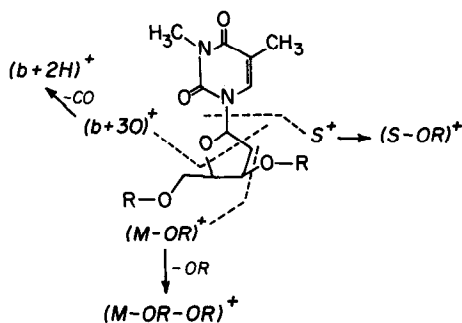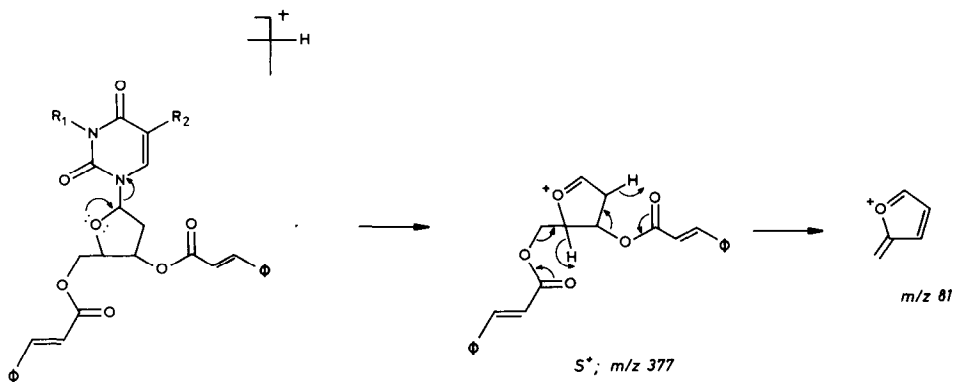
Scheme 1.

TABLE III

METHANE PICIMS OF ELECTROPHORE-LABELED NUCLEOSIDES

Values in parentheses are percentages relative abundance.

Ion	m/z						
	1	2	3	4	5	6	7
MH^+	503	683	783	517	617	631	979
	(2)	(80)	(100)	(0)	(100)	(37)	(100)
$(M-OR)^+$	335	535	585	369	419	433	781
	(9)	(68)	(12)	(46)	(11)	(21)	(68)
S^+	377	377	477	377	477	477	477
	(40)	(100)	(25)	(66)	(14)	(4)	(19)
$(S-OR)^+$	229	229	279	229	279	279	279
	(18)	(20)	(6)	(23)	(10)	(5)	(3)
$(b+30)^+$	155	335	335	169	169	183	531
	(26)	(47)	(12)	(40)	(19)	(22)	(10)
$(b+2)^+$	127	307	307	141	141	155	503
	(76)	(88)	(35)	(100)	(56)	(100)	(15)
Other	149	182		149	181	179	181
	(28)	(15)		(25)	(12)	(30)	(20)
	131	149		131			
	(29)	(23)		(24)			
	81	131		81			
	(100)	(30)		(88)			
		81					
		(54)					

The spectra of the three cinnamoyl-substituted derivatives show an abundant ion of m/z 81. This ion is absent in the other derivatives studied and, most likely, can be rationalized in terms of the furan moiety depicted in Scheme 2. A similar ion has been observed in the EI mass spectra of trifluoroacetyl derivatives of nucleosides[1,32]. The production of this ion in only the cinnamoyl derivatives is consistent with a series of six-membered ring rearrangements (Scheme 2). These processes would not be possible in the case of the pentafluorobenzyl derivatives.

Scheme 2.

Electron impact mass spectrometry

EI mass spectra were obtained for compounds 1–7 and are summarized in Table IV. As might be anticipated for these nucleosides, the spectra are composed almost solely of fragment ions, with no $M^{+\cdot}$ ions detected. The cinnamoyl esters 1, 2, and 4 are dominated by the m/z 81 ion, as discussed earlier for the PICI mass spectra. For the pentafluorobenzyl derivatives 3, 5, 6, and 7 the base peak is at m/z 181, $CH_2C_6F_5^+$. An earlier study by McCloskey[33] on the EI mass spectra of monobenzyl nucleoside derivatives reported the analogous tropylium ion at m/z 91, $C_6H_5CH_2^+$, to be the base peak.

CONCLUSION

Derivatization with electrophoric groups influences significantly the fragmentation pattern of nucleosides. In the ECNI mode, incorporation of a cinnamoyl group provides ideal conditions for formation and stabilization of a molecular anion even in the presence of a pentafluorobenzyl group. In contrast, derivatives containing a pentafluorobenzyl group alone largely form the anion $CH_2C_6F_4O^-$ with this ionization technique. On the other hand, significant fragmentation is encountered for all compounds at least under PICI and EI conditions. Together these latter spectra are well suited for structural studies.

For trace quantitative analysis, the advantages of pentafluorobenzyl derivatives of deoxynucleosides are that, for the ones examined to date, they are stable derivatives which can be detected with high sensitivity by GC–ECD[23], and certain of them give a structurally characteristic ion at M − 181 (loss of pentafluorobenzyl) with moderate

TABLE IV

EIMS OF ELECTROPHORE-LABELED NUCLEOSIDES

The values in parentheses are percentages relative abundance.

Ion	m/z						
	1	2	3	4	5	6	7
S^+	—	377 (5)	477 (4)	377 (5)	—	—	477 (2)
$C_6F_4CH_2{}^+$	—	—	181 (100)	—	181 (100)	181 (100)	181 (100)
$C_6H_5CHCHCO^+$	131 (42)	131 (40)	—	131 (30)	—	—	—
$C_6H_5CHCH^+$	103 (20)	103 (12)	—	103 (25)	—	—	—
$C_5H_5O^+$	81 (100)	81 (100)	—	81 (100)			
Other	147 (10)	306 (8)	—	77 (16)	141 (10)	155 (30)	—
				55 (14)	55 (20)	126 (17)	
						110 (10)	
						82 (15)	
						9 (12)	
						55 (35)	

abundance (*e.g.* 35% for 6 relative to the base peak) under ECNI conditions, encouraging their sensitive, specific detection by GC–ECNIMS.

REFERENCES

1 C. Hignite, in G. R. Waller and O. C. Dermer (Editors), *Biochemical Applications of Mass Spectrometry*, First Supplemental Volume, Wiley, New York, 1980, pp. 527–566.
2 J. A. McCloskey and S. Nishimura, *Acc. Chem. Res.*, 10 (1977) 403.
3 J. A. McCloskey, in P. O. Ts'o (Editor), *Basic Priinciples in Nucleic Acid Chemistry*, Vol. I, Academic Press, New York, 1974, Ch. 4.
4 A. L. Burlingame, K. Straubb and T. A. Baillie, *Mass Spectrom. Rev.*, 2 (1983) 331.
5 J. A. McCloskey, in A. L. Burlingame and N. Castagnol (Editors), *Mass Spectrometry in the Health and Life Sciences*, Elsevier, Amsterdam, 1985, pp. 521–546.
6 F. W. Crow, K. B. Tomer, M. L. Gross, J. A. McCloskey and D. E. Bergstrom, *Anal. Biochem.*, 139 (1984) 243.
7 H. R. Schulten and H. D. Beckey, *Org. Mass Spectrom.*, 7 (1973) 861.
8 W. Ens, K. G. Standing, J. B. Westmore, K. K. Ogilvie and M. J. Nermer, *Anal. Chem.*, 54 (1982) 960.
9 C. J. McNeal and R. D. McFarlane, *J. Am. Chem. Soc.*, 103 (1981) 1609.
10 D. L. Slowikowski and K. H. Schram, *Nucleosides Nucleotides*, 4 (1985) 347.
11 K. F. Faull and J. D. Barchas, in D. Glick (Editor), *Methods of Biochemical Analysis*, Vol. 29, Wiley, New York, 1983, pp. 325–383.
12 T. M. Trainor and P. Vouros, *Anal. Chem.*, 59 (1986) 601.
13 P. Vouros and T. M. Trainor, *Adv. Mass Spectrom., Part B*, (1985) 633.
14 D. F. Hunt, G. C. Stafford, F. W. Crow and J. W. Russell, *Anal. Chem.*, 48 (1976) 2098.
15 K. H. Schram and J. A. McCloskey, in K. Tsuji (Editor), *GLC and HPLC Analysis of Therapeutic Agents*, Marcel Dekker, New York, 1979, pp. 1149–1190.
16 J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger and R. N. Stillwell, *J. Am. Chem. Soc.*, 90 (1968) 4182.
17 J. Boutagy and D. J. Harvey, *J. Chromatogr.*, 156 (1978) 153.
18 A. P. DeLeenheer and C. I. Gelijkens, *Anal. Chem.*, 48 (1976) 2203.
19 M. A. Quilliam, K. K. Ogilvie, K. L. Sadana and J. B. Westmore, *J. Chromatogr.*, 196 (1980) 367.
20 C. F. Gelijkens, D. L. Smith and J. A. McCloskey, *J. Chromatogr.*, 225 (1981) 291.
21 U. I. Krahmer, J. G. Liehr, K. J. Lyman, E. A. Orr, R. N. Stillwell and J. A. McCloskey, *Anal. Biochem.*, 82 (1977) 217.
22 D. L. Smith, K. H. Schram and J. A. McCloskey, *Biomed. Mass Spectrom.*, 10 (1983) 269.
23 J. Adams, M. David and R. W. Giese, *Anal. Chem.*, 58 (1986) 345.
24 J. Adams and R. W. Giese, *J. Chromatogr.*, 347 (1985) 99.
25 G. B. Mohamed, A. Nazareth, M. J. Hayes, R. W. Giese and P. Vouros, *J. Chromatogr.*, 314 (1984) 211.
26 A. Nazareth, M. Joppich, S. Abdel-Baky, K. O'Connell, A. Sentissi and R. W. Giese, *J. Chromatogr.*, 314 (1984) 201.
27 R. C. Dougherty, *Anal. Chem.*, 53 (1981) 625A.
28 A. G. Netting and A. M. Duffield, Biomed. Mass Spectrom., 12 (1985) 668.
29 R. J. Strife and R. C. Murphy, *J. Chromatogr.*, 305 (1984) 3.
30 M. S. Wilson and J. A. McCloskey, *J. Am. Chem. Soc.*, 97 (1975) 3436.
31 T. Marunaka and Y. Umeno, *J. Chromatogr.*, 221 (1980) 382.
32 W. A. Koenig, L. C. Smith, P. F. Crain and J. A. McCloskey, *Biochemistry*, 10 (1971) 3968.
33 H. T. Cory, K. Yamaizumi, D. L. Smith, D. R. Knowles, A. D. Broom and J. A. McCloskey, *J. Heterocyclic Chem.*, 16 (1979) 585.