Mass Spectral Characterization of some Nucleoside Phosphates and Phosphoramidates

Ronald G. Smith and David Farquhar

Department of Developmental Therapeutics, The University of Texas System Cancer Center
M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, TX 77030

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Seven new synthetic nucleotide analogues have been characterized as their trimethylsilyl derivatives by recording their electron impact mass spectra. These spectra are consistent with their expected structures, most of which possess a cyclic phosphate or phosphoramidate group.

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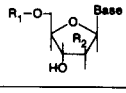
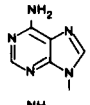
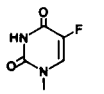
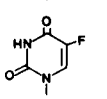
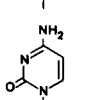
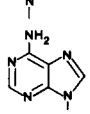
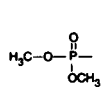
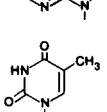
Introduction.

Recent developments in the pursuit of more effective therapy for neoplasia include the synthesis of certain nucleoside 5'-phosphates and 5'-phosphoramidates as latent precursors of antineoplastic nucleotides (1). These agents are designed to enter cells by passive diffusion and to revert subsequently to their parent nucleotides, thus overcoming cellular resistance to antimetabolites resulting from the depletion of phosphorylating enzymes (2,3).

Table I

Phosphates and Phosphoramidates



	Compound	Base	R_1	R_2
1	9-β-D-Arabinofuranosyladenine - 5' - [2 - (2 - oxo - 1, 3, 2 - dioxophosphorinane)]			OH
2	9-β-D-Arabinofuranosyladenine - 5' - [2 - (2 -oxo-1, 3, 2,- oxazaphosphorinane)]			OH
3	1- (2' - Deoxy - β - D - ribofuranosyl) - 5 - fluorouracil - 5' - [2 - (2 - oxo - 1, 3, 2 - dioxophosphorinane)]			H
4	1-(2' - Deoxy-β - D - ribofuranosyl) - 5 - fluorouracil - 5' - [2- (2- oxo - 1, 3, 2 - oxazaphosphorinane)]			H
5	1-β-D-Arabinofuranosylcytosine - 5' - [2-(2- oxo - 1, 3, 2-oxazaphosphorinane)]			OH
6	9-β-D-Arabinofuranosyladenine - 5' - dimethylphosphate			OH
7	Thymidine - 5' - [2 - (2 - oxo - 1, 3, 2 - dioxophosphorinane)]			H

The synthesis of five analogues (compounds **1-5**, Table 1) of known antitumor agents and two model compounds (compounds **6** and **7**, Table 1) were prepared as shown in Scheme I. These compounds required structural evidence to establish the presence of the 6-membered phosphate ester (or amidate) ring and to determine the position of attachment of this ring to the sugar. Mass spectrometry has the potential to provide this evidence.

A variety of derivatives have been used to characterize nucleosides (4-6) but few are sufficiently mild for the analysis of the corresponding nucleotides (7-9). Trimethylsilylation is a gentle method for derivatization that has been applied successfully to nucleotides, usually providing molecular ions and an abundance of fragment and rearrangement ions characteristic of the base, sugar, and phosphate groups. This report describes the electron impact mass spectra obtained from the trimethylsilyl derivatives of compounds **1-7** and the use of these spectra to help substantiate their chemical structures. Derivatives with trimethylsilyl-d_9 groups were also prepared to aid in the assignment of ionic compositions.

Discussion.

The mass spectra of the trimethylsilyl derivatives of compounds **1-7** exhibit many useful ions derived from the

Table II

Ions Characteristic of Base and Sugar

Ion	m/e (% Relative Intensity) 1	2	3	4	5	6	7
M^+	603 (18)	674 (5.0)	510 (0.4)	581 (3.6)	650 (.05)	591 (89)	506 (1.6)
M-CH_3	588 (48)	659 (8.8)	--	566 (7.0)	635 (1.4)	576 (82)	491 (0.2)
Base + 2H	208 (36)	208 (9.1)	--	--	184 (80)	208 (28)	199 (22)
[Base + H] — CH_3	192 (96)	192 (35)	--	187 (38)	168 (71)	192 (40)	183 (38)
Base + H + CHO	236 (82)	236 (19)	--	--	--	236 (100)	227 (5.4)
M – []	466 (5.0)[1]	466 (13)[2]	--	373 (.07)[2]	442 (0.3)[2]	465 (10)[3]	369 (0.4)[1]
	306 (5.2)	306 (1.5)	--	--	--	306 (43)	--
	376 (1.0)	376 (0.5)	--	--	--	376 (30)	--
	362 (0.5)	362 (0.2)	--	--	--	362 (17)	--
	169 (81)[4]	169 (100)[4]	81 (30)[5]	81 (19)[5]	169 (74)[4]	169 (73)[4]	81 (100)[5]
	243 (15)[4]	243 (3.0)[4]	155 (65)[5]	155 (38)[5]	243 (7.4)[4]	243 (32)[4]	155 (83)[5]
	278 (31)[1]	349 (5)[2]	--	--	--	266 (98)[3]	--

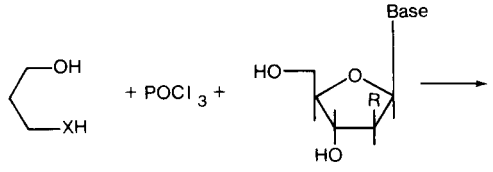
[1] X = — O $(CH_2)_3$O —
[2] X = — N$(Si(CH_3)_3)$ — $(CH_2)_3$O —
[3] X = $(CH_3O)_2$
[4] R = OSi $(CH_3)_3$
[5] R = H

0022-152X/80/081659-03$02.25

base, sugar, and phosphate moieties. Major ions possessing portions of the base and sugar are listed in Table 2. These ions are identical or analogous to those previously observed in the spectra of trimethylsilylated nucleotides (8,9) and will not be discussed here. Ions derived from the trimethylsilylated arabinose group are similar to those previously reported for the trimethylsilylated ribose of nucleotides (8,9).

Several ions have been used to distinguish nucleoside 3′-monophosphates from nucleoside 5′-monophosphates (8-10), but the differences are usually quantitative, and both isomers are required to make structural assignments. However, an intense ion at m/e 103 in the spectra of trimethylsilylated nucleosides and nucleoside 3′-monophosphates (9) is absent in these spectra. This fragment ion, assigned the structure $(CH_3)_3SiOCH_2^+$ (8), is also absent in the spectra of trimethylsilylated nucleoside 5′-monophosphates (8). This strongly supports the 5′-position of the phosphate and phosphoramidate groups in compounds **1-7**. This assignment is also consistent with the position favored by the synthetic route (11).

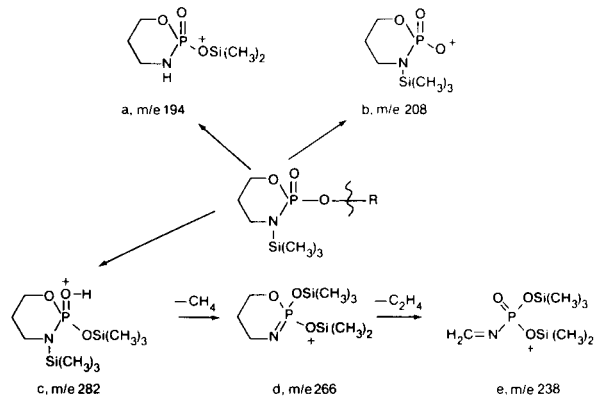
Scheme I



X = O, NH
R = H, OH

The spectra also exhibit a number of intense ions derived from the phosphate or phosphoramidate moieties. These ions are best represented as 6-membered cyclic structures containing the propylene group, as shown in Schemes II and III. The compositions, but not necessarily the specific structures shown, are supported by m/e changes of the corresponding ions in the spectra of the deuterium labeled derivatives. Ions **c** and **g** lose the elements CH_4 to form ions **d** and **h**, respectively; similar decompositions have been noted for the corresponding ions from trimethylsilylated nucleotides (9). The mass spectrum of trimethylsilylated **6** shows abundant ions (> 15% relative intensity) analogous to **f-h**, each appearing at 12 mass units lower. The ion at m/e 238 (m/e 253 for the deuterium labeled derivative) in the spectra of the cyclic phosphoramidates is assigned structure **e**, envisioned as the product of a retro Diels-Alder rearrangement of ion **d**. To facilitate this rearrangement ion **d** is assigned a structure with an endocyclic P-N double bond and a silyl group rearranged from nitrogen to oxygen. This is consistent with the migratory nature of silyl groups (12-14). Alternatively, the phosphoramidate derivatives may possess a P-N double bond before ionization and remains intact in ions **a, b, c,** and **d**.

Scheme II



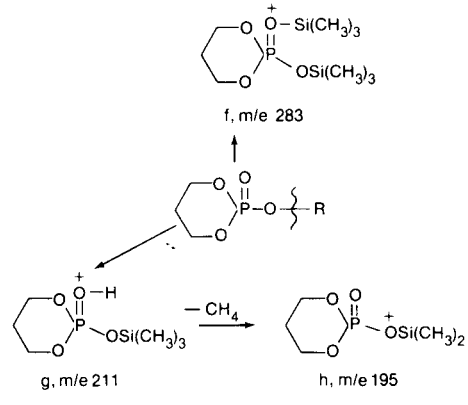
Relative Intensities of Ions a-e[1]

Cyclic Phosphoramidate

Ion	2	4	5
a	11	66	20
b	9	43	5
c	26	8.8	15
d	11	23	28
e	16	25	32

[1] Expressed as a percent of the base ion.

Scheme III



Relative Intensity of Ions f-h[1]

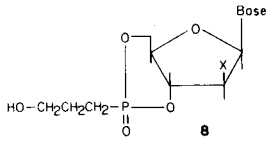
Cyclic Phosphate

Ion	1	3	7
f	8	13	18
g	90	40	48
h	87	75	76

[1] Expressed as a percent of the base ion.

The fragmentations and rearrangements shown in Schemes II and III would be extremely improbable in alternative cyclic structures such as the 3′,5′-cyclic

phosphate isomer **8**. This and other mass spectral data presented here support the proposed structures for compounds **1-7**.



8

EXPERIMENTAL

The synthesis of compounds **1-7** have been described briefly (1) and will be elaborated on elsewhere. The compounds were purified to homogeneity by high pressure liquid chromatography. Synthesis of the phosphoramidates (**2**, **4**, and **5**) generates an asymmetric center (phosphorus) and the diastereomers were separated chromatographically. As expected, the mass spectra of their trimethylsilyl derivatives were identical.

Small samples (*ca.* 100 μg.) of each compound were derivatized by the addition of 10 μl. pyridine and 10 μl. of *N,O*-bis(trimethylsilyl)trifluoroacetamide followed by heating at 60° for 15 minutes. Deuterium labeled derivatives were prepared by substituting 10 μl. of *N,O*-bis(trimethylsilyl)acetamide-d_{18} in the same procedure. Two microliters of the reaction mixture were added to the capillary sample cup and inserted into the mass spectrometer using the solids probe. Care was taken to minimize exposure of the derivatized samples to air.

Electron impact spectra were recorded at 70 eV using a Finnigan 3300F mass spectrometer interfaced with an Incos 2300 data system. When the analyzer pressure decreased below 10^{-6} torr, the instrument was scanned continuously over the range m/e 50-650 at 4 seconds/scan. Spectra of the derivatives were obtained after heating the probe to 100-180°.

Acknowledgement.

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