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Chemical ionization pyrolytical spectra of DNA

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Abstract: The chemical ionization–pyrolytical (CI–Py) spectra of DNA and deuterated DNA (Herring Sperm) are recorded. The 200–800 a.m.u. region is examined for CH_4, NH_3, ND_3, electron-capture and OH^- CI spectra. The origin of major ion species is discussed.

Keywords: *Chemical ionization MS; electron impact MS; pyrolytical MS.*

Introduction

In an earlier paper in this series [1] the authors reported the analysis of both positive (PI) and negative (NI) mass spectra of DNA recorded via the pyrolytical (Py) chemical ionization (CI) mode. 100–300 a.m.u. was set-up as a working range in which the best sensitivity for the base ion studies had been achieved. The upper range (up to 800 a.m.u.) has consequently been neglected because of the weak intensities of ion species present. In this paper is reported the analysis of DNA (Herring Sperm, Boehringer Manncheim Co.), CI spectra for the PI (CH_4, NH_3, ND_3) and the NI (OH^- and electron capture) in this area.

The electron impact (EI) spectroscopy recorded in pyrolytical mode is considered a rather drastic method. The phosphate bond of the nucleic acid or the polynucleotide is cleaved producing a nucleoside-like fragment, base or larger fragments as proposed by Loo [2] and Wiebers [3] (Scheme 1). However, several interesting structural facts about the sequence of the polynucleotide are lost.

In 1973 Schutten, using the field desorption technique [4], reported the presence of several pN_1p, pN_1pN_2 (5′-phosphate is believed to be more stable than 3′-one; for this reason the alternative structure N_1pN_2p is disregarded), pN_1pN_2p, etc. ions, where N_x is a nucleoside and p a phosphate. The presence and the fate of different ions have not been followed by more rigorous studies. The variaton of pyrolytical conditions (time, temperature) together with use of a softer ionization technique, such as CI, should enable one in principle to show down the random destruction of the polynucleotide chain and offer the possibility of its discriminatory cleavage, leading to the recording of di- and tri-nucleotide fragments.

Experimental

The Herring Sperm DNA was bought from Boehringer Mannheim Co. Of this DNA, 25 mg was deuterated with D_2O (1 ml) for 24 h then lyophylized overnight. The EI and CI measurements were performed exactly as previously described [1] on a Riber R-1010 mass spectrometer (Nermag, France). The working range of measurements was set-up at 150–800 a.m.u. The OH^- ions production was effected using the N_2O/methane method. The ammonia-d_3 (ND_3) was received from CEA Service des molécules marquées; all other gas reagents came from Air Liquide (Alpha Gaz, France).

Results and Discussion

The spectra of DNA are presented in Figs 1–5 and the intense ions within the working range are summarized in Table 1. The positive and the negative ion CI spectra represent some common features. For example, the ions corresponding to the same structure have a mass of 2 a.m.u. (+2H) higher in the PI spectrum than in the NI spectrum. Compared with the EI spectrum [1], the corresponding

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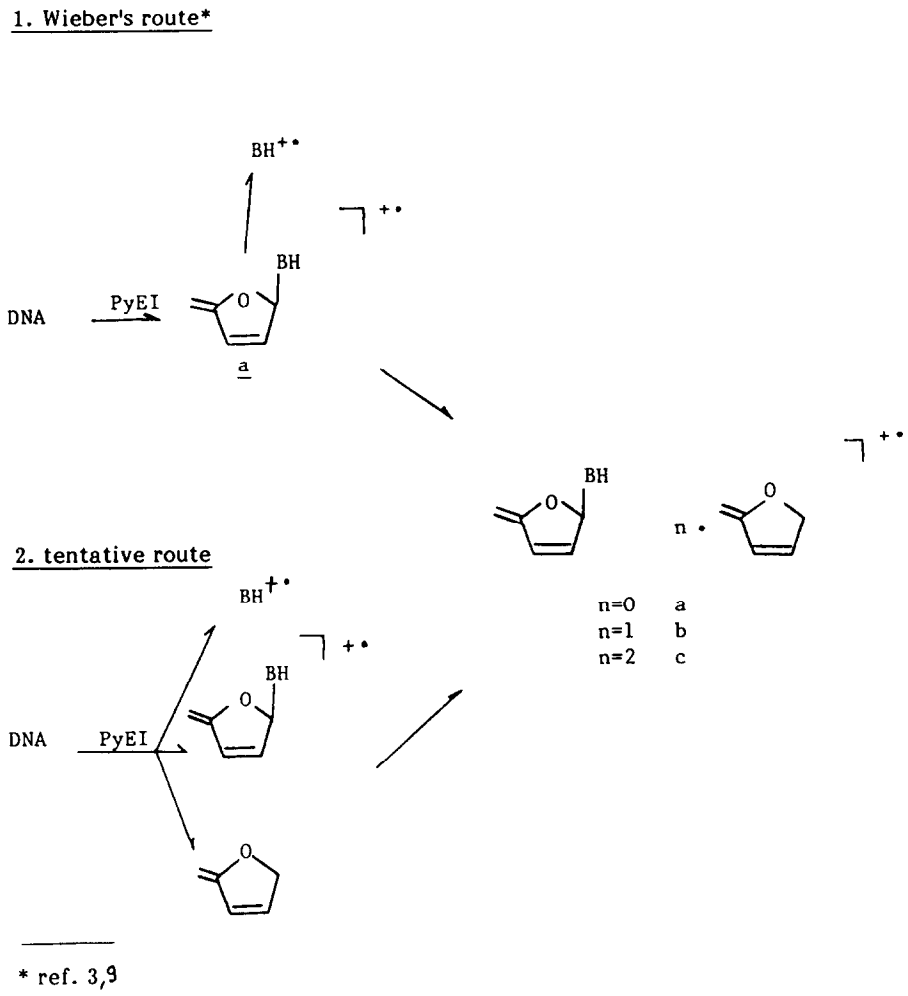

Scheme 1
Fragmentation of DNA under pyrolytical conditions (PI). (*Refs 3 and 9.)

ions display, of course, only a 1 a.m.u. difference. For example, the ion of *a* type (Scheme 1) for adenosine (A) observed at *m/z* 215 in PI–EI is shifted to 216 in PI–CI and to 214 in NI–CI. Using this criterion (of 2 a.m.u.) and the PI–EI spectrum as a reference, the authors have analysed and identified several ion species, particularly for a fragment BH+80 and BH+160. In spite of their weak intensity the corresponding fragments have already been used for analytical purposes [5].

The brief comparison of PI–CI data indicates the presence of the same ion species for methane (Fig. 1) and NH_3 (Fig. 2) spectra. In the working range, the minimum of 13 common ion pairs is present. Both NI spectra, OH^- (Fig. 3) and electron-capture (Fig. 4) display even more (15) common fragments.

All four spectra show four common ions at *m/z* 161 (deoxyribose dimer at *m/z* 161 (2 × 80 + 1) and $C_5H_{12}N_4O_2$ fragment of unknown

Table 1
Mass values of selected ions (*m/z*) derived from CI spectra of DNA*

Positive ions (PI)		Negative ions (NI)		
CH_4	NH_3	OH^-	Electron-capture	Identification
—	161	161	161	—
—	—	170	170	—
179	179	177	—	—
192	192	190	190	a(C)
216	216	214	214	a(A)
232	232	230	230	a(G)
244	243	241	241	—
272	272	270	270	b(C)
296	296	294	294	b(A)
312	312	310	310	b(G)
—	—	340	340	—
352	352	—	—	c(C)
—	—	367	367	—
376	376	376	376	—
392	392	390	390	c(G)
—	—	432	432	—
456	—	456	456	A (?)
472	472	—	472	G (?)

*The working range was set up to 800 a.m.u.

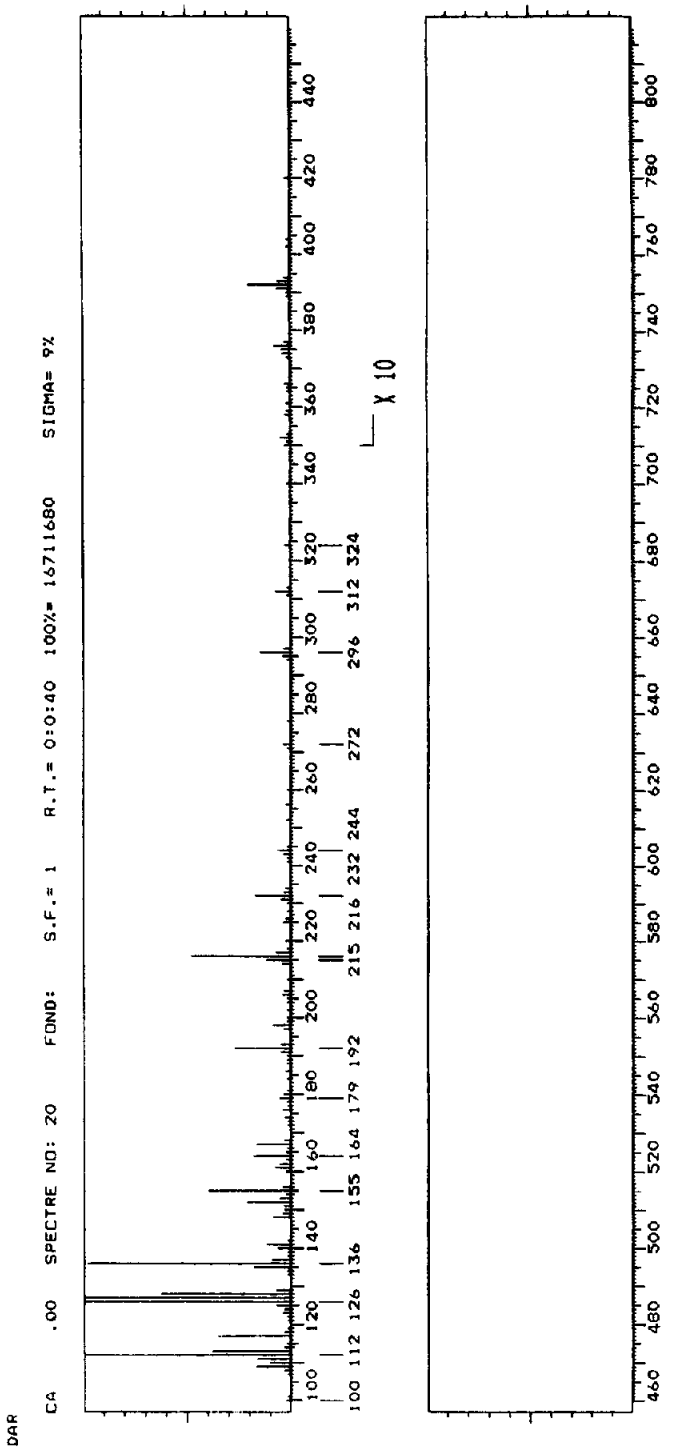

Figure 1
CH_4 PI–CI spectrum of DNA.

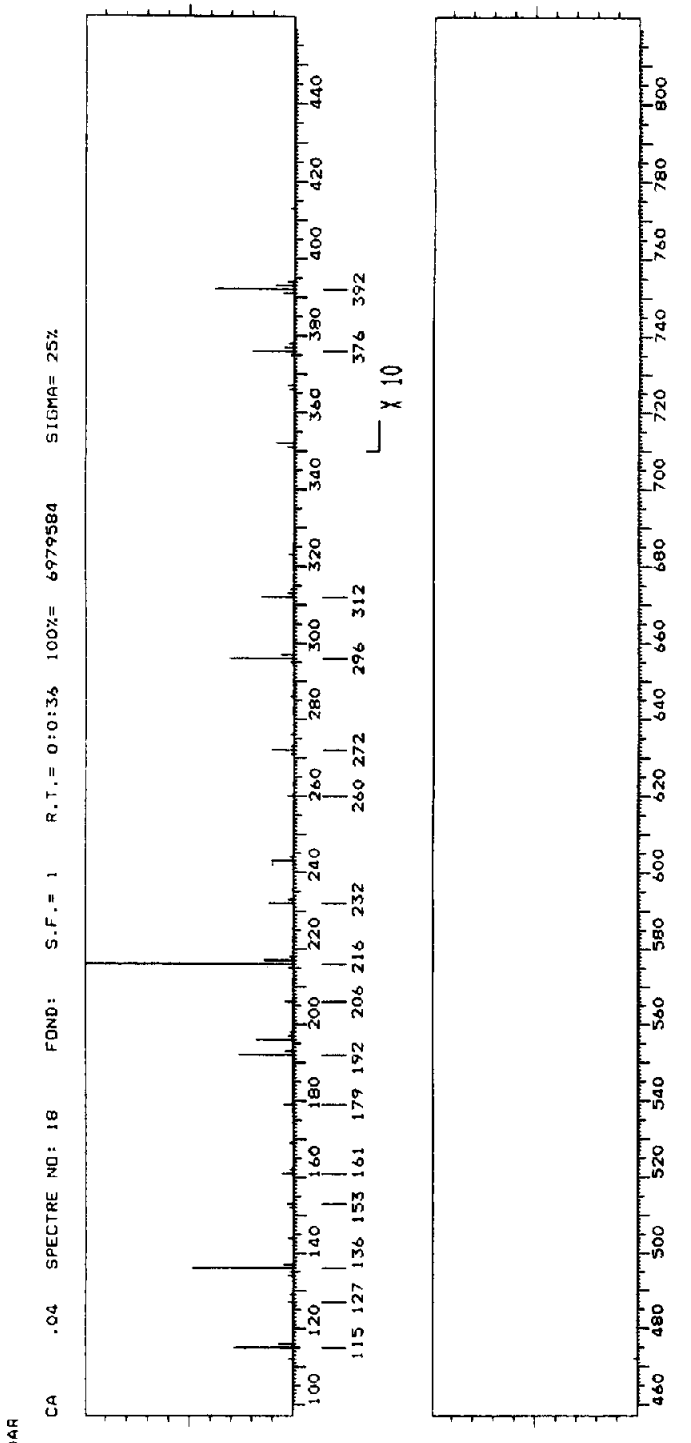

Figure 2
NH_3 PI–CI spectrum of DNA.

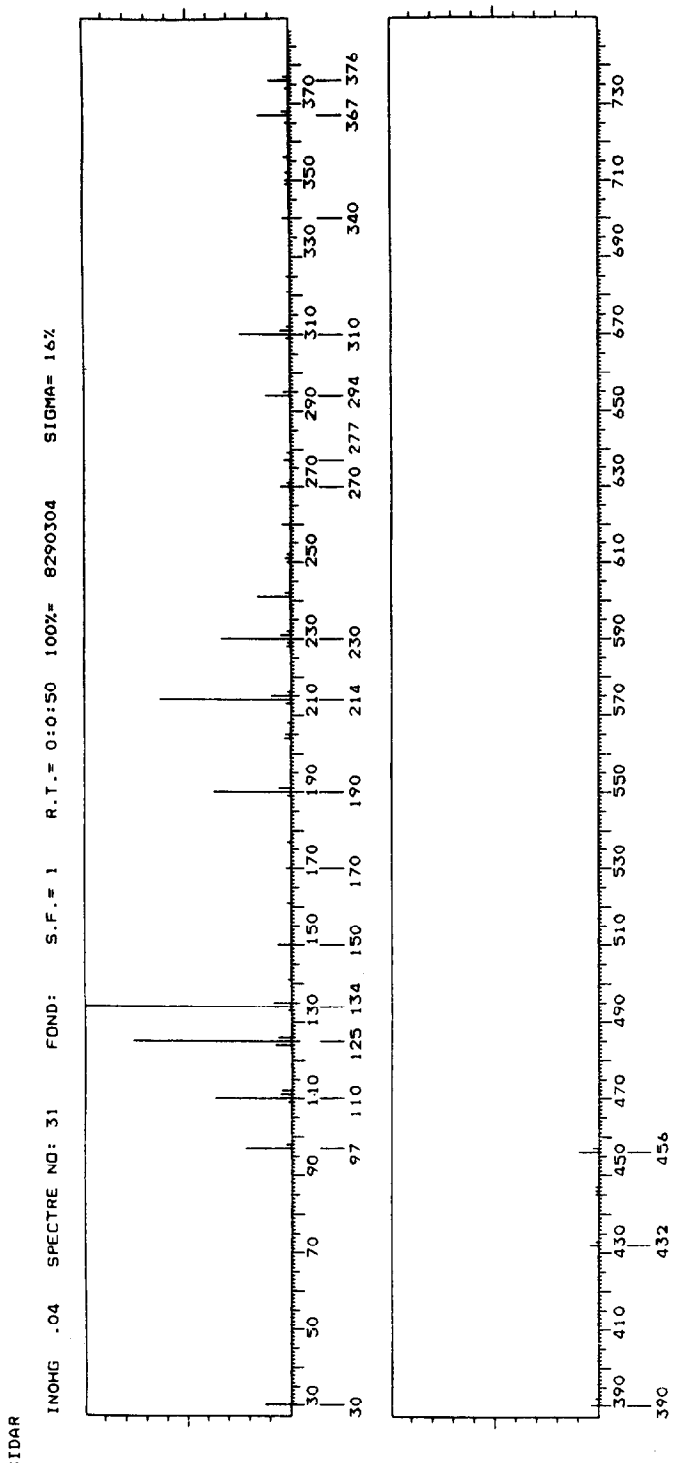

Figure 3
OH^- NI–CI spectrum of DNA.

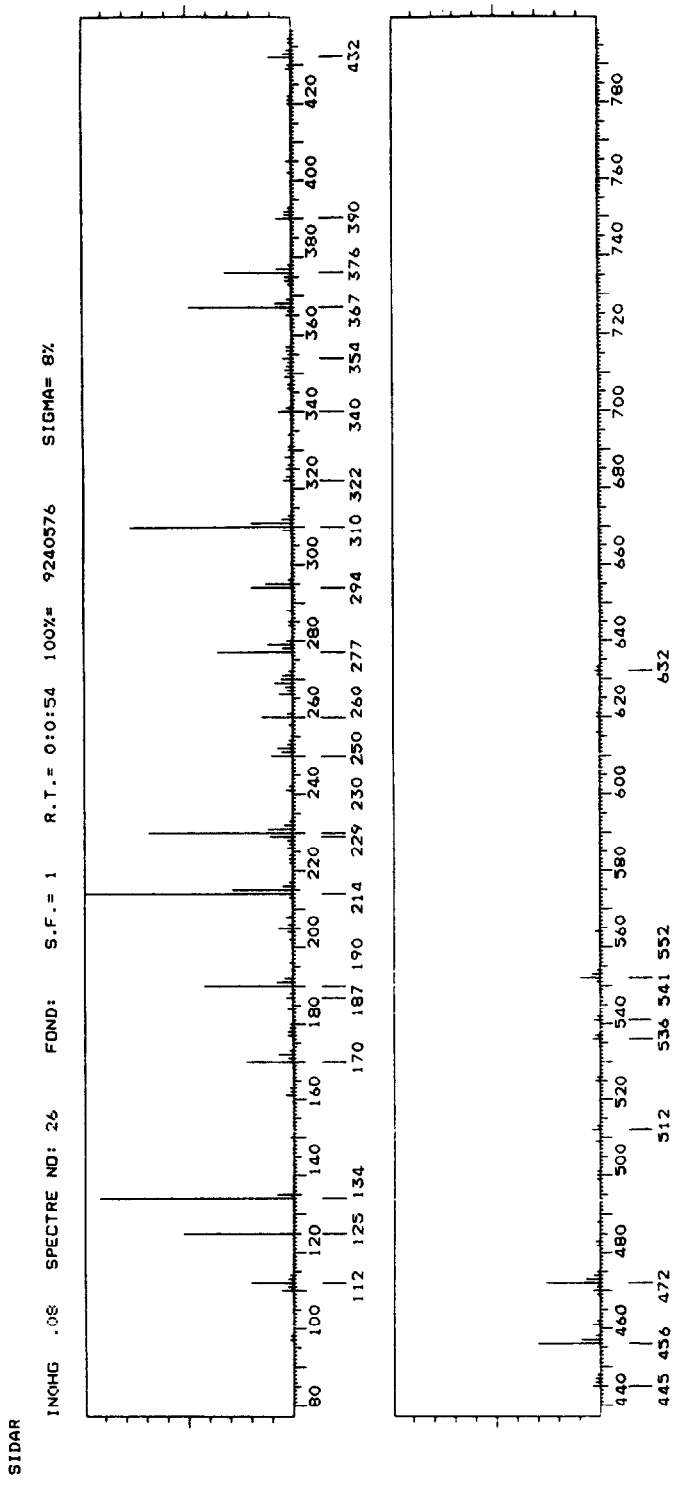

Figure 4
Electron-capture NI–CI spectrum of DNA.

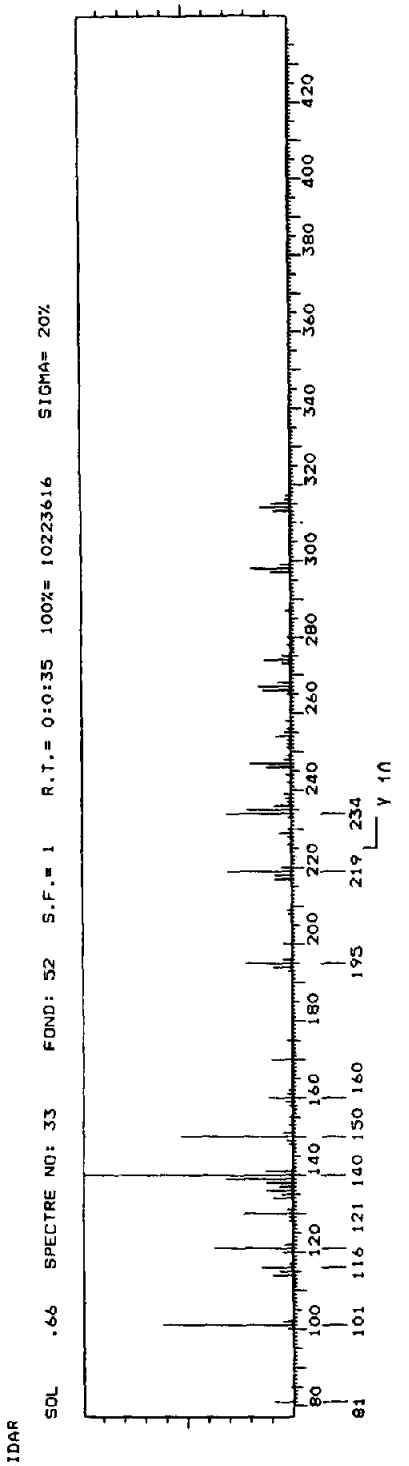

Figure 5
ND_3 PI–CI spectrum of DNA.

origin form a doublet at 160: Wiebers [9] reports a $C_8H_6N_3O$ structure), 376, 456 and 477 (these structures have been confirmed by exact mass measurement in the EI spectrum). One of these fragments, *m/z* 376, has a structure of BH plus three 2-methylfuran residues for A, as previously suggested by Loo [2]. Two remaining fragments at *m/z* 456 and 472, respectively, are BH + four 2-methylfuran residues for A and G, respectively.

In both cases, the addition or subtraction of protons is not observed. The detection of guanosine (G) fragments is quite satisfactory, e.g. *b* ion for G at *m/z* 312 in PI, and 310 in NI is present. It seems, however, that except for the base ion, the thymine (T) fragments are virtually absent. In other words, the detection of this base in DNA depends mostly on the BH (BH_2 or B) ion only.

The *m/z* 126 fragment for T is one of the strongest ions observed in the DNA spectrum (even for a low-T abundance DNA [6]). It is difficult to comment on these results. One probable indication leads to the consideration of the relatively weak aromatic character of this base and then to a weak susceptibility toward the S_E reaction with 2-methylfuran. In a heterocyclic aromatic substitution, even such an analogy is dangerous, the polymerization of five-member heterocyclic rings often stops at the trimer level [7, 8].

It is interesting to note that the B + n80 fragments for G, the base having a strong nucleophilic character, are quite intense in both NI–CI spectra, particularly *m/z* 310. This could be explained by the fact that the anion *b* is more aromatic than the corresponding cation. The tentative structure of fragments *a–c* according to Wiebers are proposed in Scheme 1 and masses of EI–PI ions in Table 2.

Eight ion-pairs undergoing the +2H variation (from NI to PI mode) have been identified and structures of *a*, *b* and *c* have been assigned.

From the spectra one can note that the intensities of base + 80, base + 160 and even base + 240 fragments are much higher than in the corresponding EI spectrum recorded at 250°C [1]. It is generally accepted that the base + 80 fragment *a* represents the nucleoside ion, and the subsequent addition of 2-methylfuran units leads to *b* and *c* ions [3]. The cleavage of DNA according to Weibers leads to the nucleoside-like fragment *a* which picks up the furan residues. From our results it is possible to suggest the parallel route: that the formation of B-ions takes place followed by the coordination of *n*-furane units (Scheme 1) (BH_2^+ in CI–PI, BH^+ in EI–PI and B^- in CI–NI).

Finally, the fragment *a* is not necessarily a result of the direct cleavage of the DNA, but could be an effect of the successive reactions between the base ion and furan residues (resulting from 2′-deoxyribose). In order to verify this hypothesis and have more evidence, the ND_3 CI–PI spectrum was run (Fig. 5).

The DNA CI spectrum with ND_3 displays some interesting features. The BH ion area shows fast HH/D exchange. The ion cluster for C, at *m/z* 114, 115 and 116 (instead of 112 in the NH_3 spectrum), means that the exchange of several active hydrogens under CI conditions takes place. The ion at *m/z* 127 for T has been replaced by a unique ion at 130 (no cluster formation). The ion at 135 for A has been replaced by a cluster centred at 140, the G ion at 152 by 150. This last result is quite amazing — and probably the nucleophilicity of this base versus that of ammonia should be accounted for.

The nucleoside-like ions for corresponding bases show clusters centred at 195 (C), 219 (A) and 234 (G), and a weak doublet at 208 and 209 for T.

This experiment shows a completely different behaviour of both types of ion, and confirms the supposition that both ions are formed simultaneously and that the BH ion does not originate from the corresponding nucleoside ion.

In order to get more information about the relationship between bases and nucleoside-like ions and to explain the ND_3—CI spectrum, the authors have run both spectra EI and NH_3—CI on perdeuterated DNA. The deuteration of DNA was performed by the simple exchange of labile protons with D_2O followed by lyophylization.

First, the EI spectrum (Fig. 6) was compared with the corresponding CI spectrum (Fig. 7).

Table 2
Masses of common ions observed in the EI–Py–PI DNA spectrum

Base	BH	*a* BH + 80	*b* BH + 160	*c* BH + 240
A	135	215	295	375
C	111	191	271	351
G	151	231	311	391
T	126	206	286	—

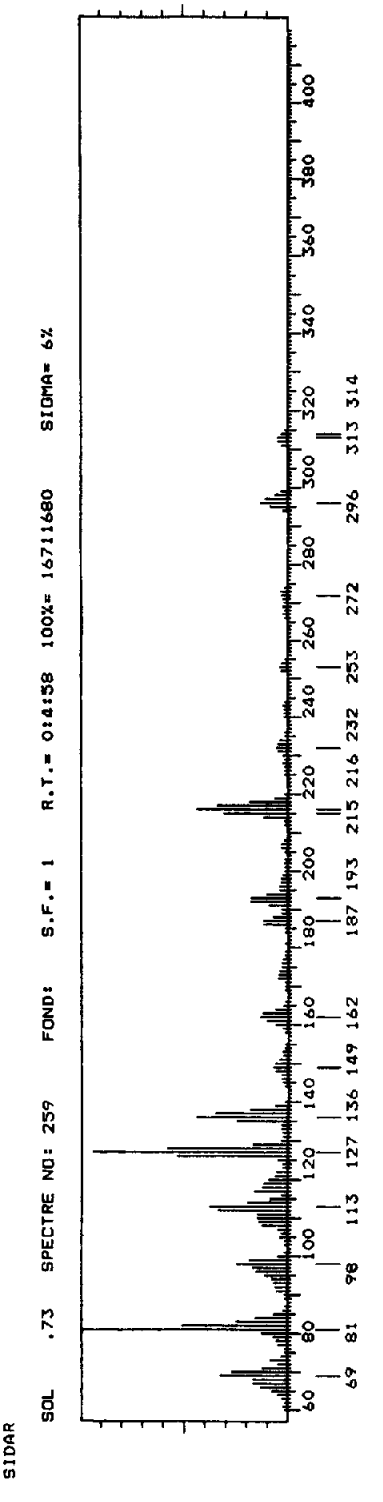

Figure 6
PI–EI spectrum of deuterated DNA.

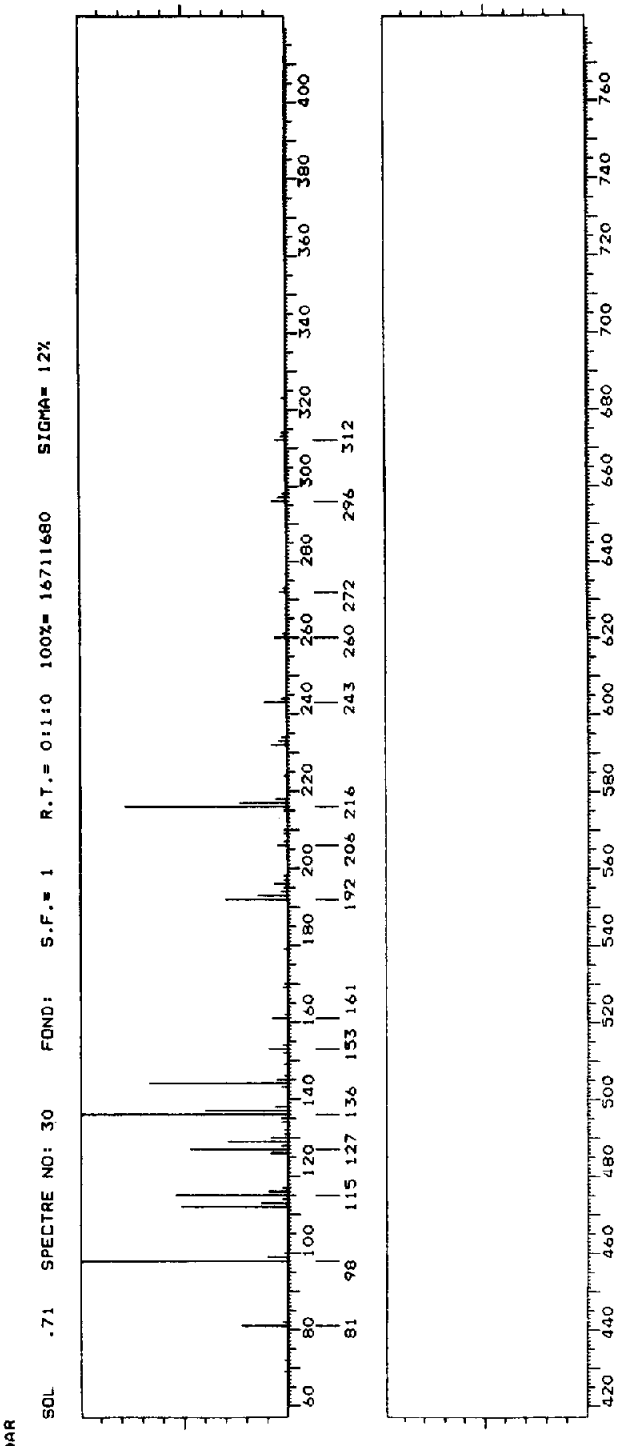

Figure 7
NH_3 PI–CI spectrum of deuterated DNA.

The base ions show a group of clusters centred at *m/z* 113, 127, 136 and 149, and then the nucleoside-like ion group at 193, 215 and 232. The T base ion at 127 is very intense; however, the corresponding nucleoside ion is almost totally absent from the spectrum. The most intense nucleoside ion is observed for A and for C while the G ion is very weak. All clusters reveal a multiple deuteration of bases.

The NH_3 spectrum run on deuterated DNA (Fig. 7) showed a fast exchange of D to H. In the BH ion family the presence of 112 and 115 for C; 127, 129 for T; and 136 and 137 for A is observed; however, the unique 153 G ion is recorded. This result should be related to the nucleophilicity of species involved in the CI processes.

The nucleoside-like ions behave in a completely different manner. The strong ions observed at 192 (C) and 216 (A) are followed by P + 1 and P + 2 ions. Two other bases, T and G display very small ions at 206 and 232, respectively. It is interesting to note this difference which cannot be accounted on, for example, sugar ring deuteration. Base ions preserve more of their deuteration, in spite of their bigger chemical activity (e.g. tautomeric). This important difference of properties seems to indicate one more time that BH ions should not originate from the nucleoside-like ions and that under the CI conditions the fragmentation of DNA proceeds via a second route (Scheme 1). It is also symptomatic that using such a wide temperature range as offered by a DCI probe (200–800°C), no increase of intensities of dinucleotide and similar fragments has been observed (especially for lower temperatures).

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References

[1] K. Jankowski, H. Virelizier and R. Hagemann, *Biomed. Mass. Spectrom.* (1983).
[2] G.A. Charnock and J.L. Loo, *Analyt. Biochem.* **37**, 81 (1970).
[3] J.L. Wiebers, *Nucleic Acids Res.* **3**, 2959 (1976).
[4] H.R. Schulten, H.D. Beckey, A.J.H. Boerboom and H.L.C. Meuzealaar, *Analyt. Chem.* **45**, 2358 (1973).
[5] K. Jankowski and F. Söler, *Eur. J. Mass Spectrom.* **1**, 45 (1980).
[6] K. Jankowski, N. Turkkan and F. Söler, *Biomed. Mass Spectrom.* **9**, 91 (1982).
[7] L. Paquette, *Modern Heterocyclic Chemistry*, p. 57. Benjamin, New York (1973).
[8] J. McCloskey in P.O.P.Ts'O (Ed.) in *Basic Principles in Nucleic Acids Chemistry*, p. 252. Academic Press, New York (1974).
[9] M.H. Bozorgzadeh, J.H. Beynon and J.L. Wiebers, *Adv. Mass Spectrom.* **8A**, 971 (1980).