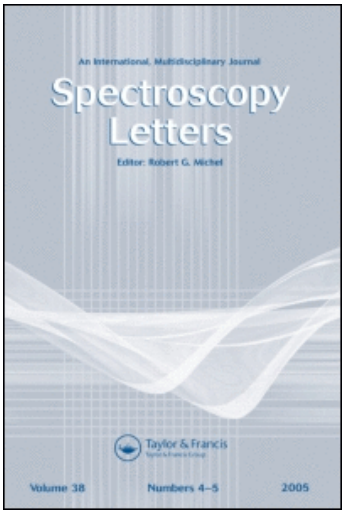
This article was downloaded by:
On: *30 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Adduct Ions Formed from DNA Under Pyrolysis Electron Impact Ionization and Chemical Ionization Using NH_3

K. Jankowski[a]; J. -C. Tabet[b]; H. Virelizier[c]
[a] Département de Chimie, Université de Moncton, Moncton, Nouveau-Brunswick, CANADA [b] Laboratoire de Chimie Organique Structurale, Université Pierre et Marie Curie, Paris Cédex, FRANCE [c] C.E.N. de Saclay, Section d'études et d'analyses isotopiue et nucléaire, Gif-sur-Yvette, FRANCE

To cite this Article Jankowski, K. , Tabet, J. -C. and Virelizier, H.(1992) 'Adduct Ions Formed from DNA Under Pyrolysis Electron Impact Ionization and Chemical Ionization Using NH_3', Spectroscopy Letters, 25: 4, 477 — 485
To link to this Article: DOI: 10.1080/00387019208021522
URL: http://dx.doi.org/10.1080/00387019208021522

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

SPECTROSCOPY LETTERS, 25(4), 477-485 (1992)

ADDUCT IONS FORMED FROM DNA UNDER PYROLYSIS ELECTRON IMPACT IONIZATION AND CHEMICAL IONIZATION USING NH_3.

K. Jankowski*

Département de Chimie, Université de Moncton, Moncton,
Nouveau-Brunswick, E1A 3W5, CANADA.

J-C. Tabet

Laboratoire de Chimie Organique Structurale,
Université Pierre et Marie Curie,
4, Place Jussieu 75252 Paris Cédex 02 FRANCE

H. Virelizier

C.E.N. de Saclay, Section d'études et d'analyses isotopique et nucléaire,
91191 Gif-sur-Yvette, FRANCE

Downloaded At: 03:54 30 January 2011

Abstract

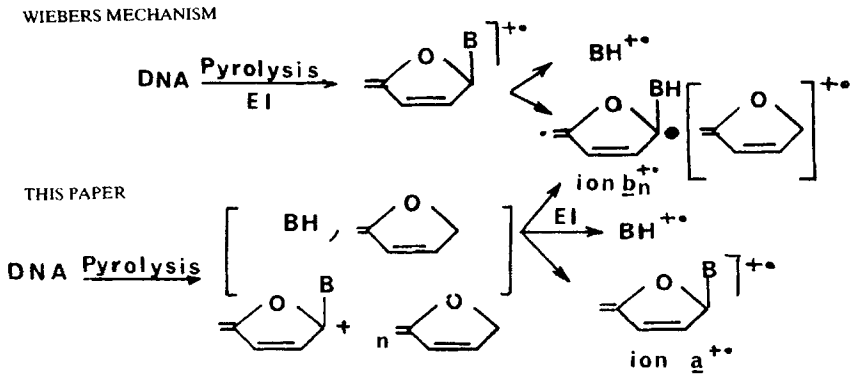
The formation of $(BH+n80)^{+\cdot}$ ions in the EI spectra of nucleic acid was investigated. The relationship between the base ions and these cluster ions as well as the nature of bonding is discussed.

INTRODUCTION

In our previous mass spectrometric studies of intact nucleic acids, particularly of several DNAs, we have looked into pyrolysis electron impact[1,2] (Pyr-EI data),ionization as well as softer ionization techniques (Pyr-DCI[3] data). In each case, DNA pyrolysis lead to various neutrals (cytosine, thymine, adenine or guanine) and corresponding to nucleoside-like fragments which contain a base B with C_5H_5O fragment (noted $[B\_C_5H_5O]$ according to the Wieber's mechanism, which are herein noted as a).

* To whom all inquiries should be addressed.

Copyright © 1992 by Marcel Dekker, Inc.



Scheme 1 *Possible processes leading to the complex* $\underline{b}_n^{+\cdot}$ *ion (where B = A, T, G on C and unresolved attachment site).*

Table 1. *Ions (m/z) observed in the pyrolysis EI mass spectra of DNAs (in paranthese, the corresponding ions in CI-*NH_4^+ *mass spectra).*

Bases	Cytosine	Thymine	Adenine	Guanine
$BH^{+\cdot}$ (BH_2^+)	111 (112)	126 (127)	135 (136)	151*
$\underline{a}^{+\cdot}$ ($\underline{a}H^+$)	191 (192)	206* (207)	215 (216)	231 (312)
$\underline{b}_1^{+\cdot}$ ($\underline{b}_1H^+$)	271 (272)	286* (287)	295 (296)	311 (312)
$\underline{b}_2^{+\cdot}$ ($\underline{b}_2H^+$)	351* (352)	366* (367)	375* (376)	391* (392)

* Ionic species characterized by low abundances.

These neutral species ionized in EI mode, yield odd-electron species such as $BH^{+\cdot}$ and adduct $[B\_C_5H_5O]^{+\cdot}$ ions (noted as $\underline{a}^{+\cdot}$, Scheme 1). Furthermore, cluster $\underline{b}_n^{+\cdot}$ ions of structure $[B\_C_5H_5O,nC_5H_4O]^{+\cdot}$ (with n = 1 or 2, Scheme 1) are easily observed in the pyrolysis EI mass spectra (Table 1, Figure 1a).

Under pyrolysis DCI in either positive or negative mode, even-electron ions such as : BH_2^+, $\underline{a}H^+$ (or B^-) and $[BH\_C_5H_5O]^+$, $\underline{b}H^+$ (or $[(B\text{-}H)\_C_5H_5O]^-$) and also protonated cluster $\underline{b}_nH^+$ are observed (Table 1 and Figure 1b).

The $\underline{b}_n^{+\cdot}$ (or $\underline{b}_nH^+$) species are thought to be formed during either the direct pyrolysis or from an ion-molecule reaction in the gas phase. No clear-cut information has been found in literature about the origin and the structure of the adduct ions $\underline{b}_n^{+\cdot}$ (or $\underline{b}_nH^+$). In fact, the molecular cohesion can be due to :

Downloaded At: 03:54 30 January 2011

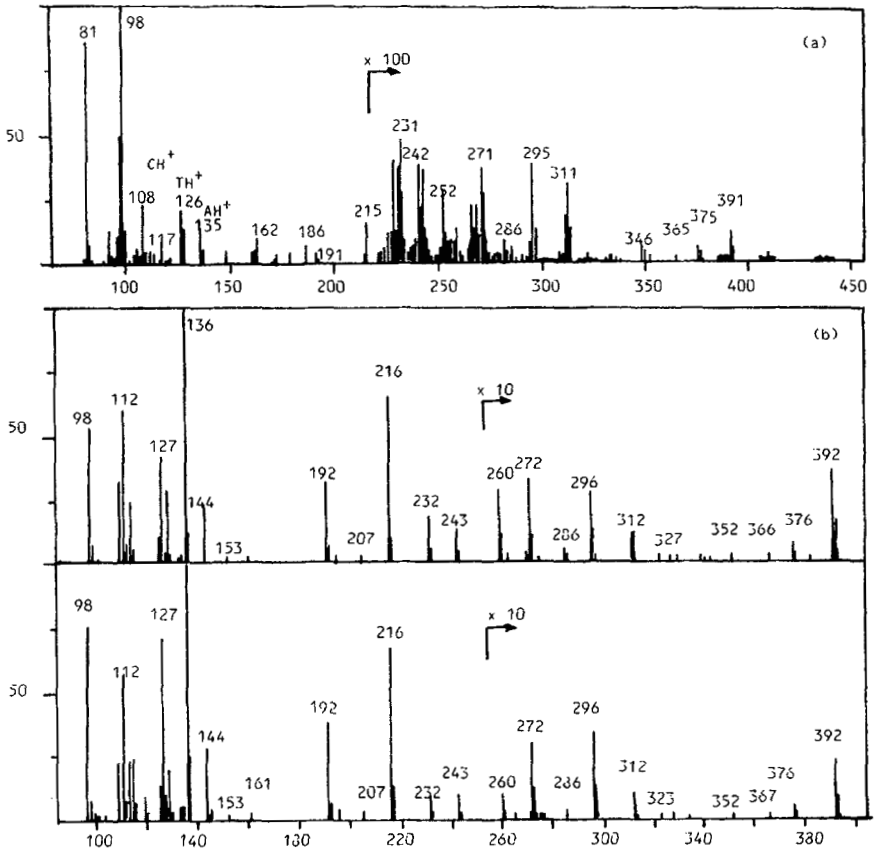


Figure 1. *Pyrolysis mass spectra of DNA under (a) electron impact and chemical ionisation conditions (b) with ammonia and (c) with 2-methylfuran.*

(i) hydrogen bonding between each unit such as observed under chemical ionization conditions for ionic reagent attachment ; or

(ii) C-C or C-O covalent bonds between the ionic species $\underline{a}^{+\cdot}$ and the 2-methylfuran neutral residues.

In order to answer these questions, we have investigated the behavior of DNA under various experimental conditions and the structure of the clusters $\underline{b}_1{}^{+\cdot}$ by analysis of their MIKE and CAD-MIKE spectra. Our approach is similar to that of Beynon[4] and Cooks[5].

Downloaded At: 03:54 30 January 2011

EXPERIMENTAL PART

The herring sperm DNA obtained from Boehringer Mannheim Co., was cut up into 3 mm pieces and placed in the centre of the filament coil or directly disposed on the coil after being poured into bidistilled water. One mm of this DNA corresponds to a 0.5 A260 unit. All reagent gases used were obtained from Air Liquide (Alpha gaz, France) ; or Matheson Coleman Co. 2-Deoxyribose, 2-Deoxythymidine and 2-Deoxcytidine have been purchased from Aldrich Chemicals (Janssen, France). The "copolymer" DNA-2-Deoxyribose was prepared by mixing equal quantities of both products, dissolving in water and stirring overnight at 25°C, then lyophylising the mixture. 2-Methylfuran was purchased from Eastman Chemicals.

A Riber R-1010 quadrupole mass spectrometer (NERMAG, France), was used in both positive and negative ionization modes. The recording and calculation of spectra were done using a PDP 8/M computer coupled to the spectrometer. Typical analytical conditions were : source temperature 200-220°C, 5 mm filament (Tungsten wire 60 μm, 6-9 turns) heated from 300-1000°C in 40s (current programmed from 200-600mA, 10 mAs^{-1}). The average lifetime of the filaments under these conditions was 20 analyses. The initial pressure of reagent gas in all mixtures was 0.3 Torr and the electron energy was maintained at 100 eV for best sensitivity. According to our previous studies, the diagnostic mass range was fixed at 100-800 Da.

Precursor $M^{+\cdot}$ ions of selected fragment m^+ ions (generated in source and in the first FFR) are determined by accelerating voltage scanning[6a] in a reversed geometry mass spectrometer (ZAB 2F, VG Analytical).

The studied fragment in ions are selected at Vo = 2 KV (accelerating voltage) and fixing : Eo = 2 KeV (for the electric sector) and B on the m/z value of the selected ion. The precursor ions are obtained by accelerating voltage scanning (from 2 KV to 8 KV) by measurement of the V/Vo ratio, which defines the $M^{+\cdot}$ ---> m^+ transition occurring in the 1st FFR.

MIKE and CAD/MIKE spectra were performed by selecting the main beam within a resolution close to 3000. For 8 KeV collision range, He target gas was used at 7.10^{-6} torr (corresponding to a lowering of main beam of 30 %, approximately single collision conditions). The produced daughter ions in the second FFR are analyzed by electric sector scanning (MIKE mode) in weaker resolution ($\sim$ 200) due to the vibrational energy release during dissociation.

Downloaded At: 03:54 30 January 2011

RESULT and DISCUSSION

In contrast to the ions observed under DCI conditions, the Py-EI mass spectrum of DNA displays odd-electron ions species only. With ionic reagents such as CH_5^+, $tC_4H_9^+$ and NH_4^+, the peaks are shifted by one Dalton when compared to pyrolysis EI mass spectra (Table 1), which correspond to protonated molecules or adduct ions[3]. These results already suggest that neutrals formed during pyrolysis are protonated as expected, even by NH_4^+ since the proton affinity of the latter reagent is lower than those of bases BH and nucleoside-like fragment **a** neutral species[7].

Note that under pyrolysis-EI, several $\mathbf{a}^{+\cdot}$ and $\mathbf{b_n}^{+\cdot}$ ions are accompanied by protonated $\mathbf{a}H^+$ and $\mathbf{b_1}H^+$ species likely due to self-ionization mechanism (or ion-molecule reactions) during pyrolysis process. However, in DCI conditions, the obtained mass spectra are more sensitive.

(a) Origin of odd-electron cluster ions $\mathbf{b_n}^{+\cdot}$.

The search for the precursor ions of the observed protonated bases $BH^{+\cdot}$ and the corresponding odd-electron ions $\mathbf{a}^{+\cdot}$ and $\mathbf{b_n}^{+\cdot}$ produced under EI conditions can be approached by analyzing of the

respective accelerating voltage scan spectra of each ionic species provided that the corresponding metastable decomposition takes place in the 1st FFR.

This method is considered sufficiently sensitive for analytical purposes[6b]; however, the research of precursor ions of the more abundant $BH^{+\cdot}$ ions (i.e. m/z 126 and m/z 135) did not add any new information since no signal was displayed in these accelerating voltage scan spectra; actually, only artefacts peaks are observed (difficult to be rationalized) rather than veritable metastable decompositions yielding either m/z 126 or m/z 135 ions. These results confirm previous results obtained by using metastable mapping of the $BH^{+\cdot}$ ions[8]. Consequently, the diagnostic $BH^{+\cdot}$ ions are not generated by unimolecular decomposition of higher mass ions displayed in Pyrolysis-EI mass spectrum of DNA. Then, *a priori* we can consider that these $BH^{+\cdot}$ ions are produced eventually *via* either ion-molecule reactions or pyrolysis prior to the ionization process.

Alternatively, complementary information can be obtained from experiments performed using a high source pressure ($\sim 3.10^{-4}$ torr) in 2-methyl-furan as Cl-reagent gas to induce ion-molecule reactions during DNA pyrolysis. Under these experimental conditions, the fragment $C_5H_5O^+$ ion is mostly observed relative to the protonated $C_5H_7O^+$ form [with $[C_5H_5O^+]/[C_5H_7O^+] \# 5$]. The structure of the reagent $C_5H_5O^+$ ion is very similar to that of the $[MH\text{-}H_2O]^+$ ion generated from hydroxy 2-methylfuran as shown by CAD spectrum analysis but it differs from the structure of 3-hydroxymethylfuran (results not reported herein).

If it is assumed that the $C_5H_5O^+$ ion structure is similar to that of the C_5H_5O moiety contained in the $\underline{a}^{+\cdot}$ and $\underline{b}_n^{+\cdot}$ ions, it is judicious to expect either a $C_5H_5O^+$ ion solvation by neutrals (e.g. BH) formed during DNA pyrolysis (Equation 1):

$$(1) \qquad C_5H_5O^+ + BH \text{ ---------> } [BH\_C_5H_5O]^+$$

or proton transfer from the protonated methyl furan to the bases (Equation 2) since PA(Bases) are higher than the PA(2-methylfuran)[7]:

$$(2) \qquad C_5H_7O^+ + BH \text{ --------> } BH_2^+ + C_5H_6O$$

Actually, mainly proton transfer occurs under these DCI-conditions (Figure 1c) such as those observed under DCI-NH_3. In addition, the solvated $\underline{a}H^+$ species are not enhanced relatively to the cluster $\underline{b}_nH^+$ ions. Since no significant differences are found between both DCI-mass spectra, it is likely that the $\underline{b}_n^{+\cdot}$ (or $\underline{b}_nH^+$) and the $\underline{a}^{+\cdot}$ (or $\underline{a}H^+$) are produced by EI (or DCI-NH_3) after the formation of neutrals like $\underline{a}$ or $\underline{b}_n$ produced by pyrolysis rather than by an ion-molecule process in the gas phase between BH and ionized $C_5H_5O^+$ form or the reverse reaction.

Downloaded At: 03:54 30 January 2011

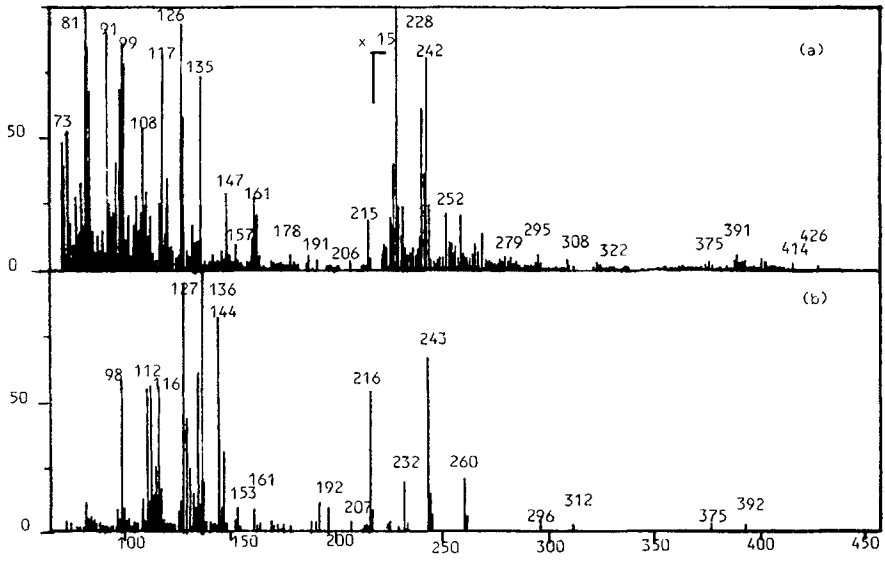


Figure 2. *Pyrolysis mass spectra of Copolymer-DNA under (a) electron impact and (b) chemical ionisation with ammonia.*

In order to verify this assomption, the behavior of the "copolymer" of 2-deoxyribose and DNA (see Experimental Part) was investigated under EI and DCI conditions (Figure 2a and 2b). Under pyrolysis EI, the mass spectra (Figure 2a) display peaks at m/z 73, m/z 81 and m/z 99 (duc to 2-deoxyribose) in large abundances. They are accompanied by signals diagnostic of BH of DNA. However, unfortunately the $\underline{a}^{+\cdot}$ and $\underline{b}_n{}^{+\cdot}$ are not enhanced, and only a few of additional signals (relative to those reported in figure 1a) are absent but they are not significant.

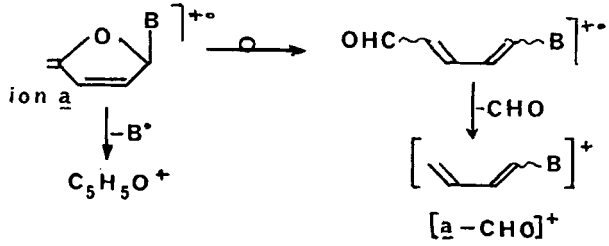
Alternatively, under pyrolysis-DCI-NH_3 (Figure 2a) conditions, DNA-copolymer yields similar diagnostic ions than those observed from DNA pyrolysis-DCI (Figure 1a), however, with a large variation in ion abundances. Other signals can be observed at m/z 116 and m/z 134 (Figure 2a). These ions are likely formed from 2-deoxyribose and correspond to $[M+NH_4\text{-}H_2O]^+$ (m/z 134) and $[M+NH_4\text{-}2H_2O]^+$ (m/z 116), respectively.

The presence of neutrals generated from copolymer did not enhance the abundances of the $\underline{b}_n\mathbf{H}^+$ (or $\underline{b}_n{}^{+\cdot}$) ions for DNA. Then, the eventual ion-molecule reactions yielding the latters ionic species must be ruled out. This also indicates that the yield of the $\underline{b}_n{}^{+\cdot}$ (or $\mathbf{b}_n\mathbf{H}^+$) ions depends directly upon the proximity of the neighboring heterocycle in the DNA sequence, and that the presence of copolymer has not a significant effect on the ion intensities. This can be explained by the long distance between the base units of

Downloaded At: 03:54 30 January 2011

Table 2. *Metastable daughter ions (m/z with relation abundance in parentheses) displayed in the MIKE spectra of different diagnostic ions (a$^{+\cdot}$ and b$_1^{+\cdot}$ odd-electron species) of DNA formed under pyrolysis-EI conditions.*

Base Units	Selected a$^{+\cdot}$ species: Precursor ions	Daughter ions -29	Daughter ions -(B)	Selected b$_1^{+\cdot}$ species: Precursor ions	Daughter ions -29	Daughter ions -81	Daughter ions -(a-H)
C	191	162(100)	81(35)	271	242(100)	190(100)	81(21)
T	206	177(100)	81(50)	286	257(100)	205(31)	81(10)
A	215	186(100)	81(55)	295	266(100)	214(35)	81(15)
G	231	202(100)	81(30)	311	282(100)	280(22)	81(8)



Scheme 2. *Formation of the fragment [a-29]$^+$ ion via assumed molecular a$^{+\cdot}$ ion isomerization.*

DNA and the deoxyribose units of copolymer, which can not enhance the yield of $b_nH^{+\cdot}$ (or b_nH^+) species.

(b) Presence of covalent bond leading to the ionic cohesion of a$^{+\cdot}$ species and cluster b$_1^{+\cdot}$ ions formed under Pyrolysis-EI conditions.

For this purpose, a better adapted approach to investigate the structure of DNA diagnostic ions is used: MIKE and CAD/MIKE spectra. Table 2 shows that :

(i) the selected a$^{+\cdot}$ ions spontaneously decompose *via* competitive routes involving losses of 29 Da radical and neutral base B units. The former radicals losses lead to : m/z : 162, 177, 186 and 202 (respectively from ions containing: cytisine, thymine, adenine and guamine bases) and the latter eliminations yield daughter [a-B]$^+$ ions (i.e. m/z 81).

Concerning the 29 Da losses, two possible neutral structures can be involved : CHO· (from ribose moiety) and CH_2=NH (from base unit). However, the neutral structure as CHO· radical is the most probable since, the abundance of these major losses are not dependant on the base structure. Furthermore, from the thymine base it is difficult ot eliminate CH_2=NH fragment, since its structure is not adapted to

Downloaded At: 03:54 30 January 2011

eliminate such neutral. The direct loss of the assumed CHO· radical likely needs an isomerization of the $\underline{a}^{+\cdot}$ ions into an open ring containing unsaturations (Scheme 2). The investigation of this possible isomerization is in progress, using labelled nucleosides.

(ii) the selected cluster $\underline{b}_1^{+\cdot}$ ions competitively lead to the daughter $[\underline{b}_1\text{-CHO}]^+$ and complementary $[\underline{b}_1\text{-}(\underline{a}\text{-H})]^+$ (m/z 81) and $[\underline{a}\text{-H}]^+$ (i.e. at m/z 190, 205, 214 and 230 according to the base unit structure).

The occurrence of the CHO· elimination as the major process rather a favourable cluster $\underline{b}_1^{+\cdot}$ separation into the $[\underline{a}\text{ -H}]^-$ and $[\underline{b}_1\text{ - }(\underline{a}\text{-H})]^+$ complementary ions indicates that only covalent bond links each unit of the $\underline{b}_n^{+\cdot}$ clusters. Indeed, if the cluster structure corresponds to that of an ion-dipole complex where electrostatic attraction gives cohesion between C_5H_5O units, then the $\underline{b}_n^{+\cdot}$ cluster decompositions should yield only fragment $\underline{a}^{+\cdot}$ or $[\underline{b}_1\text{-}\underline{a}]^{+\cdot}$ ions and not the loss of CHO·. This assomption must be ruled out since the ion productions are not consistant with the experimental results.

Under collision conditions, additionnal decompositions are displayed in CAD spectra of selected $\underline{a}^{+\cdot}$ and $\underline{b}_1^{+\cdot}$ ions. However, the additionnal decompositions of $\underline{b}_1^{+\cdot}$ (not reported herein) give no more information about linkage between the first and second C_5H_5O units and the relation between $\underline{a}^{+\cdot}$ and $\underline{b}^{+\cdot}$, since the loss of 80 Da from $\underline{b}_1^{+\cdot}$ leading to $\underline{a}^{+\cdot}$ ionic species is not observed. Furthermore, the major decomposition proceeds by loss of one CHO· radical yielding $[\underline{b}_1\text{-CHO}]^{+\cdot}$. This latter finding confirms that a covalent bonding characterizes the cohesion of different component parts of $\underline{b}_1^{+\cdot}$ rather hydrogen bonding (or electrostatic interaction) between both moieties of $\underline{b}_1^{+\cdot}$.

Note that in view of these experimental results, it is not possible to determine which position (i.e. on one C_5H_5O residue or on the base B unit) is involved in the linkage of both 2-methyl furan residues in the $\underline{b}_1^{+\cdot}$ ion structure. However, comparison of CAD/MIKE spectra of the ions $\underline{a}^{+\cdot}$ generated from (i) pyrolysis of DNA and (ii) *via* the consecutive losses of $3H_2O$ from the corresponding deoxynucleosides show that all the selected $\underline{a}^{+\cdot}$ ions are characterized by common structures. This result confirms that the pyrolysis of DNA leads directly to the $\underline{a}$ neutrals without structural modification and possibly gives rise to formation of the $\underline{b}_n$ neutrals.

The results reported in this paper show that the whole set of fragments characterizing the DNA spectra obtained by pyrolytic mass spectral techniques is essentially independant of the ionization mode used (EI or DCI). The base ions and the nucleoside-like ions are not related by the fragmentation process. The former ions result rather from the condensation of the 2-methyl furan unit with the base ions. In the era of more audacious application of reagent gases (for both CI or CAD purposes) the use of 2-methylfuran is completely justified. This highly volatile material has a capacity for polymerisation which, in principle, simulates the formation of the clusters $\underline{b}_1$ and $\underline{b}_2$. Copyrolysis of the DNA enriched in other potential source of the 2-methylfuran (C_5H_5O) unit (i.e. the 2-deoxyribose, 2-deoxythymidine or 2-deoxycytidine) does not show any possibility of ion-molecule reaction in the gas phase. Finally, the proposed series of

Downloaded At: 03:54 30 January 2011

pyrolysis experiments in the range of conditions described previously indicate that the only plausible bonding between the base and the 2-methylfuran fragment is covalent by nature.

This bonding must take place *via* several atoms :

(i) Sugar-base-sugar with two equivalent C-N bonds ; and

(ii) Sugar-sugar-base within the second family. Here we propose bonding between C_5'-C_1', C_5'-C_5 or C_1-C_1' positions (rather than 2', 3' and 4' carbons). For the moment, our preference goes to the (ii) family. However, because of the inaccessibility of simple diribonucleosides, more precise determination of this bond nature is impossible at present.

ACKNOWLEDGEMENTS. *We would like to thank, the NSERC of Canada as well as the Université de Moncton Research Council for research grants.*

REFERENCES

1- K. Jankowski, J.R.J. Paré and R.H. Wightman, Adv. Heter. Chem. 39, 79-116 (1986).

2- N. Turkkan, F. Söler and K. Jankowski, Biomed. Mass Spectrom. 9, 91-93 (1982).

3- H. Virelizier, R. Hagemann and K. Jankowski, Biomed. Mass Spectrom., 10, 559-566 (1983).

4- M.H. Bozorgzadeh, J.H. Beynon and J.L. Wiebers, Adv. Mass Spectrom., 8, 971-972 (1980).

5- A.E. Schoen, R.G. Cooks and J.L. Wiebers, Science, 203, 1249 (1979).

6- (a) J. Catalan, R.M. Claramunt, J. Elguero, J. Laynez, M. Menendez, F. Anvia, J.H. Quin, M. Tagepera, R.W. Taft, J. Amer. Chem. Soc., 110, 4105 (1988); (b) S.G. Lias, J.E. Bartmess, J.F. Liebman, J.L. Holmes, R.D. Levin, W.G. Maillard, J. Phys. Chem. Ref Data 17, Suppl. 1 (1988); (c) M. Jarman, J. Analyt. Appl. Pyrolysis, 2, 217 (1980); (d) for the review see P.F. Crain, Mass Spectrom. Rev., 9, 505 (1991).

7- (a) D. Gaudin, H. Virelizier and K. Jankowski, Spectroscopy Letters, 18, 243-9 (1988); (b) K. Jankowski, H. Virelizier, D. Gaudin and R. Hagemann, J. Pharm. Biomed. Anal., 8(1), 31-40 (1990); (c) D. Gaudin and K. Jankowski, Org. Mass Spec., 15, 78-9 (1980).

Date Received: 11/14/91
Date Accepted: 12/20/91

Downloaded At: 03:54 30 January 2011