The fragmentation pathway of the nucleosides under the electrospray ionization multi-stage mass spectrometry☆

Ruoyu Liu[1,3], Yong Ye[1,2,*], Liming Qiang[1], Xincheng Liao[1], Yufen Zhao[1,2,*]

[1]*Key Laboratory of Chemical Biology & Organic Chemistry*, *Department of Chemistry*, *Zhengzhou University*, *Zhengzhou* 450052, *China*; [2]*Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology*, *Department of Chemistry*, *Tsinghua University*, *Beijing* 100084, *China*; [3]*Chemistry & Pharmaceutical Engineering College*, *Nanyang Normal University*, *Nanyang* 473061, *China*

Received March 7, 2008

Abstract

The fragmentation pathway of the four kinds of nucleosides, adenosine, guanosine, cytidine and uridine were investigated by electrospray ionization multi-stage mass spectrometry (ESI-MS^n). Typical neutral losses correspond to the molecules CO, NH_3, CH_2CH_2, $NHCH_2$, NHCO and NH_2CN. The cleavage of the glycosidic C – N bond is characteristic for all the four compounds. The major fragmentation pathways are the ring contraction (RC) and retro-Diels-Alder (RDA). [Life Science Journal. 2008; 5(2): 37 – 40] (ISSN: 1097 – 8135).

Keywords: fragmentation pathway; ESI-MS^n; nucleosides; ring contraction; retro-Diels-Alder

1 Introduction

People have always made great efforts to discover all kinds of new drugs in order to control AIDS for a long time. Among the various diversity of these compounds, nucleosids has been used as prodrugs mainly. Much work has been done in structure modification of nucleosides to solving some problems of the nucleosides as it being anti-HIV agent[1–5], such as: toxic, side effects and drug resistance and so on. Since the nucleosides and its derivatives can be potential candidates as anti-HIV drugs, it is important to study their mass spectral fragmentation pathway. In our work, the four kinds of nucleosides: adenosine, guanosine, cytidine and uridine were analyzed by electrospray ionization multistage tandem mass spectrometry (ESI-MS^n) and the fragmentation pathway was summarized.

The ring contraction (RC) reactions of nucleosides under electrospray ionization conditions was the first reported. It should be very useful to elucidate the fragmentation of some novel nucleosides analoges and to identify the structures of them. Recently, several RC reactions under electrospray ionization conditions have been reported in the literature: e.g. some of the beta-(*N*-alkyl/arylamino)-alpha, beta-unsaturated carboxylates and alpha-(1-alkyl/arylaminoethylidene)-gamma-lactones could undergo a four-membered RC rearrangement by lose a carbon dioxide[6]. 1-[*N*-benzyloxycai-bonyl(Cbz)aminolarylmethylphosphonate monoesters underwent four-membered ring rearrangements to yield mainly nitrogen-containing fragment ions by loss of a carbon dioxide, phosphite, carbon dioxide plus phosphite, or benzyloxycarbonylphosphonate monoester[7]. Indole derivatives underwent a contraction of the six-membered ring[8]. 2,3-dihydro-4H-1,3-oxazin-4-ones underwent a novel RC reaction by loss of isocynates[9]. In the present paper, a novel RC reaction under the ESI conditions for the four compounds was found and described.

Besides the RC reaction fragmentation pathway, other reactions, such as retro-Diels-Alder (RDA) fragmentation and electrocyclic reaction (ER) under electrospray ionization conditions for the four nucleosides were also found. From this way, most RDA products can be thought as RC products. The mechanism of the ER was proposed. That

☆Supported by NNSFC (No. 20732004 and No. 20602032).
*Corresponding author. Tel: 86-371-67767050; Email: yeyong@zzu.edu.cn, yfzhao@xmu.edu.cn

means ring structure can tautomerize to a chain structure. Due to the ring strain, the ions exited mainly in chain form.

2 Materials and Methods

ESI mass spectra was acquired on a Bruker ESQUIRE-LC™ ESI ion trap spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to m/z 3000. The samples were dissolved in methanol at a concentration of 0.01 mg/ml. Samples were continuously pumped into the ESI chamber at a flow rate of 4 μl/min. The nebulizer gas was delivered at a flow pressure of 10 psi. The source temperature was maintained at 300 °C. About 5 spectrums were averaged. Adenosine, cytidine, guanosine and uridine were purchased from Baitai Co. Ltd.

3 Results

The major fragment ions of the four nucleotides are listed in Table 1 and Table 2.

Here guanosine is selected as an example to describe the detailed fragmentation mechanisms of all the four nucleotides. All of the fragment ions produced from guanosine could be rationalized by the fragmentation paths indicated in Figures 1 and 2.

Fragment ions m/z 282, m/z 284 correspond to the deprotonated/protonated molecular ions of guanosine, and ions m/z 150, m/z 152 are deprotonated and protonated of guanine. The fragmentation pathways of the guanosine are shown in Figures 1 and 2.

Protonated guanosine dissociated through decompositions of base-protonated $[B+H]^+$ ions by the cleavage of the glycosidic bonds to give the protonated bases with a sugar moiety as the neutral fragment. In the negative ion mode, it can be found that cleavage of the glycosidic bonds with charge retention on the sugar moiety eliminates the base moiety as a neutral molecule and produces sugar ions. However, not only in the positive mode, but also in the negative mode, base-protonated/deprotonated were the base peaks.

The main fragmentation pathway for the guanosine

Table 1. Positive ion tandem mass spectra of adenosine, cytidine, guanosine and uridine

Commpond	Precursor ion	Fragment ion and relative intensity percentage (%)				
adenosine	268	268(3)	136(100)			
	136	136(100)	119(61)	94(39)		
guanosine	284	284(5)	152(100)			
	152	152(100)	135(76)	110(23)	109(7)	
cytidine	244	244(29)	112(100)			
	112	112(62)	95(100)	69(29)		
uridine	245	245(23)	203(8)	185(9)	133(3)	113(100)

Table 2. Negative ion tandem mass spectra of adenosine, cytidine, guanosine and uridine

Commpond	Precursor ion	Fragment ion and relative intensity percentage (%)						
adenosine	266	266(12)	134(100)					
	134	134(100)	107(16)	92(29)				
guanosine	282	282(10)	150(100)	133(5)				
	150	150(12)	133(100)	107(8)				
	133	133(100)	105(21)					
cytidine	242	242(4)	199(5)	152(15)	126(4)	110(54)	109(100)	91(19)
	152	152(5)	124(16)	109(100)	81(4)			
	199	109(74)	91(100)					
	109	109(4)	91(100)	92(11)	81(13)	43(4)		
uridine	243	243(35)	200(100)	153(8)	152(7)	140(4)	110(20)	
	200	200(10)	152(12)	140(38)	110(100)	82(3)		
	153	153(12)	125(23)	110(100)	82(3)			
	152	124(100)	96(10)	68(10)				
	140	140(100)	122(92)	82(23)				
	110	110(21)	92(4)	82(100)	66(30)			

in the negative ion mode is the RC reaction. By this way, guanine gave rise to ions m/z 107, m/z 105 by loss smalle molecules NHCO, CO, respectively. The ion m/z 133 was generated from the ion m/z 150 by loss of NH_3. Its details were shown in Figure 3.

Molecular ion m/z 284 was protonated guanosine, and it gave rise to only one fragment ion m/z 152 in positive ion mode, and the later produced ions m/z 135, 110, 109 by loss of neutral molecules water, $NCNH_2$ and NHCHOH respectively. It occurred in the RDA and RC reaction way. Here we proposed a mechanism to elucidated the structure of the ion m/z 110. It was likely to exist in a dynamic equilibrium of the ring form and the open-chain form, and the equilibrium should predominately shift to the open-chain form because of the higher internal energy of the ring form. This phenomenone is called ER. From this way, most RDA products can be thought as RC products. The mechanism of the ER is shown in Figure 3.

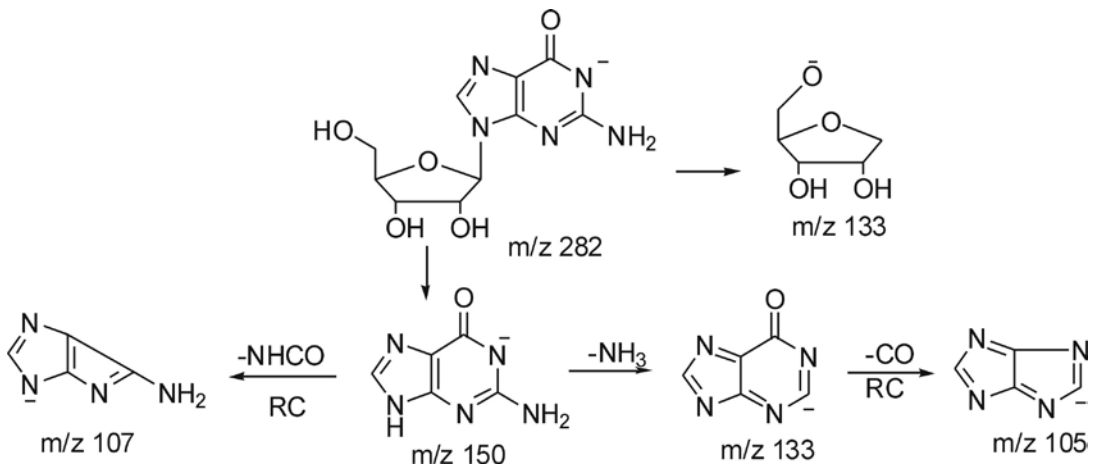

Figure 1. Fragmentation pathways of guanosine in negative ion mode.

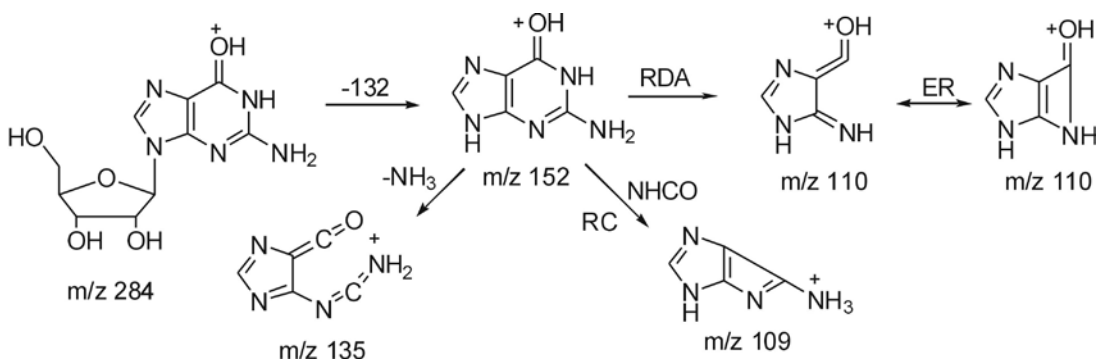

Figure 2. Fragmentation pathways of guanosine in positive ion mode.

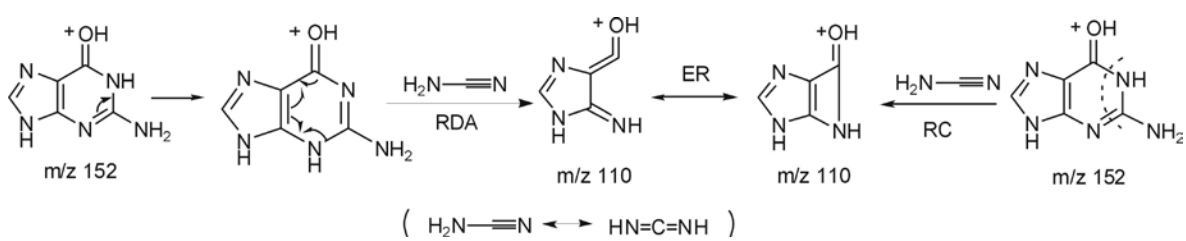

Figure 3. The mechanism of the electrocyclic reaction.

4 Conclusion

The electrospray ionization mass spectral behavior of the four kinds of nucleosides have been studied and the fragmentation pathways proposed have been supported by ESI-MS^n spectra. It has been discovered that all of the four nucleosides showed similar fragmentation pathways, that is the RDA and RC reaction. Typical neutral losses corresponding to the molecules NHCO, CO, CH_2CH_2, $NHCH_2$ were resulting from RC fragmentation pathways. Other neutral losses: NHCO, $NCNH_2$ etc. originated from the RDA fragmentation. The mechanism of the ER was proposed. From this way, most RDA products can be thought as RC products. That means ring structure can tautomerize to a chain structure.

References

1. Ueno T, Shirasaka T, Mitsuya H. Enzymatic characterization of human immunodeficiency virus type 1 reverse transcriptase resistant to multiple 2',3'-dideoxynucleoside 5'-triphosphates. The Journal of Biological Chemistry 1995; 270(40): 23605 – 11.
2. Cyrille B, Valerie ED, Naheed M, *et al*. Design, synthesis and evaluation of peptide libraries as potential anti-HIV compounds via inhibition of gp120/cell membrane interactions using the gp120/CD4/Fab17 crystal structure. European Journal of Medicinal Chemistry 2002; 37(11): 883 – 90.
3. Patrick V, Thierry C, Christophe P, *et al*. Synthesis of new covalently bound-carrageenan-AZT conjugates with improved anti-HIV activities. Journal of Medicinal Chemistry 2002; 45(6): 1275 – 83.
4. Wong A, Toth I. Lipid, sugar and liposaccharide based delivery systems. Current Medicinal Chemistry 2001; 8(9): 1123 – 36.
5. Xiao Q, Ju Y, Yang XP, *et al*. Electrospray ionization mass spectrometry of AZT H-phosphonates conjugated with steroids. Rapid Commun Mass Spectrom 2003; 17: 1405 – 10.
6. Yin W, Ma Y, and Zhao YF. The fragmentation mechanism of b-(N-alkyl/arylamino)-a,b-unsaturated carboxylates under electrospray ionization conditions. Amino Acids 2006; 31: 333 – 6.
7. Ma Y, Liu W, Zhao YF. Intramolecular four-membered ring rearrangements of 1-(N-benzyloxycarbonylamino)arylmethyl phosphonate monoesters under electrospray ionization conditions. International Journal of Mass Spectrometry 2006; 248 (1 – 2): 17 – 20.
8. Cao SX, Liu HM, Xu W, *et al*. Characterizing the electrospray ionization mass spectral fragmentation pattern of indole derivatives synthesized from 2-keto glycosides. Journal of Mass Spectrometry 2005; 40(4): 452 – 7.
9. Ma Y, Liu W, Chen Y, Zhao YF. Ring contraction reactions of 2,3-dihydro-4H-1,3-oxazin-4-ones under electrospray ionization conditions. Rapid Commun Mass Spectrom 2004; 18: 1116 – 22.