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场 解 吸 质 谱

I. 环化核苷酸类

汪聪慧　王耀新　曹开星
(燕京科学技术服务公司,北京)

张礼和　马灵台　王 序
(北京医学院药学系)

本文报道了十九个环化核苷酸及其衍生物的场解吸质谱，并对它们的谱图特点、裂解方式等作了一些探讨.

在核酸领域中应用质谱技术已有近二十年历史. 最初使用电子轰击离子化(EI)，在核苷分析上获得成功. 核苷酸因含有难挥发的磷酸单元，EI结果不满意. 为了增加样品的挥发性和获得分子离子峰，将样品三甲基硅烷化(TMS)、甲基化、酰化或酯化，其中以TMS最为有效. 但每增加一个TMS使分子量增大72，且衍生化后M^+峰的相对强度仍较低，有的甚至检测不出. Beckey[1]把场解吸(FD)电离技术应用于难挥发、热不稳定的有机化合物，为核苷酸领域的质谱分析开创了新局面. 目前，核苷[2]、核苷酸[3]、二核苷酸[4,5]、三核苷酸[6]的FD研究已有报道，甚至可用此对低聚核苷酸进行序列分析[3]. 但是，迄今为止有关环化核苷酸的FD还未见报道. 本文报道环化核苷酸及其衍生物(包括五价和三价磷)的FD质谱.

实　　验

FD数据用MAT-731型质谱计的EI/FD复合源获得. 测量条件为：分辨率1000(10%谷)，加速电压 +8kV，引出极电压 −3kV. 源温 <100°C，发射丝的最佳阳极温度(BAT)值在表中注明. 用微量注射器加样. 发射丝为直径10μ的钨丝，采用高温活化法[7]在钨丝上生长20～30μ长微针.

实验用样品为：腺嘌呤核苷-3′, 5′-环磷酸 (adenosine-3′, 5′-cyclic phosphate, *cy*-AMP, **1**, MW 329)[8]、胞嘧啶核苷-3′, 5′-环磷酸(cytidine 3′, 5′-cyclic phosphate, *cy*-CMP, **2**, MW 305)[8]、脲嘧啶核苷-3′, 5′-环磷酸 (uridine 3′, 5′-cyclic phosphate, *cy*-UMP, **3**, MW 306)[8]、腺嘌呤核苷-3′, 5′-环磷酰-*N*-环己胺 (*e*)** [adenosine 3′, 5′-cyclic-*N*-cyclohexyl phosphoramidate (*e*), **4**, MW 410][9]、腺嘌呤核苷-3′, 5′-环

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* 通讯联系人.

** *e*为横键.

磷酰-*N*-正戊胺 (*e*) [adenosine 3′, 5′-*N*-*n*-amyl phosphoramidate, **5**, MW 398][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸 (2′-*O*-tosyl adenosine 3′, 5′-cyclic phosphate, **6**, MW 483)[10]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酰-*N*-环己胺 (*e*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic-*N*-cyclohexyl phosphoramidate (*e*), **7**, MW 564][10]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酰-*N*-*n*-戊胺 (*e*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic-*N*-*n*-amyl phosphoramidate (*e*), **8**, MW 552][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酰-*N*-苯胺 (*e*) (2′-*O*-tosyl adenosine 3′, 5′-cyclic-*N*-phenyl phosphoramidate (*e*), **9**, MW 558][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸丁酯 (*e*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic-*n*-butyl phosphate (*e*), **10**, MW 539][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸丁酯 (*a*)*[2′-*O*-tosyl adenosine 3′, 5′-cyclic-*n*-butyl phosphate (*a*), **11**, MW 539][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸苄酯 (*e*) (2′-*O*-tosyl adenosine 3′, 5′-cyclic benzyl phosphate (*e*), **12**, MW 573][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸苄酯 (*a*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic benzyl phosphate (*a*), **13**, MW 573][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸乙酯 (*e*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic ethyl phosphate (*e*), **14**, MW 511][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环磷酸乙酯 (*a*) [2′-*O*-tosyl adenosine 3′, 5′-cyclic ethyl phosphate (*a*), **15**, MW 511][9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环亚磷酸 (2′-*O*-tosyl adenosine 3′, 5′-cyclic phosphite, **16**, MW 467)[10]、9-*β*-腺嘌呤阿拉伯糖苷-3′, 5′-环亚磷酸 (9-*β*-*D*-adenine arabinofuranosyl 3′, 5′-cyclic phosphite, **17**, MW 313)[10]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环亚磷酸乙硫酯 (2′-*O*-tosyl adenosine 3′, 5′-cyclic thioethylphosphite, **18**, MW 511)[9]、2′-*O*-对甲苯磺酰基腺嘌呤核苷-3′, 5′-环亚磷酰-*N*′, *N*′-二乙胺 (2′-*O*-tosyl adenosine 3′, 5′-cyclic-*N*′, *N*′-diethyl hosphoramidite, **19**, MW 522)[9]。

上述样品经熔点、元素分析、薄层层析、电泳、红外光谱及 ^{31}P 核磁共振谱分析符合要求.

结果与讨论

环化核苷酸类由于热不稳定和不易挥发,很难不经衍生化而直接进行 EI 分析. 目前仅报道了 *cy*-AMP(**1**) TMS 衍生物的 EI 谱. 看来, EI 法对这类化合物是不适宜. 使用 FD 技术则成功地得到上述大部分化合物的质子化分子峰. 表 1 为样品 **1**, **2**, **3** 的 FD 数据. 环化核苷酸的 FD 谱无 M^+ 峰而只有 (M^++H) 峰, 这种特点与核苷酸是相同的.

当制成各种衍生物后情况有所不同, 如环化腺嘌呤核苷酸制成各种酰胺衍生物 (表 2 样品 **4**, **5**) 后谱中除 (M^++H) 峰外, 还呈现 M^+ 峰, 说明环磷酸的羟基被其它基团取代时有利于 M^+ 峰的形成. 环化核苷酸糖元上 2′ 位的羟基氢为对甲苯磺酰取代时也出现 M^+ 峰, 并伴有 (M^++H) 峰, 如样品 **6**. 环磷酸的羟基和糖元上 2′ 位的羟基氢同时被取代 (表 2 样品 **8**～**15**) 时, 除 **8** 外其它样品的 $M^+/(M^++H)$ 值都较大.

* *a* 为竖键.

表 1 某些环化核苷酸的 $(M^+ + H)$ 峰

[$(M^+ + H)$ peaks of some cyclic nucleotides]

样品 (Sample)	m/z	离子类型 (Ionic form)	BAT (mA)
1	330	*cy*−AMP+H	16
2	306	*cy*−CMP+H	19
3	307	*cy*−UMP+H	16

表 2 列出的样品 **10～15** 为乙酯、丁酯、苄酯三对横、竖键立体异构体. 它们的 FD 谱没有明显差异, 只是前者的 BAT 值均高于后者, 这可能与它们的热稳定性不同有关. 表 2 所列衍生物的 M^+ 峰或 $(M^+ + H)$ 峰均属基峰, 因此, 在确定它们的分子量时显然比 EI 优越.

表 2 某些腺嘌呤核苷环磷酸衍生物的 FD 数据

(FD data of some derivatives of *cy*-AMP)

样品 (Sample)	M^+ 丰度 (Abundance of M^+) (%)	$(M^+ + H)$ 丰度 [Abundance of $(M^+ + H)$] (%)	BAT (mA)	$M^+/(M^+ + H)$
4	35	100	20	0.35
5	18	100	21	0.18
6	100	35	19	2.85
8	5	100	19	0.05
9	100	50	20	2.00
10	86	100	20	0.86
11	100	95	19	1.05
12	40	100	20	0.40
13	65	100	17	0.65
14	100	40	20	2.50
15	100	80	18	1.25

环化核苷酸本身除质子化分子峰外的碎片峰均极弱. 当制成各种衍生物后碎片峰明显增强. 从表 3 可见, 样品 **11** FD 谱中的 m/z 135 为 (B+H) 峰, 172 为 (T+H) 峰*. 483 为 $(M^+ + H)$ 离子失 C_4H_9. 404 和 136 似乎为 $(M^+ + H)$ 离子失 $C_4H_9OPO_2$ 时分别形成的峰. 实际上表 2 所列的对甲苯磺酰基衍生物均具有 m/z 135 和 172 峰, 它们分别属于腺嘌呤碱基和对甲苯磺酸根结构, 这说明与糖元 1′ 位碳原子相连的 C—N 键及 2′ 位相连的 C—O 键容易断裂, 并通过氢重排形成上述特征峰. 样品 **7～15** 的主要裂解方式见图 1.

腺嘌呤核苷环亚磷酸的对甲苯磺酰衍生物的 FD 谱与相应的五价磷有明显差异. 表 3 样品 **16** 的 FD 数据表明, M^+ 峰或 $(M^+ + H)$ 峰均不是基峰, 强峰为 m/z 420 和 421, 它可能由 $(M^+ + H)$ 峰分别失 PO 和 POH 而来, 相应的五价磷(表 2 样品 **6**)并无该特征峰. 上述过程在三价磷的样品 **17**, **18**, **19** 中更明显 (见表 3). 这三个化合物均缺少 M^+ 峰或

* B 代表腺嘌呤碱基, T 代表对甲苯磺酸根.

表 3 某些腺嘌呤核苷环磷酸衍生物的 FD 全谱*

(FD spectra of some derivatives of *cy*-AMP)

样品 (Sample)	MS(*m/z*)					BAT (mA)
11	135(4)	136(8)	172(15)	173(1)	385(4)	18
	386(4)	404(4)	405(4)	482(12)	483(6)	
	484(8)	539(100)	540(95)	541(30)		
12	573(40)	574(100)	575(37)	576(8)		20
12	135(20)	136(1)	155(30)	156(4)	170(50)	23
	171(10)	172(80)	173(15)	483(100)	484(20)	
	573(10)	574(25)	575(7)			
16	135(28)	136(2)	172(20)	173(2)	420(80)	15
	421(100)	422(20)	467(38)	468(15)		
17	266(48)	267(100)	268(15)			14
18	135(8)	136(1)	172(5)	420(36)	421(100)	20
	422(24)					
19	113(4)	114(30)	115(16)	135(24)	136(10)	18
	172(30)	173(7)	391(6)	392(6)	420(60)	
	421(100)	422(20)				

* 括号内数值为相对强度.

$(M^+ + H)$峰. 样品 **19** 的 *m/z* 421, 420 为$(M^+ + H)$峰分别失 C_4H_9NP 和 $C_4H_{10}NP$; 样品 **18** 则为$(M^+ + H)$峰失 C_2H_4SP 和 C_2H_5SP. **17** 的 FD 谱中仅有的 *m/z* 267, 266 为 $(M^+ + H)$ 峰失 PO 和 POH 的碎片离子. 看来形成上述特征碎片是一个优势的过程, 它与三价磷化合物的化学活性有关. 样品 **16**, **18**, **19** 的主要裂解过程见图 2.

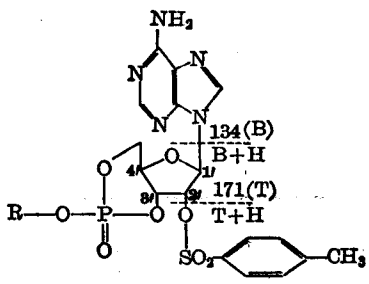

图 1 对甲苯磺酰基衍生物 P(V) 的主要裂解方式

(The main fragmentation mode of P(V) tosyl derivatives)

样品 **7** 是一种例外, FD 谱未得到 M^+ 或$(M^+ + H)$峰, 而显示$(M^+ - H_2O)$ 和 $(M^+ + H - H_2O)$ 碎片峰, 原因尚不清楚.

大部分样品的 BAT 值在 17～20 mA 之间, 少数情况下高于 20 mA. 这类化合物的热稳定性差, 过高的温度会引起样品的热解. 作者曾对样品 **12** 作过试验(结果见表 3), 过高的加热电流导致$(M^+ + H)$峰下降和热解产物离子的增加, 因而对分析不利, 故需严格控制加热电流.

与通常的有机化合物相比, 这类样品的 FD 灵敏度较低, 改用微量注射器代替常用的浸取法加样是适宜的, 它保证在钨丝的发射区域内有 1～2 μg 样品量供使用, 又避免了对离子源的不必要沾污. 当然在试验时适当地提高倍增器增益、降低滤波频率也有助于获得较佳结果.

作者曾对化合物 **1**～**19** 进行过 EI 测试, 仅有少数样品 (**4**, **5**, **10**, **11**, **14**, **15**) 获得

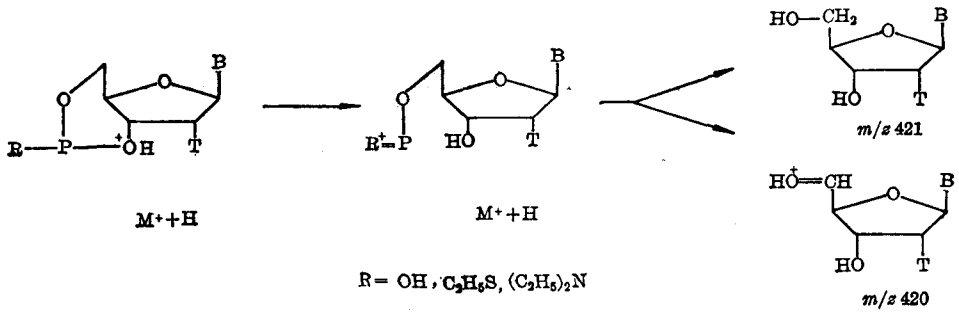

图 2　对甲苯磺酰基衍生物 P(III)的主要裂解过程

(The main fragmentation process of P(III) tosyl derivatives)

M^+ 峰，但相对强度一般低于 0.5%．由此可见 FD 技术提供环化核苷酸类分子量信息的优点是突出的，不足之处是碎片信息少．近年来发展的解吸化学离子化(DCI)、碰撞诱导离解(CID)、快原子轰击(FAB)等技术将给这类化合物的分析提供新的前景。

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FIELD DESORPTION MASS SPECTROMETRY

I. CYCLIC NUCLEOTIDES

WANG CONG-HUI WANG YAO-XIN CAO KAI-XING
(Yanjing Science and Technology Service Company, Beijing)
ZHANG LI-HE MA LING-TAI WANG XU
(Faculty of Pharmacy, Beijing Medical College)

ABSTRACT

In this paper, nineteen FD mass spectra of cyclic nucleotides are reported. The results indicated that only $(M^+ + H)$ peak presents in the spectra of *cy*-AMP, *cy*-CMP and *cy*-UMP. The derivatives of *cy*-AMP (except **7**) have M^+, $(M^+ + H)$ peaks, and the main fragments corresponding to (B+1), (T+1) peaks as shown in Fig. 1 in the Chinese Text. The adenosine 3′, 5′-cyclic phosphite and its derivatives with P(III) are somewhat different as compared with P(V). In some cases neither M^+ nor $(M^+ + H)$ is present in their spectra, but there are some characteristic peaks. Their fragmentation process has been shown in Fig. 2 in the Chinese Text.

Field desorption mass spectrometry

I. Cyclic nucleotides

WANG Cong-hui* WANG Yao-xin CAO Kai-xin

Yanjing Science and Technology Service Company, Beijing

ZHANG Li-ho MA Lin-tai WANG Xu

Faculty of Pharmacy, Beijing Medical College

Abstract The FD spectra of nineteen 3′, 5′-cyclic nucleotides and their derivatives are reported. The principal features of spectra, the fragmentation mode, and the experimental techniques are described.

Recently there has been considerable attention given to 3', 5'-cyclic nucleotides for their important biological and biochemical activities. With more and more researches done on these cyclic nucleotides, mass spectrometry has proved itself to be a highly useful technique for their structure determination both in synthetic works and in clinical analyses. MS technique has been applied to the field of nucleic acid since 1962. Biemann[1] first analyzed nucleosides with EI technique successfully, but the technique, when applied to nucleotides, is not satisfactory because of the high polarity of the phosphate components. If nucleotides are converted to their volatile derivatives by trimethylsilylation, methylation, acylation or esterification, the EI technique can be used and gives good results. The most satisfactory derivative for this technique is trimethylsilyl derivative.[2] But one trimethylsilyl group will increase the molecular weight by 72, which again creates other problems for the EI technique. Usually the peak of molecular ion (M^+) of such derivatives is very weak and sometimes even disappears. The technique of field desorption was applied to some compounds of high polarity and thermal instability by Beckey in 1969.[3] Ever since FD technique was reported, a series of FDMS of nucleosides,[4] nucleotides,[5] dinucleotides, trinucleotides[6,7,8] and determination of base sequence in oligonucleotides have been published.[5] But we have not yet found any information on the FDMS of cyclic nucleotides. In this paper, we report the FD spectra of nineteen cyclic nucleotides (including P^{5+} and P^{3+}). The spectral features, fragmentation mode and experimental techniques are also described.

Experimental

All FD spectra and data were obtained with a Varian MAT-731 with EI/FD combined source. The experimental conditions were as follows: resolution 1000 (10% valley); accelerating voltage +8kV; cathode plate voltage−3kV. The source temperature was kept below 100°C, FD emitter employed was ϕ 10μ tungsten wires activated at high temperture with microneedles of 20—30μ length.[9] The samples were applied to the emitter wire by a mic-

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rosyringe. The value of BAT of the emitter was between 14 and 21 mA. depending on different compounds.

Compounds investigated were listed in Table 1.

Table 1

Compound	Nomenclature	MW	Ref.
1	Adenosine 3′, 5′-cyclic phosphate	329	10
2	Cytidine 3′, 5′-cyclic phosphate	305	10
3	Uridine 3′, 5′-cyclic phosphate	306	10
4	Adenosine 3′, 5′-cyclic-*N*-cyclohexylphosphoramidate	410	11
5	Adenosine 3′, 5′-cyclic-*N*-*n*-amylphosphoramidate	398	11
6	2′-*O*-Tosyladenosine 3′, 5′-cyclic-*N*-phenylphosphoramidate	558	11
7	2′-*O*-Tosyladenosine 3′, 5′-cyclic-*N*-cyclohexylphosphoramidate	564	11
8	2′-*O*-Tosyladenosine 3′, 5′-cyclic-*N*-*n*-amylphosphoramidate	552	11
9	2′-*O*-Tosyladenosine 3′, 5′-cyclic phosphate	483	12
10	2′-*O*-Tosyladenosine 3′, 5′-cyclic-*n*-butylphosphate (*e*)	539	11
11	2′-*O*-Tosyladenosine 3′, 5′-cyclic-*n*-butylphosphate (*a*)	539	11
12	2′-*O*-Tosyladenosine 3′, 5′-cyclic benzyl phosphate (*e*)	573	11
13	2′-*O*-Tosyladenosine 3′, 5′-cyclic benzyl phosphate (*a*)	573	11
14	2′-*O*-Tosyladenosine 3′,5′-cyclic ethyl phosphate (*e*)	511	11
15	2′-*O*-Tosyladenosine 3′, 5′-cyclic ethyl phosphate (*a*)	511	11
16	2′-*O*-Tosyladenosine 3′, 5′-cyclic phosphite	467	12
17	9-*β*-*D*-Adenine arabinofuranosyl 3′, 5′-cyclic phosphite	313	12
18	2′-*O*-Tosyladenosine 3′, 5′-cyclic thioethylphosphite	511	11
19	2′-*O*-Tosyladenosine 3′, 5′-cyclic *N*′, *N*′-diethyl phosphoramidite	522	11
20	2′-*O*-Tosyladenosine 5′-methylphosphonate	499	(unpublished)

Results and discussion

Owing to the lower volatility and thermal instability of nucleotide cyclic phosphates and nucleotide cyclic phosphites, none of these can be introduced into the mass spectrometer without conversion to volatile derivatives. The EI spectrum of trimethylsilyl derivative of C-AMP has been reported.[2] Applying FD technique, we obtained peaks of the molecular ion (M^+) or protonated molecular ion ($M^+ + H$) of most of the compounds in Table 1. Table 2 shows FDMS data of **1, 2,** and **3.** In the spectra of cyclic nucleotides it exhibits ($M^+ + H$) only. These features are very similar to those of nucleotides.

Table 2 ($M^+ + H$) Peaks of Compound **1, 2, 3**

Compound	*m/z*	Type of ions	BAT (mA)
1	330	C-AMP$^+$+H	16
2	306	C-CMP$^+$+H	19
3	307	C-UMP$^+$+H	16

In the cases of phosphoramidates of cyclic nucleotides (**4** and **5**, Table 3), the FD spectra exhibit $M^{+\cdot}$ and $(M^+ + H)$. It indicates that when the acidic phosphate is converted to the corresponding phosphoramidate, this derivative favors the formation of $M^{+\cdot}$. The same results are obtained in the FD spectra of 2′–*O*–tosyl adenosine cyclic phosphate (**9**) and 2′–*O*–tosyl adenosine 5′–methylphosphonate **(20).** In the latter spectrum, the abundance of $M^{+\cdot}$ peak is 100% and the $(M^+ + H)$ peak is 50%. If both hydroxyl groups in phosphate and 2′ position of the sugar moiety of all the compounds in Table 1 (except **8**) are substituted, the spectra of these derivatives give $M^{+\cdot}$ in higher abundance.

Table 3 shows relative abundances of $M^{+\cdot}$ and $(M^+ + H)$ from the FD spectra of compounds **10—15.** They are the *eq* and *ax* stereoisomers of ethyl, butyl, and benzyl esters of 2′–*O*–tosyl adenosine cyclic phosphate. The stereoisomers are essentially indistinguishable in their FD spectra, only the BAT values are higher in the *eq* isomers than those of the *ax*

Table 3 FD data of some derivatives of C-AMP

Compound	Abundance of $M^{+\cdot}$ (%)	Abundance of $(M^+ + H)$ (%)	BAT (mA)
4	35	100	20
5	18	100	21
6	100	50	20
8	5	100	19
9	100	35	19
10	86	100	20
11	100	95	19
12	40	100	20
13	65	100	17
14	100	40	20
15	100	80	18
20	100	50	19

Table 4 FD spectra of some compounds

Sample	MS (*m/z*)					BAT (mA)
11	135(4*) 386(4) 539(100)	136(8) 404(4) 540(95)	172(15) 405(4) 541(30)	173(1) 482(12)	385(4) 483(6)	18
12	573(40)	574(100)	575(37)	576(8)		20
12	135(20) 171(10) 573(10)	136(1) 172(80) 574(25)	155(30) 173(15) 575(7)	156(4) 483(100)	170(50) 484(20)	23
16	135(28) 421(100)	136(2) 422(20)	172(20) 467(38)	173(2) 468(15)	420(80)	15
17	266(48)	267(100)	268(15)			14
18	135(8) 422(24)	136(1)	172(5)	420(36)	421(100)	20
19	113(4) 172(30) 421(100)	114(30) 173(7) 422(20)	115(16) 391(6)	135(24) 392(6)	136(10) 420(60)	18

* Relative abundances (%).

isomers. This phenomenon may be caused by the difference in the thermal stability of the two isomers. According to these data, all the molecular ions (or $M^+ + H$) are base peaks, so FDMS is better than the EIMS for the determination of molecular weight.

In FD spectra of cyclic nucleotides, all the peaks are very weak. But the abundance is increased considerably by using their various derivatives. Table 4 shows FD spectra of some derivatives of C-AMP.

Figure 1 shows the structure of **11** and its main fragmentation mode. They are m/z 539 ($M^{+\cdot}$), 540 ($M^+ + H$), 172 (T+H), and 135 (B+H)*. The ion m/z 483 is generated from ($M^{+\cdot} + H$) by loss of C_4H_9 and 404 is probably from ($M^+ + H$) by loss of $C_4H_9OPO_2$. In all spectra of the derivatives of cyclic nucleotide listed in Table 3, the major fragments are m/z 135 and / or 172. They can be considered as the ions of adenosine and toluene sulfonic acid. It indicates that the bonds of $C^{1'} - N$, $C^{2'} - O$ can be cleaved easily and these characteristic fragment ions are produced by hydrogen rearrangement.

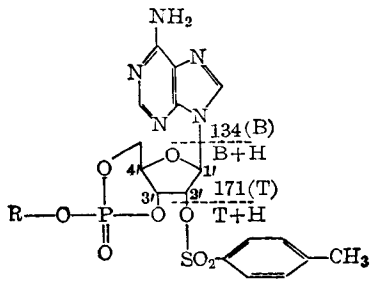

Figure 1 The main fragmentation mode of **11.**

FD spectra of the tosyl derivatives of adenosine cyclic phosphite are clearly different from those of the corresponding phosphates. The base peak is not $M^{+\cdot}$ or ($M^+ + H$). The strongest peak is m/z 421 and the second, 420. The molecular weight of **16** is 467, m/z 421 and 420 are generated from ($M^+ + H$) by loss of PO and POH. These characteristic peaks do not appear in the spectra of corresponding phosphates (such as **9**). Figure 2 shows the main fragmentation process of **16.** The same process occurs clearly in the cases of **17, 18** and **19.**

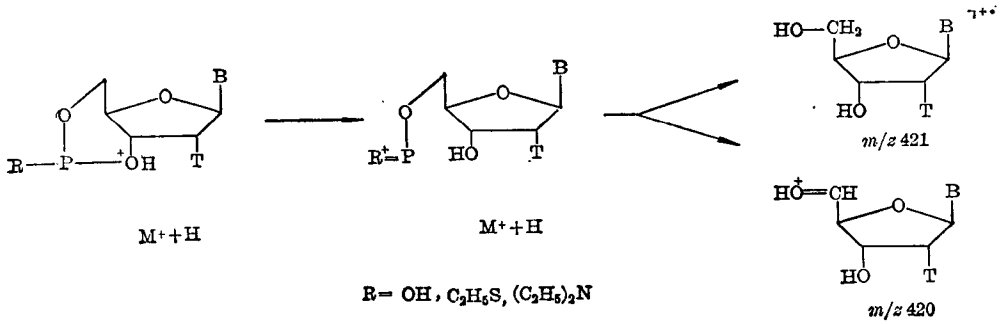

Figure 2 The main fragmentation process of **16.**

* B represents adenine, and T is *p*-tosyl group, the same below.

The spectra of these three compounds show no molecular ion, and the highest mass ions are m/z 267, 420 and 421. The ions m/z 420 and 421 in the spectrum of **19** come from $(M^+ + H)$ by loss of C_4H_9NP and $C_4H_{10}NP$, and in that of **18** from $(M^+ + H)$ by loss of C_2H_4SP and C_2H_5SP. In the spectrum of **17**, the ions m/z 266 and 267 are generated from $(M^+ + H)$ by loss of PO and POH.

The formation of characteristic ions described here is the predominant process, it may be related to the chemical activity of phosphites.

There is no molecular ion or protonated molecular ion in the FD spectrum of **7**. The highest mass of ions are M–18 and M + 1–18. The reason is still not clear.

In general, it is favorable to keep the heating current below 21 mA. Because of the thermal instability of cyclic nucleotides, thermolysis could occurs. We have recorded the FD spectra of **12** at different heating currents. At 20 mA, it gives protonated molecular ion, but with increasing heating current, the fragment ions 135 and 172 appear and the abundance of $(M^+ + H)$ is lower. When the heating current is 23 mA, the ion m/z 483 becomes the base peak and the abundance of $(M^+ + H)$ decreases to 25%. At 25 mA the abundance of $(M^+ + H)$ becomes very small and many other ions from thermolysis appear. Thus it is very important to control heating current in order to obtain an ideal FD spectrum.

In contrast to usual organic compounds, the FD sensitivity of cyclic nucleotides is low. When the emitter-dipping technique is used, the source may be contaminated by an excess of the sample deposited on non-emission region, thus the sensitivity of the instrument decreases. According to our experience the syringe technique is preferable to the former. If 1—2 μL of sample solution is applied to the needle portion with a microsyringe, 1—2 μg of sample adsorbed on the wire is enough to give these FD data, which are mentioned in this paper.

We have analyzed all compounds listed in Table 1 by using the EI technique. Among these compounds, **4, 5, 10, 11, 14** and **15** gave molecular ions, but in most cases the abundances were lower than 0.5 %.

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