A Study of the Mass Spectrum of Phenazine

Shizuko Eguchi

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730

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The mass spectrum of phenazine, a nitrogen-containing aromatic compound, was investigated in detail using a combination of ion kinetic energy spectroscopy and defocusing technique. In the fragmentation of the singly charged ions the molecular ion lost H· in the first stage to give an $[M-1]^+$ or $[M-2]^{\dagger}$ ion, which was degraded *via* the expulsion of CN· or C_2H_2 in the next stage and of HCN or C_2H_3· in a later stage. Doubly charged ions also appeared in considerable intensity, and the fragmentation sequence differed significantly from that of the singly charged ions.

Although there are some reports[1–4] about the mass spectra of phenazine and substituted phenazines, it cannot be said that the fragmentation mechanism has been fully clarified, because the investigations have usually been carried out in applying mass spectrometry to the analysis of bacterial metabolites. In the present paper, the mass spectrum of phenazine was investigated in detail by determining the elemental compositions of all the fragment ions using high resolution mass spectrometry and by sorting out the metastable ions using a combination of ion kinetic energy spectroscopy and the defocusing technique. Thus, many points which need to be corrected were found in the fragmentation pathway already reported for the phenazine nucleus.

Results and Discussion

In the ordinary mass spectrometry, phenazine, a nitrogen-containing aromatic compound, exhibited a simple spectrum which consisted of weak fragment peaks, except for the intense molecular peak at *m/e* 180, as shown in Fig. 1. For the purpose of determining the multiplicity of the peaks and the elemental composition of each ion, the high resolution mass spectrum was measured with a computer on-line system and/or with a pen-recording system under the conditions of slower magnetic scanning and narrower widths of entrance and collector slits. Seventeen fragment peaks, listed in Table 1, were confirmed to be constituted of two or three ions.

On the other hand, the IKE spectrum[5,6] of phen-

Table 1. Multiplicity of the spectral peaks and elemental composition of each fragment ion in the mass spectrum of phenazine

m/e	Elemental composition	%
180	$C_{12}H_8N_2$ (1)	100
179	$C_{12}H_7N_2$ (2)	100
178	$C_{12}H_6N_2$ (3)	100
154	$C_{10}H_6N_2$ (4)	42
	$C_{11}H_8N_1$ (5)	58
153	$C_{10}H_5N_2$ (6)	30
	$C_{11}H_7N_1$ (7)	70
152	$C_{11}H_6N_1$ (8)	94
	$C_{12}H_8$ (9)	6
151	$C_{11}H_5N_1$ (10)	100
140	$C_{10}H_6N_1$ (11)	100
129	$C_8H_5N_2$ (12)	54
	$C_9H_7N_1$ (13)	46
128	$C_8H_4N_2$ (14)	trace
	$C_9H_6N_1$ (15)	52
	$C_{10}H_8$ (16)	48
127	$C_9H_5N_1$ (17)	68
	$C_{10}H_7$ (18)	32
126	$C_9H_4N_1$ (19)	20
	$C_{10}H_6$ (20)	80
125	$C_9H_3N_1$ (21)	7
	$C_{10}H_5$ (22)	93
103	$C_7H_5N_1$ (23)	100
102	$C_7H_4N_1$ (24)	73
	C_8H_6 (25)	27
101	$C_7H_3N_1$ (26)	50
	C_8H_5 (27)	50
100	C_8H_4 (28)	100
99	C_8H_3 (29)	100
90	$C_6H_4N_1$ (30)	100
89	$C_6H_3N_1$ (31)	50
	C_7H_5 (32)	50
78	$C_5H_4N_1$ (33)	75
	C_6H_6 (34)	25
77	$C_5H_3N_1$ (35)	59
	C_6H_5 (36)	41
76	$C_5H_2N_1$ (37)	15
	C_6H_4 (38)	85
75	$C_5H_1N_1$ (39)	23
	C_6H_3 (40)	77
74	C_6H_2 (41)	100
64	$C_4H_2N_1$ (42)	67
	C_5H_4 (43)	33
63	C_5H_3 (44)	100
62	C_5H_2 (45)	100
56	C_4H_8 (46)	100
53	$C_3H_3N_1$ (47)	100
52	$C_3H_2N_1$ (48)	24
	C_4H_4 (49)	76
51	C_4H_3 (50)	100
50	C_4H_2 (51)	100

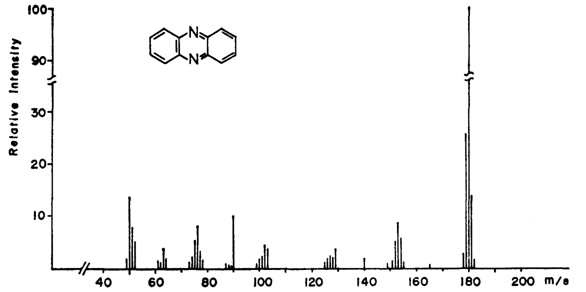


Fig. 1. Ordinary mass spectrum of phenazine.

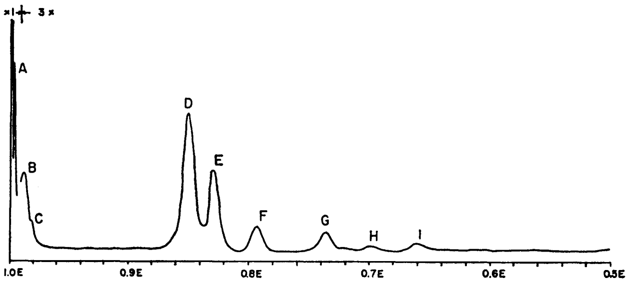


Fig. 2. IKE spectrum of phenazine from $E/2$ to E.

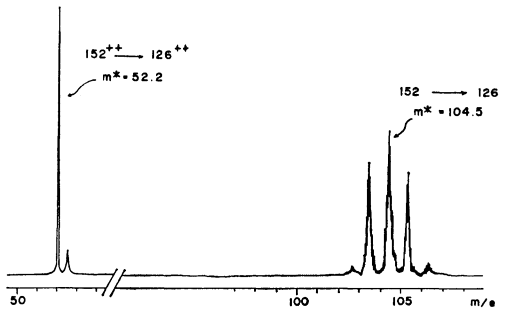


Fig. 3. Metastable ion spectrum at 0.8290E.

azine was taken over a range of electric sector voltage from 1.0E to 0.5E (Fig. 2), and then the range corresponding to the width of each peak in this spectrum was searched with magnetic current scanning at 0.0005E intervals of the electric sector voltage to determine the number of metastable ions. If some metastable ions are included in a certain peak of the IKE spectrum, they appear in general as separated peaks in the metastable ion specra, such as that shown in Fig. 3. Since each of these metastable peaks, however, slightly varies in the intensity and m/e value depending upon the electric sector voltage used, the reading of the m/e value of a certain metastable ion at the maximum intensity was determined as the mass unit of this metastable ion.[7,8] Based on such analysis, peak E in the IKE spectrum of phenazine was determined to include four metastable ions, at m/e 103.5, 104.5, 105.4, and 52.2, for each of which one or several transitions were listed in Table 2. These transitions were assigned from the elemental compositions of singly and doubly charged ions which are listed in Tables 1 and 4. As shown in Table 2, the observed metastable ion value contained several metastable ions because a metastable ion value could not measured with an accuracy better than 1000 ppm in the present work. Accordingly, a few possible transitions were proposed for the observed metastable ion value; similar results had been reported in the mass spectrum of *N*-cyclohexylguanidine.[9] Similar analyses were also carried out on the other peaks (A—D, F—I) of the IKE spectrum.

Thus, peaks A, B, and C of the IKE spectrum, which were very close to the main beam ion, were characterized as the expulsion of H· or H_2 from the main beam ion and the remaining peaks, from D to I, as the expulsion of C_2H_2, CN·, C_2H_3·, or HCN from the molecular ion or several fragment ions, as listed in Table 3. Among them, transitions due to the loss of a hydrogen molecule such as 180→178+2 and 153→151+2, have not yet been reported in the mass spectra of phenazine and substituted phenazines, nor in those of other nitrogen-containing aromatic compounds. However, no metastable ions, which would correspond to the direct expulsion of fragments such as C_2H_2, CN·, C_2H_3·, or

TABLE 2. EXACT MASSES OF THE METASTABLE IONS INCLUDED IN PEAK E OF IKE SPECTRUM AND TRANSITION ASSIGNMENT

Transition				Calcd $\gamma=E_1/E$	Calcd m^*	Obsd m^*
153		127				
$C_{10}H_5N_2^+$ (30%)	$\longrightarrow$	$C_9H_5N_1^+$ (68%)	+ CN·	0.8300(9)	105.46	
$C_{11}H_7N_1^+$ (70%)	$\nearrow$	$C_9H_5N_1^+$	+ C_2H_2	0.8300(2)	105.45	105.4
	$\searrow$	$C_{10}H_7^+$ (32%)	+ CN·	0.8301(1)	105.47	
152		126				
$C_{11}H_6N_1^+$ (94%)	$\nearrow$	$C_9H_4N_1^+$ (20%)	+ C_2H_2	0.8289(0)	104.47	
	$\searrow$	$C_{10}H_6^+$ (80%)	+ CN·	0.8289(8)	104.49	104.5
$C_{12}H_8^+$ (6%)	$\longrightarrow$	$C_{10}H_6^+$	+ C_2H_2	0.8289(2)	104.48	
$C_{12}H_8^{2+}$ (100%)	$\longrightarrow$	$C_{10}H_6^{2+}$ (100%)	+ C_2H_2	0.8289(1)	52.24	52.2
151		125				
$C_{11}H_5N_1^+$ (100%)	$\nearrow$	$C_9H_3N_1^+$ (7%)	+ C_2H_2	0.8277(5)	103.49	103.5
	$\searrow$	$C_{10}H_5^+$ (93%)	+ CN·	0.8278(4)	103.51	

Table 3. Exact masses of the metastable ions in the IKE spectrum and the transition assignment

Peak in IKE spectrum	Transition $m_1 \rightarrow m_2 + (m_1 - m_2)$	Lost element. comp.	Obsd m^*
A	$180^+ \rightarrow 179^+ + 1$	H	178.0
	$179^+ \rightarrow 178^+ + 1$	H	177.0
	$154^+ \rightarrow 153^+ + 1$	H	152.0
	$153^+ \rightarrow 152^+ + 1$	H	151.0
	$152^+ \rightarrow 151^+ + 1$	H	150.0
B	$180^+ \rightarrow 178^+ + 2$	H_2	176.0
	$153^+ \rightarrow 151^+ + 2$	H_2	149.0
	$77^+ \rightarrow 76^+ + 1$	H	75.0
	$76^+ \rightarrow 75^+ + 1$	H	74.0
	$75^+ \rightarrow 74^+ + 1$	H	73.0
C	$51^+ \rightarrow 50^+ + 1$	H	49.0
D	$179^+ \rightarrow 153^+ + 26$	C_2H_2 or CN	130.8
	$178^+ \rightarrow 152^+ + 26$	C_2H_2 or CN	129.8
	$180^{2+} \rightarrow 152^{2+} + 28$	N_2	64.2
E	$153^+ \rightarrow 127^+ + 26$	C_2H_2 or CN	105.4
	$152^+ \rightarrow 126^+ + 26$	C_2H_2 or CN	104.5
	$151^+ \rightarrow 125^+ + 26$	C_2H_2 or CN	103.5
	$152^{2+} \rightarrow 126^{2+} + 26$	C_2H_2	52.2
F	$126^+ \rightarrow 100^+ + 26$	C_2H_2	79.4
	$125^+ \rightarrow 99^+ + 26$	C_2H_2 or CN	78.4
	$128^+ \rightarrow 101^+ + 27$	C_2H_3 or HCN	79.7
	$126^{2+} \rightarrow 100^{2+} + 26$	C_2H_2	39.7
G	$103^+ \rightarrow 76^+ + 27$	C_2H_3 or HCN	56.1
	$102^+ \rightarrow 75^+ + 27$	C_2H_3 or HCN	55.1
H	$90^+ \rightarrow 63^+ + 27$	C_2H_3 or HCN	44.1
I	$77^+ \rightarrow 50^+ + 27$	C_2H_3 or HCN	32.5
	$152^{2+} \rightarrow 100^{2+} + 52$	C_4H_4	32.8

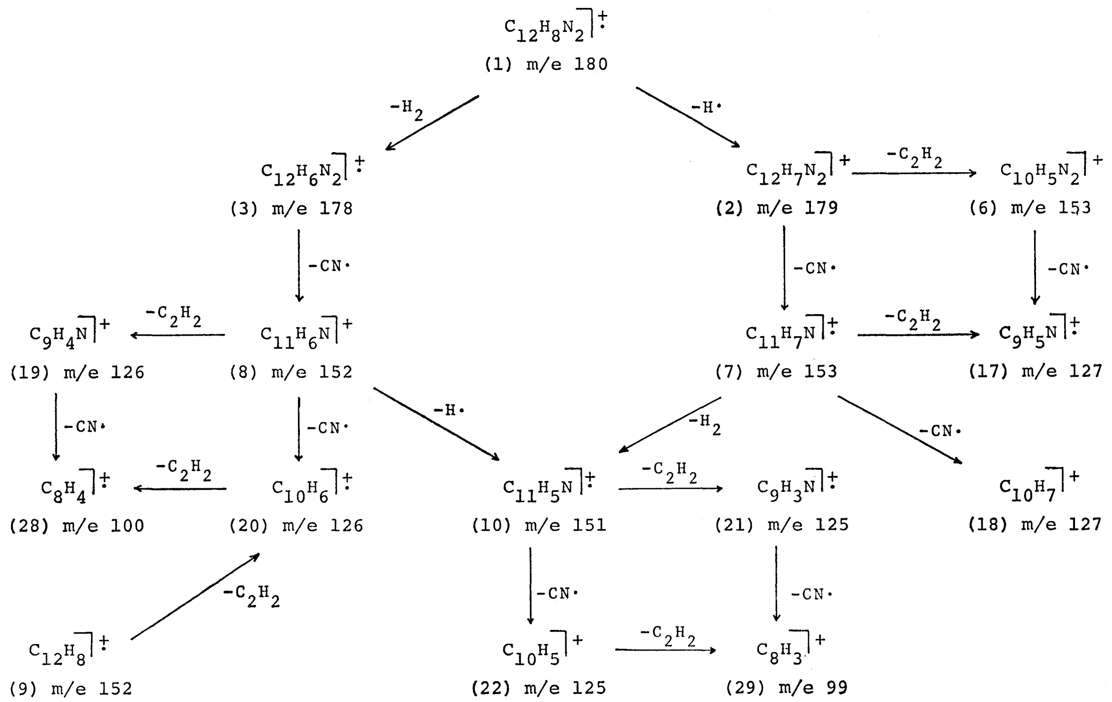
HCN from the molecular ion, could be detected. From the above observation, it may be concluded that the major fragmentation of phenazine undergoes nuclear cleavage not directly, as described by other workers,[1-4] but after the loss of H· or H_2.

On the basis of the elemental composition of the fragment ions and the transition assignment of the metastable ions, the significant fragmentation sequence of the singly charged ion in phenazine was deduced as shown in Scheme 1. The loss of H· from the molecular ion (1) yielded a $C_{12}H_7N_2$ ion (*m/e* 179) (2) as the most abundant fragment ion in the spectrum. The ion (2) gave two kinds of *m/e* 153 ions, $C_{10}H_5N_2$ (6) and $C_{11}H_7N$ (7), in a ratio of 30: 70 under expulsion of C_2H_2 and CN·, respectively. The ion (6) produced C_9H_5N (*m/e* 127) (17) under expulsion of CN·, and the ion (7) subsequently lost C_2H_2, CN·, or H_2 to be degraded to C_9H_5N (*m/e* 127, 68%) (17), $C_{10}H_7$ (*m/e* 127, 32%) (18), or $C_{11}H_5N$ (*m/e* 151) (10). There are two pathways for the C_9H_5N ion (17), but the route *via* the $C_{11}H_7N$ (7) was thought to be predominant from the above-mentioned relative intensity of the intermediate ions.

The molecular ion was further degraded *via* the second pathway, including successive losses of H_2 and CN·, to give a $C_{11}H_6N$ ion (*m/e* 152) (8), which was then degraded to a C_8H_4 ion (*m/e* 100) (28) *via* a C_9H_4N (*m/e* 126) (19) and a $C_{10}H_6$ (*m/e* 126) (20) in a ratio of 20: 80, and which lost H· to give an ion of $C_{11}H_5N$ (*m/e* 151) (10). In the formation of $C_{12}H_8$ ion (*m/e* 152, 6%) (9) which was degraded to $C_{10}H_6$ (*m/e* 126, 20%) (20) under expulsion of C_2H_2, the metastable ion corresponding to the transition from molecular ion to the ion (9) was not detected.

Furthermore, the ion (10) which was formed by loss of H_2 from the ion (7) and of H· from the ion (8) degraded to C_8H_3 (*m/e* 99) (29) *via* a C_9H_3N (*m/e* 125) (21) and a $C_{10}H_5$ ion (*m/e* 125) (22) in a ratio of 7: 93.

In the smaller fragment range of the ordinary mass



Scheme 1. Fragmentation sequence of singly charged ions of phenazine supported by metastable ion.

TABLE 4. ELEMENTAL COMPOSITIONS OF DOUBLY CHARGED IONS OF PHENAZINE

Obsd mass m/e	Mass of biradical ion $m/e \times 2$	Calcd for elemental composition	$\Delta m.u.$ (1×10^{-3})	Elemental composition
90.0344	180.0688	180.0687	0.1	$C_{12}H_8N_2$
76.0290	152.0580	152.0624	4.4	$C_{12}H_8(M-N_2)$
63.0219	126.0438	126.0468	3.0	$C_{10}H_6(M-C_2H_2N_2)$
50.0155	100.0310	100.0312	0.2	$C_8H_4(M-C_4H_4N_2)$

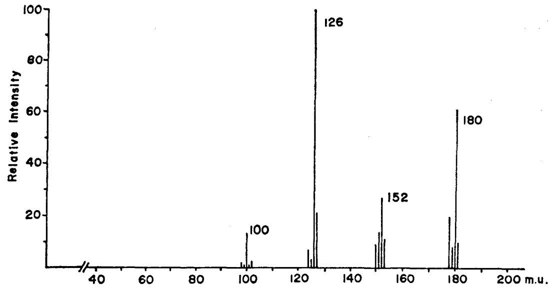
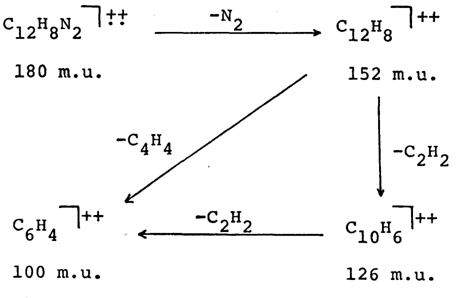


Fig. 4. 2*E* doubly charged ion spectrum of phenazine.

spectrum of phenazine, two ions appeared at *m/e* 90 and 63, and also the metastable ion corresponding to the transition between both ions could be detected at *m/e* 44.1 by the above-mentioned analysis of the IKE spectrum. However, the aromatic structure of phenazine and the fact that the metastable ion was far too weak (peak H in Fig. 2) compared with the intensities of the precursor and daughter ions in the ordinary mass spectrum were expected that doubly charged ions existed in the mass spectrum of phenazine. For these reason, the 2*E* doubly charged ion spectrum was taken by setting the electric sector voltage at twice the value used for accelerating.[6,10–12] This exhibited the characteristic ions shown in Fig. 4, whose elemental compositions were determined from the exact masses accordingly to a procedure[12] reported previously (Table 4). These observations revealed that *m/e* 90, 76, 63, and 50 ions in the ordinary spectrum were partly composed of doubly charged ions of 180, 152, 126, and 100 mass units respectively. The major fragmentation sequence of the doubly charged ions of phenazine was thus deduced to be Scheme 2.



Scheme 2. Fragmentation sequence of doubly charged ions of phenazine.

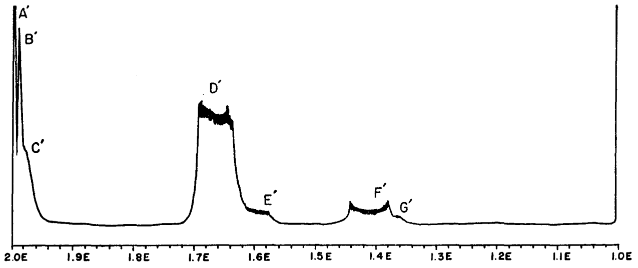


Fig. 5. High energy IKE spectrum from *E* to 2*E*.

Ion kinetic energy spectra of organic molecules show peaks corresponding to ions having energies in excess of that of the main beam ion. It has been shown that the peaks correspond to charge-exchange reactions of doubly charged ions or to decompositions of metastable clusters of ions in high energy IKE spectra of aromatic hydrocarbons.[11] The above reactions were expected from the existence of the doubly charged ions detected in the phenazine, and therefore the high energy IKE spectrum was measured over the range from *E* to 2*E* (Fig. 5). Peaks that appear between *E* to 2*E* in the high energy IKE spectrum may be due to one of two separate processes including doubly charged ions: collision-induced charge transfers and charge separation reactions. Peak A′, which appears exactly at 2.0*E*, is

TABLE 5. TRANSITIONS ASSIGNMENT OF HIGH ENERGY IKE SPECTRUM OF PHENAZINE

Peak in IKE spectrum	Transition $m_1 \rightarrow m_2 + (m_1 - m_2)$	Calcd γ[a]
A′	$180^{2+} \rightarrow 180^{+}$	2.0
	$179^{2+} \rightarrow 179^{+}$	2.0
	$178^{2+} \rightarrow 178^{+}$	2.0
	$153^{2+} \rightarrow 153^{+}$	2.0
	$152^{2+} \rightarrow 152^{+}$	2.0
	$151^{2+} \rightarrow 151^{+}$	2.0
	$150^{2+} \rightarrow 150^{+}$	2.0
	$127^{2+} \rightarrow 127^{+}$	2.0
	$126^{2+} \rightarrow 126^{+}$	2.0
	$102^{2+} \rightarrow 102^{+}$	2.0
	$100^{2+} \rightarrow 100^{+}$	2.0
B′	$180^{2+} \rightarrow 179^{+}$	1.989
	$153^{2+} \rightarrow 152^{+}$	1.987
	$152^{2+} \rightarrow 151^{+}$	1.987
	$126^{2+} \rightarrow 125^{+}$	1.984
C′	$180^{2+} \rightarrow 178^{+}$	1.974
	$152^{2+} \rightarrow 150^{+}$	1.974
D′	$180^{2+} \rightarrow 152^{+} + 28^{+}$	1.689
	$178^{2+} \rightarrow 150^{+} + 28^{+}$	1.685
	$152^{2+} \rightarrow 126^{+} + 26^{+}$	1.685
	$150^{2+} \rightarrow 124^{+} + 26^{+}$	1.653
E′	$126^{2+} \rightarrow 100^{+} + 26^{+}$	1.587
F′	$178^{2+} \rightarrow 126^{+} + 52^{+}$	1.416
G′	$126^{2+} \rightarrow 87^{+} + 39^{+}$	1.381

a) γ: Electric sector voltage ratio, E_1/E.

due to the collision-induced reaction in which doubly charged ions are converted to singly charged ions without loss of any mass units. Peak B′ is due to a reaction in which a doubly charged ion not only loses a charge but also a fragment of one mass unit:

$$m_1^{2+} \xrightarrow{\text{collision}} m_1^{+} \longrightarrow (m-1)^{+}$$

or

$$m_1^{2+} \longrightarrow (m-1)^{2+} \xrightarrow{\text{collision}} (m-1)^{+}$$

But these two processes cannot be distinguished in the present work. Similarly, peak C′ is due to a reaction in which two hydrogen atoms with one charge are lost. Other peaks, D′ to G′, were constituted of some broad dish-topped peaks which were known to be attributable to $m_1^{2+} \rightarrow m_2^{+} + (m_1 - m_2)^{+}$ type decompositions of the doubly charged ions.[13] Mass analyses of the above-mentioned peaks were carefully carried out with a interval of $0.0005E$ in the electric sector voltage to determine metastable ions. The resulted assignments of reactions and transitions are listed in Table 5.

Conclusion

The metastable ion transition, which occurred in the first free region of the mass spectrometer, was analyzed in detail by utilizing the ion kinetic energy spectroscopy and the defocusing technique. It was clarified that the molecular ion of phenazine lost H· or H_2 in the first stage of the fragmentation to give $[M-H]^+$ or $[M-H_2]^+$ ion, which was degraded step by step *via* expulsion of CN· or C_2H_2 and successive expulsion of HCN or C_2H_3·.

Doubly charged ions were also confirmed to occur to a certain extent in the mass spectrum of phenazine. The metastable transition of these ions was similarly analyzed and a fragmentation sequence was proposed.

Experimental

The high resolution mass spectra were taken using an on-line system which combined a HITAC-10 computer with a Hitachi RMU-7L double focusing mass spectrometer: ionizing energy 70 eV, ionizing current 80 μA, and accelerating voltage 3.2 kV. For determining multiplicity of peaks and elemental composition of ions, singly changed ion spectra were taken at 10 and 8 μm widths of entrance and collector slits respectively (normally 20 and 17 μm). The IKE and metastable ion spectra were examined on the RMU-7L modified with a metastable defocusing attachment.

On the other hand, the $2E$ doubly charged ion spectrum was taken at an electric sector voltage twice as large as the normally used accerating voltage, under 5×10^{-6} Torr of source pressure. For analysis of the transition from a $2E$ doubly charged ion to two singly charged ions, the high energy IKE spectrum was taken over the sector voltage from E, at which the main beam of stable ion was transmitted, to $2E$, and the mass units of the metastable ions were determined under the condition of the $2E$ spectrum measurment.

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