PULSED POSITIVE/NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY OF PYRAZINES

Anna Arnoldi*, Olga Baldi, and Alberto Griffini+

DIPARTIMENTO DI SCIENZE MOLECOLARI AGROALIMENTARI.
Università di Milano
Via Celoria, 2 - 20133 - M I L A N O - Italy -

Abstract - The mass spectrometric behavior of thirteen pyrazines under PPINICI conditions was investigated using methane as reagent gas. Differences in the mass spectra were observed depending on analyte concentration. Structural informations can be obtained, but data are highly influenced by experimental conditions.

Pyrazines have been detected in many living organisms and are important components of food flavors, in particular coffee, chocolate, fried potatoes etc. In heated or roasted foods they are formed by the complex reactions of amino acids and reducing sugar which are referred to as "Maillard Reaction"[1,2]. Some recent reviews describe the literature data on all the pyrazines isolated in foods, and, when they are known, the sensitivity thresholds for taste or smell[3,4,5], that sometimes are extraordinarily low.

Therefore mass spectrometry has an increasing importance in the detection and characterization of these compounds. The EI mass spectrometric behavior of naturally occurring pyrazines were reviewed by Brophy and Cavill[6] and Porter[7]. The fragmentation patterns depend on the substituents present and only in part arise from ring fragmentation. In the case of long alkyl substituents, the molecular ion is absent, therefore an alternative method for the detection of the molecular weight can be very important. Daishima and Iida[8] have measured the methane and isobutane chemical ionization (CI) mass spectra of some nitrogen containing heteroaromatic compounds among which pyrazine and methylpyrazine. On the contrary as far as we know, a study on

* To whom correspondence should be addressed.
+ Present address: Inverni della Beffa, Research and Development Laboratory, Via Ripamonti 99, 20141 - Milano, Italy.

Table 1 - *Analysis of pyrazines by methane chemical ionization mass spectrometry*

Number	Pyrazine	Mol. Weight	Sol.A ($mg\ ml^{-1}$)	Sol.B ($mg\ ml^{-1}$)	RT[a] (sec)
1	unsubstituted	80	0.245	2.450	474
2	methyl	94	0.103	1.030	567
3	2,5-dimethyl	108	0.105	1.049	663
4	2,6-dimethyl	108	0.147	1.473	674
5	ethyl	108	0.136	1.357	683
6	2,3-dimethyl	108	0.184	1.835	706
7	trimethyl	122	0.114	1.137	802
8	2-methoxy-3-(1-methylethyl)	152	0.123	1.231	842
9	tetramethyl	136	0.195	1.946	917
10	2-methoxy-3-(1-methylpropyl)	166	0.254	2.535	955
11	2-methoxy-3-(2-methylpropyl)	166	0.114	1.136	991
12	acetyl	122	0.129	1.289	1165
13	quinoxaline	130	0.183	1.825	1551

a. Retention Time.

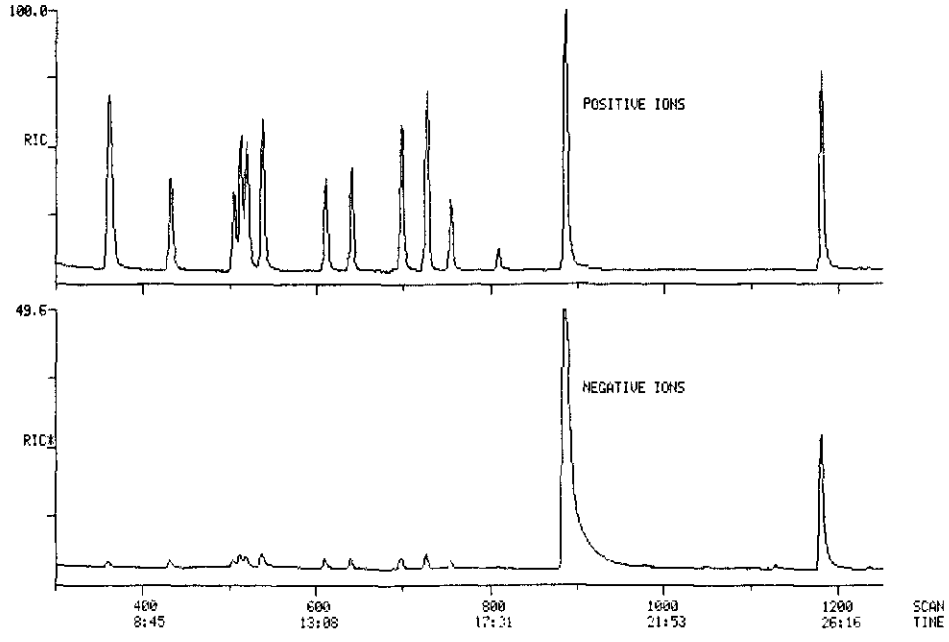

Figure 1. Methane chemical ionization positive and negative ion chromatogram of solution A

di- and trisubstituted pyrazines has not yet been reported even if these last compounds are the most important in foods and living organisms. We have used an acid reagent system as methane because of the high proton affinity of pyrazine (PA = 207.3 kcal/mole[9]) and have performed pulsed positive ion/negative ion experiments (ppinici) because the high electron deficiency of these compounds (pyrazine electron affinity EA = 0.4 eV [10]) suggested that negative ions could be formed in large amount.

As pyrazines are in general found in very complex mixtures, they were introduced by capillary GC.

EXPERIMENTAL

The pyrazines studied (Table 1) were purchased from Aldrich, their purity was higher 99% except in the case of 2-methoxy-3-(2-methylpropyl)pyrazine which contained 15% of an impurity identified as 2-methoxy-3-butylpyrazine.

Relative retention times (RRT) and response factors (RF) were calculated taking quinoxaline as internal standard. Solutions containing about 0.1 mg ml^{-1} (solution A) and 1 mg ml^{-1} (solution B) of each compound (Table 1) in dichloromethane (analytical grade, Merck) were analyzed using a Finnigan-Mat 4021 GC/MS equipped with a PPINICI unit and a Super INCOS DATA SYSTEM. A Supelcowax 10 (Supelco) wide-bore bonded phase 30 m x 0.75 mm i.d. capillary column with a 1 m thick film was used. Details about injector modifications and MS connections have been already reported[11]. Methane (99.995%, SIO-ALPHAGAZ) was introduced as make up gas.

GC conditions: carrier gas He 3 ml min^{-1} (linear rate 27.3 cm sec^{-1} at 70°); injector 250° C; oven program 70° C for 4 min., 5° C min^{-1} to 180° C, iso 180° C; interface 250° C; splitless injection mode.

MS conditions: ionizer temperature 150° C; electron energy 140 eV; conversion dynode voltage +/- 3.0 KV; electron multiplier voltage 1800 V; preamplifier sensitivity 10^{-7} A/V; MS manifold temperature 70° C. The reagent gas pressure was 0.33 torr and was measured using the ionizer Pirani head. Spectra were alternately acquired at 0.55 seconds per scan in positive and negative ion mode from 50 to 650 u (total scan time 0.986 s, total acquisition time 0.978 s and centroid sampling interval 0.200 ms).

RESULTS AND DISCUSSION

Methane Chemical Ionization Positive Ions.

The positive ion chromatogram of solution A containing about 0.1 mg ml^{-1} of each compound obtained using methane as ionizing gas is shown in Figure 1. Table 2 collects the positive mass spectra of the pyrazines studied. Unsubstituted pyrazine and alkylsubstituted pyrazines show $(M+H)^+$ as 100% of the relative abundance and the adduct ions $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$

Table 2 - Methane chemical ionization mass spectra of pyrazines at a concentration of about 0.1 mg ml^{-1} (solution A): positive ions and relative intensities in parentheses

Pyrazine	$M^{+\cdot}$	$M+H^+$	$(M+C_2H_5)^+$	$(M+C_3H_5)^+$	$(M-H)^+$	$(M-HCN)^{+\cdot}$	Other
1	-	81(100)	109(10.2)	121(3.8)	-	53(1.3)	-
2	-	95(100)	123(12.5)	135(4.04)	-	67(1.0)	-
3	108(10.3)	109(100)	137(14.1)	149(5.9)	107(2.3)	81(0.8)	-
4	108(6.7)	109(100)	137(16.1)	149(5.3)	107(1.6)	-	-
5	108(5.5)	109(100)	137(15.9)	149(4.5)	107(3.2)	-	-
6	108(4.5)	109(100)	137(15.4)	149(5.3)	107(1.6)	-	67($M-CH_3CN$)(2.1)
7	122(10.0)	123(100)	151(16.0)	163(5.7)	121(3.9)	-	-
8	152(6.3)	153(100)	181(15.38)	193(4.7)	151(7.7)	-	137(M-15)(6); 124(M-28)(1.2)
9	136(11.1)	137(100)	165(13.3)	177(4.79)	135(9.3)	-	
10	-	167(100)	195(14.1)	207(4.5)	165(11.2)	-	151(M-15, 7.5); 138(M-28, 5.0); 124(M-42, 4.1)
11	-	167(100)	195(14.1)	207(5.0)	165(11.5)	-	151(M-15, 9.2); 124(M-42, 9.5)
12	122(1.38)	123(100)	151(5.7)	163(4.7)	121(0.7)	-	57(25.6); 55(28.4)
13	130(9.2)	131(100)	159(19.0)	171(5.44)	-	103(1.1)	-

having about 10-16% and 4-6% respectively of relative abundance. The introduction of two methyl groups allows the production of a low intensity ion $(M-H)^+$ obtained by hydride abstraction from the methyl substituent. This behavior is well known for aromatic compounds[2]. This ion is particularly intense in tetramethylpyrazines (9) while it is absent in methylpyrazine (2). 2,5-Dimethyl-pyrazine, 2,6-dimethyl-pyrazine, 2,3-dimethyl-pyrazine, and ethylpyrazine spectra are very similar. 2,5-Dimethylpyrazine is characterized by a highly intense $M^{+\cdot}$ and small or totally absent $(M-HCN)^{+\cdot}$ ion. The three methoxypyrazines considered have the following features: low or lacking molecular ion, intense $(M-H)^+$, presence of a $(M-15)^+$ ion and some fragmentation by losing of 28 or 42 fragments. These ions coming from MacLafferty rearrangements are not present in alkylbenzenes, but have been already reported in methane CI spectra of 2-propylpyridine and 3-pentylpyridine[8].

Acetylpyrazine has two intense peaks at mass 57 and 55. Quinoxaline presents a low intensity $(M-HCN)^{+\cdot}$ peak.

A ten times increase in pyrazine concentration has a dramatic effect on their mass spectra. The results of the analysis of solution B (concentration about 1 mg ml^{-1}) are reported in Table 3. The spectra are characterized by the complete lacking of ion $(M+H)^+$ and by the appearance of the intense ions $M^{+\cdot}$ and $(M+2H)^{+\cdot}$.

The molecular ion is probably due the direct ionization by electron impact owing to the rather low ionization potential (unsubstituted pyrazine PI ≈ 9.5 eV[13,14]). With the exception of quinoxaline, the base peak is the ion $(M+C_2H_5)^+$ and the molecular ion has a relative intensity ranging from 40 to 80% and is accompanied by fragments, such as $(M-HCN)^{+\cdot}$ and $(M-CH_3CN)^{+\cdot}$. The ion $(M-H)^+$ is present in pyrazines with at least one alkyl substituent, even in the case of methylpyrazine, while they are lacking in low pressure EI-spectra[6,7], and are very intense in **5**, **8**, **10** and **11**, where the hydrogen can be lost by a ß-carbon.

In the case of 2-methoxypyrazines substituted in position 3 by long alkyl chains (**10** and **11**) $M^{+\cdot}$ is absent but $(M-1)^+$ and fragments derived from MacLafferty rearrangement $(M-28)^{+\cdot}$ and $(M-42)^{+\cdot}$ are very intense. Daishima and Iida[8] have studied the methane CI gas chromatography - mass spectrometry of pyrazine and 2-methylpyrazine using

Table 3 - Methane chemical ionization mass spectra of pyrazines at a concentration of about 1 mg ml^{-1} (solution B): positive ions and relative intensities in parentheses

Pyrazine	$M^{+\cdot}$	$(M+2H)^+$	$(M+C_2H_5)^+$	$(M+C_3H_5)^+$	$(M-H)^+$	$(M-HCN)^{+\cdot}$	Others
1	80(70.8)	82(38.6)	109(100)	121(36.6)	-	53(6.6)	-
2	94(53.4)	96(39.0)	123(100)	135(36.0)	93(5.7)	67(6.4)	-
3	108(48.0)	110(49.2)	137(100)	149(36.9)	107(16.3)	81(2.2)	123(2.1)
4	108(48.6)	110(39.6)	137(100)	149(35.6)	107(10.0)	81(1.0)	123(2.3); 57(5.0); 96(2.0)
5	108(42.5)	110(40.2)	137(100)	149(35.3)	107(21.8)	81(2.3)	123(1.7); 80(3.0); 56(10.8)
6	108(45.1)	110(40.4)	137(100)	149(35.1)	107(9.6)	-	123(2.1); 67(11.3); 96(0.9)
7	122(48.3)	124(47.8)	151(100)	163(37.6)	121(26.9)	-	137(2.4); 110(5.0); 81(3.2)
8	152(36.7)	154(53.1)	181(100)	193(35.6)	151(40.9)	-	139(10.3); 137(33.4); 124(8.5); 111(4.7)
9	136(70.2)	138(60.6)	165(100)	177(40.5)	135(61.1)	-	151(2.1); 124(19.4); 96(3.5); 54(18.4)
10	-	168(64.9)	195(100)	207(34.9)	165(68.6)	139(7.9)	151(46.9); 138(29.2); 124(24.7); 111(9.1)
11	-	168(63.9)	195(100)	207(40.2)	165(73.2)	139(5.6)	151(63); 124(53.4); 111(7.3)
12	122(78.6)	124(86.4)	151(100)	163(85.1)	-	95(4.8)	109(6.8); 105(7.9)
13	130(84.8)	132(100)	159(25.7)	171(75.4)	129(5.6)	103(9.6)	157(6.1); 145(5.5); 118(1.2)

1-2 mg ml^{-1} solutions, and have not observed these concentration effects. They have used a similar source and analyzer, however, they have introduced the pyrazines by a packed PEG-6000 (5%, 0.5m x 2 mm id) column. We think that, owing to the much higher efficiency, with capillary columns, temporary concentration of pyrazines in the ion source is higher and therefore undesired interactions can become very important.

In all the pyrazines studied is present an intense ion $(M+2H)^{+\cdot}$ due to reduction in the ion source. In fact the 1,4-dihydropyrazine radical-cation is a paramagnetic 7 π-electron intermediate which exhibits exceptional stability also in solution[15]. In quinoxaline, owing to the presence of the condensed aromatic ring, ion $(M+2H)^{+\cdot}$ becomes the base peak of the spectrum. As expected reduction becomes competitive at high concentration only and was not observed during the analysis of solution A.

Methane Chemical Ionization - Negative Ions.

The negative ion chromatogram of solution B is shown in Figure 1, the negative ion spectra are reported in Table 4. Solutions A and B gave similar results, except small differences in relative intesities of some ions. A large amount of negative ions are obtained only with acetylpyrazine and quinoxaline. In these cases the base peak is $(M+1)^-$, accompanied by a medium intense $(M+15)^-$.

The other pyrazine peak areas are 35-70 times smaller and their spectra are characterized by a

Table 4 - Methane chemical ionization mass spectra of pyrazines at a concentration of about 1 mg ml^{-1} (solution B): negative ions and relative intensities in parentheses

Pyrazine	$(M-1)^-$	M^-	$(M+1)^-$	Other peaks
1	79(100)	-	81(64.1)	95(3.4, M+15); 159(2M-H, 30.1)
2	93(100)	94(5.6)	95(5.6)	108(16.8, M+14)
3	107(100)	108(8.7)	109(7.6)	-
4	107(100)	108(7.9)	109(7.0)	122(27.7, M+14)
5	107(100)	108(8.9)	109(14.1)	-
6	107(100)	108(7.4)	109(4.5)	122(29.0, M+14); 93(1.6, M-15)
7	121(100)	122(10.9)	123(4.1)	136(14.1, M+14)
8	151(90.8)	152(8.0)	153(15.3)	137(100, M-15); 122(4.1, M-30); 121(10.9, M-31)
9	135(100)	136(9.4)	137(2.0)	150(10.3, M+14)
10	165(70.2)	166(5.3)	167(21.7)	151(100, M-15); 136(22.9, M-30); 135(17.2, M-31)
11	165(100)	166(6.9)	167(8.4)	151(29.7, M-15); 136(1.4, M-30); 135(7.7, M-31)
12	-	-	123(100)	124(6.3, M+2); 137(10.7, M+15); 81(2.2, M-42)
13	-	-	131(100)	132(6.1, M+2); 145(14.1, M+15)

$(M-1)^-$ base peak coming from hydrogen abstraction and a $(M+1)^-$ ion, which is very intense only in the case of unsubstituted pyrazine and medium intense in the presence of long alkyl substituents owing to the possibility of giving cyclic ions. The two 2-methoxy-3-(1-methylalkyl)pyrazines **8** and **10** have ion $(M-15)^-$ as base peak and show ions $(M-30)^{-\cdot}$ and $(M-31)^-$. These ions are formed also with 2-methoxy-3-(2-methylpropyl)pyrazine, but ion $(M-1)^-$ is the base peak *while methyl loss is less favorable.*

In conclusion in these conditions electron capture negative ion production is not a favourite process, probably due to the high analyte concentration. So the ion molecule reaction are very important in respect to low energy processes necessary for an efficient electron capture.

CONCLUSION

We have investigated the methane chemical ionization mass spectrometry of thirteen pyrazine in *ppinici conditions.*

Positive ions give a higher sensivity and the molecular weight can be easily established by the $(M+H)^+$ ion. However at concentrations of 1 mg ml^{-1} intense $M^{+\cdot}$ and $(M+2)^{+\cdot}$, due to reduction, are formed, and this can be a problem both for qualitative and quantitative analysis.

Negative ions are particulary intense in the case of acetylpyrazine and quinoxaline and therefore they can be used, for a selective detection of these two compounds in complex mixtures.

Ppinici ion source conditions must be carefully determined in order to enhance electron capture processes and the optimized concentration of reagent gas and analyte are not the same for positive and negative ion detection.

REFERENCES

1. J.E. Hodge, J.Agric. Food Chem., 1953, **1**, 928.
2. S. Kawamura, "The Maillard Reaction in Foods and Nutrition", eds. by G.R.Waller, M.S.Feather, ACS Symp. Ser. 1983, **215**, p. 3.
3. J.A. Maga and C.E. Sizer, J. Agric. Food Chem., 1973, **1**, 28.
4. J.A. Maga, "Food Flavours. Part. A. Introduction", eds. by I.D.Morton, A.J.MacLeod, Elsevier, Amsterdam, 1982, p. 283.
5. S. Fors, "The Maillard Reaction in Foods and Nutrition", *eds.* by G.R. Waller and M.S. Feather, ACS. Symp. Ser., 1983, **215**, p. 185.
6. J.J. Brophy and G.W.K. Cavill, Heterocycles, 1980, **14**, 477.
7. Q.N. Porter, "Mass Spectrometry of Heterocyclic Compounds", eds. by C. Taylor and A. *Weissberger, II edition,* John Wiley & Sons, New York, 1985, p. 715.
8. S. Daishima and Y. Iida, Shitsuryo Bunseki, 1983, 31, 281, Chem. Abstr., 1984, **101**, 45860a.
9. G.P. Ford and J.D. Scribner, J.Comput.Chem., 1983, **4**, 594.
10. I. Nenner and G.J. Schulz, J. Chem. Phys., 1975, **62**, 1747.
11. A. Arnoldi, C. Arnoldi, O. Baldi, and A. Griffini, J. Agric. Food Chem., in press.

12. M.S.B. Munson and F.H. Field, J. Am. Chem. Soc., 1967, **89**, 1047.

13. R. Gleiter, E. Heilbronner, and V. Hornung, Helv. Chim. Acta, 1972, **55**, 255.

14. P. Bischof, R. Gleiter, and P. Hoffmann, Angew. Chem. Int. Ed. Engl., 1978, **17**, 875.

15. W. Kaim, Angew. Chem. Int. Ed. Engl., 1983, 22, 171.

Received, 1st August, 1988