Solving Quality Problems in Several Flavor Aroma Chemicals

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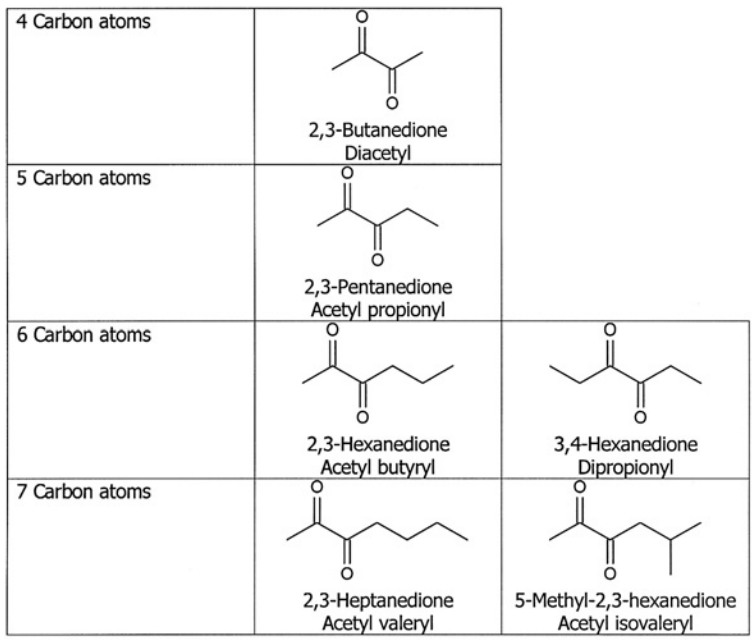
The confusion in the interpretation of some aroma chemicals' molecular structures causes confusion among the users of these products. Several important cases, including keto-enol tautomerisation of α-diketones, isomerisation of α-angelica lactone, and the exact substitution pattern of pyrazines, will be discussed. The analytical methods used to unravel the correct molecular structures were GC, GC-MS and ^{15}N-NMR spectroscopy, and particularly polarization-transfer for sensitivity enhancement (INEPT), and 2D-correlation techniques.

Figure 1. α-Diketones



α-Diketones

α-Diketones are an important group of aroma chemicals that are characterized by two adjacent keto functionalities. Their structure consists of four to seven carbon atoms, as illustrated in in Figure 1.

The flavor characteristics of α-diketones range from creamy, sweet, buttery, caramely and milky, to fruity, roasted and burnt. This group of natural products occurs in many food flavors, including butter, milk, fruits, wine, roasted products, vinegar, yogurt, coffee, raspberry and cheddar cheese.

These materials are applied in dairy flavorings (e.g. margarine, ice cream, butter, cream, etc.), in addition to strawberry, caramel, butterscotch, brown sugar, and custard flavorings. The α-diketones are also used as key raw-materials for pyrazines, including diacetyl for 2,3-dimethylpyrazine, and 3,4-hexanedione for 2,3-diethylpyrazine.

The keto-enol tautomerism of α-diketones: From personal experience, we know that α-diketones' GC analysis causes some confusion with several members of the flavor industry. The following explanation should clarify:

Aldehydes and ketones exist in solution as equilibrium mixtures of two isomeric forms — the keto form and the enol form (Figure 2). We have noticed that this equilibrium in 3,4-hexanedione especially, but also in the other α-diketones, is dependent on the GC conditions, the insert, the insert filling and age, the column, etc. It was proven that upon injecting the material in different GC conditions, this equilibrium changes, especially when using an apolar column — HP-5, for example. When a polar column (i.e. HP-FFAP) was used, only the keto form was detected; thus, both peak areas could be summed up using an apolar column. Even when using an apolar column and different inserts, the same material gave two peaks or one peak (which is the sum of both areas).

Tautomers seen in GC analysis: The enol form can react with BSTFA (Figure 3). The GC-MS of 3,4-hexanedione and the MS of 3,4-hexadione (keto form) are presented in Figures 4 and 5, respectively.

The MS of 3,4-hexanedione (enol form), GC-MS after sylilation with BSTFA and MS of sylilated enol are presented in Figures 6 to 8, respectively.

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Figure 2. The tautomers of α-diketones (keto form and enol form)

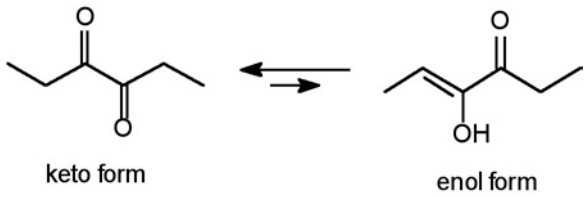


Figure 3. The enol form reacting with BSTFA

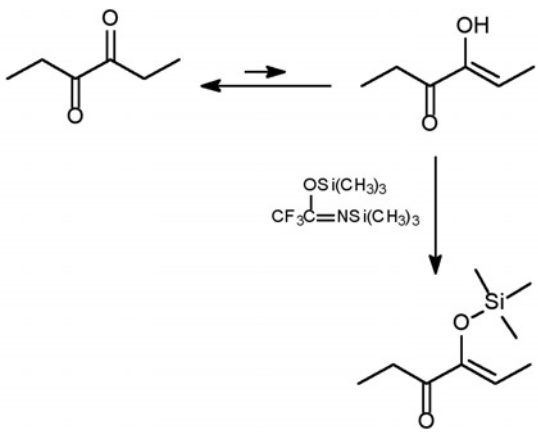


Figure 4. GC-MS of 3,4-hexanedione

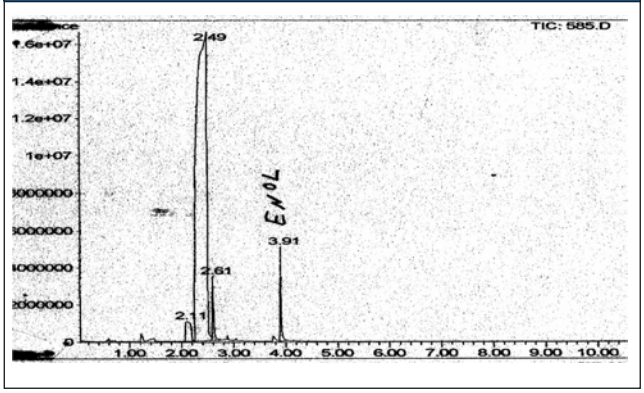


Figure 5. MS of 3,4-hexanedione — keto form

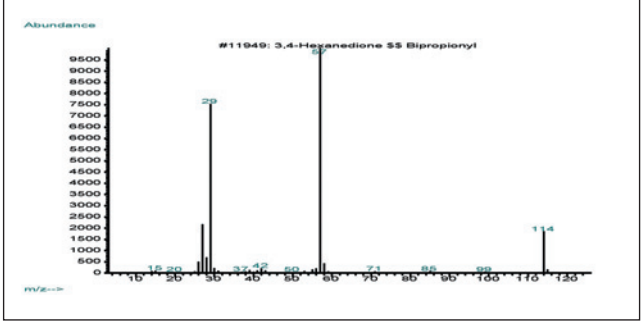


Figure 6. MS of 3,4-hexanedione — enol form

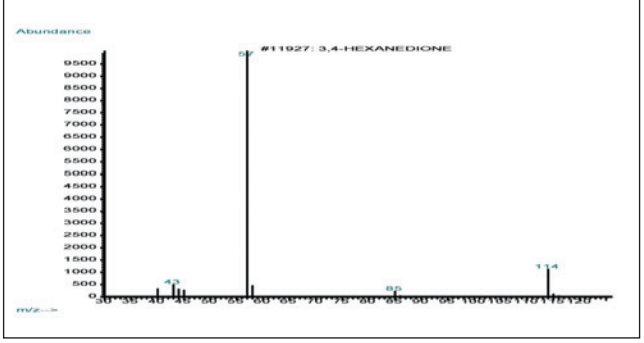


Figure 7. GC-MS after sylilation with BSTFA

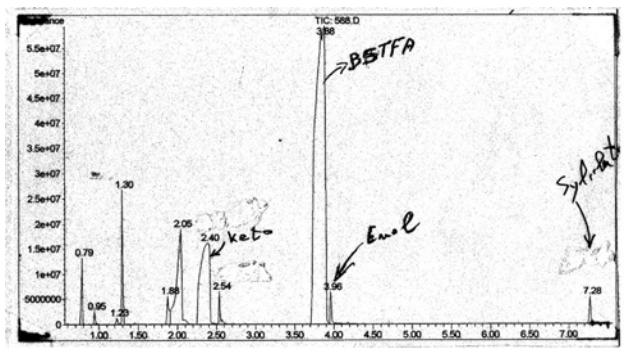


Figure 8. MS of sylilated enol

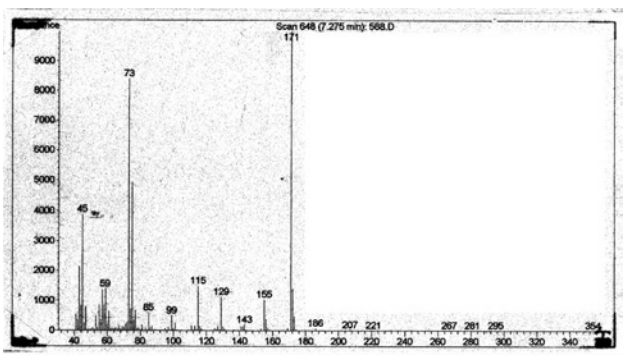
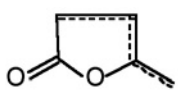


Figure 9. The structure of angelica lactone (5-methyl-2,3H-furanone)



Angelica Lactone

As a consequence of these results, one must take into account the sum of the two peaks (keto and enol), while calculating the purity of α-diketones, and not simply the major keto form peak.

α-Angelica Lactone

α-Angelica lactone occurs in grapes (dried), bread, soybeans and licorice. The material has a sweet, creamy, coconut, and vanilla flavor, with hay- and coumarin-like nuances. α-Angelica lactone is applied in aromatic and dairy formulations, and coconut, vanilla, molasses, coumarin, cream, milk, nut, and tobacco flavors. The structure of angelica lactone (5-methyl-2,3H-furanone) is illustrated in Figure 9.

This molecule is comprised of three isomers, of which α is the major (Figure 10).

As seen, there is a possibility for the double bond electrons to migrate. This phenomenon is affected by different parameters — temperature and pH, for example. When a sample of α-angelica lactone is injected to a gas chromatograph, it is influenced by the injector temperature, the form and acidity of the insert, the column conditions, its history, and other parameters. The GC and MS of angelica lactone are presented in Figure 11 and 12, respectively.

Due to its sensitivity, α-angelica lactone isomerizes during a GC run to give a certain isomeric partition, as shown in the chromatogram presented in Figure 13.

Another result of this phenomenon can be observed in the chromatogram — the cohesion of the peaks — which results from the isomerization taking place on column during the GC run. The same material was injected after injection of 5 µL diethylamine to change the column pH and to show the isomer partition presented in Figure 14.

Table 1 and Figure 15 show the isomer partition before and after diethylamine injection.

As in the former example, α-angelica lactone, one must take into account that different partition patterns of the three isomer peaks are obtained in relation to different parameters in the GC — for example, temperature and pH.

The Substitution Pattern of Pyrazines

Pyrazines are materials obtained in Maillard reactions as byproducts of the browning reaction of sugars and proteins or amino acids. These reactions occur during roasting, cooking, baking, etc., of different food products. The importance of these materials motivated organic chemists to synthesize and use them as flavor ingredients in formulations for roasted nuts and meat flavors, among others.

By 1970, the first pyrazines obtained GRAS status in the United States. However, as a result of the lack of modern analytical tools, the exact structures were not defined. For example, a mixture of isomers of methyl-methoxy-pyrazines was determined in an inexact manner, namely 2-methoxy-3(5 or 6)-methyl-pyrazine.

The problem of the exact structure of several commercially significant pyrazines arises when examining the product

Figure 10. The molecule of angelica lactone is comprised of three isomers, of which α is the major

Figure 11. GC of angelica lactone

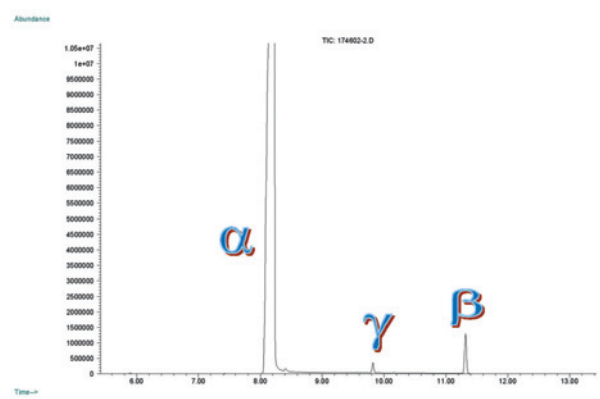


Figure 12. MS of angelica lactone

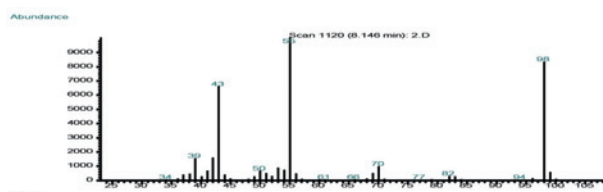


Figure 13. GC of α-angelica lactone

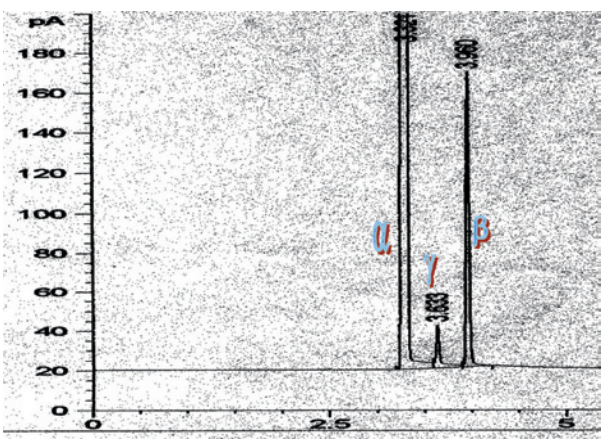


Figure 14. GC of α-angelica lactone after diethylamine injection

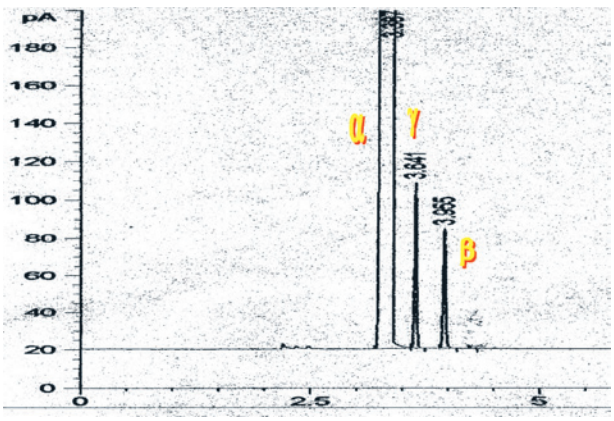


Figure 15. The isomer partition before and after diethylamine injection

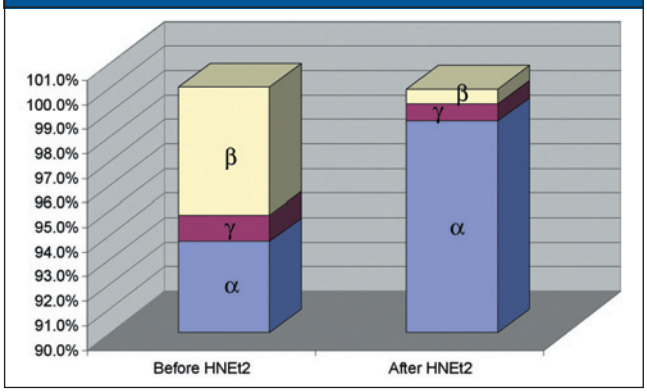


Figure 16. 2-methyl-5- or 6-methoxy- pyrazine

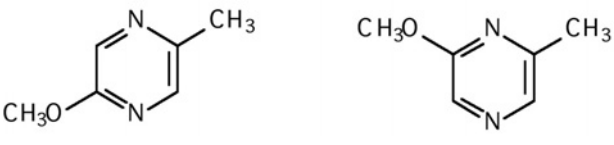


Table 1. The isomer partition before and after diethylamine injection

	α	γ	β
Before $HNEt_2$ injection	93.7%	1.1%	5.3%
After $HNEt_2$ injection	98.6%	0.7%	0.6%

lists of some important pyrazine manufacturers. The ambiguous pyrazines are mainly those that contain a substituent at position number 5 or 6, in addition to a substituent at position number 2, for example 2-methyl-5 or 6-methoxypyrazine (Figure 16).

In this work, the correct substitution pattern of disubtituted pyrazines was evaluated. The method used to elucidate the structures of these rather simple, but puzzling, molecules was the application of nitrogen NMR spectroscopy — or, to be more exact, ^{15}N-NMR spectroscopy.[1]

The INEPT spectrum of 2-methyl-5- or 6-methoxypyrazine, and heteronuclear correlated 2D NMR spectrum (HMBC) of 2-methyl-5-methoxy- or 2-methyl-6-methoxypyrazine are presented in Figures 17 and 18, respectively.

The substitution pattern of di- or trisubstituted pyrazines can be elucidated by a combination of NMR methods, especially in mixtures by gradient selected ^{1}H-,^{15}N-HMBC experiments at a natural abundance level. In the case of disubstituted pyrazines, this method allows one to distinguish between 2,3-/2,5- and 2,6-disubstituted isomers. Another advantage is the determination of ^{15}N chemical shifts, including the assignments in even relatively low concentrations. The reliability of this method was verified by experimental data resulting from ^{1}H-NMR and long-range heteronuclear correlation experiments such as FLOCK or ^{1}H-,^{13}C-HMBC experiments. Carbon-carbon coupling constants derived from INADEQUATE experiments were taken into account as additional tests. Thirty-one commercially available di- and trisubstituted pyrazines were measured in order to identify the substitution pattern and to obtain chemical-shift information.^{15}N-NMR data of di- and trisubstituted alkyl pyrazines (alkyl groups [only] place the ^{15}N chemical shifts to the range of -44 to -48 ppm) is presented in Figure 19[2].

Figure 20 illustrates the ^{15}N-NMR data of substituted alkoxy pyrazines. Alkoxy groups placed the ^{15}N chemical shifts to the range of -93 to -101 ppm.

Alkyl groups in the presence of alkoxy substituents placed the ^{15}N chemical shifts in a range of -38 to -46 ppm.

Figure 21 illustrates ^{15}N-NMR data of substituted thioalkoxy pyrazines. Thioalkoxy groups placed the ^{15}N chemical shifts in the range of -58 to -61 ppm. The alkyl groups in presence of thioalkoxy substituents placed the ^{15}N chemical shifts within the range of -46 to -54 ppm.

Figure 22 illustrates ^{15}N-NMR data of disubstituted acetyl pyrazines. Acetyl groups placed the ^{15}N chemical shifts to the range of -42 to -44 ppm. The alkyl groups in presence of acetyl substituents placed the ^{15}N chemical shifts within the range of -38 to -44 ppm.

Figure 23 illustrates ^{15}N-NMR data of di- and substituted chloro pyrazines. Chloro groups placed the ^{15}N chemical shifts to the range of -52 to -53 ppm. The alkyl groups in presence of chloro substituents placed the ^{15}N chemical shifts within the range of -37 to -53 ppm.

Figure 24 illustrates the ^{15}N-NMR data of substituted pyrazines.

To summarize, alkyl substituents cause nitrogen atoms to resonate in the range of -35 to -55 ppm.

Alkoxy groups cause nitrogen atoms to resonate in the range of -93 to -101 ppm.

Thioalkoxy substituents cause nitrogen atoms to resonate in the range of -58 to -61 ppm.

Figure 17. INEPT spectrum of 2-methyl-5- or 6-methoxypyrazine

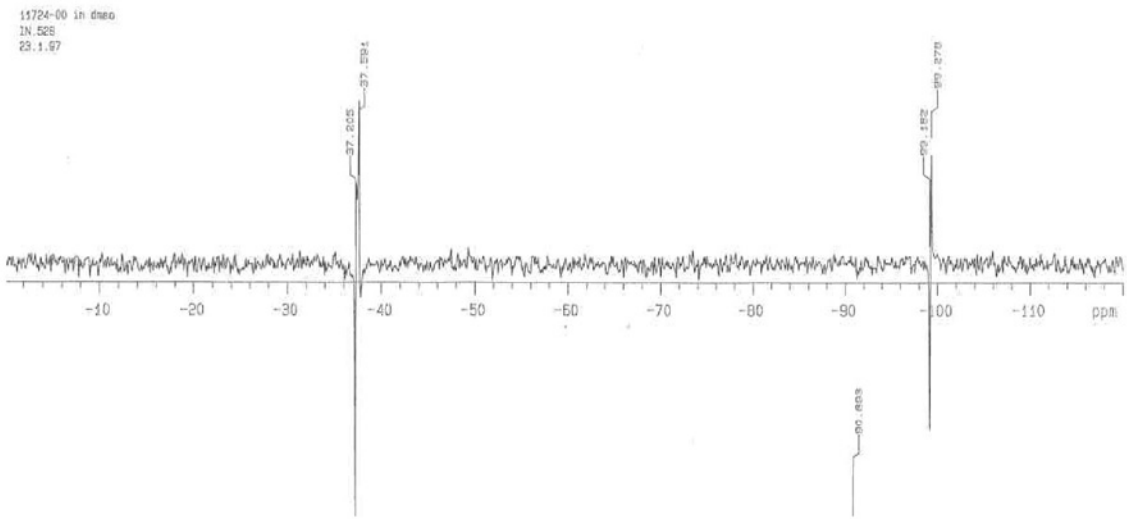


Figure 18. Heteronuclear correlated 2D NMR spectrum (HMBC) of 2-methyl-5-methoxy- or 2-methyl-6-methoxypyrazine

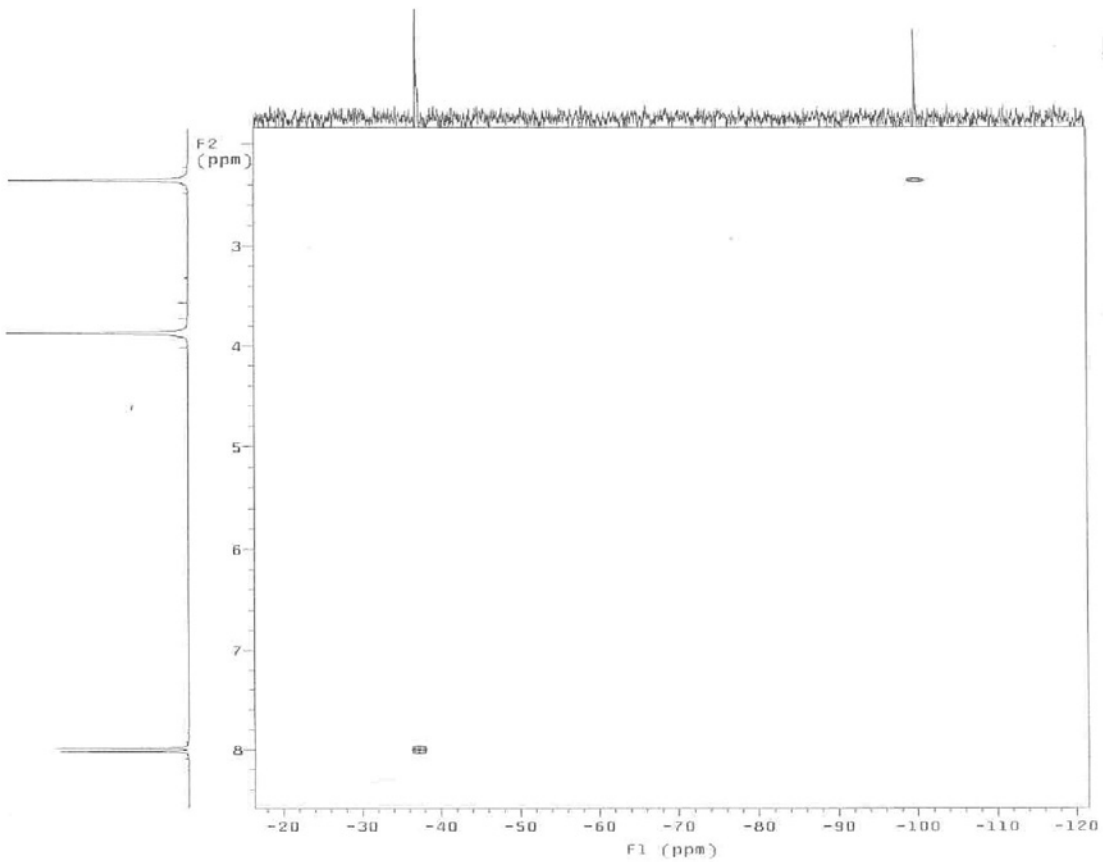


Figure 19. ^{15}N-NMR data of di- and trisubstituted alkyl pyrazines (alkyl groups (only) place the ^{15}N chemical shifts to the range of -44 to -48 ppm)[2]

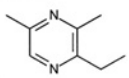
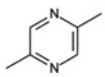
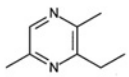
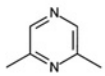
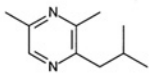
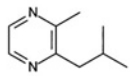
Alkylpyrazine	δ N-1	δ N-4	Alkylpyrazine	δ N-1	δ N-4
	-46.6	-46.6		-48.1	-45.6
	-45.8	-45.8		-44.8	-46.8
	-44.5	-47.2		-45.1	-46.0
	-48.1	-46.4			

Figure 20. ^{15}N-NMR data of substituted alkoxy pyrazines; alkoxy groups placed the ^{15}N chemical shifts to the range of -93 to -101 ppm; alkyl groups in the presence of alkoxy substituents placed the ^{15}N chemical shifts in a range of -38 to -46 ppm

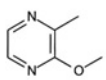
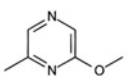
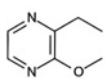
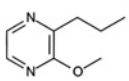
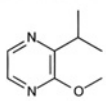
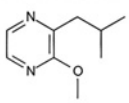
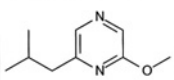
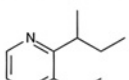
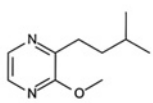
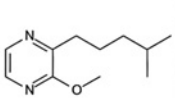
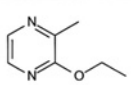
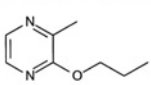
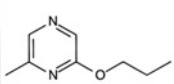
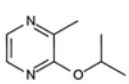
Alkoxypyrazine	δ N-1	δ N-4	Alkoxypyrazine	δ N-1	δ N-4
	-100.9	-41.7		-94.4	-37.9
	-100.9	-43.6		-100.1	-42.8
	-99.8	-45.7		-100.8	-40.3
	-94.6	-38.6		-100.3	-44.5
	-100.8	-42.6		-100.4	-42.0
	-93.5	-38.3		-100.7	-42.1
	-93.6	-38.3		-99.8	-42.4

Figure 21. ^{15}N-NMR data of substituted thioalkoxy pyrazines: thioalkoxy groups placed the ^{15}N chemical shifts in the range of -58 to -61 ppm; the alkyl groups in presence of thioalkoxy substituents placed the ^{15}N chemical shifts within the range of -46 to -54 ppm

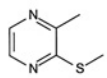
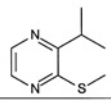
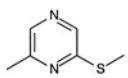
Thioalkoxypyrazine	δ N-1	δ N-4	Thioalkoxypyrazine	δ N-1	δ N-4
	-60.7	-50.0		-59.0	-54.0
	-58.4	-46.6			

Figure 22. ^{15}N-NMR data of disubstituted acetyl pyrazines: acetyl groups placed the ^{15}N chemical shifts to the range of -42 to -44 ppm; the alkyl groups in presence of acetyl substituents placed the ^{15}N chemical shifts within the range of -38 to -44 ppm

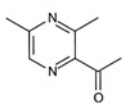
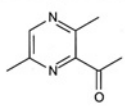
Acetylpyrazine	δ N-1	δ N-4	Acetylpyrazine	δ N-1	δ N-4
	-42.3	-40.6		-44.1	-38.6

Figure 23. ^{15}N-NMR data of di- and substituted chloro pyrazines: chloro groups placed the ^{15}N chemical shifts to the range of -52 to -53 ppm; the alkyl groups in presence of chloro substituents placed the ^{15}N chemical shifts within the range of -37 to -53 ppm

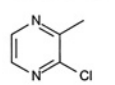
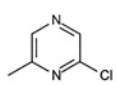
Chloropyrazine	δ N-1	δ N-4	Chloropyrazine	δ N-1	δ N-4
	-52.0	-37.0		-52.9	-35.1

Figure 24. ^{15}N-NMR data of substituted pyrazines

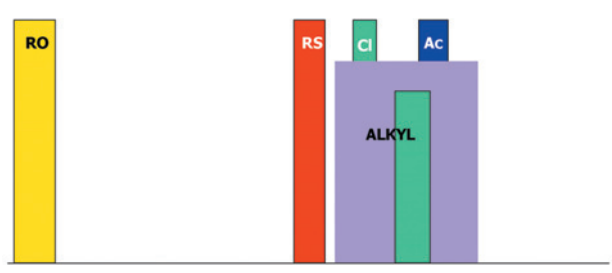


Figure 25. ^{15}N, ^{1}H and ^{13}C NMR shift data of pyrazine and monosubstituted pyrazines

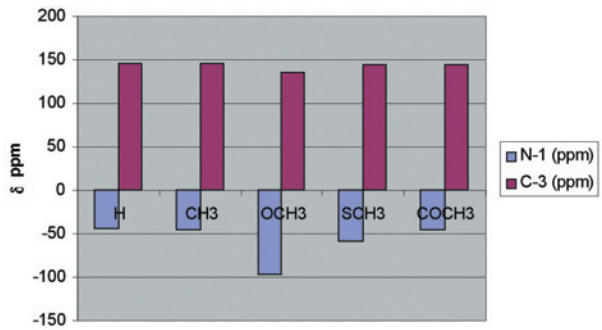


Figure 26. Effect of electronic properties of substituents on pyrazines' chemical shifts

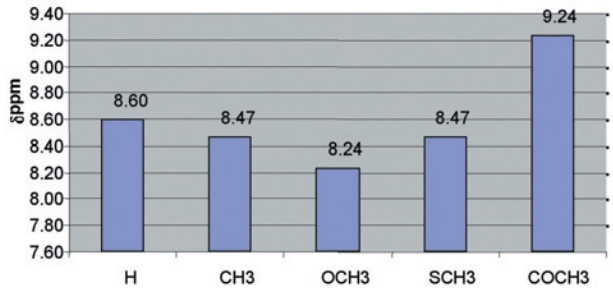
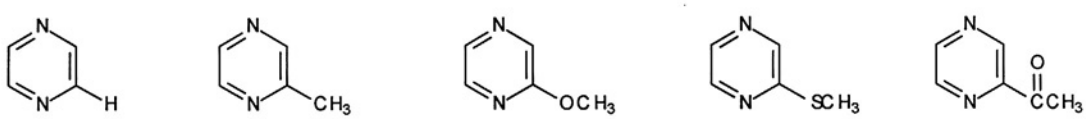


Figure 27. Effect of electronic properties of substituents on pyrazines' chemical shifts: the largest low-frequency shift of N-1 was found in 2-methoxypyrazine, which was attributed to the strong π-donating nature of the methoxy group

R	δ (^{15}N) [ppm]		δ (^{1}H) [a] [ppm]				δ (^{13}C) [ppm]				
	N1	N4	H3	H5	H6	R	C2	C3	C5	C6	R
H	-43.9	-43.9	8.60	8.60	8.60		145.2	145.2	145.2	145.2	
CH_3	-45.2	-44.1	8.471	8.382	8.462	2.576	154.2	144.9	141.9	143.9	21.6
OCH_3	-97.6	-37.3	8.236	8.117	8.089	3.971	160.6	136.0	136.5	140.6	53.5
SCH_3	-59.4	-47.4	8.465	8.192	8.365	2.574	157.7	143.5	139.1	143.8	12.6
$O=C-CH_3$	-45.6	-41.0	9.236	8.753	8.646	2.728	147.7	143.5	147.8	143.6	25.8 [b]



Acetyl groups cause nitrogen atoms to resonate in the range of -42 to -44 ppm.

Chlorine atoms cause nitrogen atoms to resonate in the range of -52 to -53 ppm.

Figure 25 illustrates ^{15}N, ^{1}H and ^{13}C NMR shift data of pyrazine and monosubstituted pyrazines. Table 2, Figure 26 and Figure 27 show the ^{15}N chemical shifts for N-1 and N-4, the ^{1}H chemical shifts for H-3, H-5, H-6 and RH, and the ^{13}C chemical shifts of C-2, C-3, C-5, C-6 and R. The largest low-frequency shift of N-1 was found in 2-methoxypyrazine, which was attributed to the strong π-donating nature of the methoxy group.

Table 3 and Figure 28 illustrate the effects of the chemical shifts of substituted pyrazines. Similar trends were observed in the chemical shift movements of H-3 (in the ^{1}H-NMR), C-3 (in the ^{13}C-NMR) and N-1 (in the ^{15}N-NMR) resonances: methyl and methoxy groups shifted upfield, thiomethoxy and acetyl groups shifted downfield.

Figure 29 illustrates the effect on chemical shifts of substituted pyrazines vs. benzenes.

When comparing the effect on the chemical shift of the *ortho* hydrogen atom of a pyrazine and a benzene ring, it seems that the effects are opposite.

Conclusion

While calculating the purity of α-diketones, one must take into account the sum of the two peaks (keto and enol), and not simply the major keto form peak.

Angelica lactone (5-methyl-2,3H-furanone) exists as an equilibrium of three isomers of the double bond, which show different GC patterns under different GC conditions.[15] N-NMR data of 31 different pyrazines were measured and evaluated.

Figure 28. The effect on the chemical shifts of substituted pyrazines

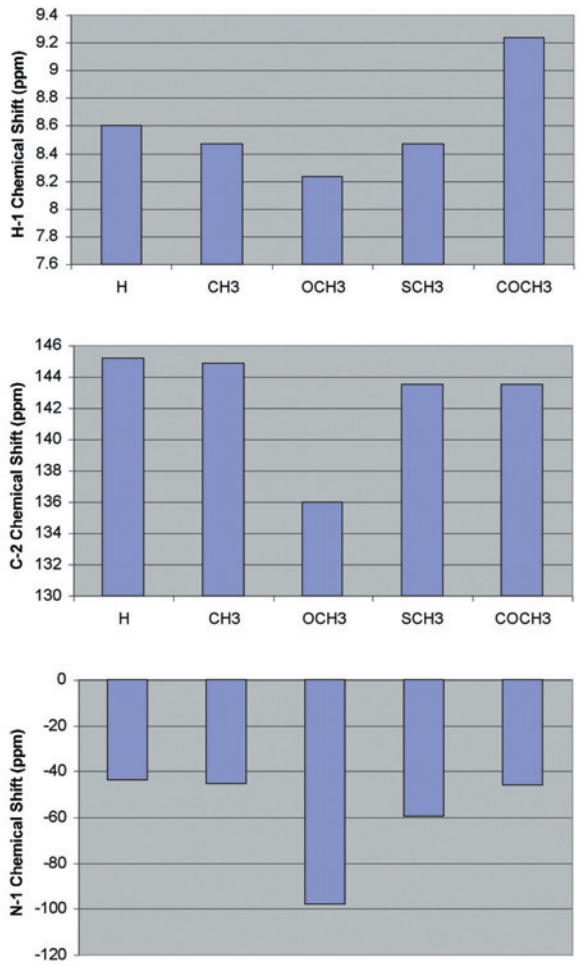


Figure 29. Effect on chemical shifts of substituted pyrazines vs. benzenes: when comparing the effect on the chemical shift of the ortho hydrogen atom of a pyrazine and a benzene ring, it seems that the effects are opposite

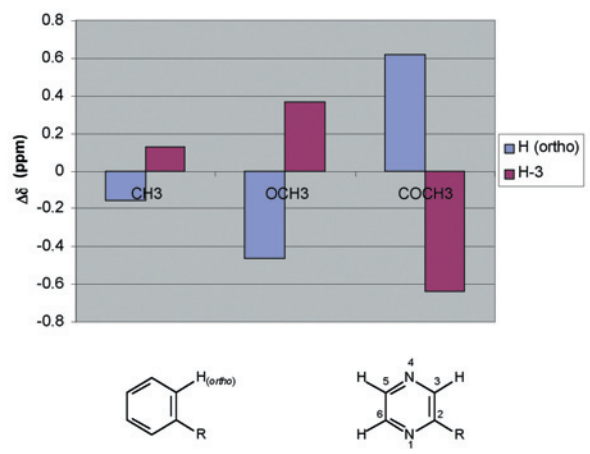


Table 2. Effect of electronic properties of substituents on pyrazines' chemical shifts

Substituent	N-1 (δppm)	C-3 (δppm)	H-3 (δppm)
H	-43.9	145.2	8.6
CH_3	-45.2	144.9	8.47
OCH_3	-97.6	136.0	8.24
SCH_3	-59.4	143.5	8.47
$COCH_3$	-45.6	143.5	9.24

Table 3 The effects of the chemical shifts of substituted pyrazines

Substituent	H-3 ^{1}H-NMR	C-3 ^{13}C-NMR	N-1 ^{15}N-NMR
CH_3	Upfield	Upfield	Upfield
OCH_3	Upfield	Upfield	Upfield
SCH_3	Downfield	Downfield	Downfield
$COCH_3$	Downfield	Downfield	Downfield

Table 4. Effect on chemical shifts of substituted pyrazines vs. benzenes: when comparing the effect on the chemical shift of the *ortho* hydrogen atom of a pyrazine and a benzene ring, it seems that the effects are opposite

Substituehnt	H (*ortho*)	Effect	H-3	Effect
CH_3	-0.16	Downfield	+0.13	Upfield
OCH_3	-0.46	Downfield	+0.36	Upfield
$COCH_3$	+0.62	Upfield	-0.64	Downfield

Chemical shifts ranges were determined for alkyl, alkoxy, thioalkoxy, acetyl and chloro substituents. The effect of electronic properties of substituents on chemical shifts of pyrazines in ^{15}N-, ^{13}C- and ^{1}H-NMR spectra was studied. These data enable us to better understand the ^{15}N-NMR of pyrazines, and to evaluate and predict their structure more accurately.

References

1. M. Zviely, A. Kern, I. Gozlan and R. Frim, *Studies Towards Structure Determination of Substituted Pyrazines*, Perfum. Flav., 23, 27, 1998.

2. H. Sommer, H.J. Bertram, G.E. Krammer, C.O. Schmidt, W. Stumpe, P. Werkhoff and M. Zviely, *Determination of the Substitution Patterns of Various Di- and Trisubstituted Pyrazines by 15N, 13C and 1H NMR Spectroscopy*, Magnetic Resonance in Chemistry, 38, 907, 2000. ■