CSIRO PUBLISHING

Aust. J. Chem.
http://dx.doi.org/10.1071/CH14273

Full Paper

2-Cyanobenzoic Acids: Tautomeric Equilibria Study

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Evidence of ring–chain tautomerism in the gas phase from mass spectrometry and in solution determined by means of ^{1}H NMR and ^{13}C NMR is reported for 2-cyanobenzoic acids. The analysis of the corresponding spectra has allowed specific assignment of fragment ions to tautomers. The predictive value of this methodology is supported by the influence of the substitution pattern of these compounds on this equilibrium.

Manuscript received: 13 March 2014.
Manuscript accepted: 19 June 2014.
Published online: 25 August 2014.

Introduction

The understanding of the nature of tautomeric equilibria is relevant to the study of processes of both organic chemistry and biochemistry.[1] Proton transfer and hydrogen bonding are two important behavioural aspects regarding structure and reactivity of simple compounds[2,3] and complex substances, from water to DNA.[4,5] Tautomerism interconversions[6,7] have been investigated by chemists during the last decades. The importance of tautomerism is evident, given that in recent years investigation about tautomerism has been the major topic in theoretical chemistry. For example, tautomerism in keto–enol,[8,9] imine–enamine[10,11] and many other systems[12] has been studied during the past decades.

Tautomerization and proton transfer within keto–enol tautomerism in heterocyclic systems with several basic centres (N, O, and S atoms) are of great interest to medicinal and biochemical applications. Understanding of the relative stabilities of heterocyclic tautomers and any subsequent conversions between tautomeric forms is also very important for both structural chemists and biologists.[13,14] Regarding this line of research, relative stabilities of various tautomeric structures of five-, six-, and seven-membered heterocyclic rings have been investigated using both theoretical and experimental tools.[15–18]

A special kind of tautomerism, which has been studied in the present work, is ring–chain tautomerism, where the hydrogen shift is followed by a change in molecular structure from an open chain to a ring. What is needed to make this tautomerism possible is that the open-chain tautomer has at least two functional groups, one containing a multiple covalent bond and the other one able to produce an addition to a multiple bond reaction;[19] 2-cyanobenzoic acids show these two characteristics.

Mass spectrometry represents a very sensitive method for the study of tautomeric equilibria because it is capable of detecting tautomeric forms that make only minor contributions and that might be undetected using other techniques.[20–23]

It has been demonstrated in the case of keto–enol tautomerism of a variety of carbonyl and thiocarbonyl compounds[24–30] that there is no significant interconversion of the tautomeric forms in the gas phase following electron-impact ionization in the ion source of the mass spectrometer before fragmentation (molecular ions, $M^{+\bullet}$, do not seem to undergo unimolecular tautomerization). Besides, for gas chromatography–mass spectrometry experiments (GC-MS), once the solvent is separated after injection in the injection port of the gas chromatograph, tautomerisation mechanisms (intermolecular or unimolecular) do not seem to take place even without chromatographic separation of the tautomers (under the experimental conditions selected). These conclusions are supported by temperature studies at the ion source (negligible effect) and at the injection port of the gas chromatograph (shifts of the relative abundances of tautomer-specific ions are in agreement with the corresponding heats of tautomerization).[30] In fact, tautomerism would take place very fast in the injection port of the GC under working conditions.

Separation of tautomers in analytical columns is usually very difficult, even though it has been carried out successfully for some compounds (previous work has reported chromatographic separation of the tautomeric forms for β-ketoesters[31]).

Consequently, the different fragmentation pathways of the tautomeric forms can be used for identification of individual tautomers.[24–30] For this reason and because of the high similarity between MS (commercial databases) and GC-MS spectra (the GC separation would not contribute to the complex distribution of internal energies of the ions formed in the ion source), analytical separation has not been considered critical for the present work. Analogously, it is thought that most of the conclusions could be useful to analyse spectra recorded with mass spectrometers equipped with direct insertion probes.

In the present work, a study on ring–chain tautomerism in 2-cyanobenzoic acids was carried out by means of mass spectrometry. The effects of substituent size are analysed.

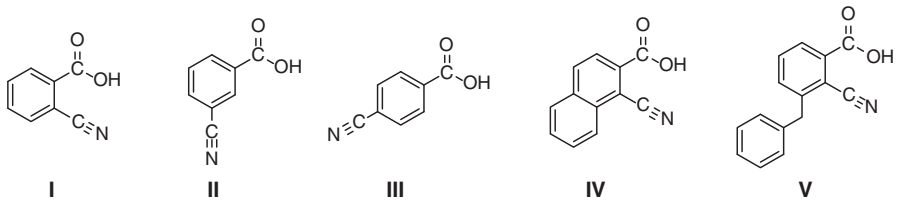


Chart 1.

In order to find additional evidence, 3-cyano and 4-cyanobenzoic acids, which would be unable to show this kind of tautomerism, were analysed as well.

Materials and Methodology

The compounds under study, 2-cyanobenzoic acid (**I**), 3-cyanobenzoic acid (**II**), 4-cyanobenzoic acid (**III**), 1-cyano-2-naphtoic acid (**IV**), and 3-benzyl-2-cyanobenzoic acid (**V**), were synthesised adapting procedures from the literature (Chart 1).[32] Given that 2-cyanobenzoic acid isomerizes to phthalimide over time, structural analysis was carried out with freshly prepared compound. The structures were confirmed by ^{1}H and ^{13}C NMR.

Structural Determinations

Gas Chromatography–Mass Spectrometry – Single Quadrupole

Mass spectra were obtained by injection of methanol solutions of the relevant compounds (1 μL, 100 μg mL^{-1}) into an HP 5890 chromatograph coupled to an HP 5972A mass selective detector (unit mass resolution). An HP5-MS capillary column (30 m × 0.25 mm × 5 μm) was used with helium as the carrier gas (0.6 mL min^{-1} in column, split ratio 1 : 30). The temperature set points were: 175°C in the split injector, 300°C in the interface, 185°C in the ion source, and the oven ramp started at 40°C (5 min) and ended at 290°C with a heating rate of 20°C min^{-1}. The electron energy was 70 eV and the pressure in the mass spectrometer was lower than 10^{-5} torr (10^{-3} Pa), thus precluding ion–molecule reactions. Isotopic exchange was performed by dissolution of the corresponding compound (10 mg) in [D1]methanol (1 mL). Mass spectra were analysed 1 h after dissolution.

Gas Chromatography–Mass Spectrometry – Ion Trap

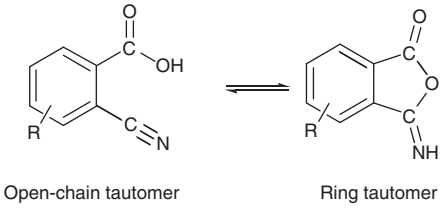
These determinations were performed by injection of methanol solutions (1 μL) in a Thermo Quest Trace 2000 coupled to a Finnigan Polaris ion-trap detector (unit mass resolution) under the same experimental conditions as mentioned for the single quadrupole GC-MS system. This instrumentation was utilised to confirm proposed fragmentation pathways by CID (collision-induced dissociation) using helium as the damping gas, a CID voltage of 4–7 eV, and an excitation energy of 0.30–0.45 eV (values were optimized for each ion transition). These experiments were done by selecting a precursor ion from the full-scan spectrum and carrying out the corresponding MS-MS product ion scan.

Nuclear Magnetic Resonance

^{1}H NMR spectra were recorded with a Varian Mercury Plus spectrometer operating at 4.7 T. The typical spectral conditions were as follows: spectral width 3201 Hz, acquisition time 4.09 s, and 16 scans per spectrum. Digital resolution was 0.39 Hz per point. Deuterium from the solvent was used as the lock and TMS as the internal standard. Sample concentration was 20 mg mL^{-1}. Measurements were performed at 25°C in deuterated dimethyl sulfoxide ([D6]DMSO).

^{13}C–proton decoupled and gated decoupled spectra were recorded with the same spectrometer from [D6]DMSO solutions at 25°C. The spectral conditions were as follows: spectral width 10559 Hz, acquisition time 1.303 s, and 1000 scans per spectrum. Sample concentration was 40 mg mL^{-1} and digital resolution was 1.29 Hz per point.

A standard one-dimensional (1D) proton NMR spectrum and a carbon spectrum with broad-band proton decoupling were run for each sample, supplemented by 2D gradient-selected NMR correlation spectroscopy (COSY) and multiplicity-edited heteronuclear single quantum coherence spectroscopy (HSQC) experiments to support the signal assignment. All 2D spectra were recorded with the same spectrometer.



Scheme 1. Ring–chain tautomerism in 2-cyanobenzoic acids.

Computational Procedure

Calculations were performed using the *GAUSSIAN 03*[33] program package. Optimum equilibrium geometries were computed for the ground states of all pertinent molecular systems and molecular ions using the density functional theory (DFT) B3LYP with 6–31G(d,p) basis sets. Numerous conformations were computed in order to ensure that the lowest-energy conformation was obtained for each molecular system and molecular ion.

Results and Discussion

Gas Chromatography–Mass Spectrometry

Scheme 1 shows ring–chain tautomerism in 2-cyanobenzoic acids. In a previous study, mass spectra were reported for the same compounds. There are major differences in the relative intensities (~10 %) between these data and the present work.[34]

Considering these differences, the measurements were repeated in triplicate and the experimental techniques (injection volume, concentration, temperature, and solvent) were improved. The values reported in the present work are the result of the average of the three measurements.

Table 1. Relevant mass spectra data (percentage relative abundance of ion fragments) of selected cyanobenzoic acids at an injection temperature of 448 K

Relative abundance error is ±10 %

Compound	$[M]^{+\bullet}$	$[M - OH]^+$	$[M - CO_2H]^+$	$[M - CO_2]^{+\bullet}$	$[M - OCNH]^{+\bullet}$	$[M - OCN]^+$
I	58.0	65.3	40.5	100	48.3	35.2
II	82.0	100	46.0	0	0	0
III	54.7	100	52.3	0	0	0
IV	100	42.5	50.3	19.0	5.2	6.0
V	8.0	72.0	19.7	9.4	21.7	8.2

Table 2. Mass spectral data of selected cyanobenzoic acids (percentage relative intensity) at different ion source temperatures at an injection temperature of 448 K

Relative abundance error is ±10 %

	Temperature [K]	$[M]^{+\bullet}$	$[M - OH]^+$	$[M - CO_2H]^+$	$[M - CO_2]^{+\bullet}$	$[M - OCNH]^{+\bullet}$	$[M - OCN]^+$
I	405	58.2	65.1	40.6	100	48.1	35.1
	458	58.0	65.3	40.5	100	48.3	35.2
	503	58.1	65.2	40.6	100	48.3	35.2
II	405	82.0	100	45.9	0	0	0
	458	82.0	100	46.0	0	0	0
	503	82.1	100	45.9	0	0	0
III	405	54.7	100	52.2	0	0	0
	458	54.7	100	52.3	0	0	0
	503	54.6	100	52.2	0	0	0
IV	405	100	42.5	50.4	19.0	5.1	6.0
	458	100	42.5	50.3	19.0	5.2	6.0
	503	100	42.5	50.3	19.1	5.1	5.9
V	405	8.1	72.0	19.7	9.3	21.7	8.3
	458	8.0	72.1	19.7	9.4	21.7	8.2
	503	8.0	72.0	19.8	9.4	21.7	8.2

A detailed study of the mass spectra of selected cyanobenzoic acids was carried out (Table 1), and fragmentation pathways for each tautomer were proposed considering the specificity of their assignments.

The relevance of spectrometric data as a predictive tool in regard to tautomeric equilibria depends mainly on the fact that the contribution due to tautomerization of molecular ions in the gas phase can be ignored or tautomerization does not take place. The importance of this point comes from the physicochemical properties of ionic and radical species, quite different from the neutral ones. As temperature changes are relevant to the determination of enthalpy differences, both sample introduction system (GC) and ion source (MS) temperatures were modified to find evidence regarding the role of neutral and ionic species in the occurrence of tautomerism. In the case of the studied compounds, no significant changes were observed when the ion source temperature was modified (Table 2) but changes did occur when changing the injection temperature (Table 3).

Further, the heats of formation for the molecule and molecular ion obtained by the DFT B3LYP method were calculated, and a good correlation with experimental data was only found in the case of the neutral molecule.

Calculations have shown that the tautomerisation energy barriers for the molecule and molecular ion are similar. They are both high and $\sim$64 kcal mol^{-1} (1 kcal mol^{-1} = 4.186 kJ mol^{-1}). This fact, added to the correlation between the experimental data and those calculated for the neutral molecule, constitute evidence in favour of the hypothesis of this paper, which is that there is no interconversion of tautomers in the mass spectrometer ion source and thus each tautomer yields distinct mass spectra.

Samples were injected into the chromatograph under different conditions, but it was not possible to achieve separation of tautomers. Coexisting tautomers are not separated by chromatography under these conditions; the observed mass spectra are the result of the superposition of the mass spectra of individual tautomers, so that accurately known fragments should be selected for proper comparison. Structures of fragments were confirmed using analysis of fragment ions of deuterated analogues and tandem mass spectrometry.

The mass spectrum of 2-cyanobenzoic acid is shown in Fig. 1.

The occurrence of the both ring and open-chain tautomers is clear from the analysis of the main fragment peaks because there are fragments ions that can only be explained through each tautomer separately.

The peaks at *m*/*z* 130 and 102 can be assigned to the open-chain form (Scheme 2).

The fragment ions at *m*/*z* 103, 104 and 105 can only be justified by the existence of the ring tautomer (Scheme 3).

The fragment ions at *m*/*z* 76 and 77 are generated from both tautomeric structures.

Table 3. Mass spectral data of selected cyanobenzoic acids at different sample introduction temperatures
Relative abundance error is ±10 %

	Temperature [K]	$[M]^{+\bullet}$	$[M - OH]^+$	$[M - CO_2H]^+$	$[M - CO_2]^{+\bullet}$	$[M - OCNH]^{+\bullet}$	$[M - OCN]^+$
I	448	58.0	65.3	40.5	100	48.3	35.2
	473	58.2	71.8	44.6	100	48.1	35.0
	498	58.6	77.5	48.8	100	48.0	34.9
	523	59.0	83.8	52.9	100	47.8	34.8
IV	448	100	42.5	50.3	19.0	5.2	6.0
	473	100	43.2	51.5	18.6	5.0	5.7
	498	100	43.8	52.9	18.0	4.7	5.4
	523	100	44.3	53.5	17.3	4.5	5.2
V	448	8.0	72.0	19.7	9.4	21.7	8.2
	473	8.1	73.2	20.0	9.4	21.9	8.1
	498	8.0	74.4	20.5	9.5	22.2	8.1
	523	8.0	75.0	20.6	9.5	22.1	8.0

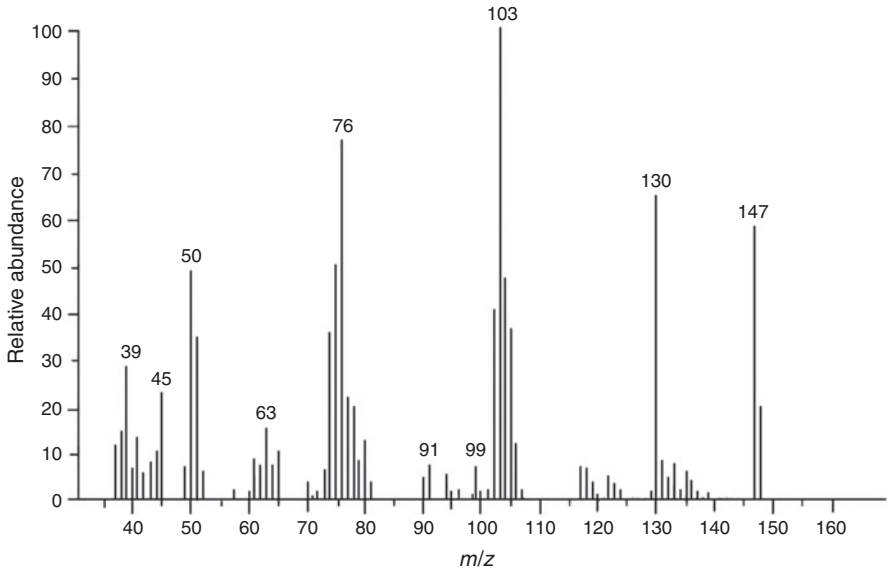
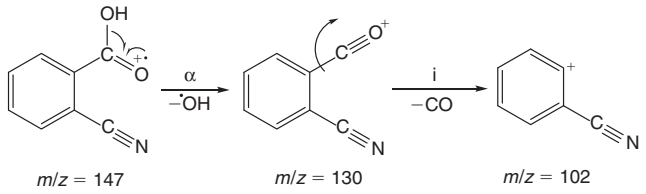


Fig. 1. Mass spectrum of 2-cyanobenzoic acid.



Scheme 2. Specific fragmentations for the open-chain tautomer (valid for 2-, 3-, and 4-cyanobenzoic acids).

In order to support the specifity of the proposed fragmentation pathway, isotopic exchange with [D1]methanol was carried out for 2-cyanobenzoic acid.

No changes were observed for the peaks at *m*/*z* 130, 105, 104, 102, and 76. The *m*/*z* 103 (base peak) became *m*/*z* 104. In the area of the molecular ion, the following peaks were observed: *m*/*z* 147, 148, 149, and 150.

Additional support for these fragmentation pathways was provided by the precursor ion scan of ions *m*/*z* 147, 130, and 105 in the mass spectrum of 2-cyanobenzoic acid.

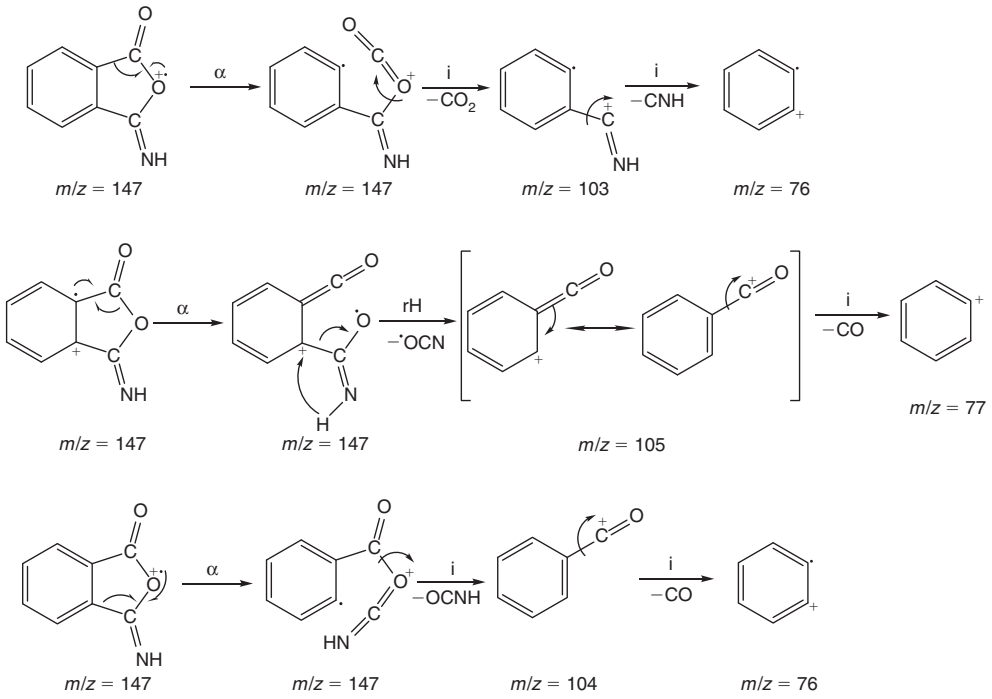
Ions at *m*/*z* 103.1 (relative abundance 100 %), 104.3 (relative abundance 23 %), 105.1 (relative abundance 48 %), and 130.1 (relative abundance 68 %) are generated from the molecular ion at *m*/*z* 147.2 (relative abundance 52 %).

It was also observed that the ion at *m*/*z* 102.3 (relative abundance 100 %) comes from the one at *m*/*z* 130.1 (relative abundance 45 %) and the ion at *m*/*z* 77 (relative abundance 53 %) comes from the ion at *m*/*z* 105 (relative abundance 80 %).

Fig. 2 shows the mass spectrum of 3-cyanobenzoic acid. In this case, as deduced from the analysis of mass spectra, the peaks corresponding to the ring tautomer are not observed (*m*/*z* 103 $[M - CO_2]^{+\bullet}$, *m*/*z* 104 $[M - OCNH]^{+\bullet}$ and *m*/*z* 105 $[M - OCN]^+$).

Fig. 3 shows the mass spectrum of 4-cyanobenzoic acid. As in the case of 3-cyanobenzoic acid, the peaks corresponding to the ring tautomer (*m*/*z* 103, 104, and 105) are not observed.

Fig. 4 shows the mass spectrum of 1-cyano-2-naphthoic acid. The main fragmentation pathways are similar to those of 2-cyanobenzoic acid (Scheme 3). As shown, the peaks at *m*/*z* 180 and 152 come from the open-chain form, whereas *m*/*z* 153, 154, 155, and 127 come from the closed-chain form.



Scheme 3. Specific fragmentations of the ring tautomer. i = inductive cleavage, α = radical site induced cleavage, rH = hydrogen rearrangement.

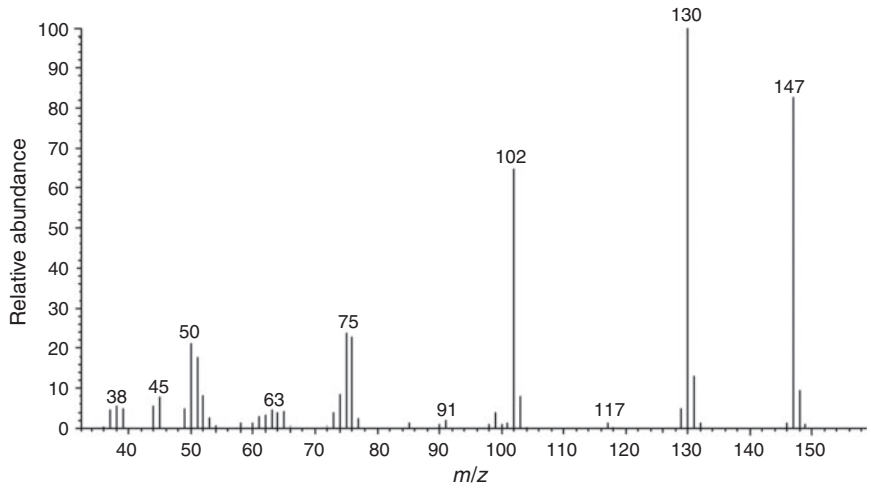


Fig. 2. Mass spectrum of 3-cyanobenzoic acid.

MS-MS product ion scans show that the ions at *m*/*z* 153.4 (relative abundance 86 %), 154.1 (relative abundance 45 %), 155.1 (relative abundance 15 %), and 180.3 (relative abundance 64 %) are generated from the molecular ion at *m*/*z* 197.2 (relative abundance 100 %).

The ion at *m*/*z* 152.3 (relative abundance 100 %) comes from the ion at *m*/*z* 180.1 (relative abundance 78 %); the ion at *m*/*z* 127.3 (relative abundance 100 %) comes from the ion at *m*/*z* 155.1 (relative abundance 78 %).

Fig. 5 shows the mass spectrum of 3-benzyl-2-cyanobenzoic acid. From analysis of the main fragment peaks, the existence of the ring form is clear because there are fragment ions that can only be explained by the tautomer (*m*/*z* 195, 194, 193) (Scheme 4).

The peaks at *m*/*z* 166, 91, 89, 75, and 63 can be explained by both tautomeric forms. The fragment ions at *m*/*z* 220 and 192 can only be justified from the open form (Scheme 5).

The base peak can be explained through both tautomers (Scheme 6).

Additional support for these routes was provided by the precursor ion scan of ions *m*/*z* 237, 220, and 195 in the mass spectrum of 3-benzyl-2-cyanobenzoic acid.

The ions at *m*/*z* 193.4 (relative abundance 36 %), 194.1 (relative abundance 45 %), and 220.3 (relative abundance 100 %) are generated from the molecular ion at *m*/*z* 237.2 (relative abundance 52 %).

The ion at *m*/*z* 192.3 (relative abundance 30 %) comes from the ion at *m*/*z* 220.1 (relative abundance 100 %) and the ion at

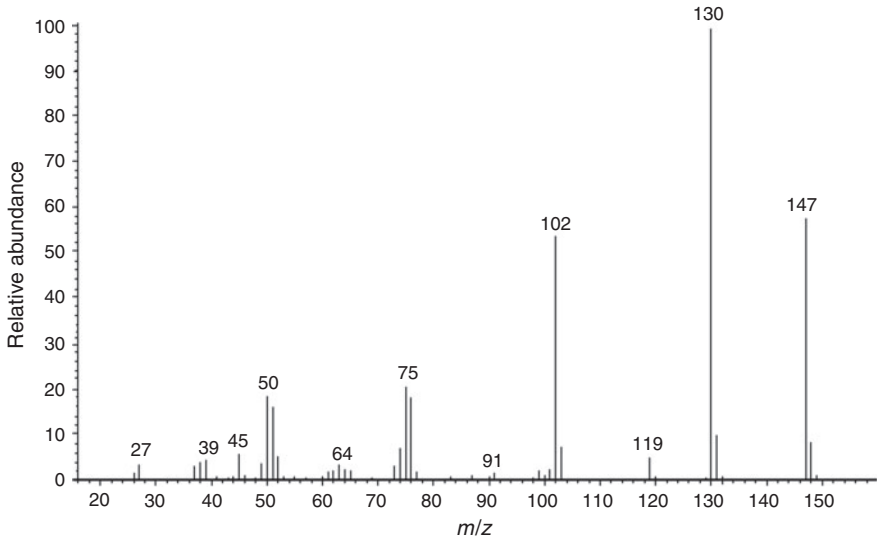


Fig. 3. Mass spectrum of 4-cyanobenzoic acid.

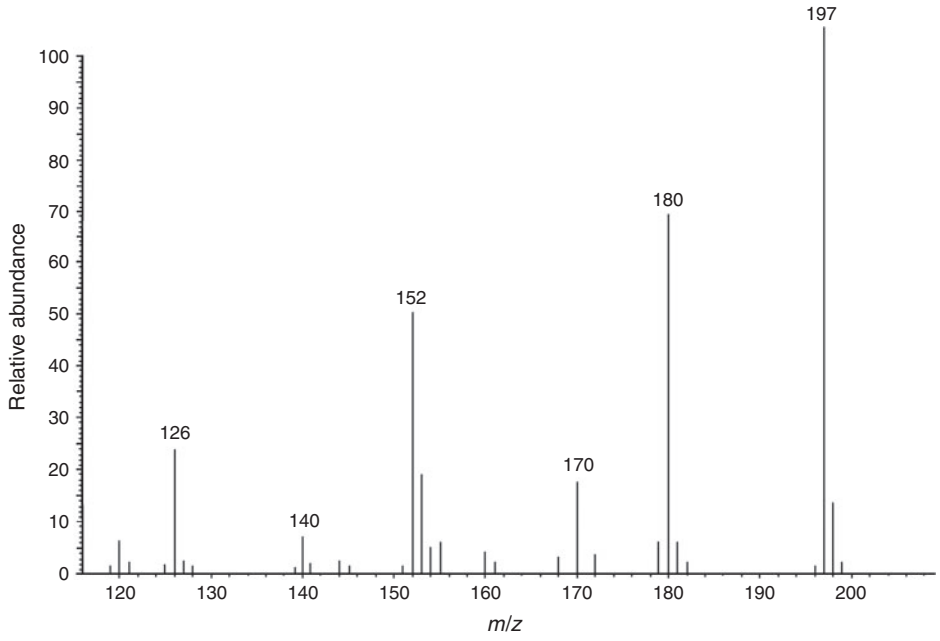


Fig. 4. Mass spectrum of 3-benzyl-2-cyanobenzoic acid.

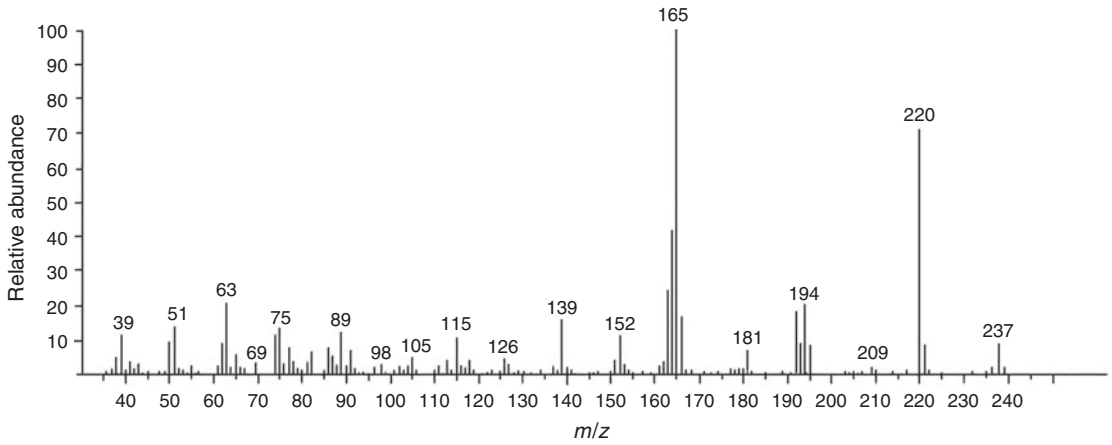
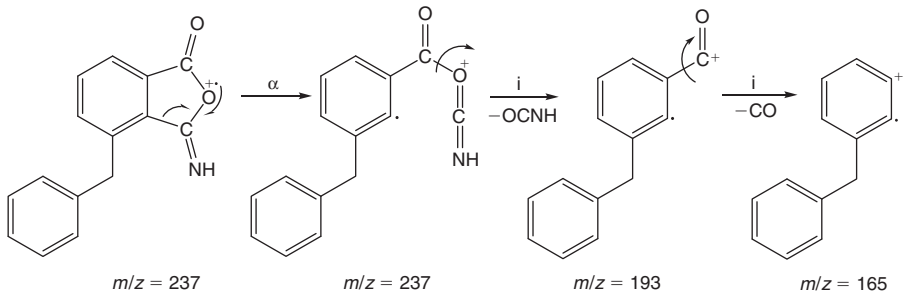
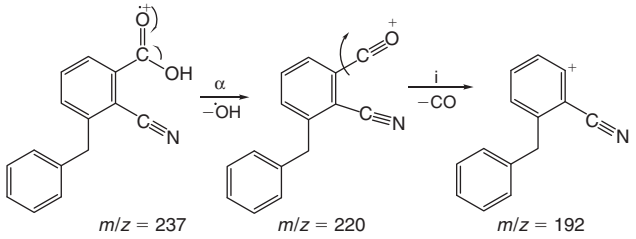


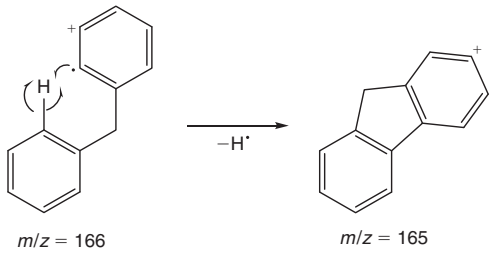
Fig. 5. Mass spectrum of 1-cyano-2-naphthoic acid.



Scheme 4.



Scheme 5.



Scheme 6.

Table 4. Relative estimation of tautomer occurrence (injection temperature 448 K)

Compound	$\frac{[M-OCNH]^{+\cdot}}{[M-OH]^{+}}$	$\frac{[M-OCN]^{+}}{[M-OH]^{+}}$	$\frac{[M-CO_2]^{+\cdot}}{[M-OH]^{+}}$	$\frac{[M-OCNH]^{+\cdot}}{[M-CO_2H]^{+}}$	$\frac{[M-OCN]^{+}}{[M-CO_2H]^{+}}$	$\frac{[M-CO_2]^{+\cdot}}{[M-CO_2H]^{+}}$
I	0.74	0.54	1.53	1.19	0.87	2.47
IV	0.12	0.14	0.45	0.10	0.12	0.38
V	0.30	0.11	0.13	1.10	0.42	0.48

m/z 167.2 (relative abundance 100 %) comes from the ion at *m/z* 195.3 (relative abundance 78 %).

As can be seen, the equilibrium position depends on the nature of the substituent, taking into account electronic and steric effects.[35,36]

From the analysis of the mass spectrometric data of selected cyanobenzoic acids (2-cyanobenzoic, 1-cyano-2-naphthoic, and 3-benzyl-2-cyanobenzoic acids), the loss of 17 Da $(M - OH)^+$ and 45 Da ($[M - CO_2H]^+$) from the molecular ion can be assigned to the open-chain form.

It is suggested that the peaks corresponding to the loss of 44 Da from the molecular ion ($[M - CO_2]^{+\bullet}$) can be assigned to the ring tautomer, as well as for the loss of 43 Da ($[M - OCNH]^{+\bullet}$) and 42 Da ($[M - OCN]^+$).

A relative estimation of the occurrence of the tautomers can be given by the following ratios: $[(M - OCNH)^{+\bullet}]/[(M - OH)^+]$, $[(M - OCN)^+]/[(M - OH)^+]$, $[(M - CO_2)^{+\bullet}]/[(M - OH)^+]$, $[(M - OCNH)^{+\bullet}]/[(M - CO_2H)^+]$, $[(M - OCN)^+]/[(M - CO_2H)^+]$ or $[(M - CO_2)^{+\bullet}]/[(M - CO_2H)^+]$ (Table 4).

The tendency to tautomerise observed for the selected cyanobenzoic acids is easily explained in terms of the steric hindrance exerted by the naphthyl moiety that is observed in Table 4 when comparing data for 1-cyano-2-naphthoic acid (**IV**) and 2-cyanobenzoic acid (**I**) (0.14 versus 0.54 for the $[M - OCN]^+/[M - OH]^+$ ratio or 0.38 versus 2.47 for the $[M - CO_2]^{+\bullet}/[M - CO_2H]^+$) ratio.

Compound **V** (3-benzyl-2-cyanobenzoic acid) exhibits a structure similar to that of **I** (2-cyanobenzoic acid), but steric hindrance to the formation of the ring form takes place owing to the presence of the benzyl group (0.11 versus 0.54 for the $[M - OCN]^+/[M - OH]^+$ ratio or 0.48 versus 2.47 for the $[M - CO_2]^{+\bullet}/[M - CO_2H]^+$) ratio.

In the analysis of the mass spectra of 3- and 4-cyanobenzoic acids, peaks corresponding to the ring tautomer are not observed. This experimental fact supports the specificity of the chosen fragmentations, given that these two compounds do not contain the structural features required to present ring–chain tautomerism. In these cases, even when they contain the two required functional groups, their spatial distribution makes it impossible for the addition to multiple bond reaction to take place.

Notwithstanding this, to support that the observed tautomeric equilibrium distributions come from the molecular species with a negligible contribution from tautomerism of molecular ions, theoretical calculations of heats of formation were carried out for both species. Table 5 shows the differences in ring and chain heats of formation for the selected molecules and corresponding molecular ions calculated by the DFT B3LYP method with the 6–31G(d,p) basis set.

In order to find evidence to support the specific ion assignment for each of the tautomeric forms, heats of reaction were determined in order to compare experimental and theoretical results.

Table 5. Ring and chain heat of formation differences for selected molecules and corresponding molecular ions ($\Delta E \approx \Delta H_{ring} - \Delta H_{open\ chain}$)

Compound	Theoretical ΔE Neutral molecule [kJ mol^{-1}]	Theoretical ΔE Molecular ion [kJ mol^{-1}]	Experimental ΔE [kJ mol^{-1}]
I	-8.37 ± 8.0	-17.5 ± 8.0	-6.81 ± 0.90
IV	-4.18 ± 8.0	-41.8 ± 8.0	-4.73 ± 0.90
V	-0.42 ± 8.0	-71.1 ± 8.0	-1.05 ± 0.21

Eqn 1 provides a simple method to determine the heat of keto–enol tautomerization for the studied compounds:

$$\ln \mathrm{K} = \ln([\text{ring form}][\text{open form}]^{-1}) = \ln([\text{f ring form}][\text{f open form}]^{-1}) = -\Delta H/\mathrm{R}T + \mathrm{C} \quad (1)$$

Table 6. ^{1}H and ^{13}C NMR chemical shifts

Compound	^{1}H NMR [ppm]	^{13}C NMR [ppm]
I	7.7–8.1 (m, 4H)	111.8(*C*–CN) 116.2 (CN) 122.5 (*C*–COOH) 128.2–132.9 (aromatic C) 170.2 (COOH)
II	7.7–8.6 (m, 4H)	112.8(*C*–CN) 117.7 (CN) 129.9 (*C*–COOH) 131.7–134.9 (aromatic C) 169.7 (COOH)
III	8.0–8.5 (m, 4H)	116.5(*C*–CN) 118.9 (CN) 122.5 (*C*–COOH) 130.7–135.2 (aromatic C) 168.7 (COOH)
IV	7.65–8.8 (m, 6H)	111.4(*C*–CN) 115.9 (CN) 123.8–133.4 (aromatic C) 169.6 (COOH)
V	4.3 (s, 2H) 7.2–8.3 (m, 8H)	112.9(*C*–CN) 116.0 (CN) 123.8 (*C*–COOH) 126.8–134.7 (aromatic C) 169.9 (COOH)

where ΔH is the enthalpy difference of reaction, R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (in K), C is a constant, and [f ring form] and [f open form] are the abundance of the fragments corresponding to the ring and open-chain forms, assuming that the concentration ratios are proportional to the ion fragment abundance ratios.

Linear correlations were found when plotting ln([ring form][open form]$^{-1}$) versus T^{-1} using the data in Table 4 for calculation of the ([ring form][open form]$^{-1}$) values at different injection temperatures. The calculated slope from the figures can be used directly to determine the enthalpy differences.

Regarding the experimental results obtained by mass spectrometry, it is interesting to observe the consistency of the calculations with the indicated fragmentation pathways. After applying the van't Hoff equation (Eqn 1) to the slopes of the figures, it can be seen that the values of the experimental heats of tautomerization are in excellent agreement with the theoretical ones (Table 5).

A reasonably good correlation with the mass spectra observations is achieved only in the case of the neutral molecules. When considering the radical ion, there is no correlation either with the experimental data or with the tendencies found experimentally (e.g. compare 2-cyanobenzoic acid (**I**) versus 1-cyano-2-naphthoic acid (**IV**), and 3-benzyl-2-cyanobenzoic acid (**V**) versus 2-cyanobenzoic acid (**I**)).

According to the abundance ratios, **I** has a higher percentage of closed chain than **IV** and **V**; therefore, the heat of formation values are only coherent with those that correspond to the neutral molecule and not to the molecular ion. Then, these findings are consistent with the occurrence of tautomerism for the neutral species, before ionization, so that tautomerization after ionization in the ion source, if it occurs at all, has a negligible effect on the position of the equilibrium. This is another indication giving support to the usefulness of mass spectrometry to predict tautomeric equilibria in the gas phase.

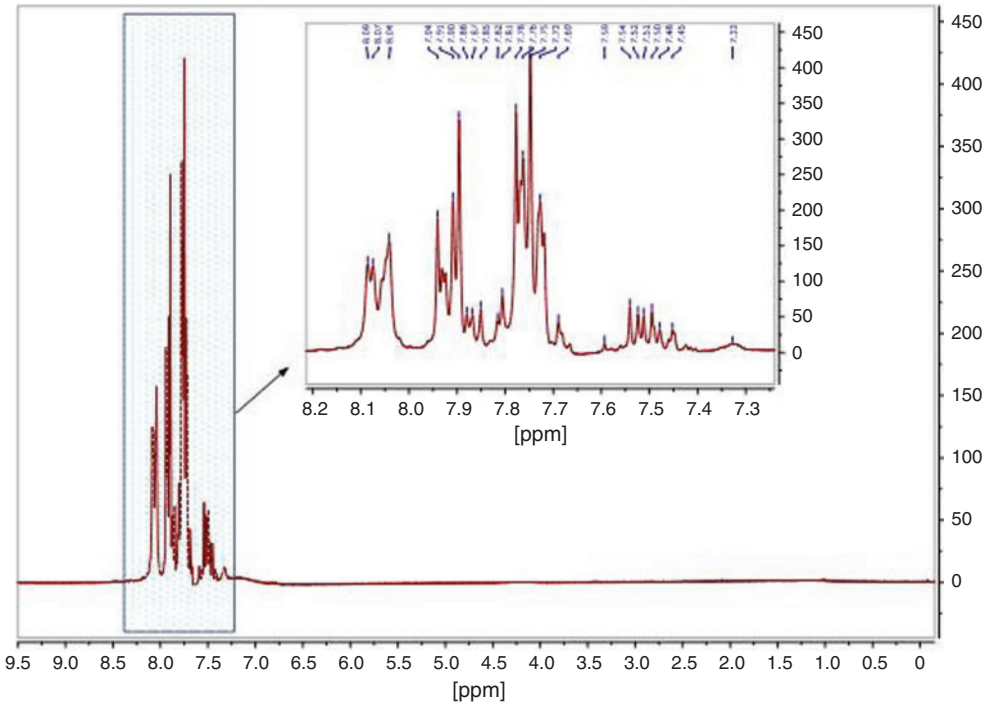


Fig. 6. ^{1}H NMR spectrum of 2-cyanobenzoic acid at 25°C in [D6]DMSO.

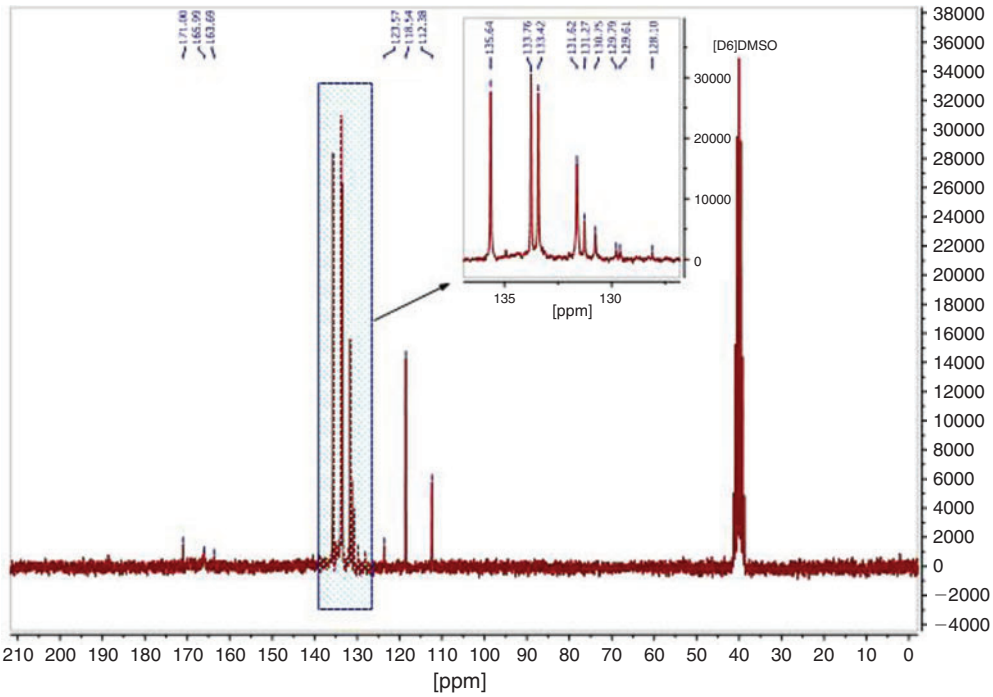


Fig. 7. ^{13}C NMR spectrum of 2-cyanobenzoic acid at 25°C in [D6]DMSO.

Nuclear Magnetic Resonance

Table 6 shows the ^{1}H NMR and ^{13}C NMR chemical shifts of the studied compounds in [D6]DMSO at 25°C. The presence of the ring tautomer is observed in the ^{1}H NMR spectrum, as shown in Fig. 6 (two groups of aromatic signals, one of higher intensity).

The spectrum shows the open-chain tautomer as the main species in solution, but small signals between δ 7.3 and 7.6 ppm are evidence of the presence of the ring tautomer. The low intensity of these signals does not allow their integration and subsequent quantification of tautomeric species. The ^{13}C NMR spectrum (Fig. 7) also shows the open-chain tautomer as the main species, but again small signals at δ 164 and δ 166 would support the presence of the ring tautomer.

Conclusion

The results found by mass spectrometry indicate that, in the gas phase, both tautomeric forms are present. The mass spectra of some 2-cyanobenzoic acids can provide valuable information regarding the ring–chain equilibrium taking place in the gas phase (fast tautomerization equilibrium in the injection port of the gas chromatograph). Results show that the ring–chain equilibrium can be studied by mass spectrometry and not only does ionization in the ion source have a negligible effect on the position of the equilibrium, but also the chromatographic conditions (with the exception of the injection port temperature) seem to exert no effect. The application of mass spectrometry techniques together with theoretical calculations provides a suitable way to analyse the occurrence of ring–chain tautomerism for the chosen set of 2-cyanobenzoic acids.

It can be concluded that both in the gas phase and in solution, the presence of the ring tautomer is evident, it being in each case the minority species present at equilibrium.

Supplementary Material

Plots of ln([ring form][open form]$^{-1}$) versus T^{-1} using the data in Table 4 for calculation of the ([ring form][open form]$^{-1}$) values at different injection temperatures are available on the Journal's website. The calculated slope from the figures can be used directly to determine the enthalpy differences.

Acknowledgements

Financial support from Facultad de Ciencias Exactas, UNLP, ANPCYT (National Agency for the Promotion of Sciences and Technology), and UNLP (La Plata National University), Buenos Aires, Argentina, is acknowledged.

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