Experimental Study of Tautomeric Equilibria of 2-Cyanobenzoic Acids in Gas, Solution and Solid Phase

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Abstract: 2-cyanobenzoic acids are very important compounds in several sciences, and it is because of this that studying their tautomeric equilibria is of great interest. Ring-chain tautomeric equilibria of 2-, 3- and 4- cyanobenzoic, 3-benzyl-2-cyanobenzoic and 1-cyano-2-naphthoic acids were studied in gas phase by means of mass spectrometry (MS). Furthermore, evidences of ring-chain tautomerism of 2-cyanobenzoic acid were found in solution using ^{1}H and ^{13}C nuclear magnetic resonance and in solid phase by infrared spectroscopy.

Keywords: Cyanobenzoic acids, mass spectrometry, NMR, infrared spectroscopy.

INTRODUCTION

Tautomers are structural isomers whose interconversion involves shifting of one atom (H or other) and it can give changes in molecular structure as a result. Tautomerism plays a very important role in Organic Chemistry, Biochemistry, Medicinal Chemistry, Pharmacology, Molecular Biology and in life itself [1].

Generally, it can be said that tautomerism is affected by: external influences (e.g. solvent [2]); internal influences, due to structural features; and protonic transfer through hydrogen bonds.

A particular kind of tautomerism, which has been studied in the present work, is the ring-chain tautomerism, where the hydrogen shift is followed by a change in molecular structure: from an open chain to a ring. To enable tautomerism it is necessary that the open-chain tautomer possessed at least two functional groups: one, containing a covalent bond of bond order higher than 1 - and the other one capable of delivering some atom(s) which could be added to this multiple bond during some pertinent chemical reaction [3]; 2-cyanobenzoic acids do have these both prerequisites.

It has been demonstrated in the case of keto-enol tautomerism of a variety of carbonyl and thiocarbonyl compounds [4-10] 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 prior to fragmentation (molecular ions, $M^{+\cdot}$, do not seem to undergo unimolecular tautomerization). Besides, for GC/MS (Gas Chromatography/Mass Spectrometry) experiments, once the solvent is separated after injection in the injection port of the gas chromatograph, tautomerism mechanisms (intermolecular or unimolecular) would not seem to take place even without chromatographic separation of the tautomers (under the selected experimental conditions). 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) [4, 8-10]. In fact, tautomerism would take place very fast in the injection port of the GC under the working conditions.

Separation of tautomers in analytical columns is usually very difficult, even when it has been carried out successfully for some compounds (previous work has reported chromatographic separation of the tautomeric forms for β-ketoesters [11]). Consequently, the different pathways of fragmentation of the tautomeric forms have to be used for identification of individual tautomers [4, 8-10]. 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), the analytical separation has not been considered critical for the present work. Analogously, it is thought that most of the conclusions could be useful to analyze spectra registered with mass spectrometers equipped with direct insertion probes.

Regarding to the study of tautomerim by means of NMR, the tautomeric equilibria of some β-

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ketobutanamides, β-ketoesters, β-diketones and β-ketonitriles in solution were investigated by ^{1}HNMR and ^{13}CNMR. Equilibrium populations of the keto and enol forms were measured. Substituent effects on the chemical shifts and the equilibrium populations were discussed [12-15].

In the present work, a study on ring-chain tautomerism in 2-cyanobenzoic acids is carried out by means of mass spectrometry (MS), nuclear magnetic resonance (NMR) and infrared spectroscopy (IR).

EXPERIMENTAL PART

The compounds under study, 2-cyanobenzoic acid and 3-benzyl-2-cyanobenzoic acid, were synthesized adapting procedures from literature. 3- and 4-cyanobenzoic acids and 1-cyano-2-naphthoic acid were not synthesized, and their MS and IR spectra were acquired from commercial databases [16].

Mass Spectrometry

These determinations were performed by injection of methanol solutions (1µl, 0.1%) in an HP 6890 Chromatograph coupled to an HP 5972A mass selective detector. The analytical column was aHP5-MS (30m x 0.25mm x 5µm) using Helium as carrier gas (0.6 ml/min). The temperatures set points were: 200ºC at the injector, 300ºC at the interface, 185ºC at the ion source and the oven ramp was 40ºC (5 min), 20ºC/min, 290ºC. The electron energy was 70 eV and the pressure in the mass spectrometer was low enough ($<10^{-5}$ torr) as to preclude ion-molecule reactions (no autoprotonation observed) and the mass range was 50-350 amu.

Nuclear Magnetic Resonance

^{1}H and ^{13}C NMR spectra were registered using a Varian Mercury Plus spectrometer, 200 MHz at 4,5T.

Typical ^{1}H NMR spectra were taken under the following conditions: Spectral width: 3201 Hz; scans per spectrum: 8-16; acquisition time: 4.09 sec; digital resolution: 0.39 Hz/point; sample concentration: 20 mg/ml; temperature: 20°C. Deuterated solvents were used and tetramethylsilane (TMS) was used as internal standard.

^{13}C nuclear magnetic resonance spectra were carried out using the same spectrometer from DMSO-d_6 solutions at 25°C. Spectral conditions were: Spectral width: 10559 Hz; scans per spectrum: 512-1000; acquisition time: 1.303 sec; digital resolution: 1.29 Hz/point; sample concentration: 40 mg/ml; temperature: 20°C.

Infrared Spectroscopy

Solid samples were prepared as follows: a certain quantity of cyanobenzoic acid and potassium bromide were finely triturated together using a mortar (in order to reduce dispersion effects from large crystals). This pulverized mixture was compressed in a mechanical screw press to form a translucid pellet that would be able to let the spectrometer ray of light pass through.

Infrared spectra were done using a Nicolet 380 FT-IR. Spectra were made in transmission mode taking 64 scans per determination.

RESULTS AND DISCUSION

Figure **1** shows ring-chain tautomerism in 2-cyanobenzoic acids.

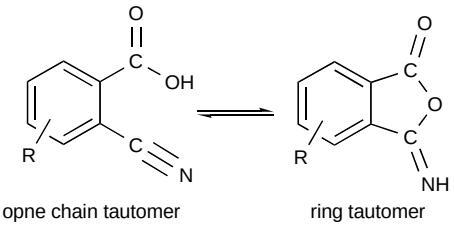


Figure 1: Ring-chain tautomerism in 2-cyanobenzoic acids.

Gas Chromatography-Mass Spectrometry

Chromatographic separation of the tautomers corresponding to 2-cyanobenzoic acid was not observed, due to the fast equilibrium between them. Figures **2-6** show mass spectra of the compounds under study.

Relevant fragmentations of mass spectra of select compounds are shown in Table **1**.

Since there was no chromathographic separation of the tautomers, the resulting mass spectrum is the superposition of the individual mass spectra of all the present isomers.

In order to establish better correlations among spectrometric data, abundances were calculaed as follows: Abundance x 1000 / ∑ Abundances.

Exhaustive analyzes of the mass spectra of the above substances have been done in order to systematize fragmentation patterns for ring-chain

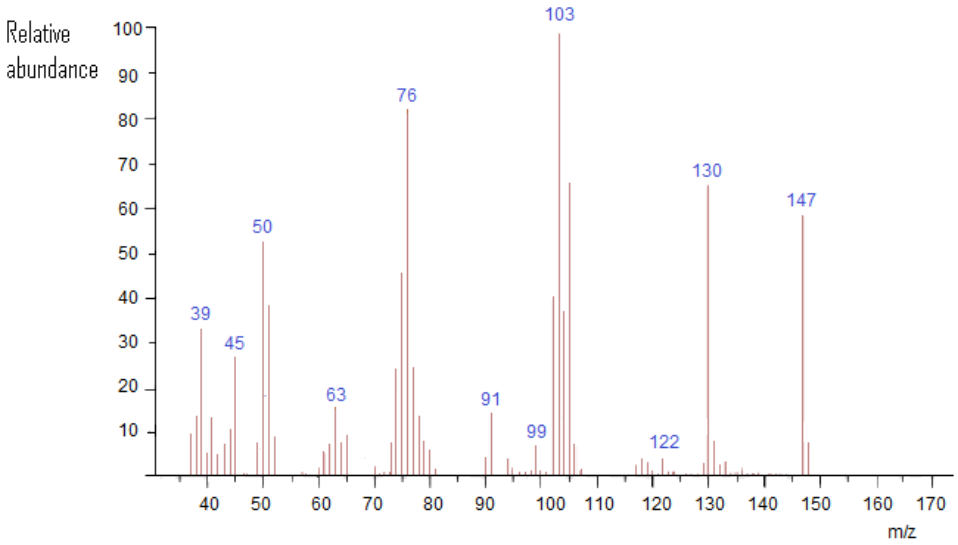


Figure 2: Mass spectrum of 2-cyanobenzoic acid.

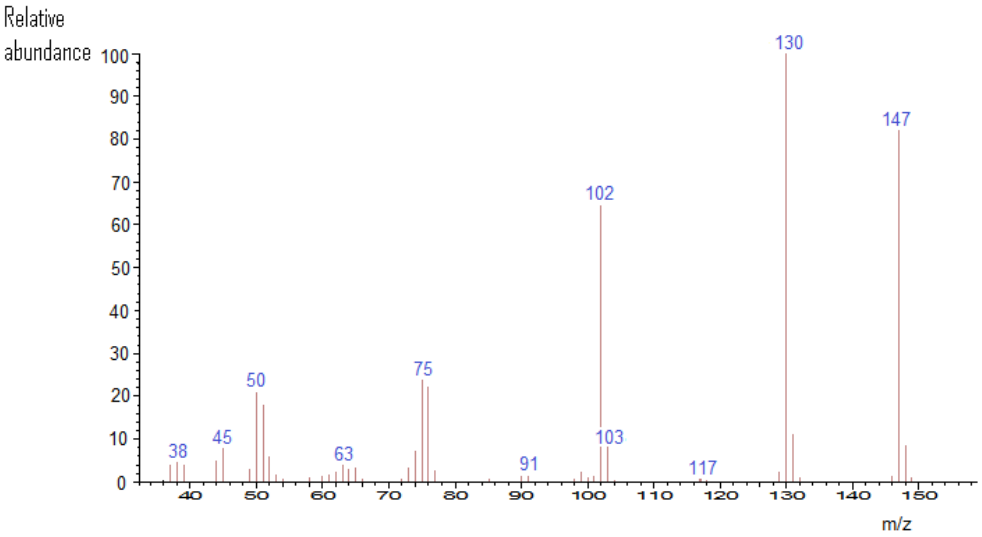


Figure 3: Mass spectrum of 3-cyanobenzoic acid.

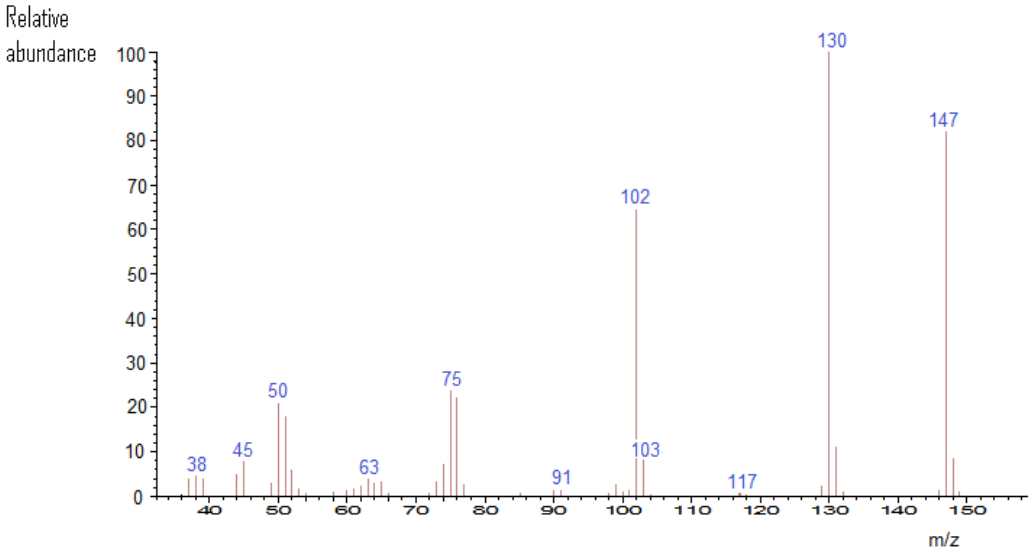


Figure 4: Mass spectrum of 4-cyanobenzoic acid.

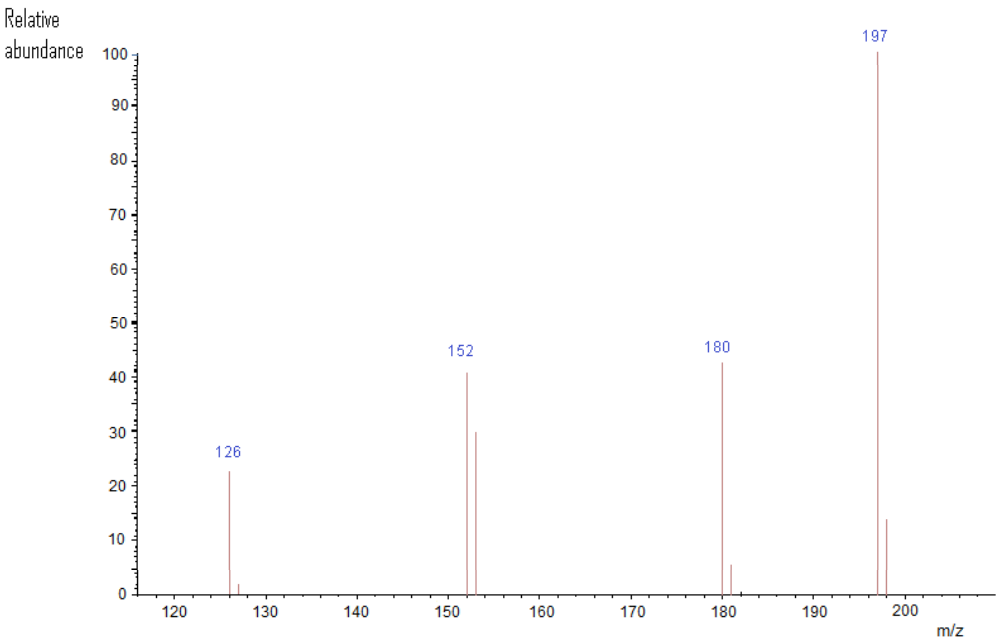


Figure 5: Mass spectrum of 1-cyano-2-naphthoic acid.

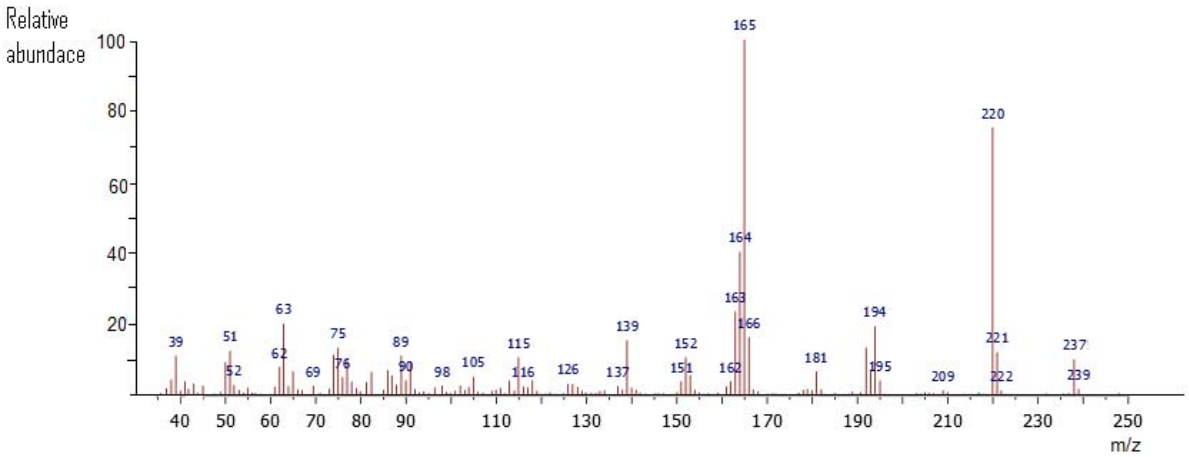
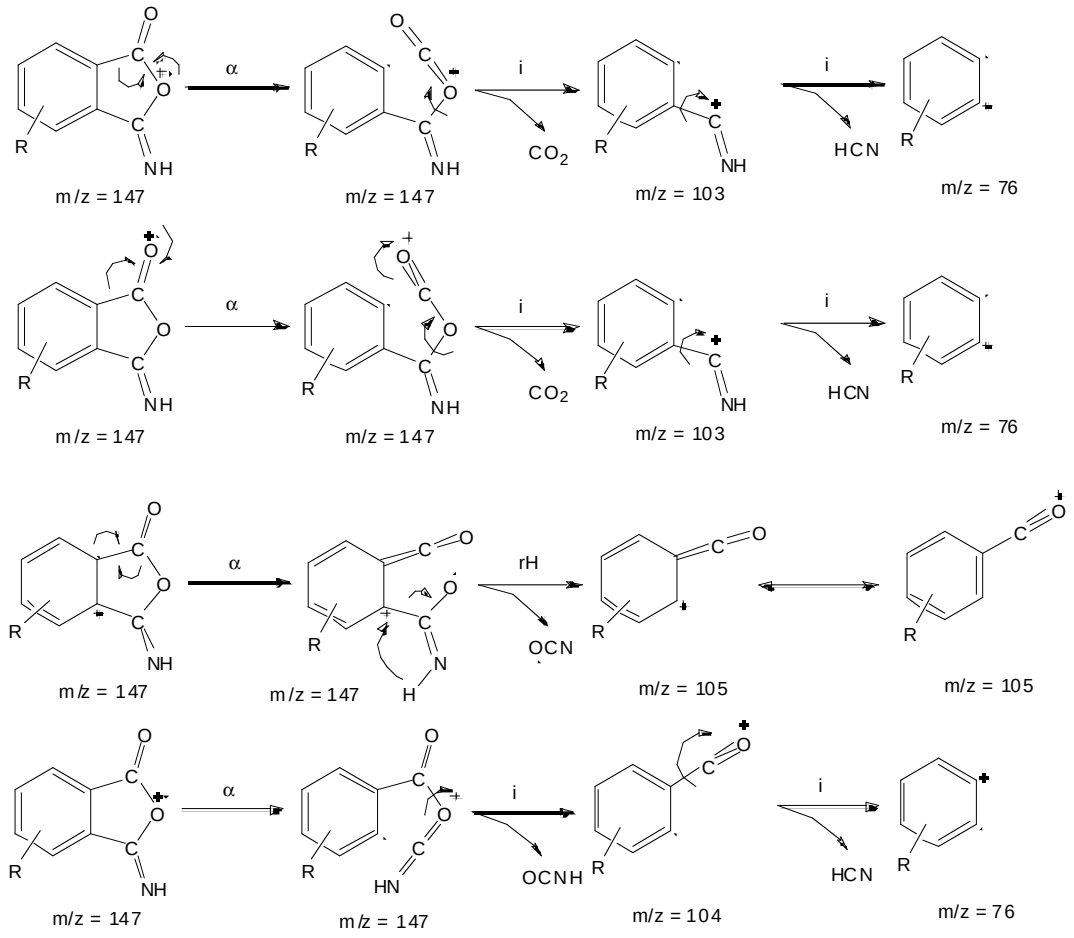


Figure 6: Mass spectrum of 3-bencyl-2-cyanobenzoic acid.

Table 1: Most Relevant Mass Spectra Data of Selected Cianobenzoic Acids

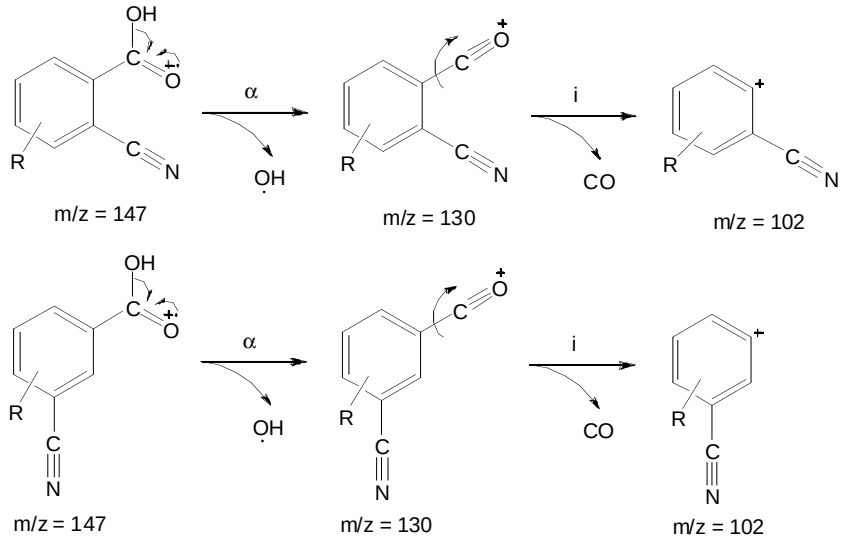
Compound	$[M]^{+\cdot}$	$[M\text{-}OH]^{+}$	$[M\text{-}CO_2H]^{+}$	$[M\text{-}OCNH]^{+\cdot}$	$[M\text{-}OCN]^{+}$	$[M\text{-}CO_2]^{+\cdot}$
2-cyanobenzoic acid	73.97	80.48	62.46	30.48	76.90	89.17
3-cyanobenzoic acid	180.79	219.72	142.40	0.00	0.00	0.00
4-cyanobenzoic acid	151.78	265.50	140.76	0.00	0.00	0.00
3-benzyl-2-cyanobenzoic acid	16.35	122.26	19.63	29.43	6.92	11.76
1-cyano-2-naphthoic acid	382.47	164.48	156.83	0.00	0.00	114.75

tautomers. In 2-cyanobenzoic, 3-benzyl-2-cyanobenzoic and 1-cyano-2-naphthoic acids it can be said that the peaks corresponding to the loss of 44 amu from the molecular ion ($[M\text{-}CO_2]^{+\cdot}$) can be assigned to the ring tautomer, as well as it can be done with the loss of 43 amu ($[M\text{-}OCNH]^{+\cdot}$) and 42 amu ($[M\text{-}OCN]^{+}$) (Scheme **1**). On the other hand, the peak corresponding to the loss of 17 amu ($[M\text{-}OH]^{+}$) and 45 amu ($[M\text{-}CO_2H]^{+}$) from the molecular ion can be assigned to the open chain tautomer (Scheme **2**).



Scheme 1: Specific fragmentations for ring tautomer.

As it can be drawn out from the analysis of mass spectra, in gas phase, in the case of 3- and 4-cyanobenzoic acids peaks corresponding to ring tautomer are not observed. This experimental fact supports the specificity of the chosen fragmentations, given that these two compounds do not accomplish the



Scheme 2: Specific fragmentations for open chain tautomer.

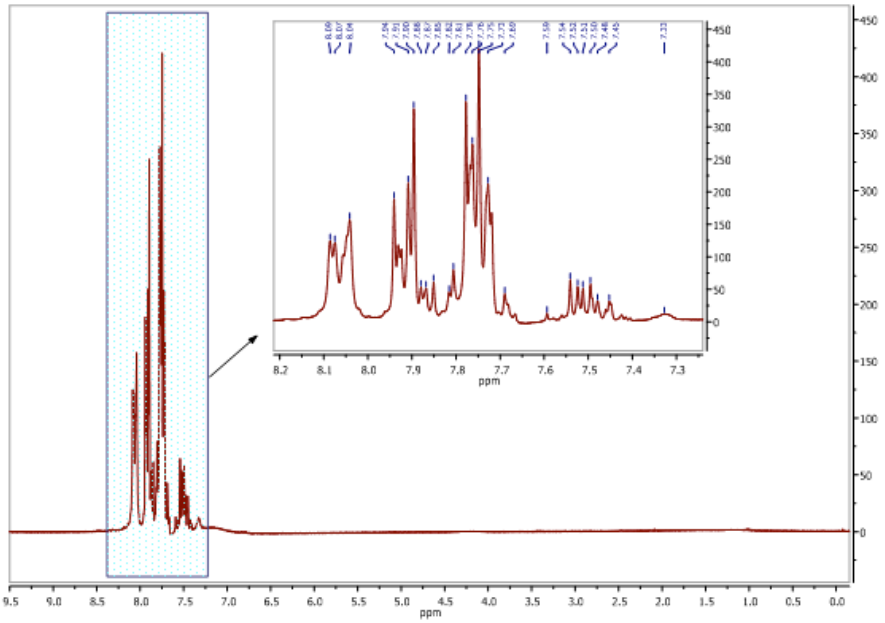


Figure 7: ^{1}H NMR spectrum of 2-cyanobenzoic acid at 25° C in DMSO-d6.

structural requirements to present ring-chain tautomerism. In these cases, even when they present the two required functional groups, their space distribution makes impossible the addition-to-multiple-bond reaction to take place.

Nuclear Magnetic Resonance

Tautomeric equilibrium in solution was analyzed for 2-cyanobenzoic acid. Figures **7** and **8** show the ^{1}H NMR and ^{13}C NMR spectra.

The spectrum shows the open chain tautomer as the main species in solution, but small signals at δ between 7.3 and 7.6 ppm should make evident the presence of the ring tautomer. The low intensity of these signals did not enable their integration and subsequent quantification of the tautomeric species. NMR spectra at 15°C and 35°C did not show considerable changes.

^{13}CNMR spectrum also shows the open chain tautomer as the main species, but again small signals

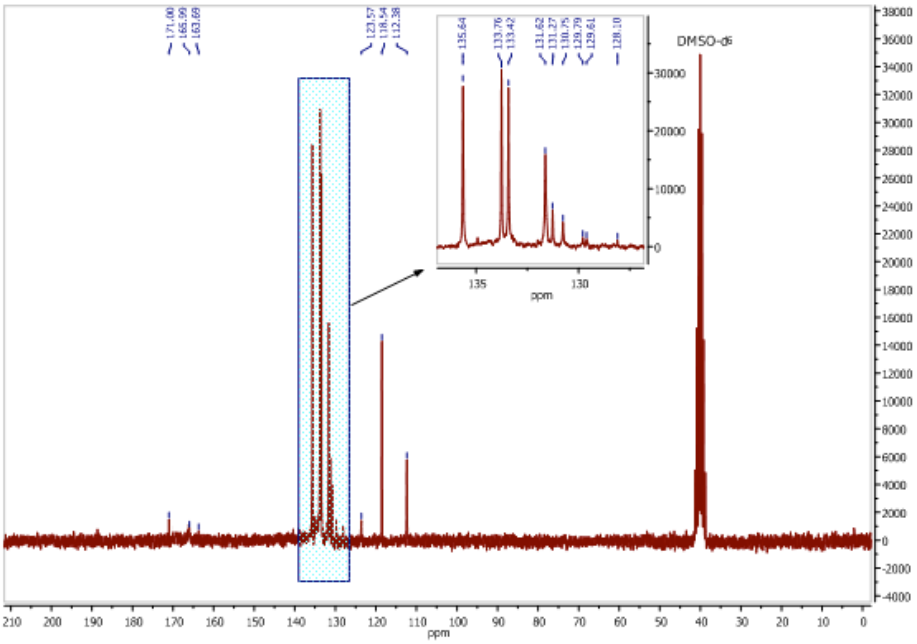


Figure 8: ^{13}C NMR spectrum of 2-cyanobenzoic acid at 25°C in DMSO-d6.

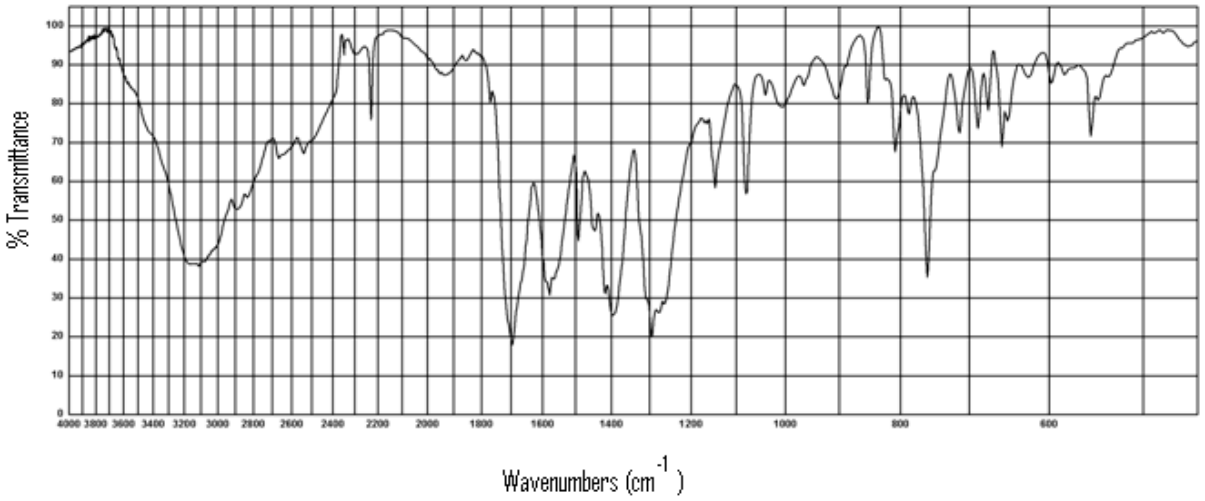


Figure 9: IR spectrum of 2-cyanobenzoic acid.

at δ 164 and 166 ppm should give evidence of the presence of the ring tautomer.

Infrared Spectroscopy

Infrared absorption spectrum of 2-cyanobenzoic acid (Figure **9**) and 3-cyanobenzoic acid (Figure **10**) were measured.

In both spectra, characteristic bands of the open chain tautomer can be observed in solid phase. The band corresponding to carboxylic OH comprises the zone between 2800 and 3000 cm^{-1}, almost overlapping with the aromatic CH band, which appears between 3070 and 3080 cm^{-1}. Thus, it can be supposed that the band observed for 2-cyanobenzoic acid in the region compressed between 2800 and 3000 cm^{-1} is the superposition of both vibrational modes but it extends to higher wavenumbers because there also would be a band corresponding to the N-H tension of the ring tautomer.

CONCLUSIONS

As shown in several papers [4-11] the usefulness of mass spectrometry (and GC/MS) to predict tautomeric behavior is demonstrated here along with additional support provided by the IR and NMR spectroscopy.

The mass spectra of cyanobenzoic acids can provide valuable information regarding the *ring-chain* equilibria taking place in the gas phase (fast tautomerization equilibrium at the injection port of the gas chromatograph). The predictive value of this methodology is supported by the influence of the nature and size of substituents on tautomeric equilibria.

Results show that the *ring-chain* equilibrium can be studied by mass spectrometry and not only ionization in the ion source has a negligible effect on the position of that equilibrium but also the chromatographic conditions (with exception of the injection port) seem to exert no effect.

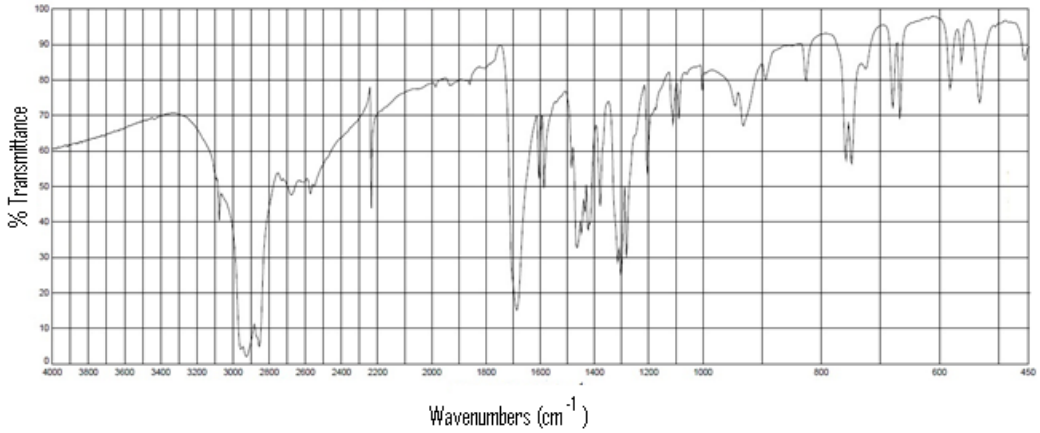


Figure 10: IR spectrum of 3-cyanobenzoic acid.

Spectrometric determinations give additional support, the analysis of NMR and IR spectra enables us to conclude that in solution and solid phase both isomers are present.

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