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April 23, 2010

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Re: The Proximity of Highly Electronegative Atoms to the Carboxyl Group of Benzoic Acid is Directly Related to an Increase in Acidity of the Molecule. Written by Alex Martin.

Dear Editor,

I would like to thank my colleagues who have taken their time to review my work and offer my revisions for your, and their examination.

Reviewer 1

1. The lack of references included in the original manuscript has been supplemented with additional research to promote the professionalism of the paper as suggested. This was a measure that I found myself in complete agreement with.
2. Several syntax and word choice issues were resolved, including the overuse of "novel", which is frowned upon by ACS journals.
3. Various formatting errors that occurred as the result of an improper file export have been resolved and originally occurred as an error.

Reviewer 2

1. The suggestion for additional discussion on the stabilization of the carboxylate form over the carboxylic acid form was highly appreciated. This issue was addressed with an expanded discussion on both the inductive and mesomeric effects and their contribution to the stabilization of the carboxylate form.
2. A more reliable determination of 2,6-dichlorobenzoic acid and 2,5-dichlorobenzoic acid's pK_a values was necessary and achieved by conducting additional titrations and determining the average pK_a measurements obtained from these titrations. This change is noted in the text.
3. Various formatting and syntax errors were resolved. I thank the reviewer for their care and attention to detail expressed in the examination of this manuscript.

Reviewer 3

1. Various formatting errors that occurred as the result of an improper file export have been resolved and originally occurred as an error.
2. Various syntax errors noted by the reviewer were corrected. I sincerely thank the reviewer for the care and attention to detail evident in the evaluation of this manuscript.
3. An critical misstatement concerning pK_a value and its relationship to protonation state *in vivo* was corrected. I sincerely thank the reviewer for catching this error as it was an oversight on my part, albeit a very important one.

I would like to thank all reviewers for their time and input and especially for the attention to detail exercised in the reviews provided. The clear, specific suggestions for improvement have helped me to strengthen this manuscript in a significant manner and their assistance is greatly appreciated.

Sincerely,

Alex Martin

The Proximity of Highly Electronegative Atoms to the Carboxyl Group of Benzoic Acid is Directly Related to an Increase in Acidity of the Molecule.

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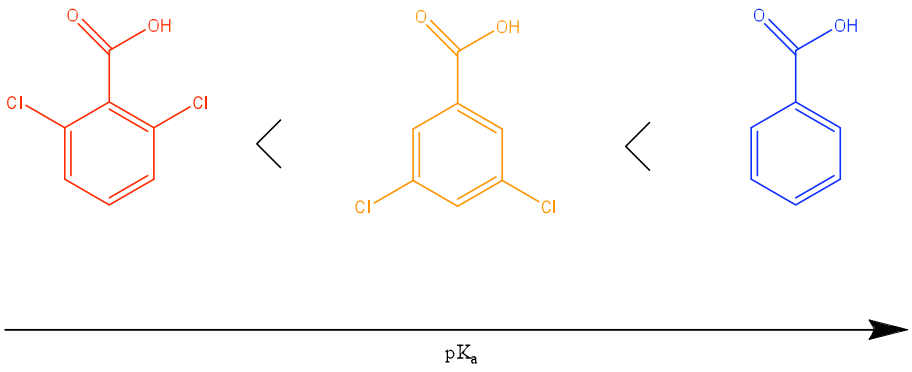
Abstract

The effect of chlorine substituent configuration on the acidity of benzoic acids was examined by synthesizing two new disubstituted benzoic acids with substituents in either the ortho or the meta positions and determining their pK_a values measured by aqueous titration. 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid were synthesized and their pK_a values were compared against previously reported pK_a values for benzoic acid, , 2-chlorobenzoic acid, 3-chlorobenzoic acid, and 2,5-dichlorobenzoic acid. The pK_a values for 3,5-dichlorobenzoic acid and 2,6-dichlorobenzoic acid were determined to be 3.54 and 1.82, respectively in water. The pK_a measurements of these disubstituted benzoic acids showed that the proximity of chlorine substituents to the carboxyl group is directly related to an increase in the acidity of the carboxyl group of benzoic acid.

Graphical Abstract



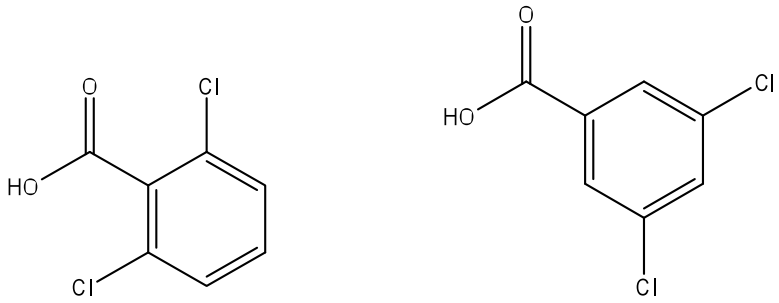
Introduction

The acidity of a solution describes the free proton concentration in solution and can have an important effect on the protonation state of molecules in that solution. Acidity is described by a pH value, which is measured as the $-\log[H^+]$ and decreases as the free proton concentration in solution increases.[1] Acidic molecules include a functional group, such as a carboxyl or an amine, that is capable of existing in a protonated or non-protonated state. Such molecules have distinct pK_a values that describe their protonation state in a specific solvent and can be used to determine the predominant form of the molecule in said solvent at a known pH. In the human body, the average pH of blood is approximately 7.2.[2] This is an important value to note because blood is the primary delivery method of orally administered drugs, which is the target delivery method for most novel drug research.

When considering molecules for drug discovery, many of the most important factors to consider concern pharmacokinetics, which includes absorption, distribution, metabolism and excretion (ADME) of the drug in the body.[3] Factors that affect ADME include lipophilicity, solubility, molecular weight and acidity.[3] These factors are the focus of new drug discovery research and the elucidation of new ways in which they can be manipulated increases the tools with which medicinal chemists have to work. Benzoic acids are a common component of candidates for novel drug discovery and further description of these molecules gives insight into more productive avenues of drug research including this molecule. Most drug discovery research involves the production of a large library of candidate molecules against which bioassays specific for the target molecule are run.[4] From this vast array of bioassays, potential candidate molecules are

identified to be further refined by experimental chemistry to find a molecule that is both safe and effective for the application being studied. This refinement process includes the addition or manipulation of substituent groups to affect molecular properties. Describing and cataloguing the effects of particular substituents on particular molecules relevant to drug research could significantly shorten drug development time.

Here we report the results of an evaluation of dichlorobenzoic acid substituent configuration on the pK_a measurement of the carboxyl group. Two new molecules were synthesized to evaluate this property: 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid as shown in Scheme 1. It was anticipated that disubstitution of chlorine atoms at the ortho positions (2,6-dichlorobenzoic acid) would produce the lowest pK_a value of the molecules tested because of the proximity of the highly electronegative chlorine atoms to the acidic oxygen. Chlorine displays a highly negative I-effect and moderately positive M-effect, which should combine to stabilize the deprotonated form of the acid by delocalizing the negative charge on the oxygen atom.[5]



Scheme 1. Chemdraw representations of 2,6-dichlorobenzoic acid on the left and 3,5-dichlorobenzoic acid on the right.

Materials and Methods

Modern medicinal chemistry requires a detailed understanding of substituents' effects on the properties of candidate drug molecules.[6] In physiological application, it is important to consider in what state they will primarily exist *in vivo*. Many modern drugs interact directly with enzymes by forming bonding interactions at the enzymes' active sites. Among these bonding interactions, hydrogen bonding plays a significant role in assuring high affinity for many targets of drug action, and the protonation state of acidic atoms at physiological pH must be taken into consideration when designing drug molecules. When the pK_a of a molecule is measured in water, it is an approximation of what the protonation state of that molecule would be at physiological pH, with higher pK_a values indicating a lower degree of protonation in the body. For this experiment, the acidic molecule benzoic acid was used as a template on which to examine the effect of substituent configuration on pK_a measurements in water.

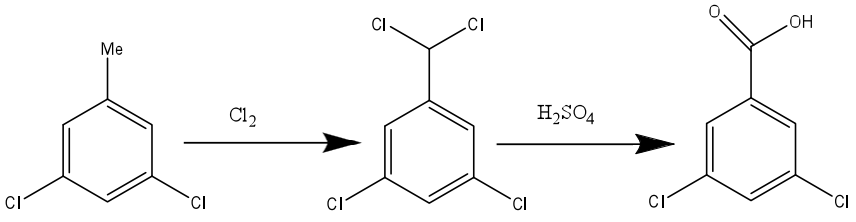
Two new disubstituted benzoic acids were synthesized in order to explore the effect of substituent configuration on pK_a measurements in water. 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid were synthesized and their pKa values measured. These molecules were chosen as targets for synthesis because they display distinct differences in substituent configuration without variation in the substituents themselves. The pK_a measurements were carried out by aqueous titrations conducted at 25°C. Using a saturated calomel electrode pH meter, two titrations were conducted of 100mL 0.01M solutions each of 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid. A 0.10M solution of sodium hydroxide was used in 0.5 mL increments to conduct the titrations. From the pH readings obtained in these titration curves, it was possible to determine pK_a values in aqueous solution for 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid. Each titration was conducted 3 times for each acid and the average of the 3 determined

pK_a values reported. The determined pK_a values for these molecules are shown in Table 1 along with monosubstituted chlorobenzoic acids and benzoic acid itself.

Table 1. pKa Values of disubstituted, monosubstituted, and unsubstituted forms of benzoic acid of interest.

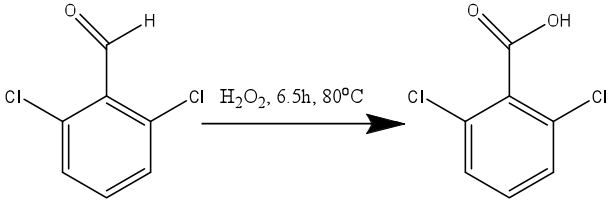
Compound Name	CAS Number	Solvent	pKa	Source[1,7]
benzoic acid	000065-85-0	Water	4.21	1
2,5-dichlorobenzoic acid	000050-79-3	Water	2.47	3
2,6-dichlorobenzoic acid	000050-30-6	Water	1.82	This Work
3,5-dichlorobenzoic acid	000051-36-5	Water	3.54	This Work
2-chlorobenzoic acid	000118-91-2	Water	2.92	1
3-chlorobenzoic acid	000535-80-8	Water	3.80	1

3,5-dichlorobenzoic acid was synthesized from 3,5-dichlorotoluene purchased from the 3B Scientific Corporation. 3,5-Dichlorotoluene is first chlorinated in heat and light before being hydrolyzed by a strong acid. This process yields 3,5-dichlorobenzoic acid in up to 90% yield from the starting materials(Scheme 2). A more detailed discussion of the synthesis of 3,5-dichlorobenzoic acid is included in the appendix.



Scheme 2. Reaction of 3,5-dichlorotoluene with Cl_2 and H_2SO_4 to form 3,5-dichlorobenzoic acid.

2,6-dichlorobenzoic acid was prepared from 2,6-dichlorobenzaldehyde in a simple oxidation reaction. 2,6-dichlorobenzaldehyde was purchased from Aldritch and treated hydrogen peroxide to oxidize the 2,6-dichlorobenzaldehyde into 2,6-dichlorobenzoic acid with 66% yield from the starting material. A more in depth discussion of this process is located in the appendix (Scheme 3).



Scheme 3. Oxidation of 2,6-benzaldehyde to 2,6-benzoic acid.

Results and Discussion

2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid were synthesized and their pK_a measured by aqueous titration with 0.10M NaOH. These titrations were conducted in aqueous solution so that their measured pK_a would be relevant in biological systems. 2,6-dichlorobenzoic acid was measured to have a pK_a value of 1.82 and 3,5-dichlorobenzoic acid was measured to have a pK_a value of 3.54. These molecules were chosen as targets for synthesis due to their exclusively ortho or meta nature of substituent position compared to the carboxyl group of benzoic acid. 2,6-dichlorobenzoic acid has both chlorine substituents situated ortho to the carboxyl functional group while 3,5-dichlorobenzoic acid has both chlorine substituents situated meta to the carboxyl functional group. This allows the specific effect of ortho or meta substituents on pK_a values to be measured.

Previously studied molecules were used as both a basis of comparison and a template upon which to build our hypothesis. Benzoic acid, 2-chlorobenzoic acid, 3-chlorobenzoic acid and 2,5-dichlorobenzoic acid have previously reported pK_a values published from research conducted by Rived *et al*.[7] In this literature, the di-chlorine-substituted benzoic acid displayed the lowest pK_a value. Of the monosubstituted benzoic acids, 2-chlorobenzoic acid displayed a lower pK_a value than 3-chlorobenzoic acid, and all chlorine-substituted benzoic acids showed lower pK_a values than benzoic acid itself. These pK_a measurements suggest that the addition of chlorine substituents reduce the pK_a value of benzoic acids, higher degrees of substitution produce increased reduction of pK_a value, and closer proximity of these substituents to the carboxyl functional group cause a greater reduction of pK_a measurements.

The measurement of the two newly synthesized dichlorobenzoic acids' pK_a values confirms that ortho-substituted chlorine atoms reduced the pK_a value to a greater degree than meta-substituted chlorine atoms. This effect is likely due to the highly electronegative nature of chlorine, which is measured at 3.096 by the method developed by Xie *et al*.[8] The electronegativity scale developed by Xie *et al.* assigns higher values to more electronegative groups and this measurement compares to values of 3.729 for oxygen and 2.465 for a methyl substituent.[8] This high electronegativity delocalizes electron density from the carboxyl oxygen, pulling electron density toward the chlorine atoms, which results in a more easily abstracted proton from the acidic oxygen. The effect of electronegativity can be well expressed chlorine's negative nductive effect (I-effect) value, which indicates a tendency to pull electron density toward the atom. In addition to this I-effect, chlorine is characterized with a positive mesomeric effect (M-effec)t, which indicates a contribution to electron delocalization across the atom by pi orbital interaction. Chlorine's positive M-effect combines with the conjugated benzene

ring to produce a highly delocalized electron density with the ability to delocalize the negative charge of carboxylate form of benzoic acid, stabilizing the deprotonated form. The characterization of substituent configuration on the pK_a values of acidic molecules can be used to help direct new drug discovery in more efficient and productive avenues.

Conclusion

2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid were successfully synthesized and their pK_a values were measured. The measured pK_a values for 2,6-dichlorobenzoic acid and 3,5-dichlorobenzoic acid were found to be 1.82 and 3.54, respectively. These dichlorobenzoic acids were shown to have lower pK_a values than benzoic acid, suggesting the chlorine substituents lowered the pK_a value. This data also shows that the closer the proximity of highly electronegative substituents to acidic functional groups, the greater the degree of acidity, as shown in a reduced pK_a value.

Further characterization of highly electronegative substituents' effect on the electronegativity of benzoic acids could produce a model by which the exact effect of a particular atom's addition to benzoic acid on the pK_a value could be determined before the synthesis was ever conducted. This line of research could be invaluable to reducing the time and resources required for effective new drug development and should be considered of vital importance. While this particular line of research concerned only the configuration of substituents' on the pK_a value, electronegativity must also play a large role, and should be further investigated.

Supplemental Material Available: The appendix contains an in-depth discussion of the synthesis for the two new molecules under examination. Also, IR spectra, mass spectra and ^{13}C-NMR spectra are included for the two newly synthesized dichlorobenzoic acids.

References

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[6] Jover, J.; Bosque, R.; Sales, Joaquim. QSPR Prediction of pK_a for Benzoic Acids in Different Solvents. *QSAR Comb. Sci.* **2007**. *5*. 563-581.

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[8] Xie, Q.; Sun, H.; Xie, G.; Zhou, J.; An iterative method for calculation of group electronegativities. *J. Chem. Inf. Comput. Sci.* **1995**. *35*. 106-109.

Supporting Information

The Proximity of Highly Electronegative Atoms to the Carboxyl Group of Benzoic Acid is Directly Related to an Increase in Acidity of the Molecule.

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Table of Contents

Synthesis of 2,6-dichlorobenzoic Acid:

2,6-dichlorobenzoic acid is produced by an oxidation reaction of 2,6-dichlorobenzaldehyde in acetonitrile solvent by treatment with hydrogen peroxide. First, a mixture of benzaldehyde (10mmol) and MPHT (10 mol%) in 3mL of acetonitrile solvent is prepared. Next, aqueous 30wt% hydrogen peroxide of 20mmol concentration was added drop wise at 80^{o}C, after which the solution was heated and stirred for an hour and a half. At the end of the reaction, excess hydrogen peroxide is deactivated by the addition of aqueous sodium bisulfate, followed by Buckner funnel filtration. The precipitate left out of solution after filtration contain 2,6-dichlorobenzoic acid but are not pure. Instead, the filtrate is dissolved in ethyl acetate and separated by 3 cycles of phase separation. Finally, the solvent was dried under a vacuum and the remaining precipitate collected as 2,6-dichlorobenzoic acid. This reaction produces 2,6-dichlorobenzoic acid in approximately 66% yield. This product of this reaction can be verified by spectroscopic evaluation. The IR spectrum is shown in Figure 1, the mass spectrum is shown in Figure 2, and the ^{13}C-NMR spectrum is shown in Figure 3.

Figure 1. IR spectrum of 2,6-dichlorobenzoic acid determined using a Nicolet 170SX or JASCO FT/IR-410 spectromter.

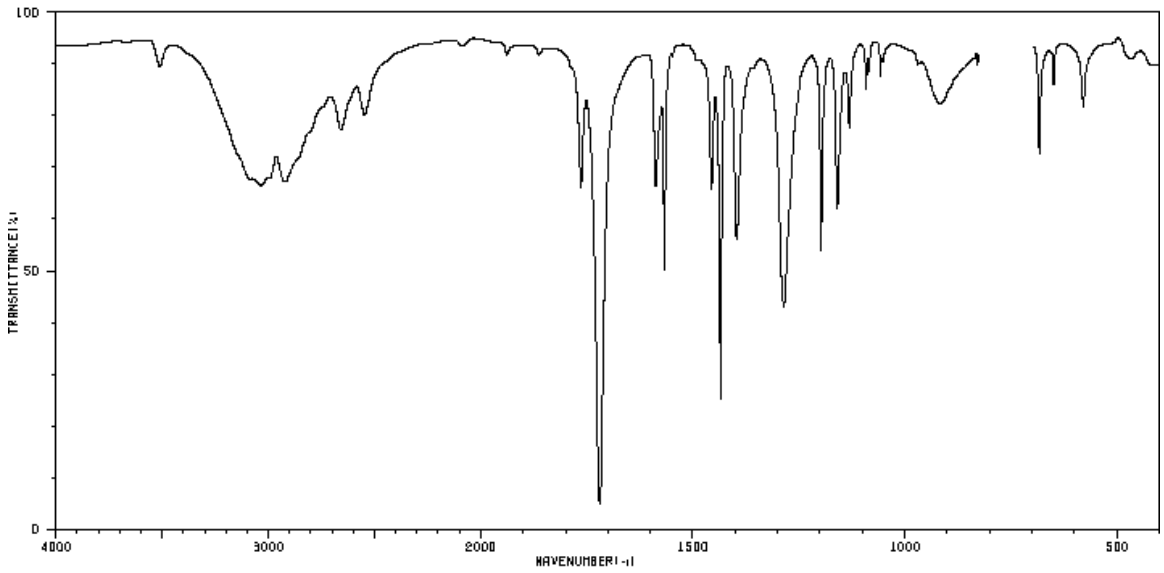


Figure 2. Mass Spectrum of 2,6-dichlorobenzoic acid with 190 nominal mass and 113 displayed peaks.

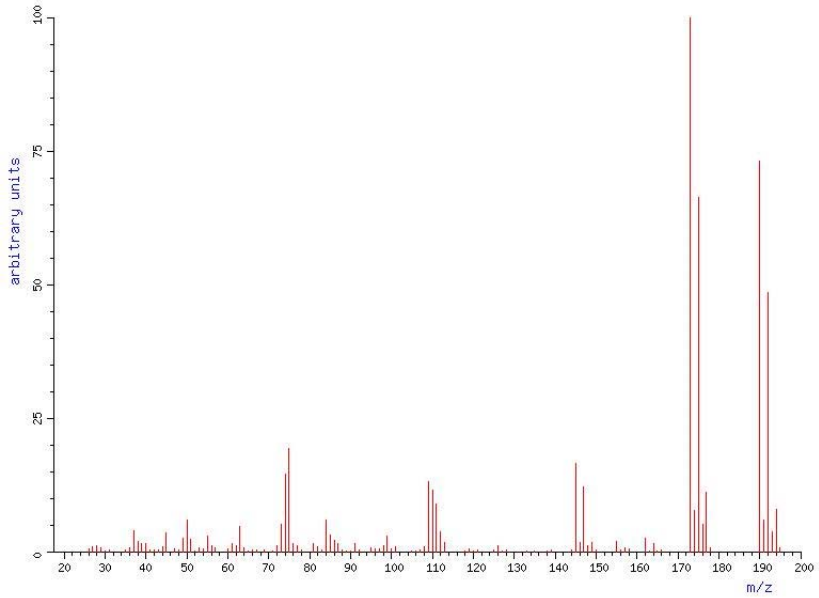
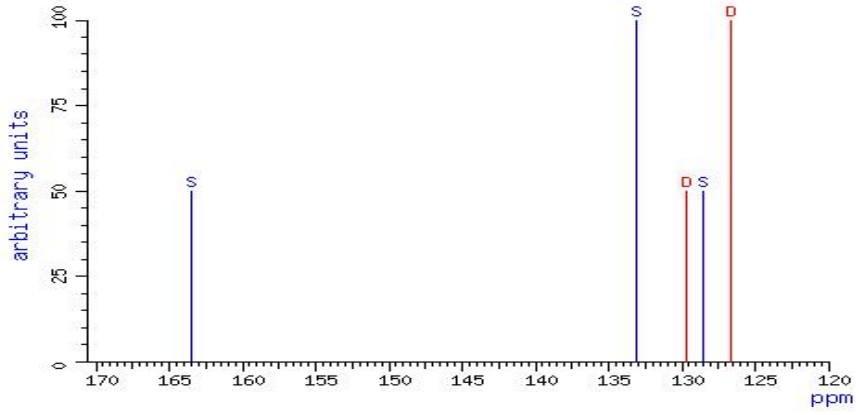


Figure 3. ^{13}C-NMR spectrum of 2,6-dichlorobenzoic acid determined using a Bruker WH-90 spectrometer in dimethyl sulfoxide-d6 solvent with a tetramethylsilane standard.



Synthesis of 3,5-dichlorobenzoic acid

3,5-dichlorotoluene is the starting material and can be purchased from the 3B Scientific Company, CAS number: 25186-47-4. In order to synthesize 3,5-dichlorobenzoic acid, 28 grams of Cl_2 must be added to a 50 gram sample of 3,5-dichlorotoluene over an 8 hour period at a temperature between 185° and 190° and under irradiation by a 150 watt lamp. After the 8 hours has passed, add 200g of 8% fuming H_2SO_4, stirring for 30 hours. After 30 hours, chill the solution to precipitate out 3,5-dichlorobenzoic acid, which can then be removed from solution easily by filtration. Figures 4, 5 and 6 shows the 3,5-dichlorobenzoic acid IR spectrum, mass spectrum and ^{13}C-NMR spectrum, respectively.

Figure 4. IR spectrum of 3,5-dichlorobenzoic acid as determined on a Nicolet 170SX or JASCO FT/IR-410 Spectrometer.

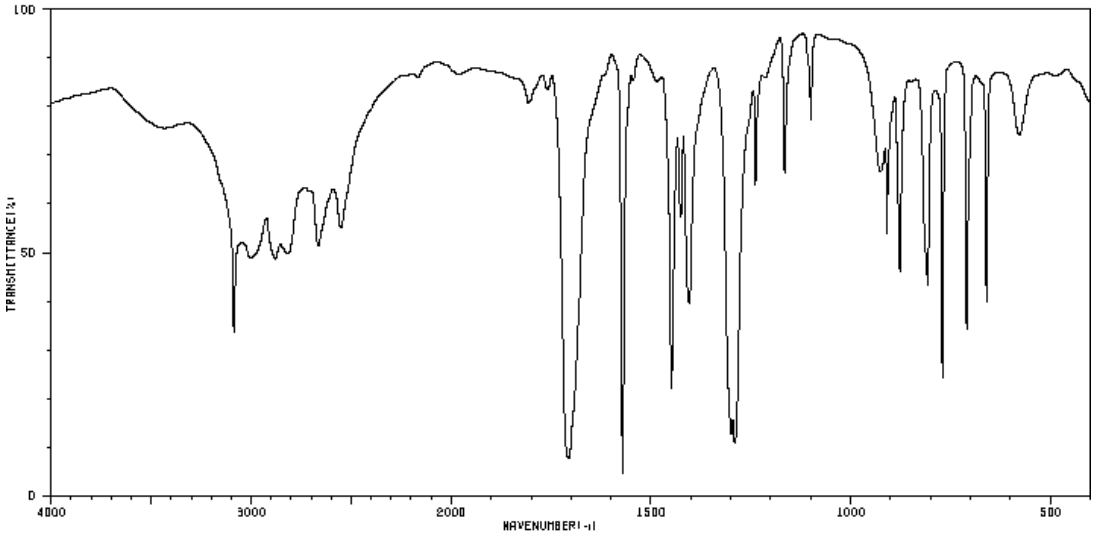

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Figure 5. Mass spectrum of 3,5-dichlorobenzoic acid determined by electron ionization, with an electron energy of 75 eV, a temperature of 180°C and a nominal mass of 190.

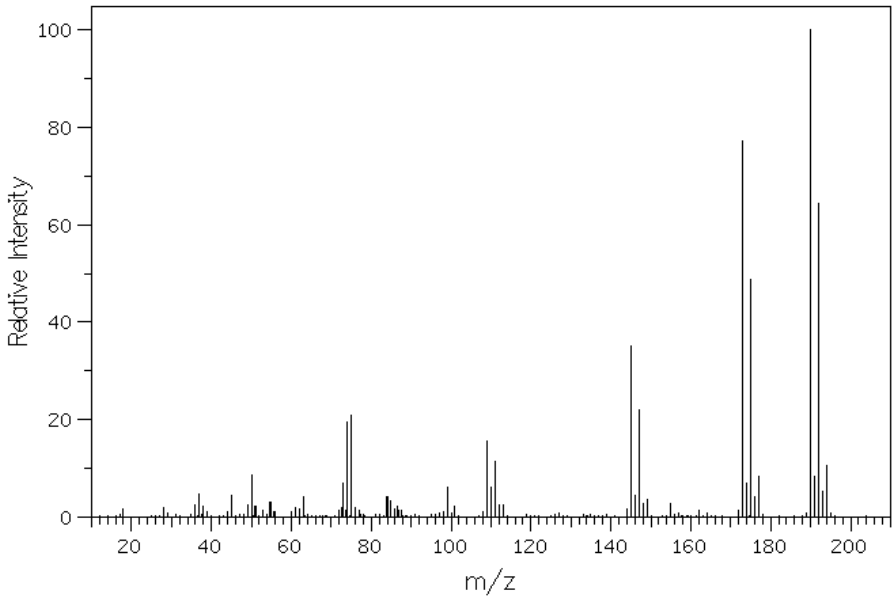
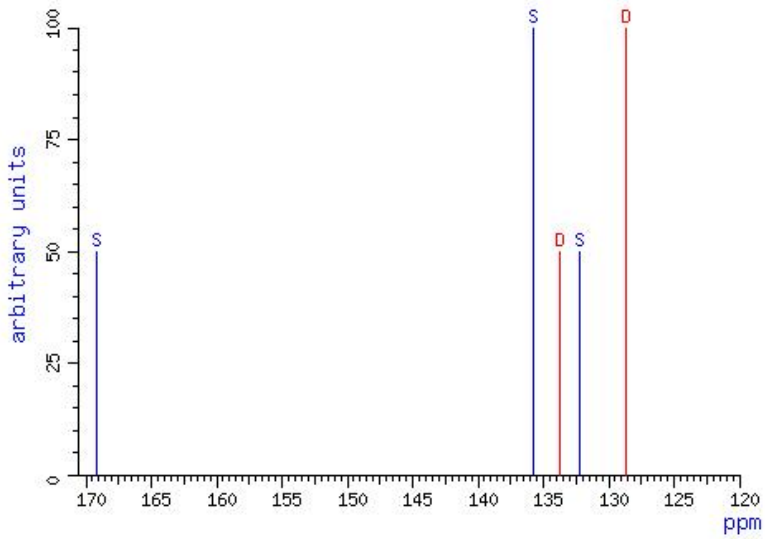


Figure 6. ^{13}C-NMR spectrum of 3,5-dichlorobenzoic acid. Images acquired with a Bruker AM-270 in a chloroform-d solvent with a tetramethysilane standard.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Appended References

2,6-dichlorobenzoic acid

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3,5-dichlorobenzoic acid

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