Mass Spectrometry of Analytical Derivatives. 2. "*Ortho*" and "*Para*" Effects in Electron Ionization Mass Spectra of Derivatives of Hydroxyl, Mercapto and Amino Benzoic Acids

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ABSTRACT

Derivatives requiring either anhydrous or aqueous reaction conditions were prepared for robust and reliable gas chromatography – mass spectrometry (GC-MS) characterization of hydroxyl, mercapto, and amino benzoic acids. Methylation and trialkylsilylation are employed for blocking the acidic function. Alkyl, trimethylsilyl, acetyl, perfluoroacyl and alkoxycarbonyl derivatization groups are introduced to hydroxyl, mercapto and amino functions. The electron ionization induced fragmentation characteristics of corresponding derivatives are explained by comparing the MS1 spectra of unlabeled compounds to their ^{2}H and ^{13}C labeled analogs, and analysis of collision-induced dissociation data from MS2 spectra. Competing fragmentation alternatives are identified and specific decomposition processes are detailed that characterize (a) *ortho* isomers due to interaction of vicinal functional substituents and (b) *para* isomers prone to forming *para* quinoid type structures. Skeletal and hydrogen rearrangements typical for methyl benzoates and the blocking groups are considered when discussing

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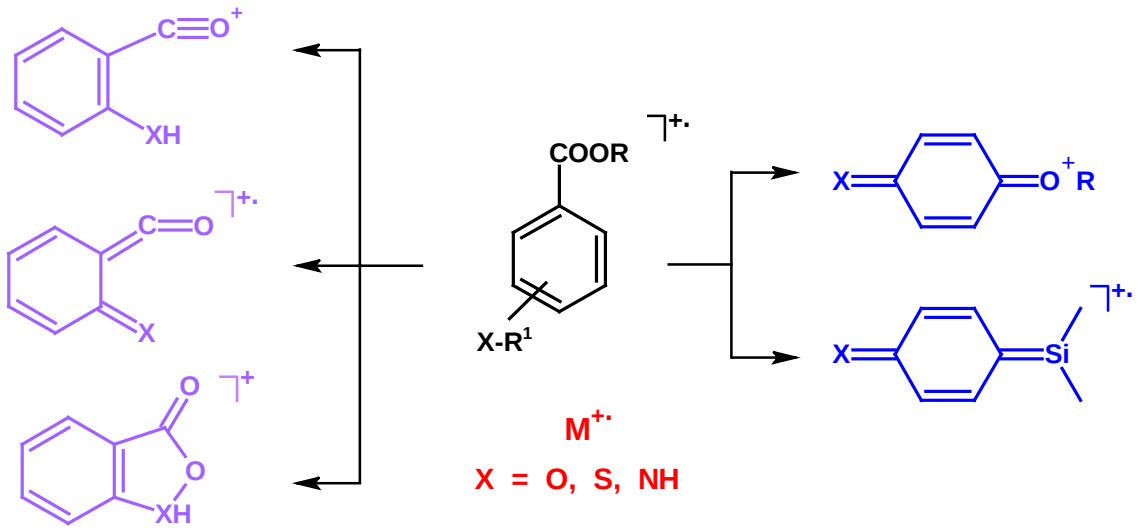
diagnostically important ions. Characteristic ions produced as a result of rearrangements in *ortho* isomers are classified, and skeletal rearrangements required to produce *para* quinoid type ions specific for para isomers are noted. Key ions for structure elucidation and differentiation of isomers for derivatives of substituted benzoic acids by GC-MS are suggested.

GRAPHICAL ABSTRACT



Novelty Statement:

Dependable GC-MS methods are developed for reliable detection of salicylic, thiosalicylic and anthranilic acids and their positional isomers by identification and characterization of diagnostically important fragmentation pathways for various of chemical modifications products of these acids.

Highlights:

- Systematic study of alkyl, trimethylsilyl, acetyl, perfluoroacyl and alkoxycarbonyl derivatives by GC-MS;
- Isomer distinction for derivatives of substituted benzoic acids by GC-MS is described;
- *Ortho* effects in EI mass spectra of derivatives of salicylates, thiosalicylates and anthranilates are described;
- *Para* effects in EI mass spectra of derivatives of 4-hydroxy-, 4-mercapto- and 4-amino-benzoic acids are described;
- Chemical modification (derivatization) prior to GC-MS analysis is detailed.

Keywords:

- Gas chromatography – mass spectrometry (GC-MS)
- Ortho effect
- Para effect
- Derivatization
- Salicylic acid
- Anthranilic acid
- Thiosalicylic acid
- Alkylation
- Silylation
- Perfluoroacylation
- Alkoxycarbonyl derivatives

1. INTRODUCTION.

Chemical modification is a valuable technique effectively used in gas chromatography – mass spectrometry (GC-MS) to extend the range of compounds amenable to analysis. The goals for chemical modifications in GC-MS vary, and the derivatization step is usually applied to modify compounds that are unsuitable for GC-MS analysis, such as compounds with thermal lability, low GC mobility and resolution, or lacking structure-specific ions in their mass spectra. Selecting suitable reaction conditions and obtaining appropriate derivatives is an important step for the analysis to achieve desired volatility for chromatographic separation, and sufficient sensitivity, selectivity and specificity under electron ionization (EI). Acquisition of reliable chromatographic and EI fragmentation patterns for derivatives of common chemicals, and knowledge of the limitations for specific derivative types are important for the successful structure elucidation of unknown materials by GC-MS. Therefore, the NIST Mass Spectrometry Data Center (NIST MSDC) continues to acquire and evaluate data for chemical modification products. The objective in increasing the number of spectra of derivatives in the NIST/NIH/EPA Mass Spectral Library [1] is to provide a comprehensive capability to identify materials through characteristic fragmentation patterns.

A systematic study of anthranilic, salicylic and thiosalicylic acids and their positional isomers has been carried out in continuation and amplification of efforts made by the NIST MSDC on the study of fragmentation processes of derivatives [2]. These isomeric hydroxyl, mercapto and amino benzoic acids are of practical interest because of their recognized anti-inflammatory properties, occurrence in nature as plant hormones,

application in cosmetic, perfume and dye industries, and use in pharmacology as building blocks in the production of antiseptic and antifungal drugs [3-5].

2. EXPERIMENTAL[2]

2.1 Materials.

"*Puriss. p.a.*" grade 2-, 3- and 4- substituted hydroxyl, mercapto and amino benzoic acids and their methyl esters were obtained from Sigma-Aldrich. The derivatization agents for alkylation (methyl, trideuteromethyl, ethyl, n-propyl and isopropyl iodides; methanol, ^{13}C-methanol, ethanol, n-propanol and isopropanol), silylation (N,O-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane), N,O-bis(tert-butyldimethylsilyl)trifluoroacetamide), acylation (acetic, trifluoroacetic, pentafluoropropionic and heptafluorobutyric anhydrides), and for the synthesis of alkoxycarbonyl-derivatives (methyl, ethyl, n-propyl and isopropyl chloroformates) were of "*derivatization*" grade available at Sigma-Aldrich. Anhydrous pyridine, chloroform, acetonitrile, dimethylformamide, powdered sodium hydroxide and 0.1 mol/L hydrochloric acid solution were also commercially available at Sigma-Aldrich. "Analytical standard" grade C7-C40 saturated alkane mixture for GC calibration was purchased at Sigma-Aldrich.

2.2 Microsynthesis.

[2] Certain commercial materials and instruments are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the identified materials are necessarily the best available for the purpose.

2.2.1 Alkoxycarbonyl derivatives.

The synthesis was carried out similarly to procedures described in [6]; 10 µL of alkyl chloroformate was added to a solution of 100 µL of 25 mmol/L aqueous hydrochloric acid, 53 µL of alcohol, 14 µL of pyridine and 1 mg of benzoic acid. Then 100 µL of chloroform containing 1 % of alkyl chloroformate (volume fraction) was added. An aliquot from the chloroform layer was separated and analyzed. Alkylation and trialkylsilylation were performed according to procedures described in [7-9]. Acylation was carried out according to [7,10], and synthesis of mixed alkyl/acyl derivatives were carried out as described earlier [2].

2.2.2 Instrumentation and Data analysis.

EI mass spectra were recorded on GC/MS systems with quadrupole analyzers (ionization energy 70 eV and ion source temperature 230 ^{o}C). Separation was achieved on a fused silica capillary column (15m, 0.25mm, 0.25 µm; non-polar stationary liquid phase: polymethylsiloxane + 5% phenyl groups) with programming oven temperature from 150 ^{o}C to 270 ^{o}C at a rate of 10 ^{o}C/min; the injection temperature was 270^{o}C. GC-MS-MS data were obtained at 5, 10, 20 and 40 eV collision energies on systems with quadrupole analyzers using nitrogen as a CID-gas. The data evaluation is based on comparison to spectra of corresponding ^{13}C and ^{2}H labeled analogs.

3. RESULTS AND DISCUSSIONS

Examination of EI mass spectral data of various chemical modification products (i.e. hydroxyl, mercapto and amino benzoic acids) has three objectives:

(1) Detection of characteristic “fingerprints” typical for specific classes of compounds.

(2) Determination of the consistency between a spectrum and the structure using ion thermo-chemistry along with the known ion decomposition rules to recognize possible exceptions to accepted organic mass spectrometry fragmentation rules.

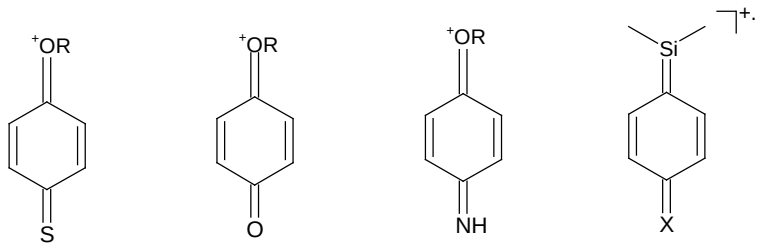
(3) Documentation of fragmentation patterns useful for structure elucidation to classify and correlate reliably recognized patterns.

Comparative analysis of EI mass spectra of alkyl, trialkylsilyl, acetyl, perfluoroacyl and alkoxycarbonyl derivatives of hydroxyl, mercapto and amino benzoic acids reveals strong *ortho* effects for derivatives of anthranilic, salicylic and thiosalicylic acids. Otherwise ubiquitous fragmentation of these “ortho compounds” is almost completely suppressed by the interaction of vicinal substituents. This interaction of neighboring groups was observed early in the history of organic mass spectrometry [11]. In contrast, the EI spectra of *meta* and *para* isomers in these series of derivatives reflect generally similar decomposition of $M^{+\cdot}$ with some remarkable exceptions: 1,4-isomers depict *para* specificity leading to the formation of ions with a *para* quinoid-type structure. A similar *para* effect was noted in the spectra of di-perfluoroacyl derivatives of bifunctional aminobenzenes [12].

*3.1 “para-E*ffect”

The 1,2- and 1,4-locations of functional groups in the chemical modification products of hydroxyl, mercapto and amino benzoic acids under EI may promote decomposition

processes leading to the formation of stable ions containing the *ortho* and *para* quinoid structure. However, for *ortho* isomers this fragmentation pathway is negligible, and competing rearrangement processes dominate due to the interaction of vicinal groups. In contrast, decomposition pathways leading to ions ***a*** - ***d*** with *para* quinoid structure predominate for all 1,4-isomers (Scheme 1), although the routes for their formation may differ.



a ***b*** ***c*** ***d***

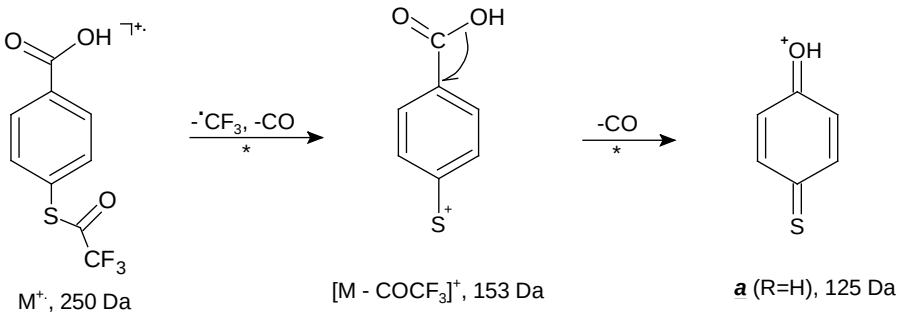
R= H, CH_3, C_2H_5, n-C_3H_7, CF_3, C_2F_5, C_3H_7
X= S, O, NH

Scheme 1. Characteristic ions for *para* isomers.

Structures of ions, their mass values and intensities of corresponding peaks in the spectra of derivatives of thiosalicylic, salicylic and anthranilic acids are given in Tables 1, 2 and 3. Mass spectral and GCRI data for all derivatives will be included in the next release of the NIST/NIH/EPA mass spectral library (http://www.nist.gov/srd/nist1a.cfm).

The formation of ions with a quinoid structure is demonstrated by considering the mass spectra of derivatives of 4-mercaptobenzoic acid.

The simplest ion ***a*** (R=H) having a possible structure of protonated 1,4-cyclohexadien-3-thione-6-one cation at 125 Da is produced from $M^{+\cdot}$ of *para*-(trifluoroacetylthio)benzoic acid as a result of a simple S-C bond cleavage followed by a skeletal rearrangement and a loss of carbon monoxide molecule. A two-step generation of ions ***a*** (R=H), presented in Scheme 2, is confirmed by CID data.



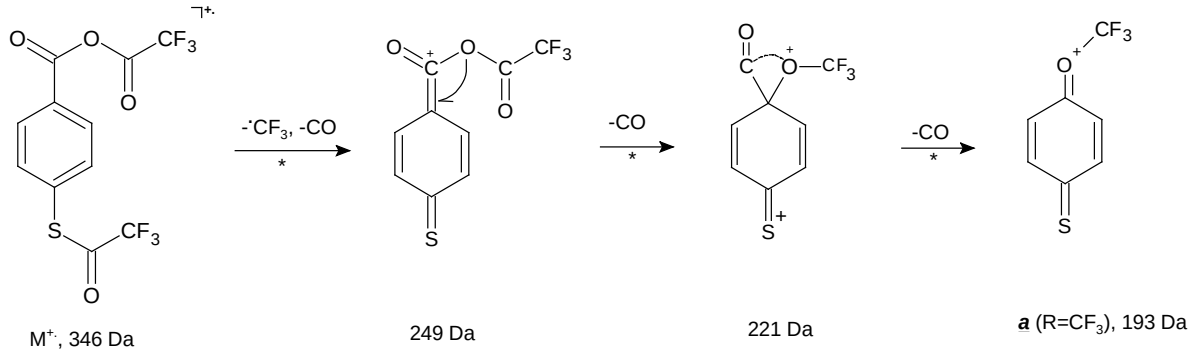
Scheme 2. Formation of protonated 1,4-cyclohexadien-3-thione-6-one cation.

The methyl ester of the same derivative (methyl 4-(trifluoroacetylthio)benzoate) generates O-methyl cations ***a*** (R=CH_3) via a similar route. The mass value of this ion at 139 Da is shifted to 142 Da in the spectrum of the corresponding trideuteromethyl ester indicating the presence in the cation ***a*** (R=CH_3) of a methyl group originated from the carbomethoxy moiety.

O-Methyl cations ***a*** (R=CH_3) are also typical for methyl 4-(alkoxycarbonylthio)benzoates (alk = methyl, ethyl, n-propyl, isopropyl), and a 1 Da mass shift is observed for this ion (from 139 Da to 140 Da) when comparing spectra of methyl and ^{13}C-methyl 4-(alkoxycarbonylthio)benzoates (Figures 2B and 2E). Ethyl 4-(ethoxycarbonylthio)benzoate and n-propyl 4-(n-propyloxycarbonylamino)benzoate are

the sources for the formation of O-ethyl and O-n-propyl cations ***a*** ($R=C_2H_5$ and $R=C_3H_7$) (Table 1).

Formation of O-trifluoromethyl cations ***a*** ($R=CF_3$) becomes characteristic for an anhydride of trifluoroacetic acid and 4-(trifluoroacetylthio)benzoic acid (Figure 2F). Successive loses of three carbon monoxide molecules from $[M\text{-}CF_3]^+$ ions (Scheme 3) are responsible for generation of cations ***a*** ($R=CF_3$) as supported by CID experiments.

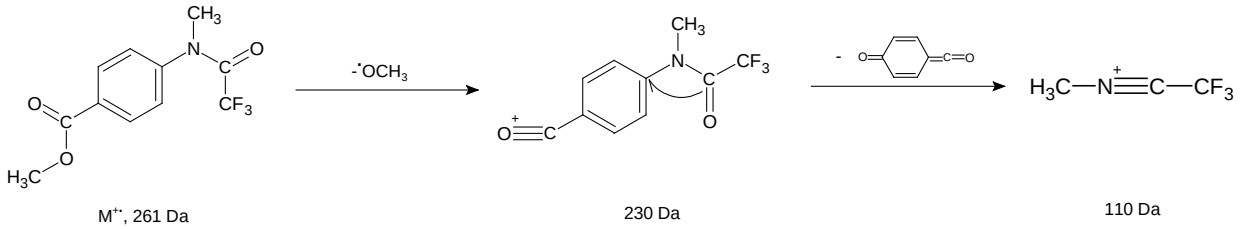


Scheme 3. Elimination of three carbon monoxide molecules from $[M\text{-}CF_3]^+$ ion.

Trialkylsilyl esters of 4-(perfluoroacylthio)benzoic acids form *para* quinoid-type ion fragments (***d***, X=S). However, there is a competing decomposition of the molecular ions to eliminate a methyl or a tert-butyl group from the trialkysilyl-function. The resulting $[M–CH_3$ (or $C_4H_9)]^+$ ions undergo skeletal rearrangement leading to the expulsion of carbon dioxide, and further elimination of another radical ($COCF_3$·) violating the 'even-electron rule' [13, 14]. As a result 1,4-cyclohexadien-3-thion-6-dimethylsilyl radical cations $[(CH_3)_2Si=C_6H_4=S]^{+\cdot}$ are produced. Relative intensities of peaks of corresponding to

these ions (***d***, X=S) in the spectra of seven various trimethylsilyl or tert-butyldimethylsilyl esters of 4-thioperfluoroacylbenzoic acids are given in Table 1.

Para isomers of methyl esters of N-methyl-N-(trifluoroacetyl)aminobenzoic acid exhibit an alternative characteristic fragmentation. The N-methyl-C-trifluoroacetylnitrilium cation $[CF_3C{\equiv}NCH_3]^+$ at 110 Da dominates the spectrum of the *para*-isomer, whereas its relative intensity is 12% and 57% for *ortho* and *meta* positional isomers, respectively. The origin of this nitrilium cation has been reported earlier [15, 16], and its formation involving oxygen rearrangement is rationalized in Scheme 4.



Scheme 4. Formation of nitrilium cations.

The ions ***a***, ***b***, ***c*** and ***d***, as well as nitrilium cations can be successfully employed for differentiation of *para* isomers from their *ortho* and *meta* counterparts.

*3.2 "ortho-E*ffect"

1,2-Location of aromatic functional groups and their interaction triggers specific decomposition reactions under EI and suppresses fragmentations characteristic for *meta* and *para*-isomers. This phenomenon is termed the '*ortho* effect', and is well-documented [17-28]. Interactions of vicinal groups in the $M^{+\cdot}$ of various derivatives of substituted benzoic acids initiate various types of hydrogen and skeletal

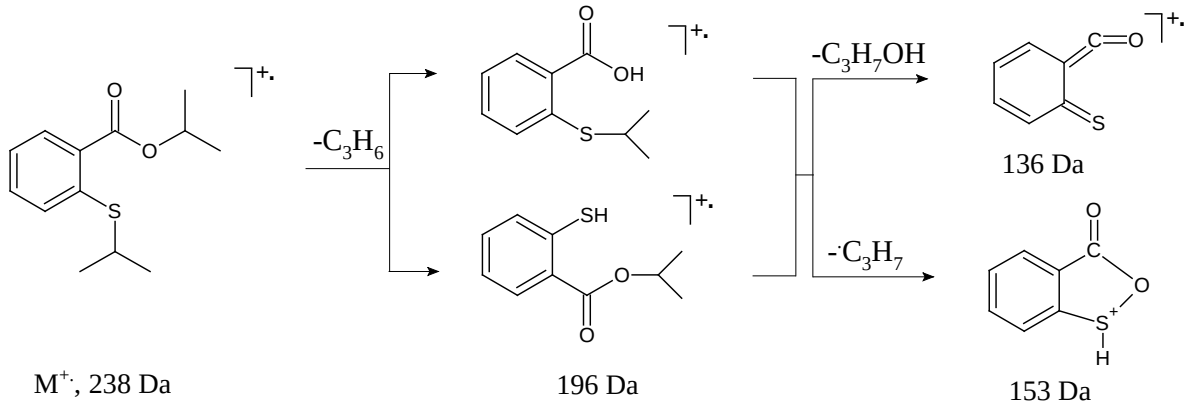
rearrangements, and generate multiple fragmentation processes. As a result, for different derivatives within each type of benzoic acid (hydroxyl, mercapto or amino) diverse competing decomposition reactions are observed. Therefore, the '*ortho* effect' recorded for each derivative type is discussed separately.

3.2.1 Alkyl derivatives

Cations $[M\text{-}CH_3\text{-}H_2O]^+$ and $[M\text{-}CH_3\text{-}H_2O\text{-}CO]^+$ are specific for the methyl ether of methyl salicylate (Figures 3C, 3D). The $M^{+\cdot}$ readily expels a methyl radical, and then 1,6-H transfers take place from the phenolic methoxy group to the carboxyl functionality; as a result, a water molecule is eliminated. The resulting cation $[M\text{-}CH_3\text{-}H_2O]^+$ at 133 Da further eliminates CO giving rise to an ion at 105 Da. Similarly, the exclusive elimination of water containing the intact hydroxyl function and hydrogen from the methyl group has been confirmed by a complete analysis of MIKE spectra along with deuterium labelling studies and semi-empirical calculations for 2-methylbenzenemethanol and 2–methyl benzoic acid [14].

Cations $[M\text{-}CH_3OH]^+$ (150 Da), $[M\text{-}CH_3OH\text{-}CO]^+$ (122 Da) and $[M\text{-}CH_3O\text{-}CH_2O]^+$ (120 Da) become diagnostically important ions useful for distinction of methyl S-methylthiosalicylate, the *ortho* isomer, (Figure 3E) from its *meta* and *para* counterparts. The unusual loss of a formaldehyde molecule from the $[M\text{-}OCH_3]^+$ cation is established both by CID experiments and comparison to the spectra of labeled analogs (Figures 3F, 3G, 3H).

Fragmentation pathways for di-alkyl derivatives become predictable when the alkyl group is longer than methyl. Thus $M^{+\cdot}$ of the di-isopropyl derivative of 2-mercaptobenzoic acid eliminates isopropene and isopropanol molecules giving rise to a base peak of an ion $[M\text{-}C_3H_6\text{-}C_3H_7OH]^{+\cdot}$ At 136 Da (Scheme 5). The fragmentation of *meta* and *para* isomers is dominated by successive losses of two propene molecules, generating $[M - 2\ C_3H_6]^{+\cdot}$ ions.



Scheme 5. Decomposition of isopropyl 2-(isopropylthio)benzoate molecular ion.

3.2.2 Trimethylsilyl esters

Partially and completely derivatized trimethylsilyl (TMS) derivatives of hydroxyl, mercapto and amino benzoic acids, and some mixed derivatives indicate an expected dependence between the observed fragmentation pathways and the extent of functional group derivatization.

3.2.2.1 TMS hydroxyl, mercapto and aminobenzoates.

Radical-cations $[M\text{-}(CH_3)_3SiOH]^{+\cdot}$ are the key ions for distinguishing the TMS salicylates and thiosalicylates from their *meta, para* positional isomers, with the corresponding

peaks among the most prominent in the spectra. These ions result from a 1,5-hydrogen shift followed by the elimination of trimethylsilylanol.

Related $[M-(CH_3)_3SiOH]^{+\cdot}$ ions are diagnostically important for differentiation of isomeric TMS aminobenzoates. Thus, TMS anthranilate – the *ortho* isomer eliminates trimethylsilanol molecule and trimethylsilyloxy radical in a 1:1 ratio. Corresponding *meta*, *para* counterparts generate only $[M-OSi(CH_3)_3]^+$ ions (Figure 4). The formation of $[M-OSi(CH_3)_3]^+$ ions is a two-step decomposition process involving successive losses of methyl radical and neutral trimethylsilyl oxide as determined by CID experiments. In contrast, trimethylsilanol is eliminated from $M^{+\cdot}$ via a single step, as a result of a 1,5 hydrogen shift rearrangement similar to the salicylate fragmentation mechanism.

3.2.2.2 TMS O(S, NH)-TMS benzoates.

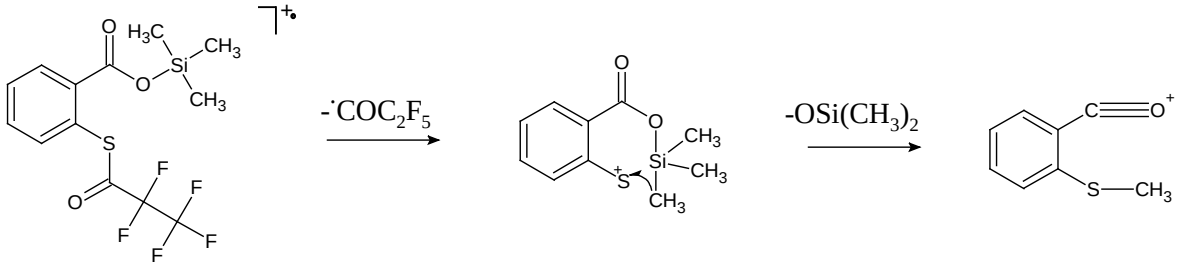
Addition of TMS to both functional groups dramatically changes the major fragmentation routes of $M^{+\cdot}$ of corresponding derivatives. Formation of an aromatic ring containing fragments, other than $M^{+\cdot}$ and $[M-CH_3]^+$, is suppressed for di(TMS) derivatives of salicylic and thiosalicylic acids. Dominant peaks in the spectra appear at 73 Da (trimethylsilyl cation) for di-TMS salicylate, and at 147 Da (pentamethylsiloxanyl cation) for di-TMS-thiosalicylate. Ions containing silyl and aryl moieties are equally generated from $M^{+\cdot}$ of di-TMS derivatives of *meta* and *para* hydroxyl and mercapto benzoic acids; prominent peaks in their spectra correspond to $[M-CH_3-CO_2]^+$ ions.

3.2.2.3 TMS O(S, NH)-perfluoroacyl benzoates.

Mixed derivatives formed using the perfluoroacyl group for blocking hydroxyl, mercapto or amino functions and trimethylsilyl for modification of the carboxyl group lead to generation of multiple fragmentation pathways. Ions produced as a result of vicinal group interactions and specific for *ortho* isomers are different for hydroxyl-, mercapto and amino benzoic acid derivatives.

Salicylates. The diagnostically important ions in the spectra of TMS O-(perfluoroacyl)salicylates formally correspond to methoxybenzoyl cation (135 Da) and a radical cation at 120 Da that is a result of $\cdot CH_3$ elimination from methoxybenzoyl cation.

Thiosalicylates. Two prominent peaks in the spectra can be successfully used for differentiation of TMS S-(perfluoroacyl)thiosalicylates from their *meta* and *para* counterparts. Thus, the spectrum of TMS S-(pentafluoropropionyl)thiosalicylate contains a base peak of $[M\text{-}C_2F_5CO]^+$ ion at 225 Da and a prominent peak of $[M\text{-}C_2F_5CO\text{-}(CH_3)_2SiO]^+$ that is a product of skeletal rearrangement and the loss of dimethylsilyl oxide from the ion at 225 Da (Scheme 6, Figure 5).



$M^{+\cdot}$, 372 Da 225 Da 151 Da

Scheme 6. Specific fragmentation of *ortho* isomers of trimethylsilyl S-perfluoroacyl mercaptobenzoates.

Anthranilates. Another ion $[M\text{-}C_nF_{2n+1}\text{-}(CH_3)_3SiOH]^+$ at 146 Da with maximum intensities in the spectra is characteristic for TMS O-(trifluoroacetyl)- and O-(pentafluoropropionyl)anthranilic acids. It can be successfully employed for differentiation of the *ortho* from its positional isomers.

3.2.2.4 TMS N-TMS-N-pentafluoropropionyl-benzoates.

Introduction of an additional perfluoroacyl substituent to the amino group in O, N-di(trimethylsilyl)anthranilic acid molecule leads to the formation of ions more useful for structure elucidation. Thus, characteristic ions for TMS ester of N-TMS-N-pentafluoropropionylanthranilic acid are: $[M\text{-}C_2F_5]^+$ and $[M\text{-}C_2F_5\text{-}Si(CH_3)_4]^+$ (Figure 6). They serve to differentiate the *ortho* isomer from its *meta*- and *para*- counterparts.

3.2.3 Methyl esters of acyl derivatives

Methyl esters of O-, S- or N-perfluoroacyl derivatives of benzoic acids exhibit fragmentation characteristics similar to their TMS counterparts.

Salicylates. The $[M\text{-}OCH_3\text{-}COC_nF_{2n+1}]^{+\cdot}$ ion at 120 Da that is generated by successive loss of two radicals dominates the spectra of methyl O-perfluoroacylsalicylates (Acyl = $COCF_3$, COC_2F_5, COC_3F_7). A similar kind of violation of the 'even-electron rule' was reported [29] when a base peak of $[M\text{-}30]^+$ was detected in the spectrum of TMS ester of methylsalicylate; it was determined that consecutive losses of two methyl radical ions resulted in the formation of this ion. The radical cations $[M\text{-}OCH_3\text{-}COC_nF_{2n+1}]^{+\cdot}$ can be used for distinguishing the *ortho* isomer since $M^{+\cdot}$ of corresponding *meta* and *para* counterparts do not exhibit these losses.

Thiosalicylates. Two ions $[M\text{-}COC_nF_{2n+1}\text{-}CH_3]^+$ at 152 Da and $[M\text{-}COC_nF_{2n+1}\text{-}CH_2O]^+$ at 137 Da are sufficient for differentiation of methyl S-perfluoroacylsalicylate from its positional isomers.

Aminobenzoates. Methyl aminobenzoates may be analyzed in two ways - as N-acyl- and N-methyl-N-acyl-derivatives, since there is a possibility for the introduction of an additional alkyl to N- atom.

(A)The presence of a base peak at 146 Da likely corresponds to the indol-2,3-dionyl cation in the spectrum of methyl N-trifluoroacetylanthranilate, and can be used for structure elucidation. This ion is a result of elimination of trifluoromethyl radical followed by the loss of methanol.

(B) The base peak at 132 Da, probably with a structure of indol-3-onyl cation, is characteristic of methyl N-methyl-N-trifluoroacetylanthranilate. Its corresponding molecular ion loses neutral CH_3OH along with $\cdot OCH_3$ radical in a 1:1 ratio; elimination of methanol is possible if the N-methyl group is recognized as a potential hydrogen donor. Further, the ions at $[M\text{-}CH_3OH]^{+\cdot}$ eliminate $\cdot COCF_3$ radical producing the base peak at 132 Da.

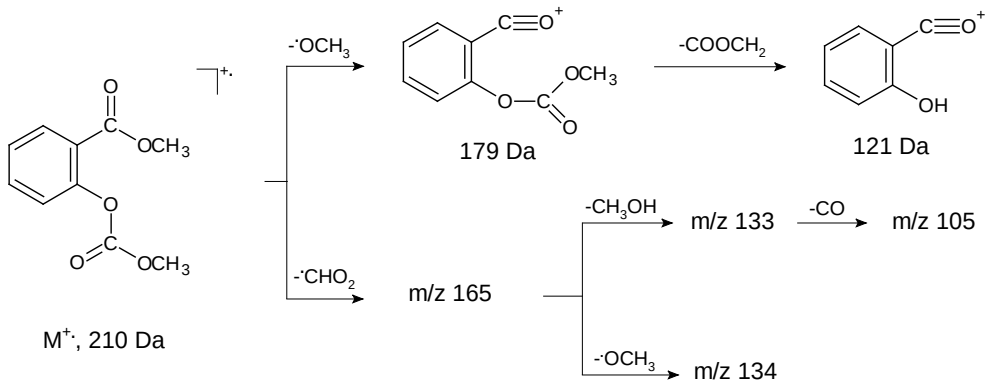
The indol-2,3-dionyl and indol-3-onyl cations can be employed for structure elucidation of methyl acylanthranilates.

3.2.4 Methoxycarbonyl derivatives

The character of 1,2-interaction between two methoxycarbonyl moieties connected to C_{Aryl} and O, S or N depends on the nature of a hetero-atom to which the

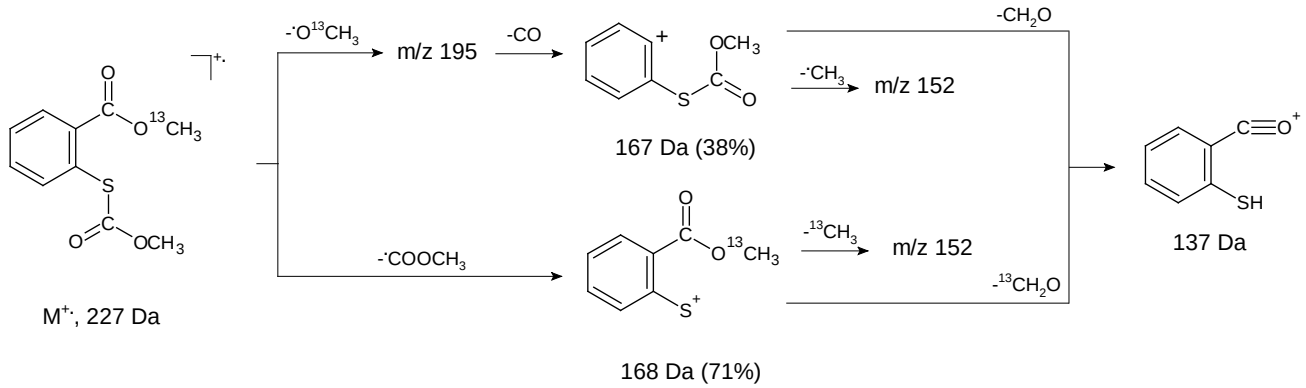
methoxycarbonyl group is attached. Decomposition of methyl esters for methoxycarbonyl derivatives of hydroxyl and mercaptobenzoic acids appear more complex than their corresponding anthranilates.

Salicylates. Peaks of four ions $[M\text{-}HCO_2]^+$ at 165 Da, $[M\text{-}HCO_2\text{-}OCH_3]^{+\cdot}$ at 134 Da, $[M\text{-}HCO_2\text{-}CH_3OH]^+$ at 133 Da and $[M\text{-}OCH_3\text{-}C_2H_2O_2]^{+\cdot}$ at 133 Da are results of complex rearrangements (Scheme 7). These ions allow reliable differentiation of methyl methoxycarbonylsalicylate from *meta* and *para* isomers.



Scheme 7. Fragmentation pathways useful for differentiation of methyl methoxycarbonylsalycilates from *meta* and *para* counterparts.

Thiosalicylates. Analysis of peak intensities and their ratios is required for differentiation of methyl thiosalicylate from its positional isomers (Figures 2A-C). Ions specific for *ortho* isomers possess the following mass values: (122, 137, 150 and 152) Da. Comparative analysis of spectra for methyl thiosalicylate and 13C-methyl analog (Figure 2D) allows differentiation of isobaric ions as denoted in Scheme 8.



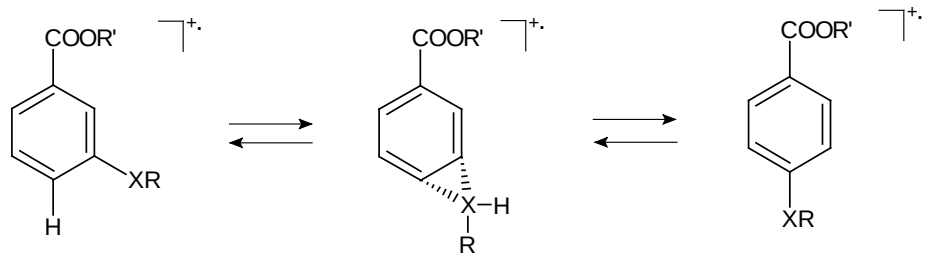
Scheme 8. Fragmentation pathways useful for differentiation of methyl S-(metoxycarbonyl) mercaptosalycilates from *meta* and *para* isomers.

Aminobenzoates. The key diagnostically important fragmentation of methyl N-methoxycarbonylanthranilate – the ortho isomer is associated with hydrogen rearrangement. It leads to the elimination of a neutral methanol molecule. The same derivative of the *meta* isomer expels both neutral methanol and a methoxy radical in a 1:2 ratio; the *ortho* and *para*-isomers eliminate only methoxy radicals. The base peaks in the spectra of all positional isomers formally correspond to the loss of hydrogen and two methoxy groups from $M^{+\cdot}$.

3.3 Meta isomerization

Molecular ions of *meta* isomers are not expected to produce ions with *ortho*- or *para*-quinoid structures, and the potential for interaction between 1,3-functional groups is unfavorable. Consequently, the fragmentation pathways for these isomers are straightforward, and decomposition of corresponding $M^{+\cdot}$ is governed by well-established ion fragmentation rules, as exemplified by the EI spectra of methyl 3-methoxybenzoate (Figure 3A), methyl 3-S-methoxycarbonylthiobenzoate (Figure 2C) and trimethylsilyl 3-pentafluoropropionylthiobenzoate (Figure 5). However, the spectra

of some derivatives contain low intensity peaks of ions characteristic for their *ortho* or *para* counterparts. Thus, a spectrum of methyl 3-S-methoxycarbonylthiobenzoate (Figure 2C) reveals a peak at 139 Da of 4% intensity vs 31% for the para isomer (Figure 2B). This may be a result of partial isomerism of *meta* -isomer to its *para* counterpart under EI [30] (Scheme 9):



Scheme 9. Possible isomerization of *meta* isomer to *para* counterpart.

The same is true for the isomerism of *meta* to *ortho*: there is a measureable peak at 133 Da (.4 %) in the spectrum of methyl 3-methoxybenzoate (Figure 3A). This peak corresponds to an ion $[M\text{-}CH_3\text{-}H_2O]^+$ and characteristic for the *ortho* isomer; the purity is controlled by GC.

Conclusion

GC-MS compatible derivatives for the identification of positional isomers of hydroxyl, mercapto and amino benzoic acids produce mass spectra with different characteristics that are useful for distinguishing among isobaric species. Methyl and trimethylsilyl esters of O, S- and N-alkyl, -trimethylsilyl, -acetyl, -perfluoroacyl and –alkoxycarbonyl derivatives of benzoic acids were analyzed and compared. General fragmentation

pathways of $M^{+\cdot}$ are described using the analysis of spectral data of labeled and unlabeled isotope analogs, supplemented by examination of CID data. Specific fragmentation processes for *ortho* isomers due to interaction of vicinal substituents are established; they mostly include additional hydrogen rearrangements. Skeletal rearrangements characteristic for *para* isomers are determined; the driving force for these rearrangements is the formation of stable *para* quinoid type ions. Knowledge of diagnostically important pathways can inform the selection of an appropriate derivative for an analysis by including the structural information required for structure differentiation. The choice of media is important as well: alkyl and acyl derivatives are prepared in anhydrous media while alkoxycarbonyl derivatives require the presence of water.

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The captions of Figures and Schemes

1) Figure 1. Molecular structures of chemical modification products of salicylic, mercaptosalicylic and anthranilic acids, and their positional isomers.

2) Figure 2. Mass spectra of methyl (A) *ortho*-, (B) *para*- and (C) *meta*-S-methoxycarbonylmercaptobenzoates, ^{13}C-methyl (D) *ortho*- and (E) *para*-S-methoxycarbonylmercaptobenzoates, and (F) anhydride of trifluoroacetic and S-trifluoroacetyllmercaptobenzoic acids.

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4) Figure 4. Mass spectra of trimethylsilyl esters of anthranilic acid and its *meta*- and *para*-isomers.

5) Figure 5. Mass spectra of trimethylsilyl esters of S-pentafluoropropionylthiosalicylic acid and its *meta*- and *para*-isomers.

6) Figure 6. Mass spectra of trimethylsilyl esters of N-trimethylsilyl-N-pentafluoropropionylanthranilic acid and its *meta*- and *para*-isomers

7) Scheme 1. Characteristic ions for *para* isomers.

8) Scheme 2. Formation of protonated 1,4-cyclohexadien-3-thione-6-one cation.

9) Scheme 3. Elimination of three carbon monoxide molecules from $[M\text{-}CF_3]^+$ ion.

10) Scheme 4. Formation of nitrilium cations.

11) Scheme 5. Decomposition of isopropyl 2-(isopropylthio)benzoate molecular ion.

12) Scheme 6. Specific fragmentation of *ortho* isomers of trimethylsilyl S-perfluoroacyl mercaptobenzoates.

13) Scheme 7. Fragmentation pathways useful for differentiation of methyl methoxycarbonylsalycilates from *meta* and *para* counterparts.

14) Scheme 8. Fragmentation pathways useful for differentiation of methyl S-(metoxycarbonyl) mercaptosalycilates from *meta* and *para* isomers.

15) Scheme 9. Possible isomerization of *meta* isomer to *para* counterpart.

Tables

1) Table 1. Thiobenzoquinoid type ions in the spectra of derivatives of para-mercaptobenzoic acids.

Compound	***m/z (Rel. %)***	***Ion structure***
4-(Thiotrifluoroacetyl)benzoic acid	125 (31)	$HO^+=C_6H_4=S$
4-(Thiopentafluoropropionyl)benzoic acid	125 (27)	
4-(Thioheptafluorobutyryl)benzoic acid	125 (24)	
4-Thiocarbomethoxybenzoic acid	125 (58)	
4-Thiocarboethoxybenzoic c acid	125 (20)	
4-Thiocarbo-n-propyloxybenzoic acid	125 (14)	
4-Thiocarboisopropyloxybenzoic acid	125 (13)	
Methyl 4-(thiocarbomethoxy)benzoate	139 (31)	$CH_3O^+=C_6H_4=S$
Methyl 4-(thiocarboethoxy)benzoate	139 (26)	
Methyl 4-(thiocarbo-n-propyloxy)benzoate	139 (26)	
Methyl 4-(thioisopropyloxy)benzoate	139 (17)	
Methyl 4-(thiotrifluoroacetyl)benzoate	139 (46)	
Methyl 4-(thioacetyl)benzoate	139 (13)	
Ethyl 4-(thiocarboethoxy)benzoate	153 (9)	$C_2H_5O^+=C_6H_4=S$
n-Propyl 4-(thiocarbo-n-propyloxy)benzoate	167 (3)	$C_3H_7O^+=C_6H_4=S$
Trifluoroacetyl 4-(thiotrifluoroacetyl)benzoyl anhydride	193 (11)	$CF_3O^+=C_6H_4=S$
Trimethylsilyl 4-(thiotrifluoroacetyl)benzoate	166 (12)	$(CH_3)_2Si=C_6H_4=S^{+\cdot}$
Trimethylsilyl 4-(thiopentafluoropropionyl)benzoate	166 (12)	
Trimethylsilyl 4-(thioheptafluorobutyryl)benzoate	166 (14)	
t.-Butyldimethylsilyl 4-(thiotrifluoroacetyl)benzoate	166 (6)	
t.-Butyldimethylsilyl 4-(thiopentafluoropropionyl)benzoate	166 (10)	
t.-Butyldimethylsilyl 4-(thioheptafluorobutyryl)benzoate	166 (11)	
Trimethylsilyl 4-(thiotrimethylsilyl)benzoate	166 (12)	

2) Table 2. Benzoquinoid type ions in the spectra of derivatives of para-aminobenzoic acids.

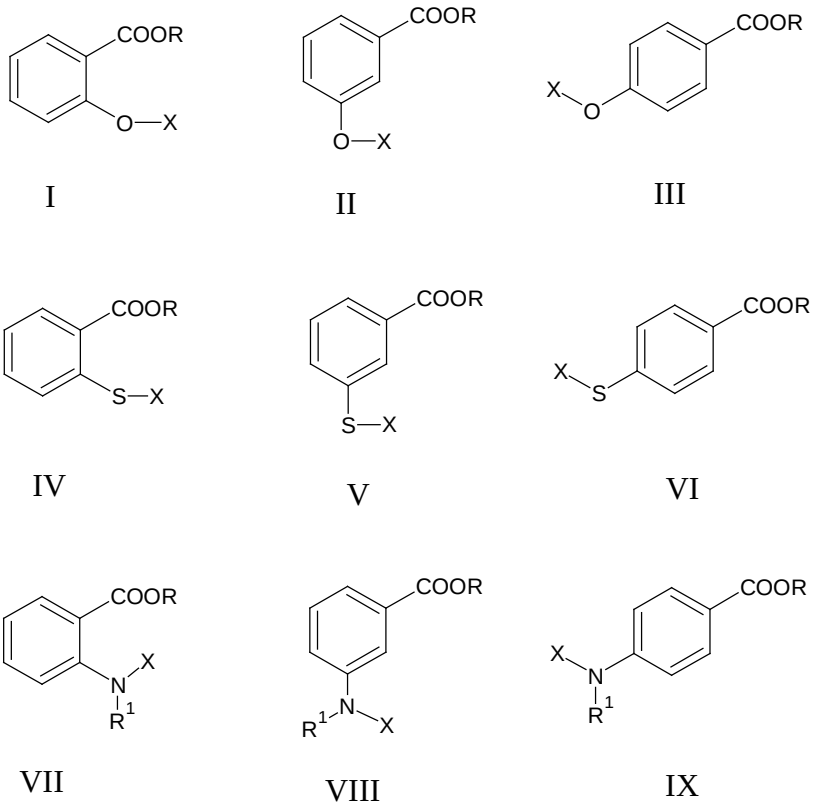
Compound	***m/z (Rel. %)***	***Ion structure***
4-Aminobenzoic acid, N-carbomethoxy-	108 (10)	$HO^+=C_6H_4=NH$
4-Aminobenzoic acid, N-carboethoxy-	108 (18)	
4-Aminobenzoic acid, N-carbo-n-propyloxy-	108 (13)	
4-Aminobenzoic acid, N-carboisopropyloxy-	108(15)	
Methyl 4-(aminocarbomethoxy)benzoate	122(19)	$CH_3O^+=C_6H_4=NH$
Methyl 4-(aminocarboethoxy)benzoate	122 (17)	
Methyl 4-(aminocarbo-n-propyloxy)benzoate	122 (13)	
Methyl 4-(aminoisopropyloxy)benzoate	122 (10)	
Methyl 4-(aminotrifluoroacetyl)benzoate	122 (8)	
Methyl 4-(aminopentafuoropropionyl)benzoate	122 (9)	
Methyl 4-(thioheptafluorobutyryl)benzoate	122 (14)	
Methyl 4-(aminoacetyl)benzoate	122 (3)	
Trifluoroacetyl 4-(aminotrifluoroacetyl)benzoyl anhydride	176 (.8)	$CF_3O^+=C_6H_4=NH$
Pentafluoropropionyl 4-(aminopentafluoropropionyl) benzoyl	226 (.5)	$C_2F_5O^+=C_6H_4=NH$
Heptafluorobutyryl 4-(aminoheptafluorobutyryl) benzoyl	276 (.5)	$C_3F_7O^+=C_6H_4=NH$

3) Table 3. Benzoquinoid type ions in the spectra of derivatives of para-hydroxybenzoic acids.

Compound	***m/z (Rel. %)***	***Ion structure***
4-(Oxytrifluoroacetyl)benzoic acid	109 (10)	$HO^+=C_6H_4=O$
4-Hydroxybenzoic acid, N-carbomethoxy-	109 (10)	
4-Hydroxybenzoic acid, N-carboethoxy-	109(4)	
4-Hydroxybenzoic acid, N-carbo-n-propyloxy-	109 (3)	
4-Hydroxybenzoic acid, N-carboisopropyloxy-	109 (1)	
Methyl 4-(oxycarbomethoxy)benzoate	123 (8)	$CH3O^+=C_6H_4=O$
Methyl 4-(oxycarboethoxy)benzoate	123 (5)	
Methyl 4-(oxycarbo-n-propyloxy)benzoate	123 (7)	
Methyl 4-(oxyisopropyloxy)benzoate	123 (6)	
Methyl 4-(oxytrifluoroacetyl)benzoate	123 (5)	
Methyl 4-(oxypentafluoropropionyl)benzoate	123 (10)	
Methyl 4-(oxyheptafluorobutyryl)benzoate	123 (15)	
Methyl 4-(oxyacetyl)benzoate	123 (3)	
Trifluoroacetyl 4-(oxytrifluoroacetyl)benzoyl anhydride	177 (1)	$CF3O^+=C_6H_4=O$
Pentafluoropropionyl 4-(oxypentafluoropropionyl) benzoyl	227 (.5)	$C_2F_5O^+=C_6H_4=O$
Heptafluorobutyryl 4-(oxyheptafluorobutyryl) benzoyl	277 (0.4)	$C_3F_7O^+=C_6H_4=O$

Figures

Figure 1. Molecular structures of chemical modification products of salicylic, mercaptosalicylic and anthranilic acids, and their positional isomers.

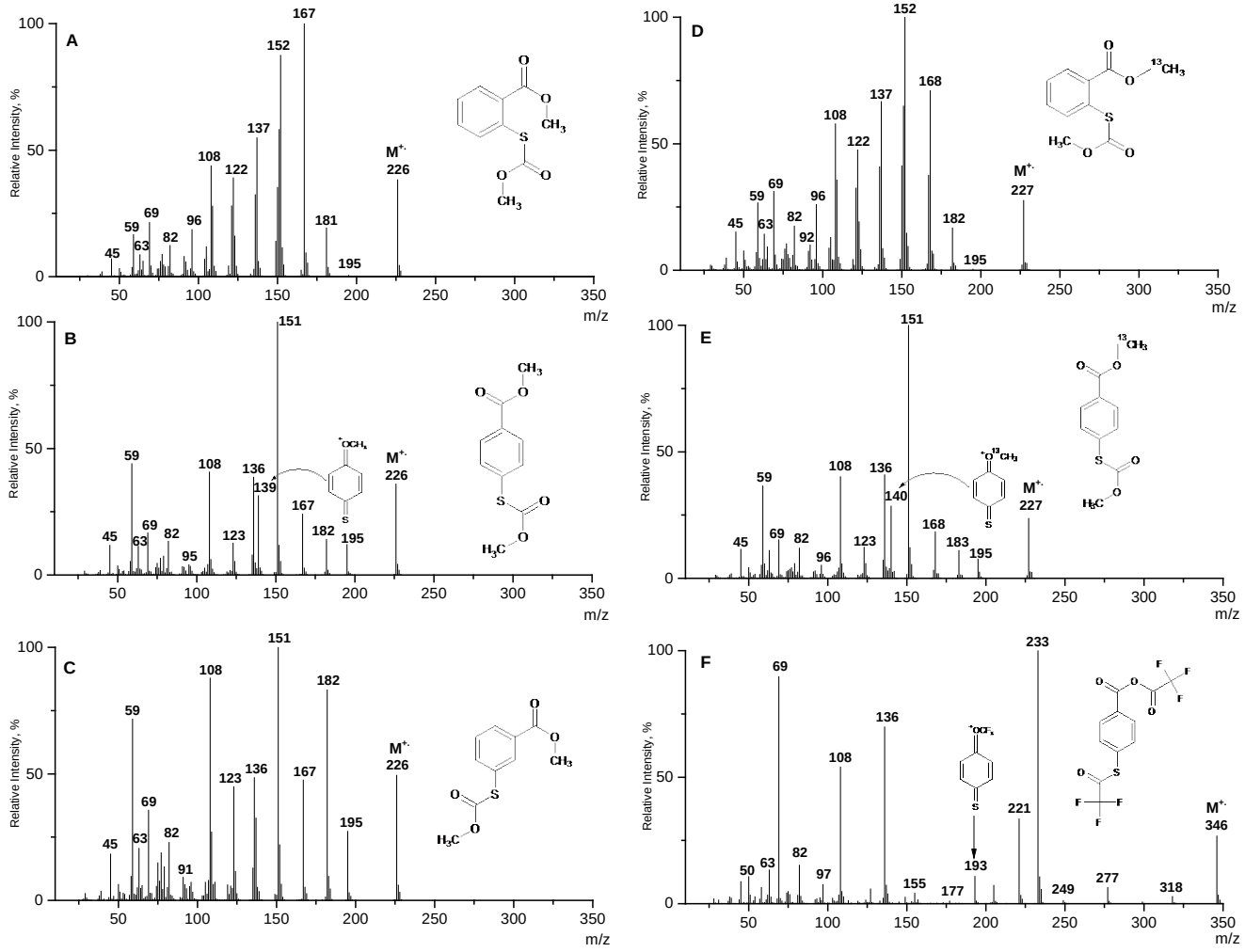


R = H, CH_3, $COCF_3$, $Si(CH_3)_3$, $Si(CH_3)_2(t\text{-}C_4H_9)$

R^1 = H, CH_3

X = H, CH_3, $COCH_3$, $COCF_3$, COC_2F_5, COC_3F_7, $COOCH_3$, $COOC_2H_5$, $COOC_3H_7$-n, $COOC_3H_7$-i, $Si(CH_3)_3$

Figure 2. Mass spectra of methyl (A) *ortho*-, (B) *para*- and (C) *meta*-S-methoxycarbonylmercaptobenzoates, ^{13}C-methyl (D) *ortho*- and (E) *para*-S-methoxycarbonylmercaptobenzoates, and (F) anhydride of trifluoroacetic and S-trifluoroacetyllmercaptobenzoic acids.



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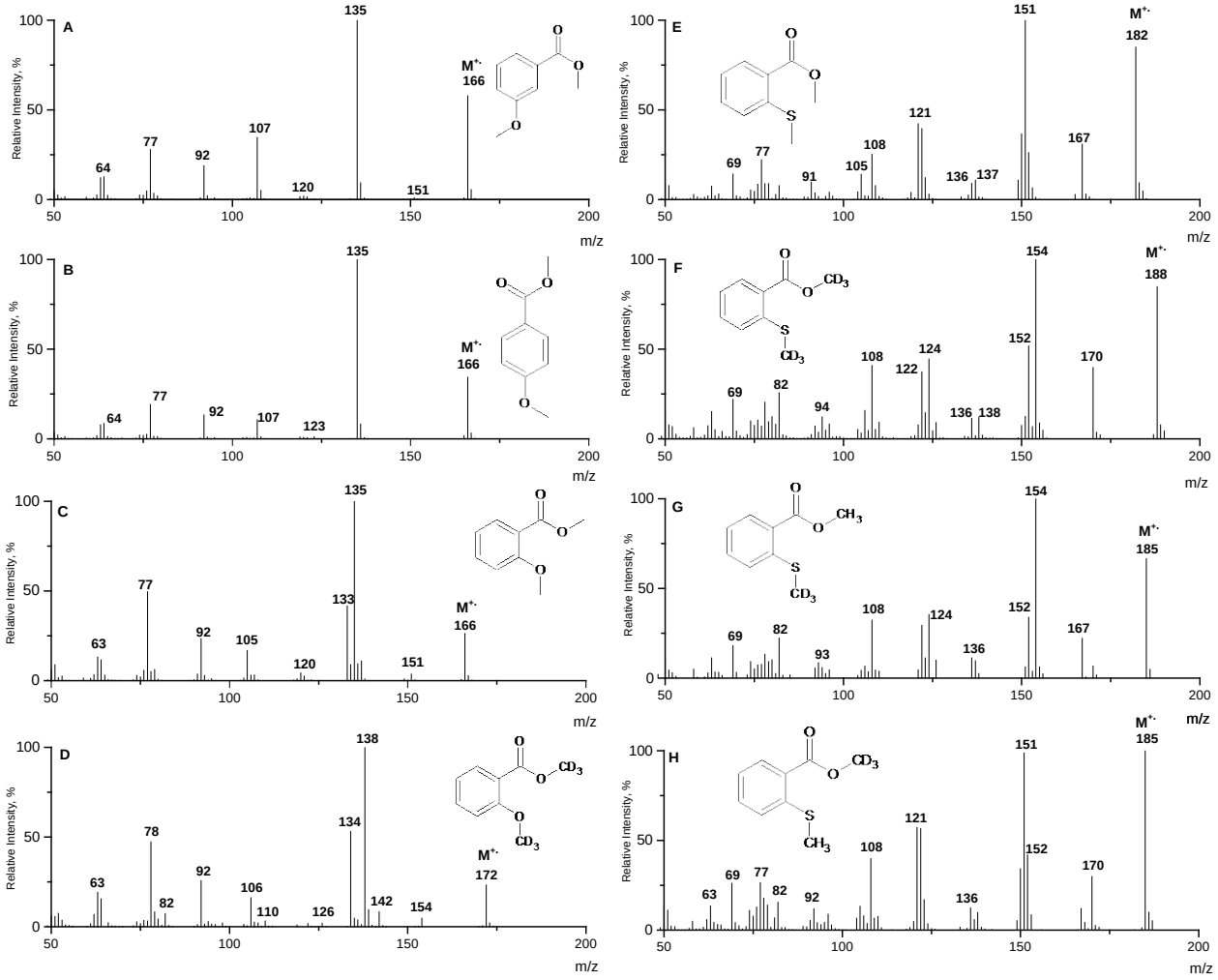

Figure 4. Mass spectra of trimethylsilyl esters of anthranilic acid and its *meta*- and *para*-isomers.

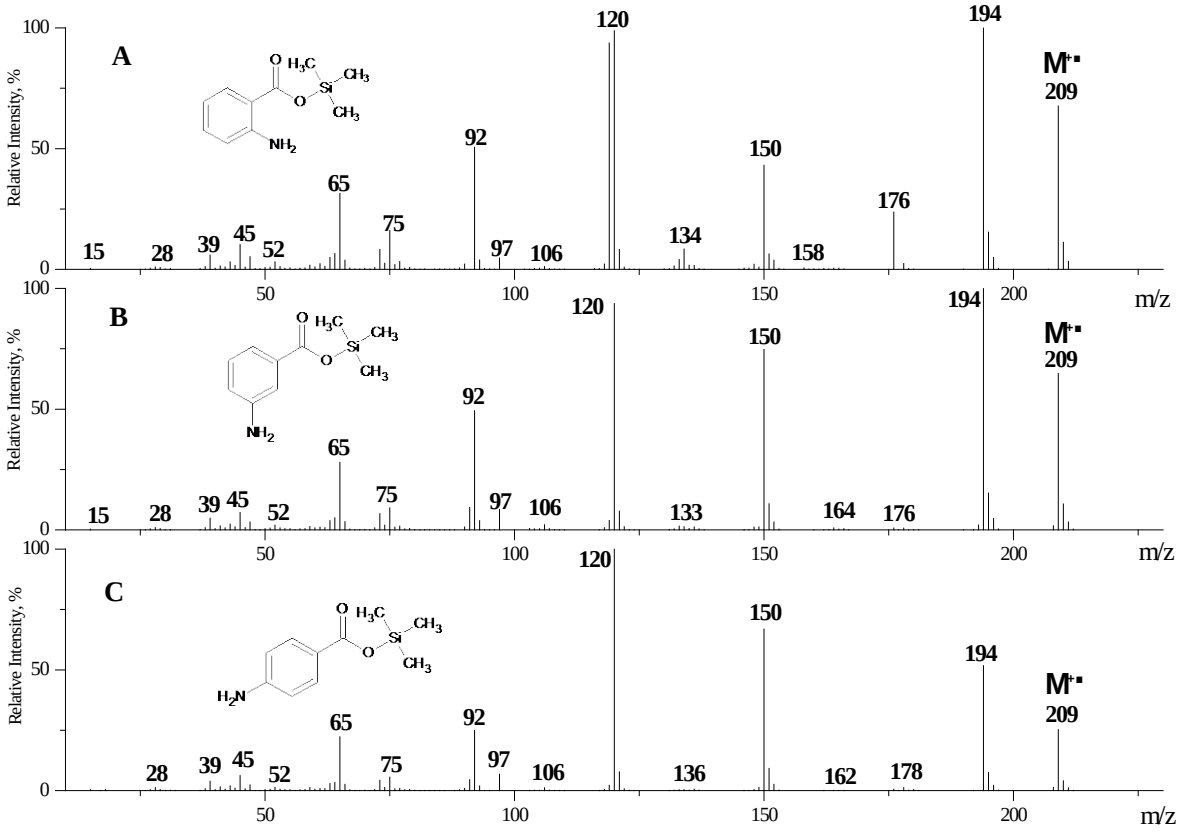

Figure 5. Mass spectra of trimethylsilyl esters of S-pentafluoropropionylthiosalicylic acid and its *meta*- and *para*-isomers.

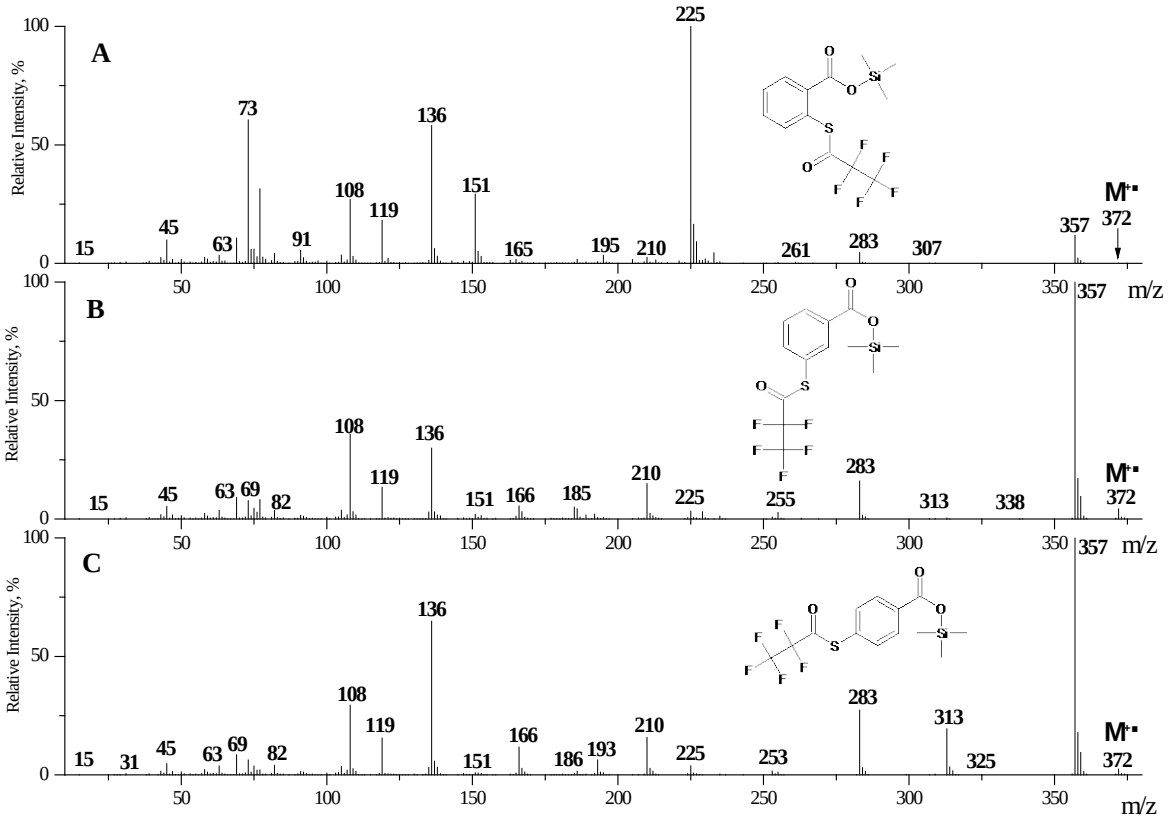

Figure 6. Mass spectra of trimethylsilyl esters of N-trimethylsilyl-N-pentafluoropropionylanthranilic acid and its *meta*- and *para*-isomers