Ortho Effect in Electron Ionization Mass Spectrometry of *N*-Acylanilines Bearing a Proximal Halo Substituent

Freneil B. Jariwala, Margaret Figus, and Athula B. Attygalle

Center for Mass Spectrometry, Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey, USA

Electron ionization (EI) mass spectra are not very helpful for characterizing *ortho*, *meta*, and *para* isomers of underivatized haloanilines since their spectra are virtually identical. In contrast, when the amino group of chloro-, bromo-, or iodoanilines is transformed to an *N*-formyl, *N*-acetyl, or *N*-benzoyl derivative, the spectra of the derivatives reveal a highly dramatic loss of a halogen radical, instead of an HX elimination usually expected from an "ortho effect." For example, the spectra of *N*-formyl, *N*-acetyl, and *N*-benzoyl derivatives of *ortho* isomers of chloro-, bromo-, and iodoanilines show a very prominent peak at m/z 120, 134, and 196, respectively, for the loss of the corresponding halogen atom. (J Am Soc Mass Spectrom 2008, 19, 1114–1118) © 2008 American Society for Mass Spectrometry

Haloanilines are widely used as synthetic raw materials for azo dyes, or intermediates in the manufacture of agrochemicals, drugs, laboratory reagents, and many other industrial chemicals [1]. Because haloanilines are often discharged as industrial waste into the environment, they constitute a significant group of pollutants [2, 3]. Undoubtedly, isomer identification of haloanilines is an important analytical problem, particularly for metabolic studies. Gas chromatography–mass spectrometry (GC/MS) is the preferred analytical procedure because of its high specificity and sensitivity. Although retention times are useful, characterization of haloaniline isomers solely by their mass spectra is not straightforward since the spectra are virtually identical. We describe here the practical value of a rather underutilized, but useful, ortho effect, which can be used to determine the substitution pattern of chloro-, bromo-, and iodoaniline derivatives.

Experimental

Reagents and Sample Preparation

All reagents and solvents were purchased from Aldrich Chemical Co. (St. Louis, MO, USA) and used as obtained.

Address reprint request to Prof. Athula B. Attygalle, Stevens Institute of Technology, Center for Mass Spectrometry, Department of Chemistry and Chemical Biology, Castle Point on Hudson, Hoboken, NJ 07030. E-mail: athula.attygalle@stevens.edu

Derivatization

For the preparation of the formyl derivatives, each haloaniline was treated with concentrated formic acid and the mixture was heated to 75 °C for 1 h. The excess formic acid was removed with a stream of N₂. A similar procedure was used for the synthesis of acetyl derivatives. A 1:1 mixture of acetic acid and acetic anhydride was added to each haloaniline. After 30 min, the samples were dried with a stream of N₂ to remove the excess acetic acid. The benzoyl derivatives were formed by mixing each haloaniline with aqueous NaOH and benzoyl chloride. For the synthesis of 2,4,6-tribromoaniline, aniline was mixed with 10% Br₂/H₂O and after 10 min, the product was then extracted into diethyl ether. The *N*-formyl, *N*-acetyl, and *N*-benzoyl derivatives of 2,4,6-tribromoaniline were formed using the methods described above. All of the derivatives formed were crystalline in nature and were dissolved in dichloromethane for GC/MS analysis.

EI Mass Spectrometry

Electron ionization (EI) mass spectra were recorded using an HP 5989B quadrupole mass spectrometer linked to a Hewlett Packard 5890 Series II gas chromatograph. Positive-ion electron-ionization mass spectra were acquired from m/z 35 to 450. Samples (1.0 mg/mL in dichloromethane) were introduced by splitless injection to a 30 m × 0.25 mm capillary column coated with a 0.25 μm film of XTI-5 (5% diphenyl/95% dimethyl polysiloxane; Restek, Bellefonte, PA, USA). The oven temperature was maintained at 40 °C for the initial 2 min and increased at 15 °C/min to a final temperature

of 270 °C. After reaching 270 °C, the GC oven was kept isothermal for 10 min.

Results and Discussion

Haloanilines are a group of volatile compounds amenable to GC/MS analysis. However, unequivocal iden-

tification of the *ortho*, *meta*, and *para* isomers of haloanilines by EI/MS is not straightforward since their spectra are virtually identical (Supplementary Figure S1, which can be found in the electronic version of this article supplementary figure). Although there are minor differences in the relative intensities of each peak cluster that are sufficient for an advanced pattern-recognition

Figure 1. Positive-ion electron-ionization (70 eV) spectra of *N*-formyl derivatives of *o*-, *m*-, and *p*-fluoroaniline (a), *o*-, *m*-, and *p*-chloroaniline (b), *o*-, *m*-, and *p*-bromoaniline (c), and *o*-, *m*-, and *p*-iodoaniline (d).

algorithm to search against a spectral library and find a correct match, unfortunately the quality of spectra in most commercial libraries is poor. Consequently, a search against existing libraries failed to correctly identify the isomers of haloanilines. However, when the spectra of haloanilines depicted in Supplementary Figure S1 were included in a user database, and used for haloaniline isomer identification, significantly better matches were obtained.

Generally, EI mass spectra of many 1,2-disubstituted aromatic compounds are distinguishably different from those of their *meta* or *para* isomers as the result of a phenomenon known as the ortho effect [4]. According to the conventional definition of the ortho effect [5–7], a labile hydrogen is transferred from a donor functional group attached to an *ortho* position of an aromatic ring, via six-membered transition state [4, 8]. Consequently, a neutral molecule is eliminated to give an odd-electron diene product that usually bears the charge [9]. More general definitions given to the ortho effect cover all aspects of interactions, including the participation of higher-member transition states and transfer of atoms and molecules other than hydrogen [4].

Unfortunately, no ortho effect is noticeable in the spectra of haloanilines (Supplementary Figure S1). However, when the amino group of chloro-, bromo-, or iodoanilines is transformed to an *N*-formyl, *N*-acetyl, or *N*-benzoyl derivative, the spectra of the derivatives revealed a highly dramatic “ortho effect,” in which the halogen is lost as a radical. For example, the base peak of the *N*-formyl-*o*-chloroaniline (**1**) spectrum is observed at *m/z* 120. In contrast, a peak is absent at *m/z* 120 in the spectra of the corresponding *meta* and *para* isomers (Figure 1b). Similarly, the *m/z* 120 is the most abundant peak in the spectra of *N*-formyl-*o*-bromoaniline (**2**, Figure 1c), and *N*-formyl-*o*-iodoaniline (**3**, Figure 1d) (Scheme 1) as well. Besides the *m/z* 120 peak that is characteristic for the *ortho* isomer, all other peaks appear to be common to all three isomers (Figure 1b–d).

However, it is not surprising that the *N*-formyl derivatives of the fluoroanilines fail to show a peak for the loss of the halogen atom since the C–F bond is much stronger than that of other carbon–halogen bonds (Figure 1a). The mechanism for the loss of the halogen presumably follows a path similar to that proposed for *ortho*-halogenated phenylureas, thioureas, carbamates [10, 11], formamidines [12, 13], and phenylhydrazones and 2,4-dinitrophenylhydrazones of benzaldehydes and acetophenones [14]. Investigations by Grützmacher and coworkers have established that the fragmentation follows a two-step addition/dissociation mechanism via a σ -complex (**10**) leading to a benzoxazolium ion (**11**, Scheme 1) [11, 13].

A similar halogen loss was observed with *N*-acetyl-*o*-haloanilines, except those from *o*-fluoroanilines (Figure 2a). However, the assistance provided by the neighboring methyl group for the elimination of the halogen atom is much less than that provided by the hydrogen in the *N*-formyl group. Consequently, the intensity of the *m/z* 134 peak in the spectrum of *N*-acetyl-*o*-chloroaniline (Figure 2b) is about 33% compared to the 100% peak at *m/z* 120 in the spectrum of *N*-formyl-*o*-chloroaniline (Figure 1b). The spectra of bromo derivatives corroborate this conclusion. Although bromine is a better leaving group than chlorine, the intensity of the *m/z* 134 peak in the spectrum of *N*-acetyl-*o*-bromoaniline is still 77% (Figure 2c). On the other hand, the *m/z* 134 peak in the spectrum of *N*-acetyl-*o*-iodoaniline is 96%, given that iodine is the best leaving group among halogens (Figure 2d). Furthermore, the intensity of the peak for the halogen loss is less intense for the *N*-benzoyl derivatives as well (Supplementary Figure S2). Apparently, the electron-withdrawing or -donating character of the R substituent has a direct effect on the intensity of the resulting peak. Thus, an electron-withdrawing group is expected to facilitate the ipso electrophilic attack of the charged carbonyl group to the benzene ring leading to electrophilic aromatic substitution and consequent halogen radical loss [15].

Scheme 1. Fragmentation of *o*-haloaniline derivatives.

Figure 2. Positive-ion electron-ionization (70 eV) spectra of *N*-acetyl derivatives of *o*-, *m*-, and *p*-fluoroaniline (a), *o*-, *m*-, and *p*-chloroaniline (b), *o*-, *m*-, and *p*-bromoaniline (c), and *o*-, *m*-, and *p*-iodoaniline (d).

To demonstrate the applicability of the generalizations, the spectra of 2,4,6-tribromoaniline derivatives were recorded. In fact, the intensity of the peak originating from the bromine loss could be semi-quantitatively predicted by our rationalizations (Supplementary Figure S3). The intensity of the peaks centered around m/z 292 and 354

for the bromine loss from the *N*-acetyl and *N*-benzoyl derivatives, respectively (Supplementary Figure S3c and d), is less than that observed at m/z 278 for the *N*-formyl derivative (Supplementary Figure S3b).

Generally, the ortho effect leads to a loss of a neutral molecule, although sometimes a loss of a radical has also

been noted. For example, the loss of a halogen radical has been recognized as an indicator of 2,2', 6,6' substitution in dihalobiphenyls. Presumably, the halogen loss leads to the formation of a planar, bridged halonium ion intermediate [16], somewhat similar to the six-membered ring intermediate we have proposed here. Similarly, a loss of CH_3O has been noted by Blachut et al. in the spectra of *o*-methoxybenzylimine derivatives [17], and a significant loss of H $\cdot$ has been reported from the spectra of *N,N*-dimethyl-*N'*-phenylformamidines by Grützmaier and Kuschel [18]. Compared to previously reported ortho effects, the examples given here are highly dramatic because the ion produced by the halogen loss, in most cases, represents the base peak of the spectrum. Although this effect had been observed as early as 1967 [10, 19, 20], unfortunately it has not received much attention. For example, no textbook on mass spectral interpretation uses spectra of *N*-substituted haloanilines as typical examples for demonstrating the ortho effect. Even a recent review on mass spectrometry and gas-phase chemistry of anilines failed to mention this strong ortho effect of acylanilines [21]. Hopefully, results presented here will encourage wider acknowledgment and practical usage of this dramatic effect.

Acknowledgments

This research was supported by a Stevens Institute of Technology Technogenesis[®] grant for undergraduate research to Freneil B. Jariwala.

References

- Kouris, C. S.; Northcott, J. Aniline and Its Derivatives. In *Encyclopedia of Chemical Technology* (2nd ed.), vol. II, Kirk, R. E.; Othmer, D. F., Eds.; John Wiley & Sons: New York, 1963; p. 411.
- Games, L. M.; Hites, R. A. Composition, Treatment Efficiency, and Environmental Significance of Dye Manufacturing Plant Effluents. *Anal. Chem.* **1977**, *49*, 1433–1440.
- Livingston, A. G.; Willacy, A. Degradation of 3,4-Dichloroaniline in Synthetic and Industrially Produced Wastewaters by Mixed Cultures Freely Suspended and Immobilized in a Packed-Bed Reactor. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 551–557.
- Schwarz, H. Some Newer Aspects of Mass Spectrometric Ortho Effects. *Top. Curr. Chem.* **1978**, *73*, 231–263.
- Gross, J. H. Ortho Elimination (Ortho Effect). In *Mass Spectrometry*; Springer: Berlin, 2004; p. 304.
- McLafferty, F. W.; Tureček, F. *Interpretation of Mass Spectra* (4th ed.); University Science Books: Mill Valley, CA, 1993; p. 77.
- Sparkman, O. D. *Mass Spec, Desk Reference*; Global View Publishing: Pittsburgh, 2000; p. 22.
- Barkow, A.; Pilotek, S.; Grützmaier, H. F. Ortho Effects: A Mechanistic Study. *Eur. J. Mass Spectrom.* **1995**, *5*, 525–537.
- McLafferty, F. W.; Golke, R. S. Mass Spectrometric Analysis—Aromatic Acids and Esters. *Anal. Chem.* **1959**, *31*, 2076–2082.
- Baldwin, M. A.; Loudon, A. G.; Maccoll, A.; Smith, D.; Ribera, A. Formation of a Five-Membered Ring Ion on Electron Impact. *Chem. Commun.* **1967**, *7*, 350–351.
- Baldwin, M. A.; Loudon, A. G.; Maccoll, A.; Webb, K. S. The Nature and Fragmentation Paths of the Molecular Ions of some Arylureas, Arylthiureas, Acetanilides, Thioacetanilides, and Related Compounds. *Org. Mass Spectrom.* **1976**, *11*, 1181–1193.
- Kuschel, H.; Grützmaier, H. F. Mechanisms of Mass Spectrometric Fragmentation Reactions. XIII. Influence of Dissociation Energy on the Intramolecular Aromatic Substitution Reactions of Molecular Ions of *N,N*-Dimethyl-*N'*-Phenylformamidines. *Org. Mass Spectrom.* **1974**, *9*, 408–417.
- Kuschel, H.; Grützmaier, H. F. Mechanisms of Mass Spectrometric Fragmentation Reactions. XI. Effects of Substituents on the Formation of Cyclic Fragment Ions in the Mass Spectra of *N,N*-Dimethyl-*N'*-2-Chlorophenylformamidines and 2-Chloroformanilides. *Org. Mass Spectrom.* **1974**, *9*, 395–402.
- Cable, J.; Kagal, S. A.; MacLeod, J. K. Ortho-Effects in Mass-Spectrometric Fragmentation Processes. *Org. Mass Spectrom.* **1972**, *6*, 301–307.
- Kotaiho, T.; Shay, B. J.; Cooks, R. G.; Eberlin, M. N. Electrophilic Aromatic Cl^+ Addition and CO^+ Substitution in the Gas Phase. *J. Am. Chem. Soc.* **1993**, *115*, 1004–1014.
- Sovocool, G. W.; Mitchum, R. K. Use of the "Ortho Effect" for Chlorinated Biphenyl and Brominated Biphenyl Isomer Identification. *Biomed. Environ. Mass Spectrom.* **1987**, *14*, 579–582.
- Blachut, D.; Danikiewicz, W.; Olejnik, M.; Czarnocki, Z. Electron Ionization Mass Spectrometry as a Tool for the Investigation of the Ortho Effect in Fragmentation of Some Schiff Bases Derived from Amphetamine Analogs. *J. Mass Spectrom.* **2004**, *39*, 966–972.
- Grützmaier, H. F.; Kuschel, H. Mechanisms of Mass Spectrometric Fragmentation Reactions. V. Substituent Effects on the Intramolecular Substitution of the Phenyl Radical in the Mass Spectra of *N,N*-Dimethyl-*N'*-Phenyl-Formamidines. *Org. Mass Spectrom.* **1970**, *3*, 605–622.
- Baldwin, M. A.; Loudon, A. G. Relationship between Activation Energies and Relative Intensities for Fragmentations under Electron Impact. *Org. Mass Spectrom.* **1969**, *2*, 549–550.
- Benezra, S. A.; Bursey, M. M. Ortho Effects on Ordering Factors in Mass Spectral Rearrangements. Loss of Ketene from Halogenated Phenyl Acetates and Acetanilides. *J. Chem. Soc. B.* **1971**, *7*, 1515–1520.
- Eberlin, M. N.; Augusti, D. V.; Augusti, R. Mass Spectrometry and Gas-Phase Chemistry of Anilines. In *The Chemistry of Anilines*, Part 1, Zvi Rappoport, Ed.; John Wiley & Sons: West Sussex, UK, 2007; p. 293.