Research Article

Rapid Communications in Mass Spectrometry

Received: 15 September 2010 Revised: 8 November 2010 Accepted: 9 November 2010 Published online in Wiley Online Library: 00 Month 2011

Rapid Commun. Mass Spectrom. **2011**, *25*, 423–428
(wileyonlinelibrary.com) DOI: 10.1002/rcm.4869

The mechanism of Sandmeyer's cyclization reaction by electrospray ionization mass spectrometry

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Using electrospray ionization (tandem) mass spectrometry (ESI-MS(/MS)) spectrometric experiments, the Sandmeyer reaction was monitored on-line, and key intermediates were intercepted and characterized for the first time. The mechanistic information provided by on-line ESI-MS(/MS) is in accordance with Sandmeyer's proposal, and was made possible by coupling a microreactor on-line to the ESI ion source, which allowed reactions to be screened from 0.7–2.0 s, identifying and characterizing all intermediates that were formed and consumed during the reaction. **Copyright © 2011 John Wiley & Sons, Ltd.**

Isatins (**1**, Scheme 1) are heterocycles with great applicability in medicinal chemistry, and they are normally used as building blocks in the preparation of various heterocyclic systems, including quinoline and indole derivatives. Electrophilic aromatic substitution at carbons C-5 and C-7 of isatins can be performed, along with NH alkylations, selective reduction reactions, and condensations of the carbonyl moieties.[1,2] Natural and synthetic isatin derivatives have been reported to possess antiviral, anti-inflammatory, anticonvulsant, anticancer, and other bioactivities.[3–6]

There are several methods for the synthesis of isatins,[7–12] but Sandmeyer's protocol is the most applicable, since the starting materials are easy to prepare, inexpensive, safe to handle, stable, and no inert atmosphere is required to achieve the production of substituted isatins. This method consists of the use of isonitrosoacetanilide (**3**) (obtained from aniline (**2**)), followed by cyclization using H_2SO_4 in aqueous media (Scheme 1). However, the mechanism of Sandmeyer's cyclization is not clear and has been discussed in a few publications, generating doubts about the intermediaries and the possible pathways.[11,13,14]

We chose to study the reaction by identifying the produced intermediaries, therefore giving new insights about the reaction mechanism. In this work, we applied on-line monitoring ESI-MS(/MS) of isatin preparation from isonitrosoacetanilides. Since ESI normally releases ions preformed in solution,[15–18] we expected that transient ionic species such as protonated **4**–**7** (Scheme 1) should be detectable by ESI-MS from the reacting solution. ESI is also known for the mild conditions used to form the gaseous ions; even very labile molecules can be transferred to the gas phase.[19–21]

In the reaction initially proposed by Sandmeyer, imine intermediate **4** leads to isatin upon reaction with water (paths (a) and (b), Scheme 1). On the other hand, Piozzi and Favini also suggested the formation of intermediate **4**, and proposed the formation of further intermediaries **5**–**7** (paths (c)–(e), Scheme 1). For the first step, the authors suggested a dehydration of isonitrosoacetanilide to form nitrile **5** (path (c)), which then would be attacked by water to form **6** (path (d)). Furthermore, intermediate **6** was proposed to be in equilibrium with **7** (via tautomerization, path (f)). Moreover, according to the proposal of Piozzi and Favini, **6** is the intermediate that produces imine **4** through path (e), as depicted in Scheme 1.[13,14] It is also known that acidic media plays an important role in the Sandmeyer cylization.

Comprehensive mechanistic investigations usually require kinetic data gained by monitoring over long timescales, usually by UV, IR, or NMR spectroscopy. However, these techniques are often not suitable for the short timescales required for investigating transient species as unequivocal assignments of UV and IR bands or NMR peaks and couplings, particularly for more complex or transient structures, may be challenging and sometimes unfeasible. Electrochemical techniques have also been used to monitor short-lived species, but they provide limited structural information on the detected intermediates. On the other hand, during the last two decades there has been considerable growth in the development of electrospray ionization (ESI)[22–24] for molecular analysis by mass spectrometry (MS) as a practical method in the study of reaction mechanisms. This method allows the interception and characterization of molecules and supramolecules[25,26] of high polarity and molecular complexity. ESI is an interesting 'ion-fishing' technique because it gently transfers preformed ions directly from solution to the gas phase.[23] ESI-MS (and its tandem version ESI-MS/MS) is rapidly becoming the technique of choice for fast screening of intermediates directly from solution in chemistry and biochemistry[27–30] and in high-throughput screening of homogeneous catalysis reactions, providing hitherto unavailable chemical information to mechanistic studies.[15,18] In this context, we were interested

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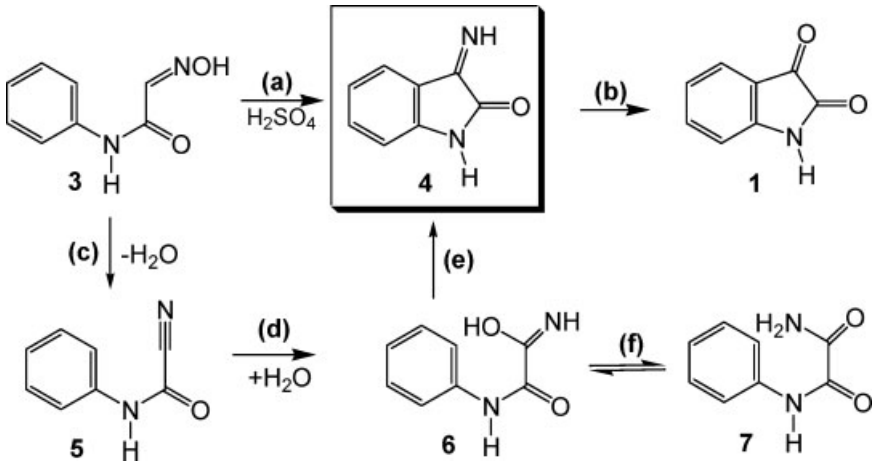

Scheme 1. Original reaction proposed by Sandmeyer for the formation of isatins through paths (a) and (b), and the Piozzi-Favini proposal for the reaction by paths (c)–(f) and (b).

in employing the on-line ESI-MS technique to trap and characterize the transient species that are involved in the synthetic route for isatin **1**, under Sandmeyer's conditions.

EXPERIMENTAL

Chemicals were used as purchased unless otherwise noted. Acetonitrile was distilled from calcium hydride immediately prior to use. The reaction progress was monitored by thin-layer chromatography (TLC) on silica gel (aluminum foils) and spotted under UV light, followed by staining with ethanolic 25% phosphomolybdic solution or aqueous $KMnO_4$. Purification by column chromatography was carried out with silica gel (70–230 or 230–400 mesh). ^{1}H NMR spectra were measured at 200 MHz and the ^{13}C NMR spectra at 50 MHz, in $CDCl_3$ at room temperature. Chemical shifts (δ) were reported in ppm and the coupling constants (^3J) in Hertz (Hz). Signal multiplicity was assigned as singlet (s), doublet (d), double doublet (dd), triplet (t), double triplet (dt), quartet (q), quintuplet (qt), multiplet (m) and broad (br).

Preparation of isonitrosoacetanilide (3)

A mixture of sodium sulfate (130 g, 0.915 mol), distilled water (120 mL), chloral hydrate (18.0 g, 0.109 mol), hydroxylamine sulfate (13.0 g, 0.079 mol) dissolved in 60.0 mL distilled water, 8.6 mL of concentrated hydrochloride acid and 0.1 mol of aniline (9.3 g) was heated gradually to 70°C. After adding 100 mL of ethanol, the mixture was heated under reflux until TLC indicated that formation of isonitrosoacetanilide was complete. The mixture was poured onto ice/H_2O and the solid isonitrosoacetanilide formed was collected by filtration and washed with water. The isonitrosoacetanilide **3** was obtained in 87% yields. ^{1}H NMR ($CDCl_3$, 200 MHz), δ: 11.95 (s, 1H), 9.64, 7.61 (d, *J* 8.0 Hz, 2H), 7.55 (s, 1H), 7.23 (t, *J* 8.0 Hz, 2H), 7.01 (t, *J* 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz), δ: 159.1, 142.4, 136.7, 127.2, 122.5, 118.6. ESI-MS: *m/z* calcd. for $[C_8H_8N_2O_2 + H]^+$ 165.0664; found 165.0660.

Preparation of 4-methylisonitrosoacetanilide

A mixture of sodium sulfate (130 g, 0.915 mol), distilled water (120 mL), chloral hydrate (18.0 g, 0.109 mol), hydroxylamine sulfate (13.0 g, 0.079 mol) dissolved in 60.0 mL distilled water, 8.6 mL of concentrated hydrochloride acid and 0.1 mol of 4-methylaniline (10.7 g) was heated gradually to 70°C. After adding 100 mL of ethanol, mixture was heated under reflux until TLC indicated that formation of isonitrosoacetanilide was complete. The mixture was poured onto ice/H_2O and the solid isonitrosoacetanilide formed was collected by filtration and washed with water. The 4-methylisonitrosoacetanilide was obtained in 60% yield. ^{1}H NMR ($CDCl_3$, 200 MHz), δ: 10.02 (s, 1H), 8.48 (s, 1H), 7.44 (s, H), 7.37 (d, *J* 8.3 Hz, 2H), 7.02 (d, *J* 8.3 Hz, 2H), 2.20 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz), δ: 159.6, 142.6, 134.3, 129.5, 128.8, 125.5, 124.3, 122.2, 16.8. ESI-MS: *m/z* calcd. for $[C_9H_{10}N_2O_2 + H]^+$ 179.0821; found 179.0812.

Isatin (1)

The isonitrosoacetanilide **3** (3.0 g, 18 mmol) was added slowly to concentrated sulfuric acid (9.0 mL) under magnetic stirring at room temperature. After 1 min, the reaction mixture was poured over crushed ice and the solid isatin was collected by filtration and washed with water. The isatin **1** was obtained in 77% yield. ^{1}H NMR (DMSO, 200 MHz), δ: 11.03 (s, 1H), 7.57 (td, *J* 2.0 and 8.0 Hz, 1H), 1H), 7.48 (d, *J* 8.0 Hz, 1H), 7.04 (td, *J* 2.0 and 8.0 Hz, 1H), 6.89 (d, *J* 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz), δ: 184.9, 159.8, 151.2, 138.9, 125.2, 123.3, 118.3, 112.7. ESI-MS: *m/z* calcd. for $[C_8H_5NO_2 + H]^+$ 148.0399; found 148.0396.

4-Methylisatin

The 4-methylisonitrosoacetanilide (3.0 g, 17 mmol) was added slowly to concentrated sulfuric acid (9.0 mL) under magnetic stirring at room temperature. After 1 min, the reaction mixture was poured over crushed ice and the solid 5-methylisatin was collected by filtration and washed with water. The 4-methylisatin was obtained in 85% yields. ^{1}H NMR ($CDCl_3$ + DMSO, 200 MHz), δ: 10.64 (s, 1H), 7.21 (s, 2H), 6.73 (d, *J* 8.0 Hz, 1H), 3.30 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz), δ: 182.8, 157.6, 146.9, 136.9, 130.2, 123.0, 115.8, 110.3, 18.5. ESI-MS: *m/z* calcd. for $[C_9H_7NO_2 + H]^+$ 162.0555; found 162.0545.

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ESI-MS microreactor monitoring

ESI-MS and ESI-MS/MS experiments in the positive ion mode were performed in a high-resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Q-TOF Micro, Waters-Micromass, UK) with a constant nebulizer temperature of 100°C, and the cone and extractor potentials were set to 15 and 4.5 V, respectively, with a scan range of *m/z* 70–1000. Samples were directly infused into the ESI source at flow rates of 5–20 μL min^{-1} by means of a microsyringe pump via the microreactor. A solution of **1** (6.10 μmol, 1.0 mg) in H_2O (2.0 mL) was mixed with H_2SO_4 (18 M) using a dual-syringe pump operating at a flow rate of 5-10 μL min^{-1} in an effective micromixer that was coupled directly to the ion source of the mass spectrometer (Fig. 1), and the solution was fed continuously into the mass spectrometer. The flow rate could be varied from 5 to 10 μL min^{-1}. By connecting the microreactor directly to the spray capillary, reaction times from 0.7 to 1.4 s

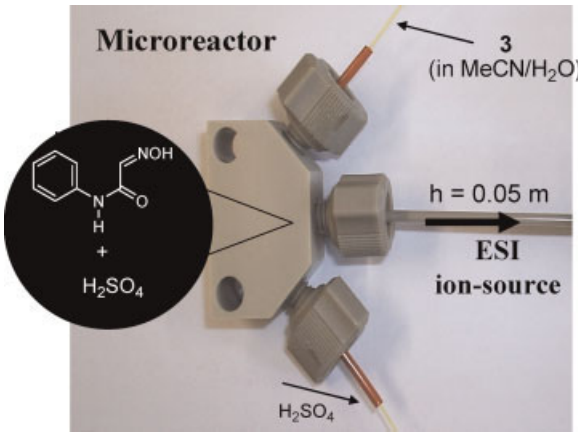

Figure 1. Microreactor coupled to ESI-MS employed for on-line screening of the Sandmeyer cyclization reaction.

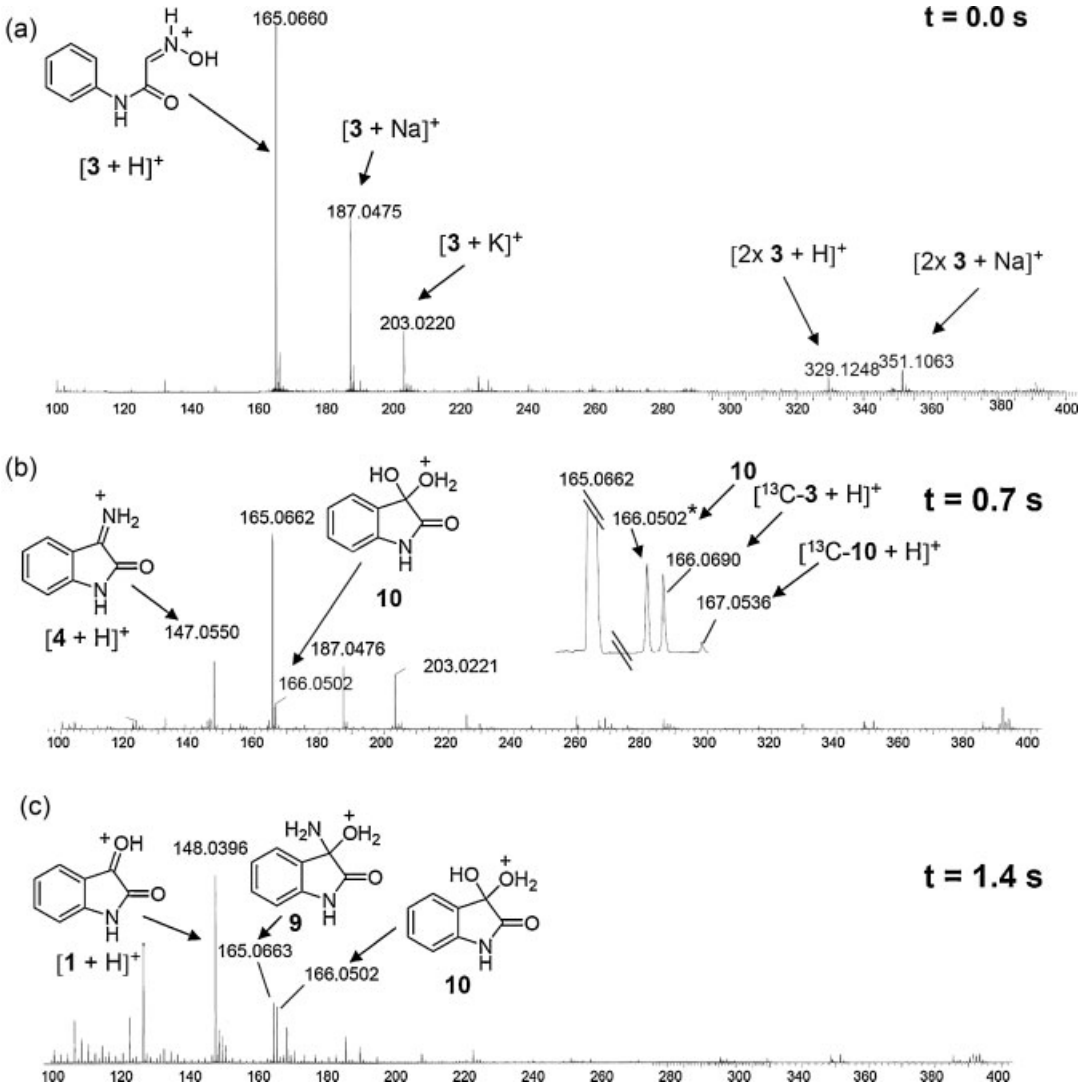

Figure 2. On-line screening of isatin cyclization reactions from isonitrosoacetanilides using microreactor coupled to ESI-MS: (a) ESI-MS of **3** in H_2O:MeCN; (b) on-line monitoring by microreactor-ESI-MS of reaction of **3** and H_2SO_4 at 0.7 s; (c) on-line monitoring by microreactor-ESI-MS of reaction of **3** and H_2SO_4 at 1.4 s. *Note that the ion **10** is not the $[^{13}C\text{-}\mathbf{3} + H]^+$ isotope.

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could be covered. MS/MS experiments were carried out by mass selection of a specific ion in Q1 which was then submitted to collision-induced dissociation (CID) with argon in the collision chamber. The product-ion MS analysis was accomplished with the high-resolution orthogonal TOF analyzer.

RESULTS AND DISCUSSION

Proof-of-principle reactions

We monitored the reaction via ESI mass (and tandem mass) spectrometric experiments trying to intercept ionic intermediates and therefore to collect mechanistic information that could guide the optimization of reaction conditions. Via ESI-MS operating in the positive ion mode, we monitored the reaction of **3** and a H_2SO_4/H_2O solution by direct infusion with reaction times around 20 s. However, spectra as depicted in Fig. 2(c) were observed and no intermediates were detected, mainly due to the short life-time of these intermediates. Thus, direct ESI-MS was discarded as the technique for this mechanistic study.[31,32]

Microreactors coupled to an ESI-MS ion source have been introduced into organic reaction mechanistic studies, thus allowing the interception of transient and short life-time compounds.[15–17] Since ESI-MS is able to transfer ions directly from solution to the gas phase with efficiency and gentleness (no or little dissociation), reaction times around 0.7 s can be screened through coupling the microreactor depicted in Fig. 1, which provided therefore proper snapshots of the ion composition of reaction solutions in short reaction times. We have been using this technique extensively to investigate the mechanisms of several reactions.[19–21] Thus, to study the Sandmeyer cylization reaction we employed a microreactor coupled to ESI-MS trying to fish the previously proposed intermediates (Fig. 1 and Scheme 1).

Figure 2(a) shows the ESI-MS spectrum of **3** in neat H_2O/MeCN (1:1). Major ions clearly detected in the mass spectrum correspond to the protonated reactant $[\mathbf{3}+H]^+$ of *m/z* 165 along with sodium and potassium adducts of **3** (*m/z* 187 and 203, respectively). Also observed were the proton and sodium dimers of **3** (*m/z* 329 and 351). Each of these ions was then mass-selected and structurally characterized via CID with argon in ESI-MS/MS measurements. Figure 3(a) shows the ESI-MS/MS spectra for the ion $[\mathbf{3}+H]^+$ of *m/z* 165 [The adducts of *m/z* 187, 203, 329 and 351 afforded neutral losses of **3** as the main fragmentation pathway (spectra not shown).] Then, we set out to investigate the formation of the intermediate species in a reaction solution that was prepared by on-line mixing of **3** with aqueous H_2SO_4 via the microreactor (Fig. 1) following the Sandmeyer procedure.[11,12] The reaction of isonitrosoacetanilide (**3**) solution with H_2SO_4/H_2O using the microreactor[15,17,19] resulted in a very clean spectrum (Fig. 2(b)) displaying three cationic species that were easily identified by their absolute masses at reaction times of 0.7 s: $[\mathbf{4}+H]^+$ (*m/z* 147.0550, calculated 147.0558), $[\mathbf{10}]^+$ (*m/z* 166.0502, calculated 166.0504), and protonated **3** of *m/z* 165 (Fig. 2(b)). The overall appearance of the spectrum and relative intensities of these ions changed very little from 0.0 to 0.7 s of reaction in solution, as indicated by on-line ESI-MS monitoring.

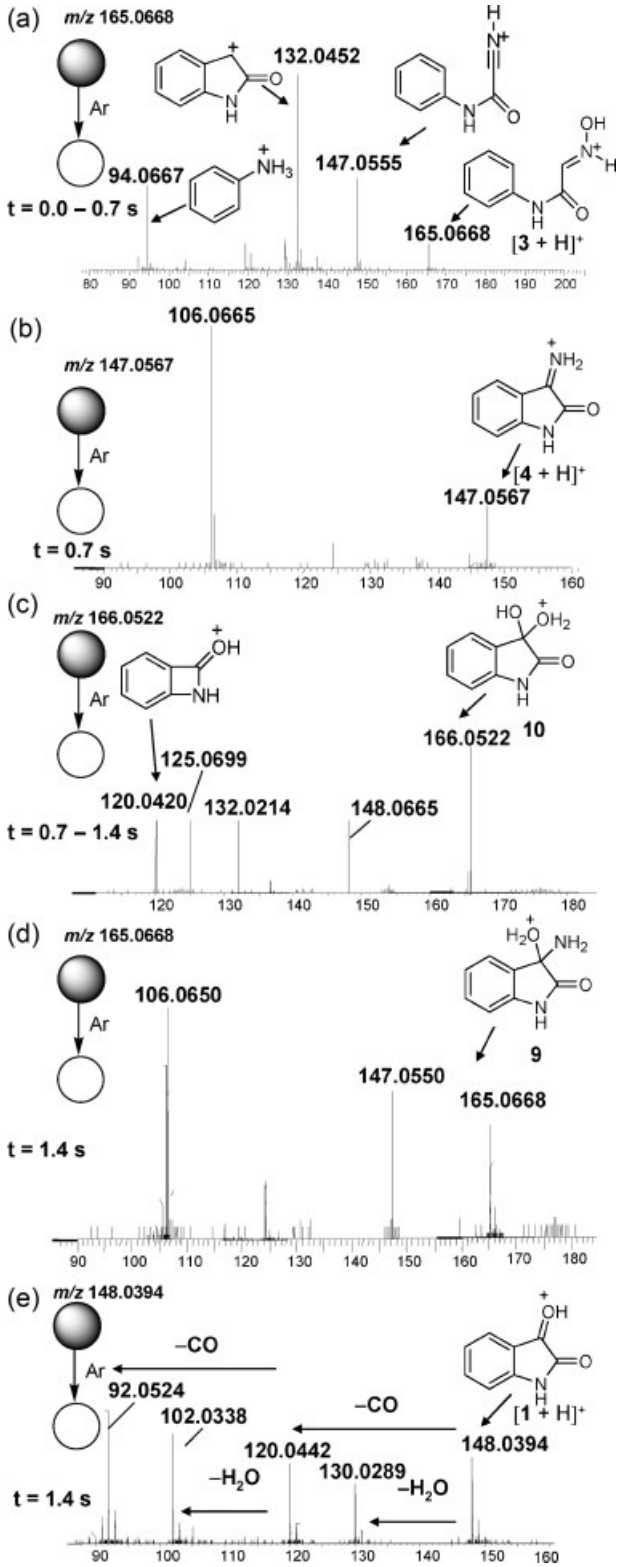

Figure 3. Species intercepted and characterized by ESI-MS/MS through on-line microreactor monitoring of Sandmeyer cyclization reaction with time: (a) t = 0 s; (b) t = 0.7 s; (c) t = 1.4 s; (d) t = 0.7–1.4 s; (e) t = 1.4 s.

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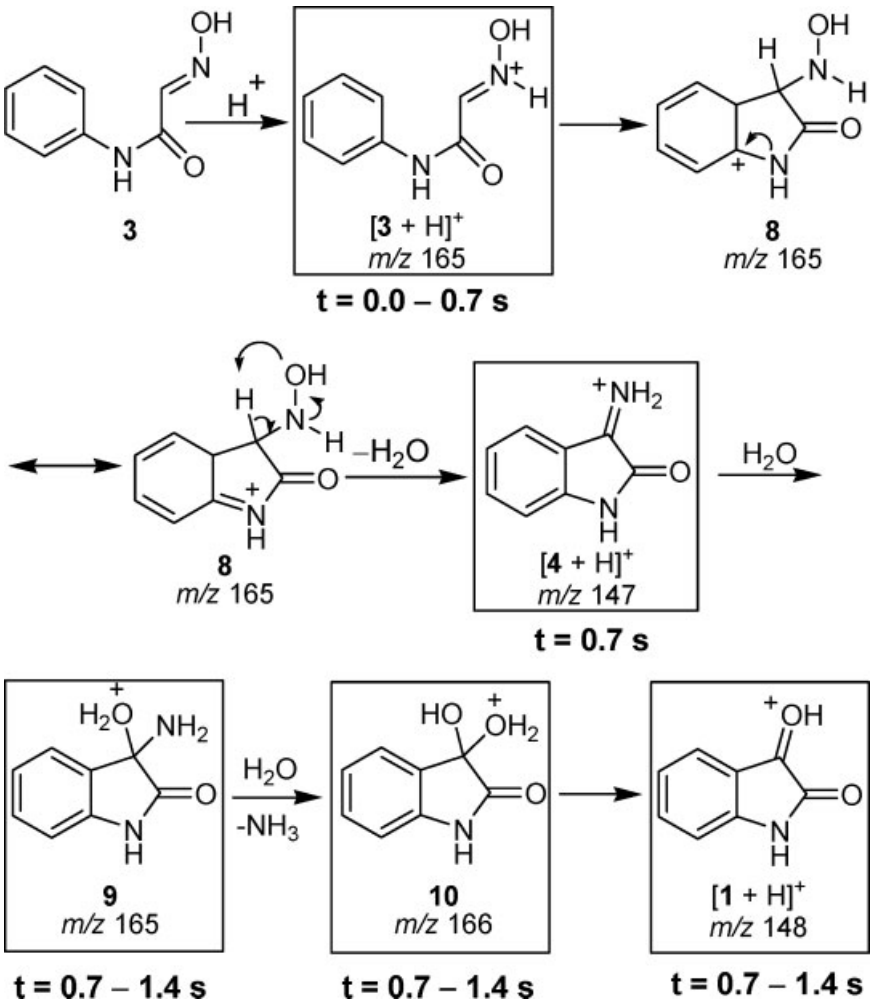
Figures 3(a)–3(c) show the ESI-MS/MS spectra of the ions of *m/z* 165 ($[\mathbf{3}+H]^+$), *m/z* 147 ($[\mathbf{4}+H]^+$), and *m/z* 166.0522 (**10**). The MS/MS experiments of $[\mathbf{4}+H]^+$ gave a fragment ion of *m/z* 106, as depicted in Fig. 3(b). The ions **10** and $[^{13}C\text{-}\mathbf{3}+H]^+$ have the same nominal masses (see insert in Fig. 3(b)). However, as noted in Fig. 3(b), it is possible to distinguish these signals with time. The ion of *m/z* 166.0690 dissociates in the mass spectrometer by CID mainly into fragment ions of *m/z* 148, 133, and 95 that are formed in the gas phase from $[^{13}C\text{-}\mathbf{3}+H]^+$ (see Supporting Information), whereas dissociation to ions of *m/z* 148, 132, 125, and 120 is evidence for the presence of **10** of *m/z* 166.0522 (Fig. 3(c)). After 0.7 to 1.4 s of reaction (Fig. 2(c)), however, the ion of *m/z* 166.0502 for **10** prevails in the spectra. Furthermore, a new fragmentation behavior is seen for the species of *m/z* 165.0663 after 1.4 s (Figs. 2(c) and 3(d)). This temporal change in product-ion distribution of *m/z* 165 confirms that in a later stage of the reaction, intermediate **9**, rather than $[\mathbf{3}+H]^+$, becomes a dominant species, as identified in Fig. 2(c) and characterized in Fig. 3(d). These ions show dissociation behaviors that fully match proposed structures as indicated by structural assignments of fragment ions displayed in each respective mass spectrum.

Scheme 2 presents the proposed cyclization mechanism of **3** to isatin **1** based on the ESI(+)-MS and ESI(+)-MS/MS spectra. This mechanism is based on previous mechanistic interpretations,[7–14] but now shows four authentic cationic intermediates ($[\mathbf{3}+H]^+$, $[\mathbf{4}+H]^+$, **9** and **10**) that have been properly detected and characterized by ESI-MS(/MS).

Thus, the ion $[\mathbf{3}+H]^+$ of *m/z* 165 is generated by *N*-protonation in sulfuric acid (Scheme 2). Then, the π-electrons of the aromatic ring attack the electrophilic carbon bonded to the protonated nitrogen affording the ion of *m/z* 165 (**8**), which undergoes a rearrangement and releases water, generating the ion $[\mathbf{4}+H]^+$ of *m/z* 147. This ion is attacked by water producing a new intermediate species of *m/z* 165 (**9**), which, through attack by another H_2O molecule, releases NH_3 to give **10** of *m/z* 166. Finally, isatin $[\mathbf{1}+H]^+$ is formed in high yields after 1 min of reaction time. A similar experiment was performed by using 4-methylisonitrosoacetanilide and the expected similar species were detected, confirming that a mechanism as proposed in Scheme 2 is ongoing.



Scheme 2. Mechanism for Sandmeyer cyclization of isonitrosoacetanilide **3** to isatin **1** corroborated by on-line ESI-MS(/MS) monitoring.

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CONCLUSIONS

In conclusion, the ESI-MS and ESI-MS/MS experiments described herein have led to new insights into the reaction mechanism of the Sandmeyer cyclization reaction by mass spectrometric characterization of its key reaction intermediates. The species **4**, **9** and **10** have been transferred to the gas phase, isolated by mass selection, and for the first time detected and then structurally characterized by MS and MS/MS experiments. The detection of **9** and **10** fully supports the mechanism proposed for the Sandmeyer cyclization reaction of isonitrosoacetanilide **3** to isatin **1** (Schemes 1 and 2). Nitrile species **5**, and intermediates **6** and **7**, were not observed, as had been proposed by Piozzi-Favini.[13,14] It is worth mentioning also that **5** has been synthesized in our laboratory and did not produce isatin (**1**) upon applying the Sandmeyer protocol. Of course, this result is another indication that the reaction does not proceed through intermediate **5**, as suggested by Piozzi-Favini. Our results on the Sandmeyer cylization reaction illustrate once more the suitability of coupling microreactors to ESI-MS^n techniques in order to isolate and characterize short-lived key intermediates that may be directly transferred from solution to the gas phase, a vast but still under-explored field. This report is another example of the successful application of atmospheric pressure ionization (API) in revealing, elucidating, and helping to consolidate previously proposed reaction mechanisms. Similar applications of the ESI-MS technique are expected to find broad use in organic and organometallic chemistry in probing many of their reaction and catalytic mechanisms.[15–18]

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Acknowledgements

L.S.S. thanks FONDECYT (Project No. 1085308) for support of research activity at Talca University. CAPES and CNPq are acknowledged for financial support (B.V.S., F.A.V., A.C.P). We thank Prof. Dr. Peter Bakuzis for further corrections in the manuscript.

REFERENCES

[1] B. V. Silva, N. M. Ribeiro, M. D. Vargas, M. Lanznaster, J. W. M. Carneiro, R. Krogh, A. D. Andricopulo, L. D. Dias, A. C. Pinto. *Dalton Trans.* **2010**, *39*, 7338.
[2] N. M. Ribeiro, B. V. Silva, F. A. Violante, C. M. Rezende, A. C. Pinto. *Org. Prep. Proc. Int.* **2005**, *37*, 265.
[3] K. L. Vine, L. Matesic, J. M. Locke, M. Ranson, D. Skropeta. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 397.
[4] M. E. Matheus, F. A. Violante, S. J. Garden, A. C. Pinto, P. D. Fernandes. *Eur. J. Pharmacol.* **2007**, *556*, 200.
[5] G. Zapata-Sudo, L. B. Pontes, D. Gabriel, T. C. F. Mendes, N. M. Ribeiro, A. C. Pinto, M. M. Trachez, R. T. Sudo. *Pharmacol. Biochem. Behavior* **2007**, *86*, 678.
[6] M. D. Hall, N. K. Salam, J. L. Hellawell, H. M. Fales, C. B. Kensler, J. A. Ludwig, G. Szakács, D. E. Hibbs, M. M. Gottesman. *J. Med. Chem.* **2009**, *52*, 3191.
[7] P. G. Gassman, B. W. Cue, Jr, T.-Y. Luh. *J. Org. Chem.* **1977**, *42*, 1344.
[8] A. Taylor. *J. Chem. Res. (S)* **1980**, 347.
[9] G. Loloiu, O. Maior. *Rev. Roumaine Chimie* **1997**, *42*, 67.
[10] A. C. Pinto, A. A. M. Lapis, B. V. Silva, R. S. Bastos, J. Dupont, B. A. D. Neto. *Tetrahedron Lett.* **2008**, *49*, 5639.
[11] T. Sandmeyer. *Helv. Chim. Acta* **1919**, *2*, 234.
[12] C. S. Marvel, G. S. Hiers. *Org. Syn. Coll.* **1941**, *1*, 327.
[13] G. Favini, F. Piozzi. *Atti. Accad. Naz. Lincei Cl. Sci. Fis., Mat. Nat. Rend.* **1955**, *19*, 44.
[14] F. Piozzi, G. Favini. *Atti. Accad. Naz. Lincei Cl. Sci. Fis., Mat. Nat. Rend.* **1955**, *18*, 647.
[15] L. S. Santos, *Reactive Intermediates: MS Investigations in Solution*, Wiley-VCH, Weinheim, **2010**.
[16] L. S. Santos. *Eur. J. Org. Chem.* **2008**, 235.
[17] L. S. Santos, L. Knaack, J. O. Metzger. *Int. J. Mass Spectrom.* **2005**, *246*, 84.
[18] P. Chen. *Angew. Chem. Int. Ed.* **2003**, *42*, 2832.
[19] L. S. Santos, J. O. Metzger. *Angew. Chem. Int. Ed.* **2006**, *45*, 977.
[20] L. S. Santos, J. O. Metzger. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 898.
[21] L. S. Santos, G. B. Rosso, R. A. Pilli, M. N. Eberlin. *J. Org. Chem.* **2007**, *72*, 5809.
[22] C. M. Whitehouse, R. N. Dreyer, M. Yamashita, J. B. Fenn. *Anal. Chem.* **1985**, *57*, 675.
[23] J. F. de la Mora, G. J. Van Berkel, C. G. Enke, R. B. Cole, M. Martinez-Sanchez, J. B. Fenn. *J. Mass Spectrom.* **2000**, *35*, 939.
[24] R. B. Cole, *Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications*, (2nd edn), John Wiley, New York, **2010**.
[25] Z. Takats, S. C. Nanita, R. G. Cooks. *Angew. Chem. Int. Ed.* **2003**, *42*, 3521.
[26] L. S. Santos, B. A. D. Neto, C. S. Consorti, C. H. Pavam, W. P. Almeida, F. Coelho, J. Dupont, M. N. Eberlin. *J. Phys. Org. Chem.* **2006**, *19*, 731.
[27] K. J. Koch, F. C. Gozzo, S. C. Nanita, Z. Takats, M. N. Eberlin, R. G. Cooks. *Angew. Chem. Int. Ed.* **2002**, *41*, 1721.
[28] A. Kamal, N. Markandeya, N. Shankaraiah, C. R. Reddy, S. Prabhakar, C. S. Reddy, M. N. Eberlin, L. S. Santos. *Chem. Eur. J.* **2009**, *15*, 7215.
[29] J. Griep-Raming, S. Meyer, T. Bruhn, J. O. Metzger. *Angew. Chem. Int. Ed.* **2002**, *41*, 2738.
[30] J. B. Domingos, E. Longhinotti, T. A. S. Brandao, L. S. Santos, M. N. Eberlin, C. A. Bunton, F. Nome. *J. Org. Chem.* **2004**, *69*, 7898.
[31] C. D. F. Milagre, H. M. S. Milagre, L. S. Santos, M. L. A. Lopes, P. J. S. Moran, M. N. Eberlin, J. A. R. Rodrigues. *J. Mass Spectrom.* **2007**, *42*, 1287.
[32] L. S. Santos, C. H. Pavam, W. P. Almeida, F. Coelho, M. N. Eberlin. *Angew. Chem. Int. Ed.* **2004**, *43*, 4330.

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