

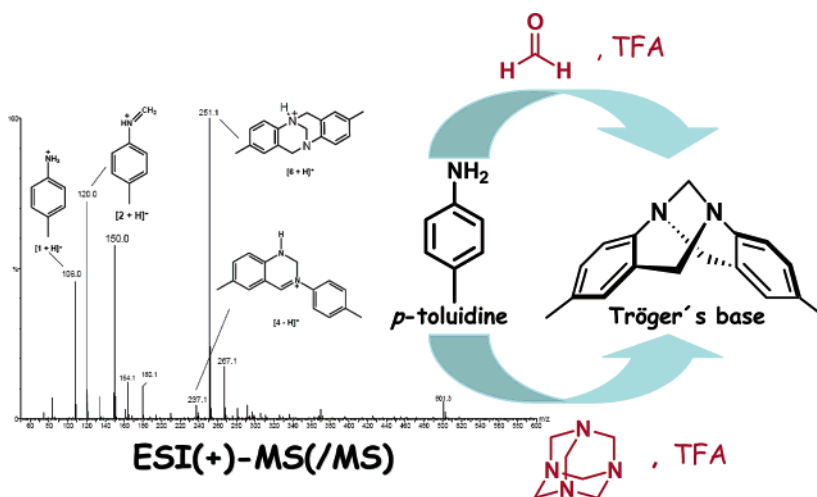
The Mechanism of Tröger's Base Formation Probed by Electrospray Ionization Mass Spectrometry

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Using direct infusion electrospray ionization mass and tandem mass spectrometric experiments [ESI-MS(/MS)], we have performed on-line monitoring of some reactions used to form Tröger's bases. Key intermediates, either as cationic species or as protonated forms of neutral species, have been intercepted and characterized. The role of urotropine as the methylene source in these reactions has also been accessed. Reaction pathways shown by ESI-MS(/MS) have been probed by gas-phase ion/molecule reactions, and an expanded mechanism for Tröger's base formation based on the mass spectrometric data has been elaborated.

Introduction

In 1887, Julius Tröger¹ described the formation of a class of fascinating molecules² known today as Tröger's bases. These relatively simply but geometrically rich V-shaped bicyclic molecules, such as the chiral 2,8-dimethyl-6*H*,12*H*-(5,11)-

methanodibenzo[*b,f*][1,5]diazocine shown in Scheme 1, are formed under acid catalysis by the condensation of anilines (*p*-toluidine for instance) with formaldehyde. Tröger's bases were the first amines to be optically resolved,³ and their dissymmetry results from the impossibility of nitrogen inversion.

Although known for more than a century, it was only in the 1980s that interest in Tröger's bases increased greatly since they started to be used as building blocks of molecular receptors.⁴ As evidenced by the pioneering work of Wilcox et al.,⁵ these bases provide relatively rigid chiral frameworks for the construction of chelating and biomimetic systems. The essential characteristic of a small molecule receptor is concavity,

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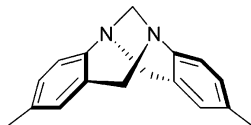
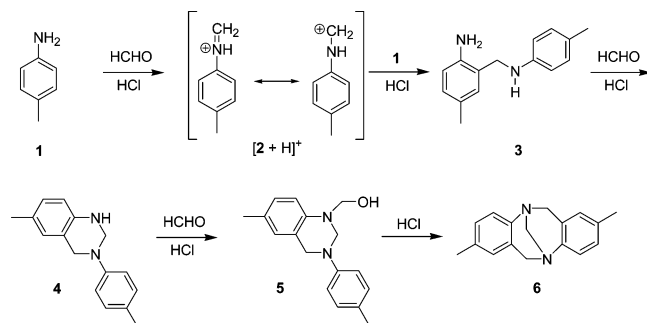
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SCHEME 1. Tröger's Base from *p*-Toluidine and Formaldehyde

SCHEME 2. Original Mechanism Proposed by Wagner for the Formation of Tröger's Bases


and the majority of synthetic receptors have used macrocyclic rings to enforce the formation of concave surfaces. For Tröger's bases, however, their rigid chiral grooves are maintained by conformational constraints intrinsic to their unique architecture and are produced without recourse to macrocyclic structural elements. Tröger's bases have been used therefore mainly as synthetic receptors but also as chiral solvating agents,⁶ as fluorescent compounds,⁷ and for biological⁸ and catalytic activity.⁹ Recently, Tröger's base interactions with DNA have been explored, as exemplified by the work of Yashima et al. who used a Tröger base derived from chiral bis(1,10-phenanthroline) for DNA recognition.¹⁰ Demeunynck et al. also used both the unique geometry and chirality of Tröger's bases with the DNA binding properties of acridines to form a new family of C₂ chiral DNA binding molecules.¹¹ The binding of the proflavine-based Tröger base has also been shown to be both enantio- and sequence-specific.¹² Asymmetric Tröger bases containing the two well-characterized DNA binding chromophores, proflavine and phenanthroline, have also been formed.¹³ The proflavine moiety was shown to intercalate

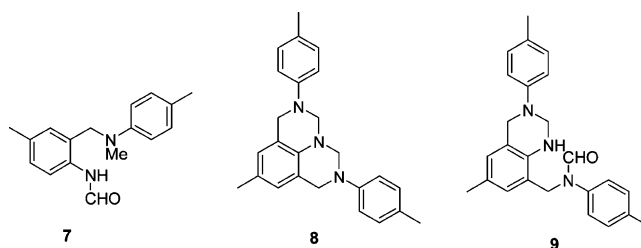
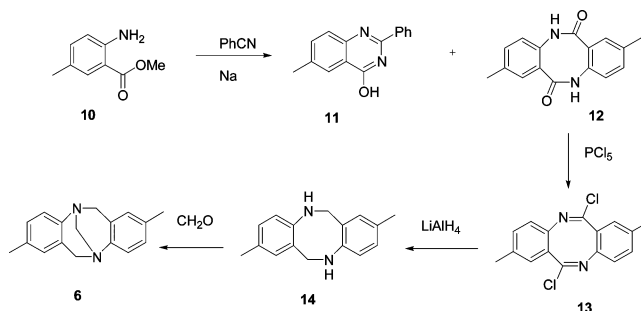


FIGURE 1. Intermediates proposed by Farrar for the formation of Tröger's bases and its byproduct **8**.

SCHEME 3. Intermediate Proposed by Cooper and Partridge to Explain the Formation of Tröger's Base


between DNA base pairs, and the phenanthroline ring occupied the DNA groove. Tröger's bases have been used also as molecular tweezers by extending their aromatic surfaces via fusion of the methanodiazocine core with other Tröger's bases. Examples of such molecular tweezers are bis-Tröger's bases¹⁴ and tris-Tröger's base analogues.¹⁵

The first attempts to establish the structures of Tröger's bases by Löb,¹⁶ Goecke,¹⁷ Lepetit,¹⁸ and Eisner and Wagner¹⁹ were incorrect, but in 1935, Spielman²⁰ succeeded in determining their correct and unique V-shaped structures. The angle formed by the least-square planes containing the two aryl rings changes depending on the ring substituents and varies from 88 to 113°. The mechanism of Tröger's base formation was extensively studied by Wagner in the 1930s,²² and Scheme 2 depicts his first proposal.

In the classical reaction conditions (Scheme 2), the first step involves the acid-catalyzed reaction of *p*-toluidine **1** with formaldehyde, which acts as the source of methylene to give imine **2** as the first key intermediate. Acid-catalyzed condensation of [2 + H]⁺ with **1** followed by two concomitant methylene additions accompanied by cyclizations gives Tröger's base **6**

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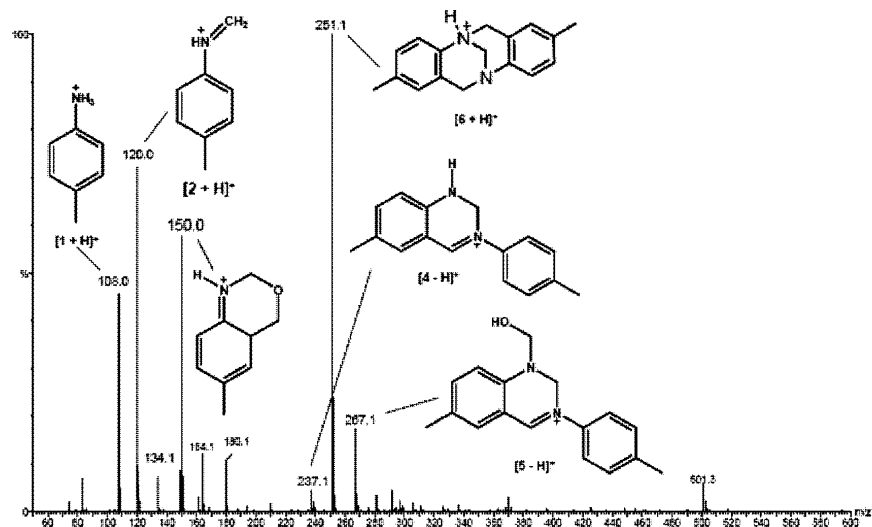


FIGURE 2. ESI-MS for the reaction solution of **1** and formaldehyde in neat TFA after 3 min of reaction.

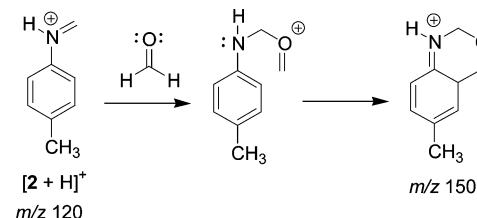
via intermediates **3**–**5**. Intermediate **5** has been, however, questioned, for instance, by Farrar,²³ who proposed instead the participation of intermediate **7** (Figure 1). Farrar also isolated a byproduct identified as diazajulolidine **8**, which led him to propose **9** as an additional intermediate that leads to **8**.

Cooper and Partridge²⁴ demonstrated that Tröger's bases could also be obtained via intermediate **14** (Scheme 3), which they were able to synthesize. Tetrahydrophenomazine **14** was shown to react readily with formaldehyde to yield Tröger's base **6**.

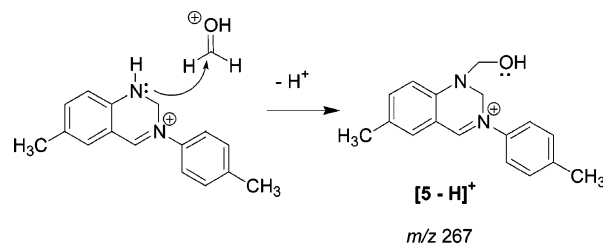
In general, it is now accepted that the mechanism for Tröger's base formation involves a series of in situ Friedel–Crafts reactions of **2**, but the detailed course of the reaction has not yet been fully established. In trying to elucidate major mechanistic aspects, different methylene sources as well as different anilines have been used to form Tröger's bases.

In this study, we used direct infusion electrospray ionization mass and tandem mass spectrometric experiments (ESI-MS/MS) to monitor Tröger's base formation from different anilines (*p*-toluidine and 4-aminoveratrol),²⁵ using both formaldehyde and urotropine as the methylene source. ESI-MS/MS has been selected since it is rapidly becoming an important technique for mechanistic studies of chemical reactions in solution.^{26–28} Since ESI is known to be highly efficient in transferring ions to the gas phase in a gentle way that most often causes little or no dissociation, providing therefore rapid snapshots of the ionic population in solution, we expected that ESI-MS could efficiently “fish” major intermediates, as well as reagents and

SCHEME 4. Proposed Mechanism for the Formation of the Ion of *m/z* 150



SCHEME 5. Proposed Mechanism for the Formation of $[5 - H]^+$ of *m/z* 267



products (Scheme 1), directly from solution to the gas phase, either in their cationic or protonated forms (expected to be abundant in the acid medium), so they could be properly characterized via both ESI-MS and ESI-MS/MS experiments.²⁹

Results and Discussion

Formaldehyde: First in our mechanistic investigation, we performed a classical reaction for Tröger's base formation, that is, that of *p*-toluidine in neat TFA as solvent with formaldehyde

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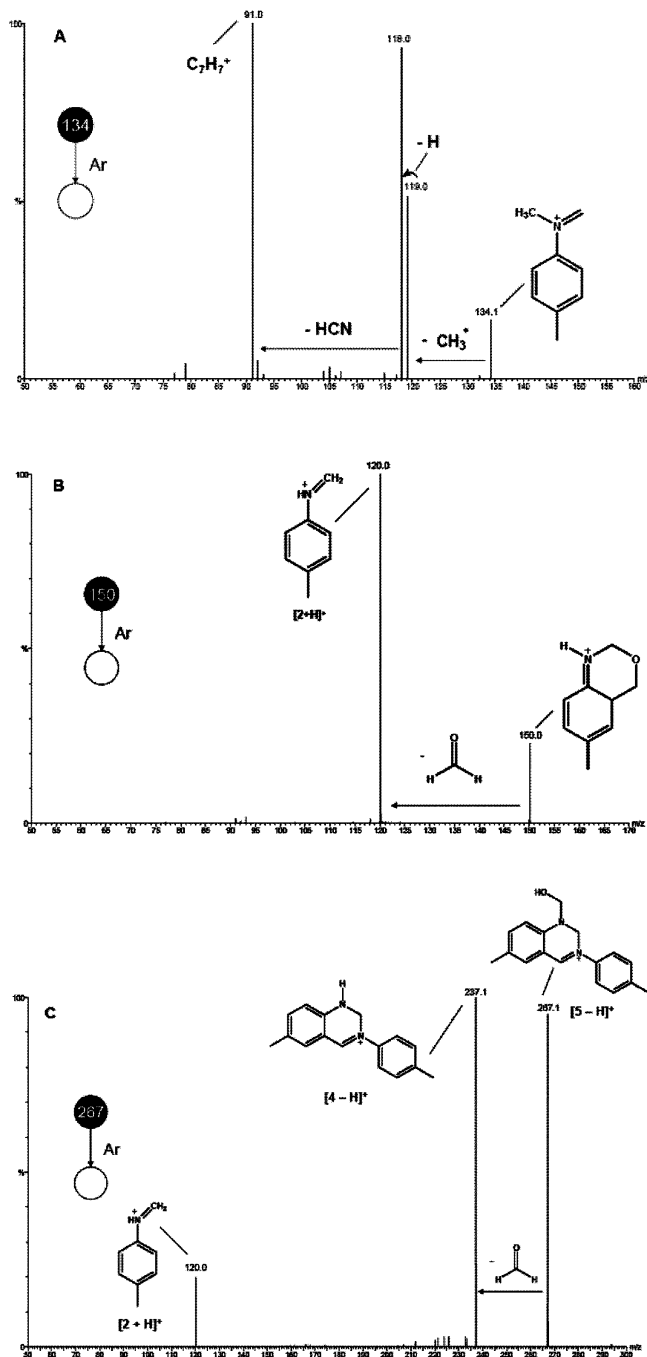
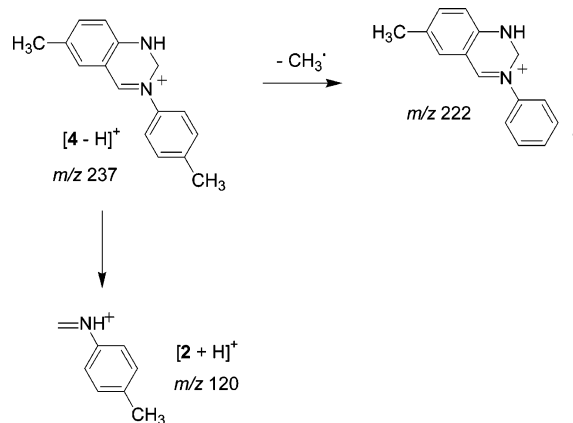


FIGURE 3. ESI-MS/MS for the ions of m/z 134, 150, and 267.

as the methylene source. At room temperature, a clear reaction solution was formed and directly injected to the ESI source operating in the positive ion mode. Approximately 3 min after mixing, ESI-MS (Figure 2) detects four cationic species attributed to key participants of the reaction: the protonated reactant $[1 + H]^+$ (Scheme 2) of m/z 108, $[2 + H]^+$ of m/z 120, $[4a - H]^+$ of m/z 237 (the species preceding **4**), and the final product, that is the protonated Tröger base $[6 + H]^+$ of m/z 251. These key species were characterized by collision-induced dissociation (CID) via ESI(+)-MS/MS experiments (see discussion below for the reaction with urotropine).

After a few more minutes (5 min), ESI-MS shows that most of **2** had been consumed and $[6 + H]^+$ of m/z 251 was now detected as by far the major ion, with some residual $[4 - H]^+$

SCHEME 6. Proposed Routes to the Dissociation of $[4 - H]^+$



of m/z 237. ESI-MS(/MS) monitoring has been able therefore, in the reaction of *p*-toluidine and formaldehyde in neat TFA, to intercept and characterize in cationic forms two key intermediates, **2** and **4** (Scheme 2). The ion of m/z 237 is presented as resulting from a hydride loss from **4**. We believe that **4** is oxidized in the ESI source,³⁰ and that the driving force for this oxidation is the formation of the stable $[4 - H]^+$ displaying a highly resonance-stabilized positive charge.

The ions of m/z 134, 150, and 267, which appeared at first to have no direct relation to the formation of Tröger's bases, were also isolated and dissociated (Figure 3A–C). The ion of m/z 134 dissociates mainly to $C_7H_7^+$ of m/z 91 as well as by methyl and HCN loss and, from this dissociation behavior, we propose the ion to be the protonated form of *N*-methylated **2**. The mechanism for *N*-methylation of **2** is, however, unclear to us at the moment.

The ion of m/z 150 seems to result from the incorporation of a molecule of formaldehyde into **2** (Scheme 4). Its ESI-MS/MS (Figure 3B) corroborates this possibility as the proposed bicyclic ion dissociates mainly by the loss of formaldehyde.

The ion of m/z 267 (Figure 3C) is attributed to the interception of another key intermediate for Tröger's base formation (Scheme 2), that is, $[5 - H]^+$ (Scheme 5). The ion dissociates mainly by formaldehyde loss to afford likely $[4 - H]^+$ of m/z 237. Interestingly, a compound structurally related to $[5 - H]^+$ has been proposed by Wagner²² (Scheme 2) as an advanced intermediate for Tröger's bases. Note that, in addition to the ESI-MS/MS characterization of the intercepted species, high-accuracy m/z measurements also corroborate the proposed structures (see Supporting Information).

Urotropine: To gain further insights of the mechanism of Tröger's base formation, we performed the reaction of *p*-toluidine with urotropine (**15**) as an alternative methylene source (instead of formaldehyde) again in neat TFA at room temperature. A clear reaction solution was formed, which was monitored by on-line ESI-MS initiated approximately 20 s after mixing. From 30 s up to 1 min of reaction, ESI-MS detects only the two starting reactants in their protonated forms, that is, $[1 + H]^+$ of m/z 108 and $[15 + H]^+$ of m/z 141, affording ESI-MS similar to that shown in Figure 4a. After 2 min, the same imine intermediate **2** (as for Figure 2) in its protonated form $[2 + H]^+$ of m/z 120 shows up clearly (Figure 3B). When

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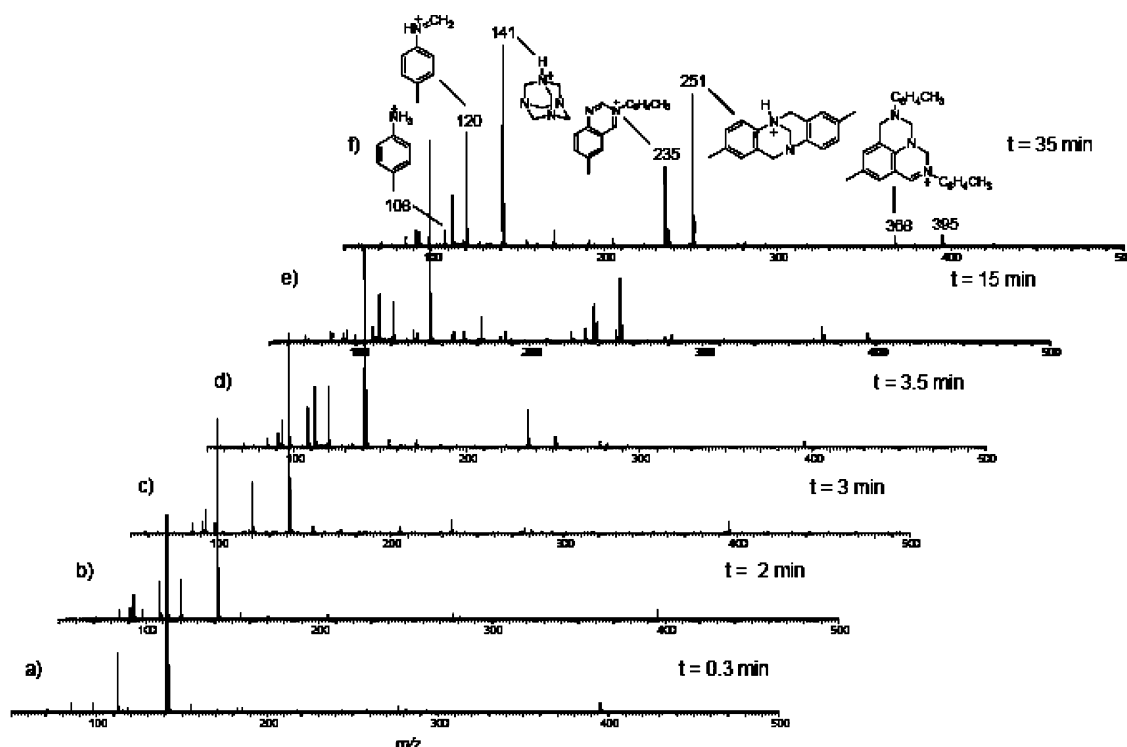
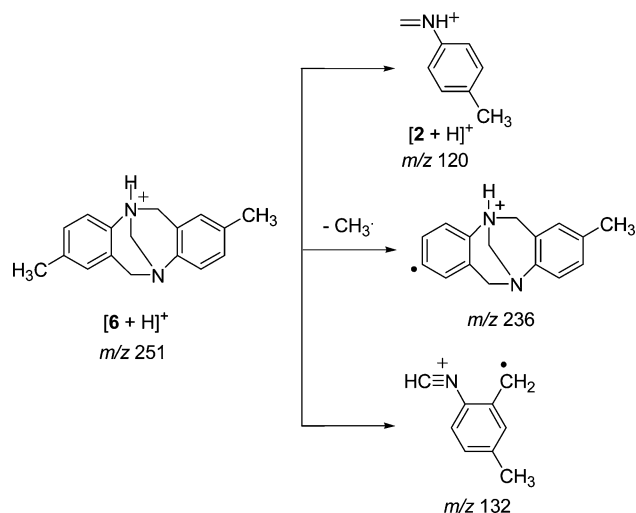


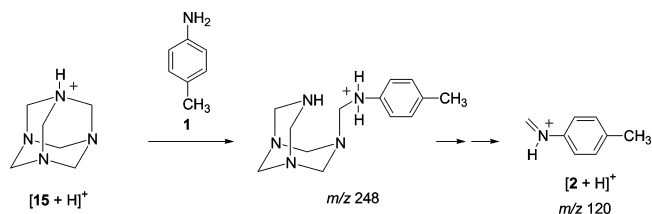
FIGURE 4. ESI(+)-MS acquired for the reaction solution in neat TFA during the on-line monitoring of the reaction of *p*-toluidine and urotropine (**15**) after (a) 20 s; (b) 2 min; (c) 3 min; (d) 3.5 min; (e) 15 min; and (f) 35 min of reaction.

SCHEME 7. Proposed Routes for the Dissociation of the Protonated Tröger's Base [6 + H]⁺



this ion of m/z 120 was selected (by quadrupole filtering) and dissociated (Figure S1a, see Supporting Information), it was found to dissociate mainly by the loss of $\text{CH}_2=\text{NH}$ to form a C_7H_7^+ ion of m/z 91. This ion displayed therefore the same CID behavior as that of $[2 + \text{H}]^+$ of m/z 120 intercepted in the reaction using formaldehyde as the methylene source. After 3 min of reaction, an additional cationic species of m/z 235 was detected (Figure 4c). This new intermediate was characterized by ESI-MS/MS (Figure S1b). Dissociation proceeds mainly by loss of neutral methylbenzopyrimidine to give a C_7H_7^+ ion of m/z 91 and by CH_3 radical loss to produce the distonic cation of m/z 220. The ion of m/z 235 is rationalized to be formed in the reaction medium via the abstraction by urotropine of benzylic

SCHEME 8. Proposed Mechanism for the Action of Urotropine as a Methylene Source in Tröger's Base Formation



hydrogens from $[4 - \text{H}]^+$ to form the aromatic pyrimidine cationic derivative $[4 - 3\text{H}]^+$. Finally, after 3.5 min of reaction (Figure 4d), the Tröger's base final product showed up as $[6 + \text{H}]^+$ of m/z 251. From this moment on, as exemplified in Figure 4e,f by the ESI-MS acquired at 15 and 35 min of reaction, the abundance of $[6 + \text{H}]^+$ increased significantly, whereas the $[2 + \text{H}]^+/[4 - 3\text{H}]^+$ abundance ratio decreased proportionally. Interestingly, a byproduct attributed to **8**, which has been isolated previously by Farrar and Johnson,^{8,23} was also detected by ESI-MS as $[8 - \text{H}]^+$ of m/z 368 after 15 min of reaction and characterized by CID (Figures 4e and S1e). The $[8 - \text{H}]^+$ dissociates mainly by loss of neutral tolylimine ($\text{CH}_3-\text{C}_6\text{H}_4-\text{N}=\text{CH}_2$) to form the ion of m/z 249. It also dissociates to the iminium ion of m/z 120 (Figure S1e). We do not observe any dissociation of $[8 - \text{H}]^+$ to form an ion of m/z 251. The reduced form of ion $[8 - \text{H}]^+$ has already been detected and isolated from the reaction medium by Johnson⁸ and Farrar.²³ Once formed, this compound is not converted to the Tröger base.⁸

Furthermore, in regard to the ESI-MS/MS characterization of the intercepted species, intermediate $[4\text{a} - \text{H}]^+$ of m/z 237 (Figure S1c) contravenes the even-electron rule²⁶ to lose a CH_3 radical, thus forming the fragment ion of m/z 222. Scheme 6

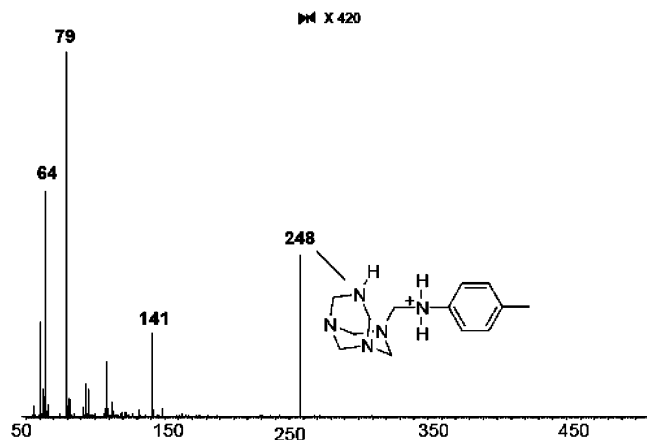


FIGURE 5. ESI-MS screening for the reaction solution formed by mixing TFA solutions of *p*-toluidine (**1**) with urotropine (**15**) on-line using a microreactor coupled just before the ESI source and reaction time of ca. 2.0 s and not 0.7 s.

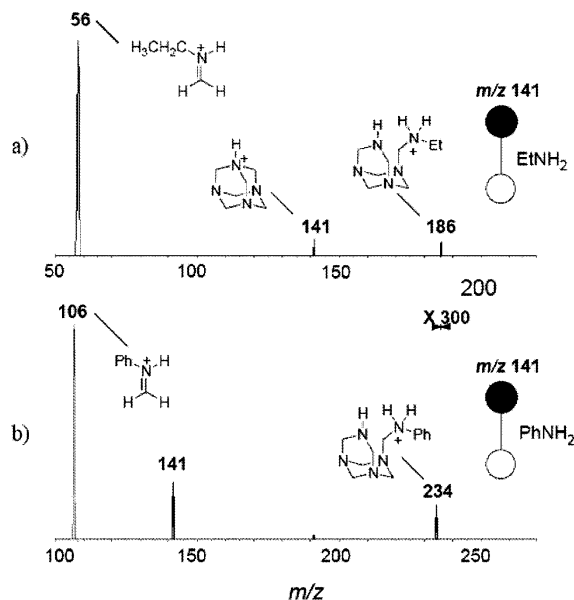


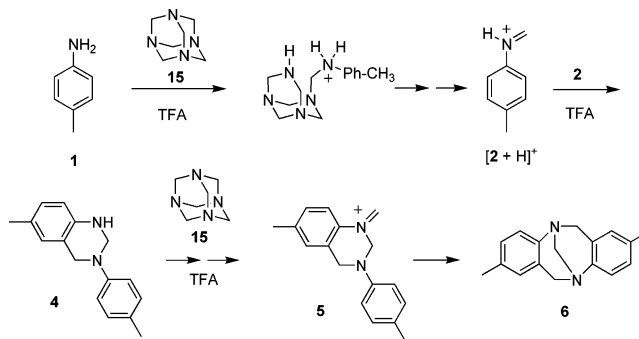
FIGURE 6. ESI-MS/MS for gas-phase ion/molecule reactions of protonated urotropine of *m/z* 141 with (a) ethylamine and (b) aniline.

rationalizes this loss, which forms a relatively stable distonic radical cation,^{31,32} but the main dissociation pathway for $[4a + H]^+$ is by retro-addition to form the species $[2 + H]^+$ of *m/z* 120 (Figure S1c).

The protonated Tröger's base, that is, $[6a + H]^+$ of *m/z* 251, also dissociates in a structurally diagnostic fashion (Figure S1d): as for $[4a + H]^+$, it loses a CH_3 radical to form the ion of *m/z* 236 (Scheme 7), by the loss of a neutral imine $CH_3-C_6H_4-N=CH_2$ to afford an ion of *m/z* 132, as well to the iminium ion **2a** of *m/z* 120.

In Tröger's base formation, urotropine has been proposed to act as an efficient methylene source due to its hydrolysis to formaldehyde in the acidic TFA medium.⁸ The same imine

SCHEME 9. Mechanism for the Formation of Tröger's Base as Probed by ESI(+)-MS(/MS) and Ion/Molecule Reactions



intermediate **2** was indeed detected (in its protonated form of *m/z* 120) in reactions of *p*-toluidine **1** with both formaldehyde (Figure 2) and urotropine (**15**, Figure 4), but no intermediate that could be attributed to any stage of urotropine hydrolysis was detected. In trying to intercept early reaction intermediates that could reveal exactly how urotropine acts as a methylene source, TFA solutions of **1** and urotropine (**15**) were mixed in a microreactor placed very close the ESI source; hence, in this way, the reaction solution was electrosprayed just ca. 2.0 s after mixing. Under these conditions, the ESI-MS of Figure 5 was acquired. Interestingly, a new cationic intermediate of *m/z* 248 was observed, that is, $[1 + \text{urotropine} + H]^+$. ESI-MS/MS of this ion shows dissociation to occur mainly by the loss of neutral tolylamine to give protonated urotropine of *m/z* 141 (Figure S1f). This rather transient species that escaped detection without the use of the microreactor provides therefore a clue for the action of urotropine as a methylene source in Tröger's base formation: without the need of hydrolysis to formaldehyde, it transfer methylene directly to **1** via acid-catalyzed nucleophilic attack (Scheme 8).

To gain further evidence for the methylene transfer step, gas-phase ion/molecule reactions of protonated urotropine (**15**) with two volatile amines were performed. We used a hybrid linear ion-trap equipment (2000 QTrap Applied Biosystems) in which N_2 gas was replaced with reactive neutrals by needle valve adaptors that allowed the introduction of reactive gases into the collision cell (Figure 6). Interestingly, **15** of *m/z* 141 was indeed found to react with ethylamine and aniline to form directly the respective iminium ions of *m/z* 56 and 106 (Figure 6). Figure 6a shows that the imine $C_2H_5-NH=CH_2^+$ (as for **2**) of *m/z* 56 is formed in high yield in the ion/molecule reactions. Herein, the adduct of *m/z* 186, the first transient intermediate leading to methylene transfer, is obtained from nucleophilic addition of the amine to protonated urotropine. Furthermore, in reactions with aniline (Figure 6b), the corresponding imine of *m/z* 106 (as for **2**), that is, $Ph-NH=CH_2^+$, is formed as an abundant ion, and the transient adduct of *m/z* 234 is detected as a low abundance ion. When aminoveratrol was used as the aniline source and following the same procedure as described above, the reaction showed the same behavior observed for *p*-toluidine.²⁵

On the basis of the mechanistic data collected from on-line ESI-MS(/MS) monitoring and the gas-phase ion/molecule reactions just described, an experimentally probed mechanism for the formation of Tröger's bases using either formaldehyde or, more particularly, urotropine as the source of methylene is presented (Scheme 9). This mechanism proposes the participa-

(31) Tomazela, D. M.; Sabino, A. A.; Sparrapan, R.; Gozzo, F. C.; Eberlin, M. N. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1014.

(32) For an example concerning similar dissociation patterns, see: Santos, L. S.; Padilha, M. C.; Neto, F. R. D.; Pereira, A. D.; Menegatti, R.; Fraga, C. A. M.; Barreiro, E. B.; Eberlin, M. N. *J. Mass Spectrom.* **2005**, *40*, 815.

tion of all of the intermediates intercepted by ESI-MS and properly characterized by ESI-MS/MS.

Conclusions

Key intermediates for Tröger's base formation in reactions of amines and aldehydes have been transferred and measured directly from the reaction solution to the gas-phase environment of a mass spectrometer by ESI-MS followed by characterization via ESI-MS/MS. The participation of urotropine as a direct source of methylene in Tröger's base formation has also been demonstrated by gas-phase ion/molecule reactions of its protonated molecule with aniline and ethylamine. The urotropine action has also been shown in solution, via the ESI-MS interception and ESI-MS/MS characterization of a key reaction intermediate leading to methylene transfer. A concise and experimentally probed mechanism for the formation of Tröger's base has been therefore elaborated.

Experimental Section

General Procedures. All reactions were performed under an inert atmosphere of dry nitrogen and were followed by ESI-MS. Glassware was flame dried before use. Standard syringe techniques

were applied to transfer dry solvents and reagents. The preparation of samples and the setup of the microreactor were carried out using two syringe pumps.²⁵ Electrospray ionization mass spectra (ESI-MS) and accurate mass measurements were carried out with a QTOF-I instrument. TFA and other reagents were used as received without further purification. Ion/molecule reactions were performed in a 2000 QTRAP system replacing N₂ gas with reactive neutrals using needle valve adaptors that allowed the introduction of reactive gases into the collision cell through the residual vacuum of the equipment, which was measured by a vacuum gauge and varied from around 9×10^{-6} to 5×10^{-5} torr depending on the neutral introduced into the equipment.

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Supporting Information Available: Figure S1, ESI(+)-MS/MS of major intermediates intercepted in reactions of *p*-toluidine (**1**) and urotropine (**15**) in TFA. Spectra (a), (c), and (d) were nearly the same when acquired for the reaction using formaldehyde as the methylene source. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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