Ionic Liquids

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On the Formation of a Protic Ionic Liquid in Nature**

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Dedicated to Professor Charles M. Lukehart

Abstract: *The practical utility of ionic liquids (ILs) makes the absence (heretofore) of reported examples from nature quite puzzling, given the facility with which nature produces many other types of exotic but utilitarian substances. In that vein, we report here the identification and characterization of a naturally occurring protic IL. It can be formed during confrontations between the ants S. invicta and N. fulva. After being sprayed with alkaloid-based S. invicta venom, N. fulva detoxifies by grooming with its own venom, formic acid. The mixture is a viscous liquid manifestly different from either of the constituents. Further, we find that the change results as a consequence of formic acid protonation of the N centers of the S. invicta venom alkaloids. The resulting mixed-cation ammonium formate milieu has properties consistent with its classification as a protic IL.*

With its especially painful bite, the fire ant (*Solenopsis invicta*) is a South American native that has become a serious nuisance species in North America.[1] Another nonnative, the tawny crazy ant (*Nylanderia fulva*), has now spread[2] into areas of the United States that have existing fire ant populations, bringing the two into competition. Significantly, their territories also overlap in the regions of South America from which they originate, and as a consequence they have shared evolutionary histories.

In areas where invasions are occurring, *N. fulva* is making substantial headway in displacing established *S. invicta* populations.[3] It has been observed that *N. fulva* captures 93% of resources contested with *S. invicta*.[3] Furthermore, this is in spite of the apparent advantage provided to the latter by its highly insecticidal venom, composed of more than 95% water-insoluble 2-methyl-6-alkyl/alkenylpiperidines (solenopsins, Figure 1), the alkyl/alkenyl side chains of which are lipidic in character.[4]

In a recent paper, LeBrun et al. reported that the key to the ability of *N. fulva* to dominate its opponent is the successful detoxification of *S. invicta* venom to which it is exposed.[5] *N. fulva* accomplishes this by mixing its own venom (formic acid) with that sprayed onto it by *S. invicta*.[5] Insofar as the mechanism of detoxification is concerned,

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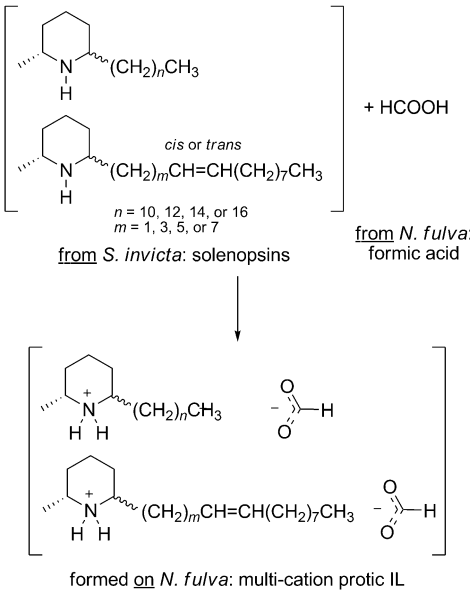

Figure 1. Structures of major solenopsin alkaloids known to comprise *S. invicta* venom, and the proposed products of their reaction with formic acid (*N. fulva* venom).

LeBrun postulated that it may be due to the denaturation of very small quantities of proteins present in the *S. invicta* venom by formic acid.[5] Offering an alternate theory, Meinwald suggested[6] that acid–base chemistry between the alkaloids and formic acid may be instrumental in detoxification, perhaps by interfering with the ability of the highly lipophilic alkaloids to penetrate cell membranes. While we presently take no stance as to mechanism, it was apparent to us from a strictly physicochemical standpoint that alkaloid protonation was probable and that the resulting reaction product would likely be an ionic liquid. Whereas synthetic ILs employing naturally occurring ions are well known, we believe the present report to be the first to describe a naturally occurring protic ionic liquid (PIL).

Venom from *S. invicta* was isolated using an established protocol.[4b,c] Formic acid was then added to it, and after thorough mixing, excess acid was removed in vacuo. Further vacuum treatment at ambient temperature resulted in no further change in the quantity of formic acid retained, suggesting a strong association of the acidic and basic venom constituents. The material remaining after the excess formic acid was removed, was a liquid much higher in viscosity than either of the original free-flowing constituent venoms (Figure 2). Using Raman microscopy, ^{1}H and

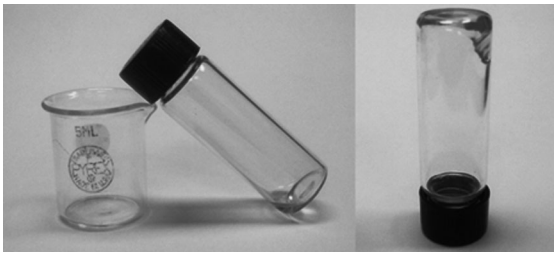

Figure 2. Left: Venom from *Solenopsis invicta*, a free-flowing liquid composed of a mixture of lipidic piperidine alkaloids, at 21 °C. Right: Also at 21 °C, the *S. invicta* venom after treatment with and removal of excess formic acid (*N. fulva* venom). The viscous but liquid nature of the reaction product is apparent.

^{13}C NMR spectroscopy, and ESI ion-trap mass spectrometry, we then established that this change was coincident with the protonation of the *S. invicta* alkaloids by formic acid to form a milieu of mixed solenopsinium cations paired with formate anions. Note that ILs composed of mixed cations are attracting increasing interest since such ion mixing can have salutary effects on the properties of the resulting ILs.[7]

The venom–formic acid product was examined by Raman microscopy, using Argon-ion laser excitation at 488 nm through a 50X microscope objective.[8] No crystallinity was evident. Instead, the deposit had a decidedly greasy appearance when viewed through the microscope. Note that the formation of such "soft" phases by protic and formate ILs is a known phenomenon.[9–11]

The Raman spectrum of the mixed venom product is shown in Figure 3, as is that of formic acid. A key difference is the presence of doublets for both C–O and C=O stretching in the mixed venoms, and a pronounced red shift of the C=O

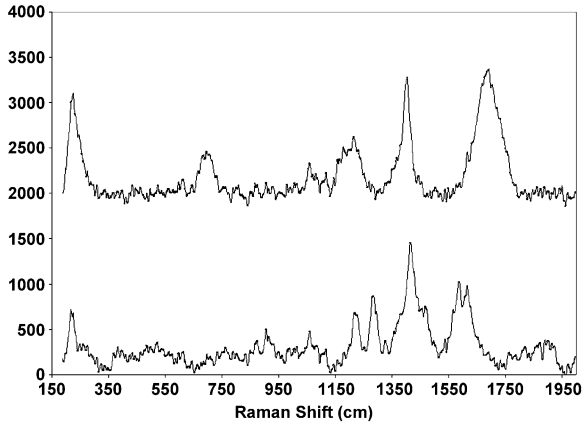

Figure 3. Raman spectra of acidified fire ant venom (lower) and formic acid (upper).

stretching vibrations. The doublets are likely due to C–O and C=O bonds from formate anions associated with other species in differing chemical environments,[12] and are consistent with the known C=O stretching mode of formate (1596 cm^{-1}).[14] Also, the C=O stretching band is a composite of two bands, the relative intensity and shapes of which are temperature- and environment-dependent.[13,14] Further, the Raman spectrum of the present PIL formate bears similarities to those for the known PILs 2-methylbutylammonium formate and pentylammonium formate.[15] Consequently, these results are consistent with the presence of the formate anion and its interaction (e.g., by H bonding)[16] with other species present in the treated venom matrix. This would be expected after a reaction between the venom alkaloids and formic acid to form a protic IL.

The venom sample and pure (2*R*,6*S*)-2-methyl-6-undecylpiperidine (prepared using a known procedure)[17] were then independently treated with deuterated formic acid, DCO_2D, and analyzed by mass spectrometry. Despite the use of an aprotic solvent and weak ionization conditions (see the Supporting Information), piperidines are detected with excellent sensitivity, indicating that they react with residual water in the solvent and/or source region. Consequently, the alkaloids were treated with excess deuterated formic acid (DCO_2D).

The ESI-MS study showed that the pure venom alkaloid (2*R*,6*S*)-2-methyl-6-undecylpiperidine reacts with DCO_2D to form the D^+ adduct at *m*/*z* 255 (H^+ adduct at *m*/*z* 254). In turn, the reaction with the far weaker acid, D_2O, proceeds to a lesser extent (the ratio of 255/254 is 0.65 vs. 1.29 for DCO_2D). Also noteworthy is the absence of peaks from materials other than those arising from alkaloid protonation/deuteration.

Critically, when the actual *S. invicta* venom is likewise treated with DCO_2D (Figure 4a), equivalent results are observed. The alkaloid ions detected have been deuterated by DCO_2D, and no additional products (i.e. peaks not present in Figure 4b) are detected. This is consistent with the clean conversion (no side reactions) of the Solenopsin alkaloids into their respective formate salts. Consequently, ESI-MS data fully comport with an acid–base protonation reaction occur-

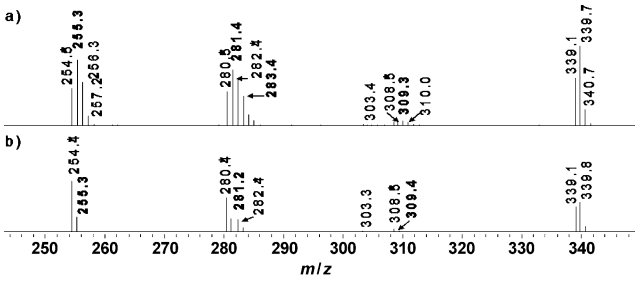

Figure 4. ESI ion-trap mass spectra: a) *S. invicta* venom with DCO_2D, b) *S. invicta* venom. * indicates the nominal mass corresponding to alkaloids previously identified.[4b,c] Relevant isotopomers ($^{13}C_1$ and/or D_1, in bold face) and increase in relative abundance after D^+ transfer from acid to alkaloid.

ring when the alkaloid-rich venom of *S. invicta* is challenged with the formic acid venom of *N. fulva*.

Diagnostic changes in the ^1H and ^{13}C NMR spectra ($CDCl_3$) of the *S. invicta* venom (which are paralleled in the pre-/postacidification spectra of pure (2*R*,6*S*)-2-methyl-6-undecylpiperidine) are also observed upon treatment with formic acid. The N–H resonance in the original venom, a broad peak of intensity one at 2.32 ppm disappears and is replaced by a broader peak of approximate intensity two at 9.7 ppm, consistent with N–H protons in known PILs.[18] The postacidification presence of formate is also confirmed by the presence of peaks at 8.48 ppm (^1H) and 167 ppm (^{13}C NMR).

Other key changes are also seen in the NMR spectra. First, the piperidine-ring methyl groups (isomers apparent), which appear at 1.07 ppm preacidification, shift to 1.36 ppm afterwards (^1H). Likewise, the ^{13}C resonances from these methyl groups shift, from ca. 19 and 21 ppm to 16 and 17 ppm, respectively. Also, the H atoms on the piperidine-N-adjacent ring carbons shift from 2.97 and 3.08 ppm to 3.22 and 3.47 ppm, respectively, upon acidification. These carbon signals likewise change (^{13}C), from 45.87 and 50.86 to 47.37 and 51.30 ppm, respectively, after exposure of the venom to formic acid. All of these changes, including the upfield shift of the N-adjacent methine ^1H resonances upon protonation, comport with those computationally predicted (ChemNMR) for protonated solenopsin alkaloids.

Clinching the proposed formation of salts by the formic acid protonation of *S. invicta* alkaloids, we acquired a single-crystal X-ray structure of (2*R*,6*S*)-2-methyl-6-undecylpiperidinium formate (Figure 5). The 2,6-dialkylpiperidine element of the material is N-protonated, and the counterion is readily identifiable as formate. Since the head groups of most of the other solenopsin alkaloids in the venom are identical to the one in (2*R*,6*S*)-2-methyl-6-undecylpiperidine,[4b,c] it seems reasonable to expect they too would form analogous salts.

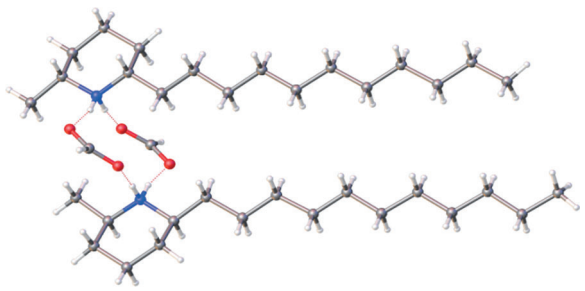

Figure 5. Cations and anions in the unit cell of the solenopsin alkaloid salt (2*R*,6*S*)-2-methyl-6-undecylpiperidinium formate.

Having established that the alkaloids comprising the venom of *S. invicta* are protonated by the venom of *N. fulva*, i.e., that the product is indeed ionic, we turned to the matter of whether the product is in fact a liquid. Certainly the simplest evidence of this is visual (Figure 2); the mixture very clearly flows under the force of gravity. Nevertheless, using differential scanning calorimetry (DSC) we quantified its thermochemical behavior as well as that of the untreated venom and the formate salt of (2*R*,6*S*)-2-methyl-6-undecylpiperidine.

The DSC of pure (2*R*,6*S*)-2-methyl-6-undecylpiperidinium formate shows it to undergo a solid-to-solid phase transition between 10.6 and 16.0 °C, before melting into an isotropic liquid beginning at 57.9 °C, well below the T_m = 100 °C criterion commonly used in defining ionic liquids. In turn, the thermal behavior of the pure venom and its formate salt is shown in Figure 6. The venom exhibits a broad melting

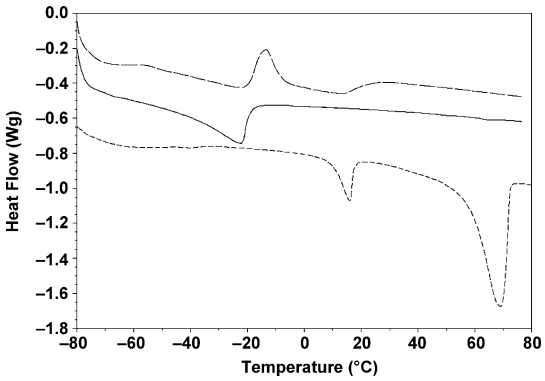

Figure 6. DSC curves for the pure fire ant venom (solid line), the formate of the fire ant venom (long dashed line) and (2*R*,6*S*)-2-methyl-6-undecylpiperidinium formate (short dashed line). The curves were measured under nitrogen at a ramp rate of 10 °C min^{-1}. They have been shifted on the *y* axis for clarity, but not rescaled.

signature, as would be expected for a complex mixture. It begins around −60 °C, and is complete by approximately −20 °C. The venom formate shows a similar trend in the lower temperature region, but then exhibits an exotherm, due to crystallization or ordering, at −20 °C. This then overlaps with an endothermic melting event, which is not complete until ca. 25 °C. The latter behavior is common to species with high internal degrees of freedom. This includes lipidic ILs[19] where the chain motion below the T_m can allow amorphous solids to rearrange into crystalline or more ordered structures.

Altogether, the present data indicate that a PIL forms when the pure (but structurally representative) solenopsin venom alkaloid (2*R*,6*S*)-2-methyl-6-undecylpiperidine is combined stoichiometrically with formic acid (*N. fulva* venom). The data is likewise consistent with categorizing, as a PIL, the stoichiometric combination of *N. fulva* venom

(formic acid) and *S. invicta* venom, which consists of more than 95% of a mixture of solenopsin alkaloids. Having said that, it is important to bear in mind that these experiments were conducted in a laboratory setting. In nature there would be many environmental variables prevailing during ant encounters, ranging from differences in humidity and temperature to nonstoichiometric venom mixing. Of these, the one perhaps most directly bearing on the formation of an IL is nonstoichiometric mixing. Yet, even in that circumstance the matrix created would likely be a "protic ionic liquid solution" as described by Lopes and Rebelo[20a] and likewise discussed by MacFarlane and Seddon as a "mixture of ionic liquids with neutral compounds."[20b] So, even with taking a conservative view of the present findings, it seems reasonable to conclude that at least a (perhaps sizeable) subset of the vast number of daily *N. fulva* and *S. invicta* encounters in nature occur under conditions that result in the outright formation of PILs, or minimally, protic ionic liquid solutions.[20,21]

In closing, we note that the biological effects of ionic liquids are a topic of considerable interest and increasingly apparent importance.[22] Perhaps surprisingly, bio-IL studies to date have not only found situations in which an IL has a deleterious effect on a biomolecule or organism exposed to it.[22] Rather, there are instances in which the bio-impact of a PIL is decidedly positive. The stabilization of certain proteins by PILs has been observed, as has a case in which the viability of a virus was prolonged by storage in a PIL versus a standard aqueous medium.[23] So, given that ionic liquids can and do have biological effects ranging from the level of individual biomolecules up to entire organisms, the possibility that naturally occurring ILs exist to play specific biological roles cannot be dismissed if, when, and wherever they might be found.

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