# Isolation and Stabilization of a Pheromone in Crystalline Molecular Capsules

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S *Supporting Information*

ABSTRACT: The active monomer form of the male-produced pheromone of the Mediterranean fruit fly can be isolated selectively from its equilibrating trimer species by encapsulation within a calixarene pocket built into a hydrogen-bonded framework from guanidinium 4-sulfocalix[4]arene. Encapsulation of the Δ^1-pyrroline guest significantly perturbs the assembly of the quasihexagonal two-dimensional guanidinium-sulfonate network of the guest-free framework, to the extent that guanidinium ions are excluded from some sites to accommodate the steric requirements of the guest. Nonetheless, single crystal X-ray diffraction reveals the preservation of a layered structure in which the calixarene capsules stack in an antiparallel configuration. These observations illustrate that the binding of the pheromone monomer by the calixarene is sufficiently strong to overcome the loss of guanidinium-sulfonate hydrogen bonds, which is corroborated by the strong binding constants measured in solution. The solid-state encapsulation stabilizes the otherwise volatile unstable monomer form, suggesting an effective strategy for the storage, application, and controlled release of an important agricultural adjuvant.
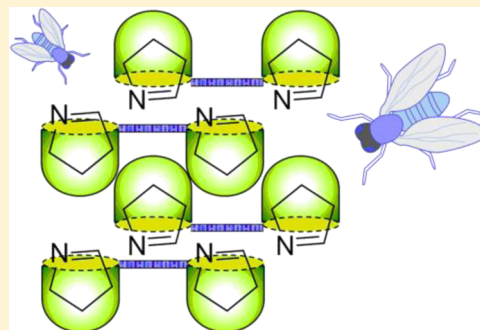
The Mediterranean fruit fly (Medfly), one of the most destructive fruit pests, has a record of infestation throughout South Europe, Africa, Australia, Hawaii, Central and South America, and portions of North America.[1] The potential damage to agriculture caused by the Medfly in California alone can exceed $1 billion every year.[2] Consequently, substantial effort has been spent to control the spread and infestation of the Medfly.[3] One approach to mitigate infestation relies on trapping the female Medfly, using one of the male-produced pheromones,[4] 3,4-dihydro-2H-pyrrole (Δ^1-pyrroline), as an attractant. The Δ^1-pyrroline coexists with its corresponding trimer species in both the solution phase and in neat liquid form (by ^{1}H NMR),[5] but only the Δ^1-pyrroline monomer is active as a pheromone for the Medfly.[6] The pheromone, however, is unstable at ambient temperature, which has been attributed to polymerization of the monomeric form.[7] Consequently, there exists a need for approaches to isolate the active monomer from its trimer, while stabilizing the monomer long-term so that it can be handled, stored, and applied as an agricultural adjuvant.
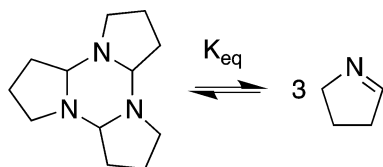
Our laboratory has reported a large library of low-density molecular frameworks constructed from a two-dimensional quasi-hexagonal hydrogen-bonding network of guanidinium (G) cations and organosulfonates (S). Through judicious selection of various organic residues of organomonosulfonates and organodisulfonates, the size, shape, and chemical character of inclusion cavities flanked by the organosulfonate "pillars" can be controlled in a rational manner.[8−14] This attribute enabled separation of molecular isomers through highly selective inclusion, during crystallization.[15] This suggested that the Δ^1-pyrroline pheromone monomer, which is much smaller than the trimer (77 Å^3 vs 221 Å^3), could be isolated from the trimer by size exclusion in properly designed GS frameworks. To date, however, our attempts to trap Δ^1-pyrroline in various pillared GS frameworks have afforded only guest-free material. Although the absence of inclusion compounds containing Δ^1-pyrroline is surprising given the proclivity of GS frameworks to trap guest molecules, our experience has taught that guests with hydrogen-bonding groups can interfere with their formation.

Our laboratory recently reported GS frameworks decorated with 4-sulfocalix[4]arene "capsules" (Figure 1),[16] in which the four sulfonate groups on the upper rim of the calixarene occupied the customary positions in the GS sheet. These capsules have an internal volume of approximately 50 Å^3, which is somewhat smaller than the molecular volume of Δ^1-pyrroline (77 Å^3). The same framework, however, was used to trap 4,6-dimethyl-α-pyrone (132 Å^3) inside the calixarene capsules, although with some rearrangement of the guanidinium-
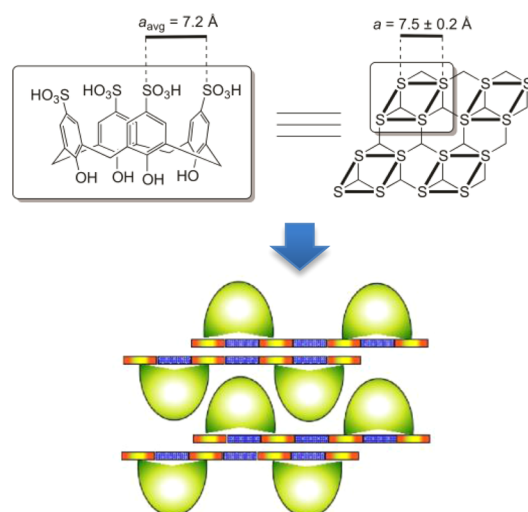
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Figure 1. Molecular structure of 4-sulfocalix[4]arene (upper left) and a schematic representation of the GS hydrogen-bonded sheet (upper right). The sulfonate S···S distances on the upper rim of the calixarene, which range from 6.52−8.06 Å (determined from 51 crystal structures obtained from the Cambridge Structural Database version 5.34, 2012), overlap the range of sulfonate S···S distances observed for the GS sheet, permitting incorporation of the sulfonated calixarene into the GS sheet. The lower panel illustrates the antiparallel packing of the calixarene capsules in guest-free guanidinium 4-sulfocalix[4]arene. Adapted from ref 16. Copyright 2009 American Chemical Society.

sulfonate hydrogen-bonding motif to accommodate the larger size,[17] illustrating the adaptive and robust character of the guanidinium-sulfonate hydrogen bonds. Herein, we describe the selective encapsulation of the Δ^1-pyrroline monomer by guanidinium 4-sulfocalix[4]arene (GSC), which stabilizes the volatile pheromone monomer in the crystalline state.

Slow evaporation at ambient temperature of an aqueous solution containing 0.067 mM 4-sulfocalix[4]arene (acid form), 0.27 mM guanidine hydrochloride, and 0.27 mM pheromone produced platelike crystals. ^{1}H NMR and elemental analysis obtained for the dissolved crystals were consistent with 1.5 equivalents of guanidinium and one effective equivalent of Δ^1-pyrroline monomer for each 4-sulfocalix[4]arene, suggesting a formula $G_{1.5}SC \supset \Delta^1$-pyrroline. Single crystal X-ray diffraction was consistent with the monoclinic space group $C2/m$ (a = 21.15 Å, b = 18.25 Å, c = 15.74 Å, β = 109.56°), differing from the guest-free structure ($P\bar{1}$, a = 12.32 Å, b = 13.08 Å, c = 14.36 Å, α =90.70°, β = 92.57°, γ = 93.54°). A Δ^1-pyrroline monomer was trapped in each calixarene capsule, with no evidence of a trimer (Figure 2). This was corroborated by infrared spectroscopy of $G_{1.5}SC \supset \Delta^1$-pyrroline, which revealed a sharp peak at 1667 cm^{-1}, characteristic of the $\nu_{C=N}$ stretch assignable to the Δ^1-pyrroline monomer. The C=N double bond, distinguished by its shorter length (1.35 Å) compared with the C−C and C−N single bonds (~1.48 Å), was situated at the upper rim of the calixarene capsule. The observed orientation is not unexpected as it places the less polar portion of the guest in the less polar region of the calixarene pocket, with the more polar C=N group at the upper rim in the vicinity of the guanidinium and sulfonate ions. The structure was refined with the C=N bond disordered about a mirror plane, as the scattering factors for carbon and nitrogen are nearly identical. X-ray refinement revealed only 1.2 equivalents of guanidinium in the framework. The remaining 0.3 equivalents could not be located from the
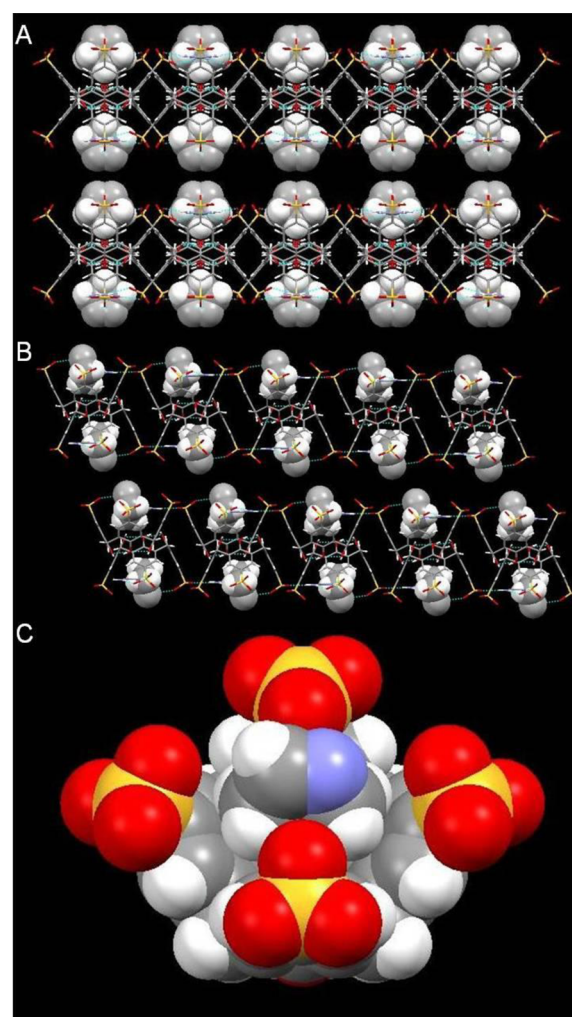

Figure 2. Molecular packing in $G_{1.5}SC \supset \Delta^1$-pyrroline as viewed (A) along the a axis and (B) along the b axis. The Δ^1-pyrroline pheromone is depicted as space-filling and water molecules between the layers have been removed for clarity. (C) Δ^1-pyrroline trapped in the calixarene capsule.

differential Fourier maps, suggesting substantial disorder of the guanidinium ions. Whereas attempts to synthesize inclusion compounds with guanidinium mono- and disulfonate frameworks were unsuccessful, $G_{1.5}SC \supset \Delta^1$-pyrroline forms readily, suggesting that the tetravalency of calixarene outweighs competitive hydrogen bonding by the Δ^1-pyrroline guest through a kind of chelate effect.

The ^{1}H NMR spectrum of crystals dissolved in D_2O revealed both monomer and trimer, demonstrating equilibration of the monomer with its trimer form on the timescale of crystal dissolution and measurement. The ^{1}H NMR peaks assigned to the monomer and trimer were shifted upfield, indicating these species remained associated with the calixarene pocket in solution. The calixarene:pheromone ratio was 1:1 based on the total amount of monomer and trimer determined from ^{1}H NMR. Collectively, X-ray diffraction, infrared spectroscopy, and NMR spectroscopy demonstrate that the pheromone monomer was encapsulated selectively in a stoichiometric ratio during crystallization from an equilibrium mixture of the monomer and trimer.

The crystal structure of $G_{1.5}SC \supset \Delta^1$-pyrroline reveals a bilayer architecture (Figure 2A). Within each bilayer, the

calixarene capsules are packed in an antiparallel configuration (Figure 2B). The encapsulation of the guest molecule in the calixarene pocket perturbed the customary quasi-hexagonal hydrogen bond motif found in the guest-free structure (Figure 3). A singular common feature of guest-free G_4SC and $G_{1.5}SC$
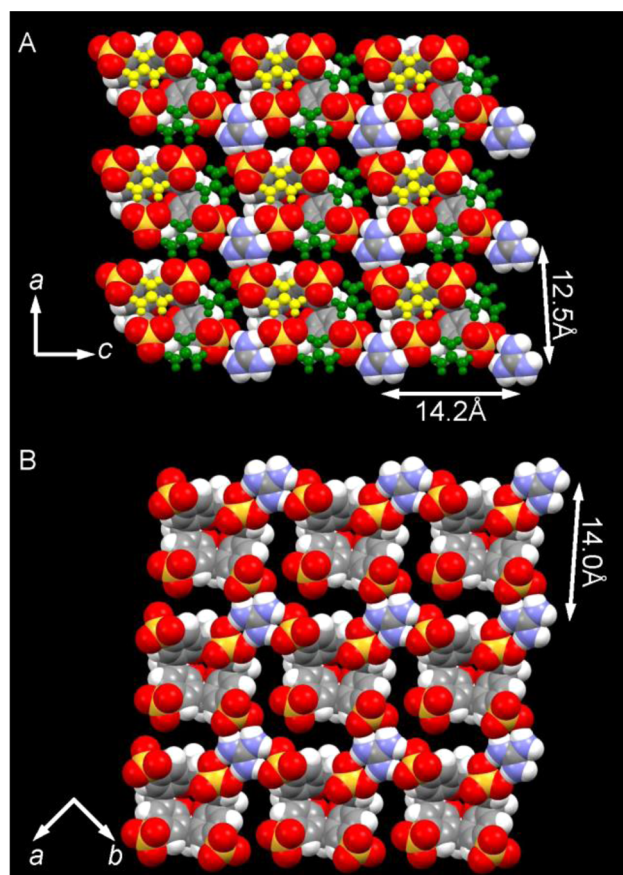

Figure 3. (A) Hydrogen bond motif of guest-free G_4SC. G ions bridging three sulfonates at the upper rim of each 4-sulfocalix[4]arene are depicted as yellow balls-and-sticks. G ions bridging three sulfonates on two neighboring sulfocalixarenes are depicted as green balls-and-sticks. G ions bridging three sulfonates on three neighboring sulfocalixarenes are depicted as spacefilled. (B) Hydrogen bond motif of $G_{1.5}SC \supset \Delta^1$-pyrroline.

$\supset \Delta^1$-pyrroline is the presence of G ions, bridging three sulfonate groups on three neighboring calixarenes, a natural consequence of the 3-fold symmetry of the G ion (Figure 3, depicted as spacefilled). These G ions define a rhombus with dimensions of 14.0 × 14.0 Å, reflecting a significant expansion compared with guest-free G_4SC, in which the G ions define a parallelogram with dimensions of 12.5 × 14.2 Å, suggesting an expansion of the 2D sheet. The G ions that otherwise cap the top rim of the calixarene in guest-free G_4SC (Figure 3, yellow ball-and-stick) are absent, their positions occupied by the encapsulated Δ^1-pyrroline. Inclusion of the Δ^1-pyrroline guest also distorts the calixarene (S−S distances of 6.98 Å and 7.35 Å on the upper rim compared with the guest-free values of 6.96 and 7.08 Å), which frustrates incorporation of a G ion at a third site occupied in guest-free G_4SC (Figure 3, green ball-and-stick). The absence of a G ion at the upper rim of the calixarene illustrates that the binding of the Δ^1-pyrroline guest by the calixarene is sufficiently strong to overcome the loss of

guanidinium-sulfonate hydrogen bonds that ordinarily complete the GS sheet.

The deficiency of guanidinium ions in $G_{1.5}SC \supset \Delta^1$-pyrroline suggests the presence of other countercations to balance the four negative charges of the 4-sulfocalix[4]arene anion. Refinement of the crystal structure found only one water molecule per 4-sulfocalix[4]arene anion, associated through hydrogen bonding with one of the sulfonate groups. Thermogravimetric analysis (TGA) upon heating to 200 °C, however, revealed a mass loss corresponding to approximately five equivalents of water in the crystal and one equivalent of Δ^1-pyrroline (Figure S9 of the Supporting Information). The NMR data did not reveal any species other than water and no other source of cations was present in the crystallization medium, arguing for charge compensation by hydronium ions in the crystal lattice. This was supported by the observation that 15 mg $G_{1.5}SC \supset \Delta^1$-pyrroline crystals in 1 mL of water afforded acidic solutions with pH = 2 (the calculated pH based on 2.5 equivalents of hydronium is 1.5), whereas the pH for the guest-free compound was nearly neutral (pH = 6).

In accordance with previous reports,[4] the Δ^1-pyrroline monomer is stable in DMSO for the four days and even less in aqueous solution. The intensities of the original NMR peaks declined noticeably over four days in D_2O. Upon standing at ambient temperature in D_2O for two weeks, the characteristic NMR peaks for both monomer and trimer were no longer observed, due to polymerization. The pure liquid pheromone gradually converts to a dark solid because of polymerization after four months storage in neat liquid form. In contrast, the monomer can be stored indefinitely in the $G_{1.5}SC$ framework with no decomposition evident from NMR, IR, and single crystal X-ray data. This illustrates that polymerization of the otherwise unstable Δ^1-pyrroline monomer was prevented by its sequestration in the solid state. Moreover, crystals of $G_{1.5}SC \supset \Delta^1$-pyrroline become opaque within ten minutes upon standing in open air. TGA revealed that all the lattice water was lost within 10 min at 30 °C. Upon further heating of the remaining solid, Δ^1-pyrroline exits the solid at ∼40 °C. When sealed in humid air, however, the crystals were stable indefinitely.

^{1}H NMR spectroscopy revealed that the equilibrium between the Δ^1-pyrroline monomer (M) and its trimer (T) is achieved immediately upon adding the pheromone to an aqueous solution. The equilibrium constant at ambient temperature (298 K) was $K_{eq} = 1.3 \times 10^{-2}$ M^2, calculated from [M]:[T] = 1.0:0.6 from integration of the monomer and trimer ^{1}H NMR peaks. Variable temperature ^{1}H NMR revealed that the [M]:[T] ratio increased with increasing temperature (ΔS = +313 J mol^{-1} K^{-1}). Upon addition of one equivalent of G_4SC to a 0.06 M (based on monomer) aqueous solution of the pheromone, the NMR peaks assigned to the monomer and trimer shifted upfield, consistent with association of both species with the calixarene pocket and in agreement with the ^{1}H NMR spectrum observed for dissolved crystals of $G_{1.5}SC \supset \Delta^1$-pyrroline (see above). No free pheromone, in either the monomer or the triplet form, was apparent. This illustrates that the calixarene binds the monomer and trimer equally well in the solution phase. During crystallization, however, only the monomer is encapsulated due to the prohibitive size of the trimer (Figure 4).

These observations illustrate the separation of an active pheromone monomer from its inactive trimer form through sequestration in an appropriately sized molecular capsule in a solid-state framework, adding to emerging discoveries that
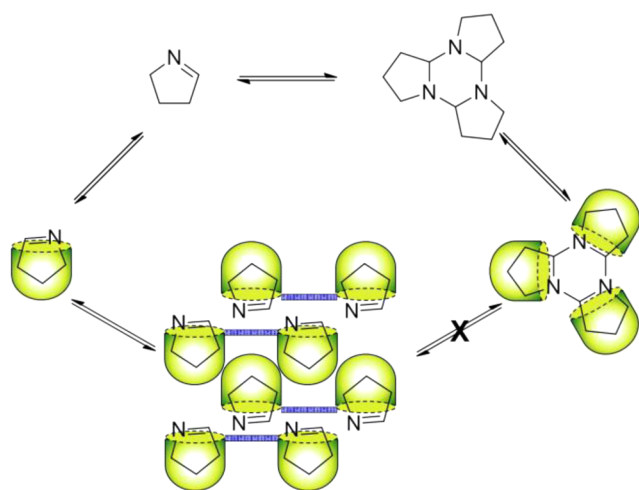
Figure 4. Schematic representation of binding of the pheromone monomer and trimer by 4-sulfocalix[4]arene (yellow) in solution and the selective encapsulation of the monomer during crystallization of $G_{1.5}SC \supset \Delta^1$-pyrroline. The blue lines denote the guanidinium cation located by X-ray diffraction in the solid state.

capitalize on crystal engineering strategies for trapping small molecules in the solid state for structural characterization that otherwise is evasive.[18,19] The encapsulation stabilizes the otherwise volatile unstable monomer form but releases the pheromone above ambient, suggesting an effective strategy for the storage, application, and controlled release of an important agricultural adjuvant.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Experimental details, characterization, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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