DANSing with *Caenorhabditis elegans*

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The emergence of natural product research over the past decades has largely been driven by the discovery of biologically active metabolites for drug discovery programs (1). A broad array of organisms, including bacteria and fungi, and plants and animals have provided such important antibiotics as erythromycin, tetracycline and the penicillins, anticancer agents such as doxorubicin, mitomycin C, and taxol, the antimalarial agents quinine and artemisinin, the "statin" cholesterol-lowering agents, and the *Conus* shell-derived peptides used in the treatment of chronic pain. Bioassay-guided fractionation of cell extracts, and isolation of individual, pure compounds for complete structure elucidation has been the operational paradigm for the field. In this way, thousands of complex molecules have been characterized and tested in a growing number of screening systems developed for drug targets against important diseases in humans.

Two important issues have remained underexplored in this dominant model that underlies the study of natural products. First, what is the endogenous role for the metabolite discovered from its native host? Second, does synergy exist in the function of structurally related metabolites that might be overlooked when assessing the biological activity of these materials as pure chemical entities? This is particularly significant when considering that most natural product biosynthetic systems produce groups of compounds that share a common core structure, but vary in the degree and extent of tailoring to provide a natural series of analogs (2). The study of these compounds in situ has been hindered by the limitations of modern analytical methods in deconvoluting 3-dimensional structures as complex mixtures. Recently, significant progress in NMR methodology has enabled new solutions to this daunting task (3). In turn, these approaches have enabled opportunities to address key questions relating to both endogenous function and synergy in the action of small-molecule metabolites.

In a groundbreaking report in this issue of PNAS, Pungaliya et al. use differential analysis by 2-dimensional NMR spectroscopy (DANS) to study small-molecule signals that regulate larval development and mating in the nematode *Caenorhabditis elegans* (4). In response to crowding, starvation, and increased temperature, *C. elegans* larvae arrest as an alternative larval stage called dauer (5). In 1982, Golden and Riddle reported the existence of an activity purified from organic extracts of conditioned *C. elegans* growth medium that promotes *C. elegans* dauer arrest, referred to as dauer pheromone (6). The molecular components of dauer pheromone have eluded characterization until recently, when 2 groups used activity-guided fractionation to characterize related ascarosides with dauer-inducing

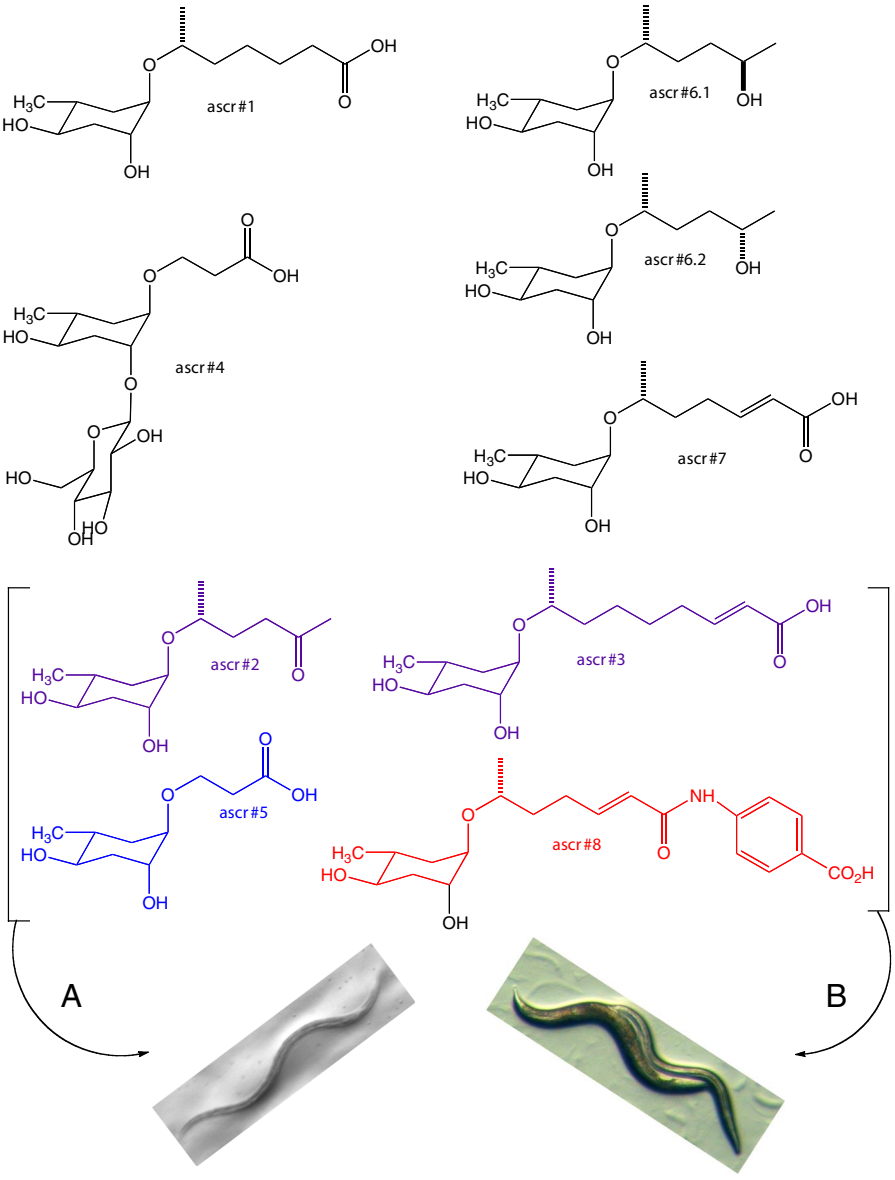

Fig. 1. *C. elegans* ascaroside metabolites involved in signaling for dauer arrest (*A*) and as pheromones for mating (*B*). Color coding relates to unique (red [mating]/blue [dauer]) or cross-over (purple) biological roles for individual molecules; ascr#2, ascr#3, and ascr#5 synergize to induce dauer arrest (9), and ascr#2, ascr#3, and ascr#8 act synergistically as a male mating attractant (4, 10).

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activity (7, 8). Initial purification demonstrated that the dauer-inducing activity of purified fractions was much lower than crude extract from the conditioned medium (7). A detailed analysis of other ascarosides present in crude extract revealed the presence of multiple distinct but related ascarosides with dauer-inducing activity (8). Notably, although individual ascarosides induce dauer arrest, combinations of ascarosides have additive or synergistic effects (8, 9). Furthermore, the identity of ascarosides purified from crude extracts varies depending on growth conditions (9), suggesting that ascaroside profiles may reflect specific information about environmental conditions. Interestingly, the same ascarosides that induce dauer arrest also function synergistically as male mating attractants when present at low concentrations (10) (Fig. 1).

In retrospect, the complexity of *C. elegans* ascaroside biology highlights limitations intrinsic to activity-based purification strategies that may have contributed to early difficulties in structure elucidation of dauer pheromone components. Like many small-molecule natural products involved in signaling and regulation activities, dauer pheromone is a mixture of components that act synergistically (9). In such situations, activity-based purification schemes are biased against molecules that do not have significant biological activity as individual chemical entities, but which may synergize with other molecules in vivo. Furthermore, activity-based purification schemes may preclude identification of labile molecules with biological activity. In this context, the article by Pungaliya et al. represents a significant advance (4). They use DANS to compare small-molecule profiles of wild-type *C. elegans* and a *daf-22* mutant, which is deficient in dauer pheromone biosynthesis (11, 12). In addition to detecting all 4 ascarosides previously identified by using activity-based approaches (referred to as ascr#1, ascr#2, ascr#3, and ascr#5), they found 4 novel ascarosides that are also *daf-22*-dependent. One of these, referred to as ascr#8, is labile, possibly explaining why it was not isolated by using activity-based purification strategies (Fig. 1). Addition of ascr#8 to ascr#2 and ascr#3 fully reconstitutes the male attractant activity present in wild-type extracts. Thus, Pungaliya et al. have confirmed previous findings on active components of dauer pheromone while providing novel insights into *C. elegans* dauer and male mating pheromone biology.

DANS can detect novel, biologically active small molecules in extracts from whole animals.

This work illustrates the power of using DANS in a model system amenable to genetic analysis and provides proof of principle that DANS can detect novel, biologically active small molecules in extracts from whole animals. In principle, this approach can be used to analyze any biosynthetic pathway for which mutants exist. For example, a recent report described the characterization of long-chain ascaroside species with weak dauer-inducing activity that are present in *daf-22* and *dhs-28* mutants (both of which are defective in pheromone biosynthesis) but not in wild-type animals (12). These are likely to be biosynthetic precursors of ascarosides previously identified as active components of dauer pheromone. DANS of extracts from wild-type, *daf-22* mutant, and *dhs-28* mutant animals should provide further insights into mechanisms of dauer pheromone biosynthesis. DANS could also illuminate mechanisms of the biosynthesis of dafachronic acids (DAs), which are steroid ligands for the nuclear receptor DAF-12 (13); recent work has identified 3 mutants that are likely to be deficient in DA biosynthesis (13–15). Steroids also influence *C. elegans* lifespan (16, 17), and the use of DANS on extracts from long-lived and short-lived *C. elegans* strains could potentially reveal the molecular nature of steroids or other small molecules that influence longevity.

Ultimately, one can envision the application of DANS to more complex model systems in which defined mutants exist, such as *Drosophila* or mice, or even to diseases in humans such as cancer. Recently, Chinnaiyan and colleagues performed metabolomic profiling of multiple clinical samples related to prostate cancer and identified sarcosine, an *N*-methyl derivative of glycine, as a differential metabolite that is a marker of prostate cancer progression and a potential contributor to the invasive phenotype of prostate cancer cells (18). Applying DANS to cancer-related clinical samples could lead to the identification of other metabolites that play critical roles in cancer progression and/or pathogenesis. In due time, organisms throughout the evolutionary spectrum may have the opportunity to go DANSing and reveal their fascinating spectrum of biologically active metabolites from both primary and secondary metabolism.

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