BIOCHEMICAL EVOLUTION IN FIRE ANT VENOMS

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(Received 1 August, 1972)

ABSTRACT

The distribution of 2,6-dialkyl(and alkenyl-)piperidine alkaloids in the venom of fire ant workers of Solenopsis xyloni, S. geminata, S. richteri, and S. invicta has been compared with the occurrence of these compounds in the venom of alate queens of the same species. Whereas the venoms of workers of S. invicta and S. richteri contain piperidines with C13 or C15 side chains, the queens of these species produce venoms in which these compounds are essentially lacking. A comparison of the ratio of cis-2-methyl-6-n-undecylpiperidine to trans-2-methyl-6-n-undecylpiperidine in all of these venoms, together with the qualitative differences of other alkaloidal components, particularly in workers of S. richteri and S. invicta, has led to the proposal that the venoms of S. xyloni and S. geminata are similar to the ancestral type, whereas those of S. richteri and S. invicta are more highly evolved.

It is generally accepted (and certainly true in our experience) that the sting of both imported fire ant species, Solenopsis invicta and S. richteri, causes more discomfort than the sting of either S. xyloni or S. geminata, two species indigenous to the southern U.S.A. The difference in the reaction of human beings to the sting of workers of S. invicta and S. xyloni has been pointed out by Blum, Roberts, and Novak (1961). These stings are similar only during the first few hours, after which time the sting of S. xyloni usually causes only mild itching. Blum and others (1961) suggested that these facts strongly indicate that the venoms of these two species of Solenopsis differ chemically although they are both basic and contain secondary amines.

An investigation of the chemical nature of the venom of S. invicta established the presence of various 2,6-dialkylpiperidine alkaloids (MacConnell, Blum, and Fales, 1971). A comparative analysis of these piperidine alkaloids in the four main North American forms of Solenopsis (S. invicta, S. richteri, S. geminata, and S. xyloni) has shown that the venom components of each species are indeed characteristic of the species (Brand, Blum, Fales, and MacConnell, 1972).

To our knowledge almost all previous studies on hymenopterous venoms have utilized pooled samples of venom. Certainly, previous work on Solenopsis venom has been carried out on pooled samples collected from many worker ants. Recently we have refined our experimental techniques in order to analyse the piperidine alkaloids present in the venom of individual ants, and during these studies we have sampled both workers and alate queens of these four species of Solenopsis. The results obtained have shown that the venoms of workers of S. invicta and S. richteri contain alkaloidal components which are not present in their alate queens or in either the workers or alate queens of S. xyloni or S. geminata. We have used these results to propose a model for the evolutionary development of the various alkaloidal components in the venom of Solenopsis species.
MATERIALS AND METHODS

Worker ants and alate queens of *S. xyloni*, *S. geminata*, *S. richteri*, and *S. invicta*, and worker ants only of an unidentified species in the *S. geminata* group from South America (hereafter termed *S. sp.*) were utilized for venom analyses. Venom was collected by either one of two procedures, depending upon whether or not the ant could be milked easily with a capillary. In cases where the yield of venom was low, the poison gland was removed, placed in 10 µl CS₂ and the extract injected into the gas chromatograph (Carbowax 20 M, 180° C.). Generally, however, ants were milked exhaustively with a 1-µl. capillary, the venom was transferred to a 1-µl. syringe and injected directly into the gas chromatograph.

![Gas chromatograms](image)

**Fig. 1.**—Gas chromatograms of the venom alkaloids from worker ants of four species of fire ant.
RESULTS

Typical chromatograms of the major alkaloidal components in the venom of worker ants and alate queens of four species of *Solenopsis* are presented in Fig. 1A, B, C, D, and the mean ratios of the two compounds, *cis*- and *trans*-2-methyl-6-n-undecylpiperidine, common to both castes of the four species, are presented in Table I. A comparison of

<table>
<thead>
<tr>
<th>Species</th>
<th>Alate Queens</th>
<th>Workers</th>
<th>Soldiers</th>
</tr>
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<tbody>
<tr>
<td><em>S. xyloni</em></td>
<td>17·6:1 (7)</td>
<td>3·97:1 (10)</td>
<td>—</td>
</tr>
<tr>
<td><em>S. geminata</em></td>
<td>3·36:1 (10)</td>
<td>1·50:1 (40)</td>
<td>4·94:1 (40)</td>
</tr>
<tr>
<td><em>S. richteri</em></td>
<td>1·86:1 (6)</td>
<td>&lt;0·1:1 (10)</td>
<td>—</td>
</tr>
<tr>
<td><em>S. invicta</em></td>
<td>3·24:1 (27)</td>
<td>&lt;0·1:1 (10)</td>
<td>—</td>
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</table>

Numbers in parentheses indicate the number of individuals sampled. Queens were taken from a minimum of 3 colonies per species.

the chemical nature of these piperidine alkaloids in the various venoms indicates a most interesting series of these compounds.

In the venom of workers of *S. xyloni* and *S. geminata* (Fig. 1A, B), and in soldiers of *S. geminata*, only *C*$_{11}$ components* (i.e., *cis*- and *trans*-2-methyl-6-n-undecylpiperidine) are abundant, while *C*$_{13}$ components (i.e., *cis*- and *trans*-2-methyl-6-n-tridecyl (and trideceny1-*C*$_{13}$) piperidine) are present in trace amounts. We have found no indications of *C*$_{15}$ components (i.e., *cis*- and *trans*-2-methyl-6-n-pentadecyl (and pentadeceny1-*C*$_{15}$) piperidine) in these venoms. In both of these species *cis*-*C*$_{11}$ is considerably more abundant than *trans*-*C*$_{11}$, and the trace amount of *C*$_{13}$ present is virtually all *cis*-*C*$_{13}$ and *cis*-*C*$_{13}$:1. In contrast to these results, the venom of workers of *S.* sp. contains *trans*-*C*$_{11}$ as the major component.

Workers of *S. richteri* (Fig. 1C) contain both *C*$_{11}$ and *C*$_{13}$ components, the latter being considerably more abundant, and the *trans*-form of all components dominating. In this species the *trans*-*C*$_{13}$:1 is extremely abundant compared with *trans*-*C*$_{13}$. In the venom of *S. invicta* (Fig. 1D) the *C*$_{11}$ components are quantitatively small, while both the *C*$_{13}$ and *C*$_{13}$:1 components and the *C*$_{15}$ and *C*$_{15}$:1 components are abundant. For all three groups of compounds the *trans*-isomers dominate and *cis*-isomers are present only as trace constituents.

The venom of the queens of these four species (Fig. 2A, B, C, D) contains essentially only *C*$_{11}$ components and, in all cases, *cis*-*C*$_{11}$ is twice or more as abundant as *trans*-*C*$_{11}$.

DISCUSSION

The venom characteristics of the workers of these five species form a phenocline suggesting one of two series of evolutionary events, proceeding either from Fig. 1D to

* The terms *C*$_{11}$, *C*$_{13}$, *C*$_{13}$:1, *C*$_{15}$, and *C*$_{15}$:1 are employed to indicate the carbon length and the presence or absence of unsaturation in the 6-alkyl substituent on the piperidine ring and do not signify the total number of C-atoms in the molecule. The prefixes *cis*- and *trans*- give the orientation of the 2-methyl and 6-alkyl groups on the piperidine ring.
Fig. 1A or from Fig. 1A to Fig. 1D. If Fig. 1D represents the ancestral condition of the alkaloidal components, then evolution proceeded first by a loss of C₁₅ components, then by a drastic reduction of C₁₃ components, and finally by a switch from abundant trans-C₁₁ to abundant cis-C₁₁. If Fig. 1A represents the ancestral state, then the evolution of the venoms would have begun with a condition in which the venom contained abundant C₁₁ and minute traces of C₁₃, with the cis-isomers dominating and the trans-isomers at a much lower level. The evolutionary steps in this case would have been first a switch from abundant cis-C₁₁ to abundant trans-C₁₁, followed by the evolution of

![Gas chromatograms of the venom alkaloids from alate queens of four species of fire ant.](image)

Fig. 2.—Gas chromatograms of the venom alkaloids from alate queens of four species of fire ant.
abundant \textit{trans-C}_{13}, and then the addition of \textit{trans-C}_{15} accompanied by a reduction in the production of \textit{trans-C}_{11}.

If there was available a highly probable phylogeny of the genus \textit{Solenopsis}, the above chemical data could be arranged according to the phylogenetic scheme and the direction of evolution of these alkaloidal compounds established. Unfortunately, no such phylogeny is available and the present diagnoses of the species (Creighton, 1950; Buren, 1972) are based on differences that do not lend themselves readily to deciphering phylogeny. We thought that characters of the male genitalia might shed light on the problem of evolutionary direction, but the four species for which we have males (\textit{S. xyloni}, \textit{S. geminata}, \textit{S. richteri}, and \textit{S. invicta}) have virtually identical structures. It therefore appears that the chemical characters of the venoms are the only ones so far discovered that can give any indication of evolutionary direction.

We wish to emphasize that in deciding which are the ancestral and the derived states of a character, it is most helpful to compare the states in the taxon studied with similar character states in related taxa. This is impossible in the case of the piperidine alkaloids, as they have not been identified in the venoms of ants in any other genus. The question as to whether the alkaloidal complex in either Fig. 1A or Fig 1D represents the ancestral state of \textit{Solenopsis} must therefore be resolved, if possible, from information available on the species examined.

\textbf{The Probable Ancestral Alkaloidal State}

Indications concerning the ancestral state of these alkaloids come from two sources: (1) Thermodynamic considerations of the production of \textit{cis}- and \textit{trans}-isomers; and (2) The relative proportion of \textit{cis}- and \textit{trans}-isomers in workers and queens.

1. In other stinging ants the venom is largely proteinaceous, whereas in \textit{Solenopsis} the amount of protein present is extremely small. It appears, therefore, that in \textit{Solenopsis} there has evolved a new type of venom component as yet found in no other ants, and that this has replaced a more primitive proteinaceous constituent. The evolutionary newness of the alkaloid suggests that an arrangement of the different alkaloidal states in the order of their complexity would mirror the pattern of evolution. On this basis, \textit{S. xyloni} and \textit{S. geminata} would appear to be the most primitive as they contain primarily \textit{cis-C}_{11} and only traces of \textit{cis-C}_{13}. The chemical synthesis of these 2,6-dialkylpiperidines described by MacConnell and others (1971) gives, at best, a ratio of the \textit{cis}-isomer to the \textit{trans}-isomer of 6:1. Such a situation occurs in \textit{S. geminata} and \textit{S. xyloni}; in \textit{S. sp.}, however, \textit{cis-C}_{11} is greatly reduced and \textit{trans-C}_{11} is the predominant form. This reversal involves a switch from a probable normal production ratio of \textit{cis}- and \textit{trans-C}_{11} to a thermodynamically highly unfavourable decrease in \textit{cis}- and an increase in \textit{trans}-isomers. Such a switch would seem to be a derived condition, undoubtedly through the evolution of a new enzymatic system and/or the restriction of an old one. After this switch had occurred, first \textit{trans-C}_{13} then \textit{trans-C}_{15} components were added to the venom arsenal. In this connexion it is appropriate to mention the presence of 2-methyl-6-n-undecyl-\textit{\Delta^{1,3}}-piperidine in the venom of \textit{S. xyloni} workers (Brand and others, 1972). This compound could be either a precursor of the \textit{cis}- or \textit{trans}-isomers or an intermediate in the conversion of the one isomer to the other.

2. A comparison of the ratio of \textit{cis-C}_{11} to \textit{trans-C}_{11} between queens and workers (\textit{Table I}) brings out a most surprising circumstance. Of the four species for which both workers and queens are available, we found that in the workers of \textit{S. xyloni} and \textit{S.}
geminata cis-C_{11} was much more abundant than trans C_{11}, whereas in workers of S. richteri and S. invicta, cis-C_{11} was at a much lower level than trans-C_{11}. In queens of all four species, however, cis-C_{11} was always more abundant than trans-C_{11}. A possible significance of this difference is suggested by the different behaviour patterns of queens and workers. With extremely few exceptions, when an ant nest is disturbed the workers are the aggressive defenders. The queens (certainly in Solenopsis) move as far as possible from the point of disturbance; even if cornered and caught they seldom attempt to sting but rather to evade and hide. With this difference in behaviour there would be a strong positive selection pressure for the production of a more effective venom in the workers, but probably little if any selection pressure for a more potent venom in the queens. It therefore seems reasonable that the more primitive condition was one in which the workers and queens had roughly the same venom composition (as in S. geminata and S. xyloni), and that increasing differences in venom chemistry between queens and workers indicate evolutionary progression. Along this line of thinking, there is a difference in the effect of the various venoms on human subjects. In our collective experience, stings by S. invicta queens and S. xyloni workers produce little pain and seldom a discernible pustule or necrotic tissue; stings of S. invicta workers produce considerable pain, subsequent irritation, pustule formation, and eventually well-developed necrosis. One other item of information supports the idea that the venom composition represented in Fig. 1 A, B is the ancestral state of the alkaloidal characters. Of the species studied, only S. geminata has a true soldier caste. As soldiers occur commonly throughout the subfamily Myrmicinae, to which Solenopsis belongs, it is highly probable that S. geminata represents one of the ancestral species in the genus. This indication of phylogenetic position supports the idea that the venom components of S. geminata are also ancestral.

On the basis of the above considerations, we would like to suggest the following model for the evolution of the alkaloidal compounds in Solenopsis. In the precursor of the genus, piperidine alkaloids were added to the original proteinaceous venom and later essentially supplanted these proteinaceous elements. The venom of the ancestral species of the genus as we know it contained principally C_{11} compounds and a trace of C_{13} compounds, with a predominance of cis-isomers to trans-isomers. At this stage the soldier caste was present and the worker, soldier, and queen venoms were essentially similar. The next evolutionary step was the loss of the soldier caste, with little change in worker and queen alkaloidal chemistry. The first significant change in alkaloidal chemistry occurred in the lineage leading to S. sp., in which cis-C_{11} decreased and trans-C_{11} became the abundant compound in the venom. In the next step trans-C_{13} and C_{13;1} became the predominant venom components, resulting in an ancestral form much like the venom of S. richteri. This ancestor then gave rise to a little-changed lineage represented by S. richteri, and a lineage in which trans-C_{15} and C_{15;1} were added to the venom, resulting in a species like S. invicta. This progression of chemical changes was accompanied by an increase in the effectiveness of the worker's stinging action as a defensive or offensive mechanism.

Although this model appears logical, it is based on only five species in a group of the genus that contains many species for which no biochemical analysis of the venom is available. We feel that the greatest value of this model is in presenting a hypothetical construct in biochemical evolution that invites further investigations and testing. Two obvious questions which remain unanswered are (1) the biosynthetic origin of the
piperidine alkaloids, and (2) the basis of their differential composition in queens and workers of the more highly evolved species.

ACKNOWLEDGEMENTS

The authors are grateful to Margaret R. Barlin for her technical assistance. This research was partially supported by a grant from the U.S.D.A.

REFERENCES


Key Word Index: Fire ants, Solenopsis, venoms, piperidine alkaloids, biochemical evolution.