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Article

Defensive Alkaloids from Ants

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Os constituintes da glândula venenosa da formiga têm sido objeto de muitas investigações, tendo sido demonstrado que são normalmente protéicos. Entretanto, em alguns grupos de formigas, estas proteínas do veneno têm sido substituídas por alcalóides cíclicos, atuando como substâncias de defesa. Dois grupos de alcalóides de formiga, denominados solenopsinas e tetraponerinas, são apresentados. As solenopsinas são um grupo de 2,6-dialquilpiperidinas produzido pelas "formigas de fogo" (*Solenopsis spp*), uma praga importante em muitas partes do sul dos EUA. As tetraponerinas são alcalóides tricíclicos diaminados isolados do veneno da formiga da Nova Guiné *Tetraponera sp.* A elucidação dos caminhos biossintéticos de ambos os grupos de alcalóides é discutida.

The constituents of the poison gland in ants have been the subject of many investigations, and it has been demonstrated that they are usually proteinaceous. However, in some group of ants, these venom proteins have been superceded by cyclic alkaloids acting as defensive substances. Two groups of ant alkaloids, namely the solenopsins and the tetraponerines, are presented. The solenopsins are a group of 2,6-dialkylpiperidines produced by "fire ants" (*Solenopsis spp*), which are significant pests in many parts of the southern United States. The tetraponerines are diaminated tricyclic alkaloids isolated from the venom of the New Guinean ant, *Tetraponera sp.* The elucidation of the biosynthetic pathways of both groups of alkaloids is discussed.

Keywords: *alkaloids,* Solenopsis spp, Tetraponera sp, *ants*

Introduction

Ants are highly evolved social insects, often organized into large colonies. Members of the ant colony coordinate their activities by means of a complex communication system, mostly based on chemical signals. These are produced in specialized glands located in different parts of the body of the insect. Besides this complex intra-species communication system, many ant species use chemical secretions for defensive and offensive purposes. In this context, the poison gland which is attached to the stinger plays a major role. The venom gland constituents have been the subject of many investigations which have been reviewed several times[1-4].

The type of compounds present in the different subfamilies of Formicidae are presented in Table 1. The ants of the subfamily Formicinae do not possess a functional sting, but have a venom containing very high concentrations of formic acid (about 50% aqueous solution) which provides these ants with a very effective defensive weapon. In the other subfamilies that have been chemically studied, the constituents of the poison gland are usually proteinaceous. However, in some species of the subfamilies Myrmicinae and Pseudomyrmecinae, these proteinic venoms have been superceded by alkaloids acting as toxins. These alkaloids are cyclic amines that may be divided into several categories, according to the ring system from which they are derived. Some of these ring systems are presented in Fig. 1. The ant alkaloids have been the subject of numerous structural and synthetic studies[4,5], but their biosynthesis remains rather unknown. Our purpose is to present some of the experiments we have performed to clarify the biogenetic origin of the defensive alkaloids of the *Solenopsis* ant and that of a *Tetraponera* ant from Papua New Guinea.

The *Solenopsis*, called "fire ants" after the pain elicited by their sting, are New World species that occur primarily in tropical and subtropical areas. In the beginning of this century some species were accidentally introduced into the southern United States. Since then, they have emerged as significant pests.

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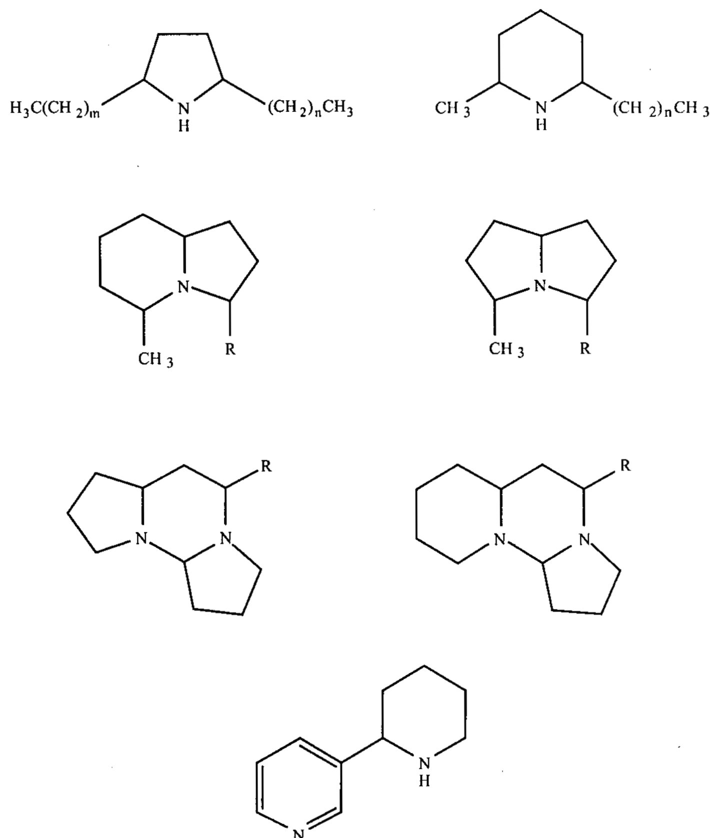

Figure 1. Examples of ant defensive alkaloids.

Table 1. Venom constituents of the Formicidae.

Family Formicidae	
Subfamilies	Venom constituents
Formicinae	Formic acid
Myrmeciinae	Proteins
Ponerinae	Proteins
Dorylinae	Proteins
Myrmicinae	Proteins or alkaloids
Pseudomyrmecinae	Proteins or alkaloids

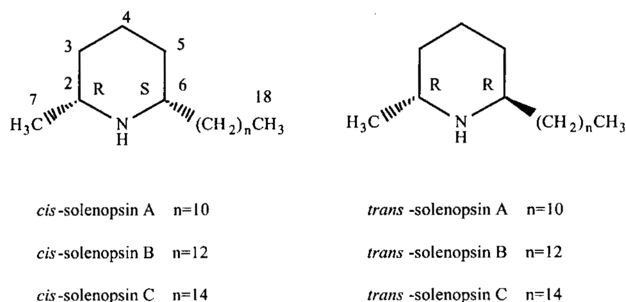

Figure 2. Structure of the major alkaloids of the Solenopsis

The fire ant venom has a very low protein content (0.1% by weight). These proteins are responsible for the allergic reactions which their sting causes in humans[6]. The remaining portion of the venom consists of a complex mixture of 2-methyl-6-alkylpiperidines, accompanied, in some cases, by *N*-methylated and/or unsaturated derivatives[7]. These piperidine alkaloids have been assigned the trivial name solenopsins. They differ in the relative configuration of their substituents and the length of the alkyl chain. The structures of the major alkaloids of *Solenopsis* venoms are represented in Fig. 2. They have no role in the allergic response but do have cytotoxic, hemolytic, necrotic, antibacterial, insecticidal and antifungal activities. They are also responsible for the pain associated with the sting[7]. The relative proportions of the solenopsins in the venom may differ between castes within a species, as well as between pooled samples of different species[8].

Recently, we have established that the absolute configuration of the *trans*-solenopsins is always *(2R,6R)* while that of the *cis*-solenopsins is *(2R,6S)* [9].

Insofar as a structural relationship can be considered to be a guide to biogenesis, the skeleton of the solenopsins might be thought to originate from the linear combination of nine, ten or eleven acetate units. This hypothesis is illustrated in Fig. 3 for *cis*- and *trans*-solenopsin A. To test the acetate origin of *cis*- and *trans*-solenopsin A, tracer experiments were performed by feeding 3000 to 4000

Solenopsis geminata ants with aqueous solutions of sodium[$1-^{14}C$]- and [$2-^{14}C$]acetate[10]. This ant species was chosen because its venom is essentially contituted of *cis*- and *trans*-solenopsin A, in contrast with other *Solenopsis* species whose venoms are much more complex, being mixtures of the different solenopsins.

Both feeding experiments yielded a radioactive mixture of *cis*- and *trans*-solenopsin A which was diluted with racemic synthetic material as the carrier, and degraded, as illustrated in Fig. 4, to determine the distribution of radioactivity in these molecules. This led to the isolation of compounds **1** (containing C-7), **2** (containing C-7 + C-2 to C-5), **3** (containing C-7 + C-2 to C-6), **4** (containing C-6 + C-8 to C-18), and **5** (containing C-8 to C-18). The distribution of label within the degradation products of *cis*- and *trans*-solenopsin A after feeding with sodium[$1-^{14}C$]- and sodium[$2-^{14}C$]acetate is reported in Table 2. Comparison between the observed and expected radioactivity clearly supports, within experimental error, the hypothesis that the solenopsins A are formed from an eighteen-carbon polyacetate chain resulting from the condensation of acetyl-coenzyme A with eight subsequent units of malonyl-coenzyme A (see Fig. 4).

The New Guinean ant *Tetraponera sp* is characterized by a defensive mechanism which is unique in the morphol-

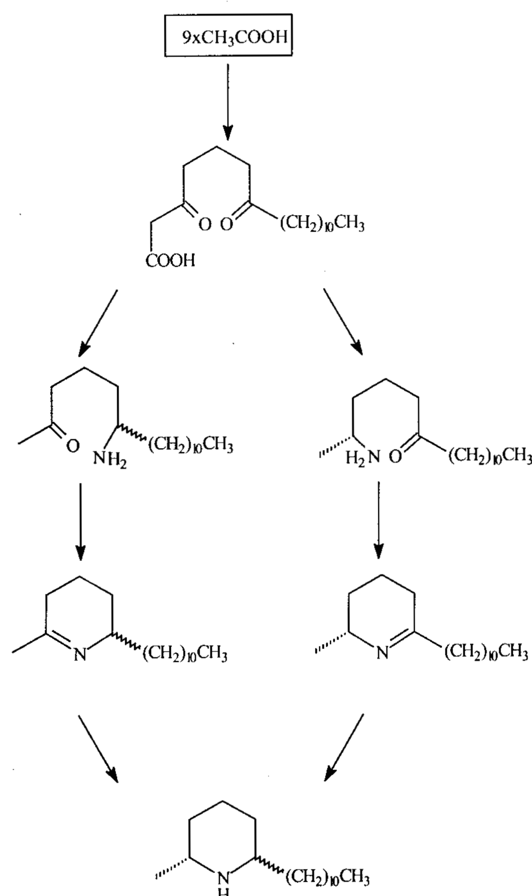

Figure 3. Hypothetical biogenetic scheme of Solenopsin A.

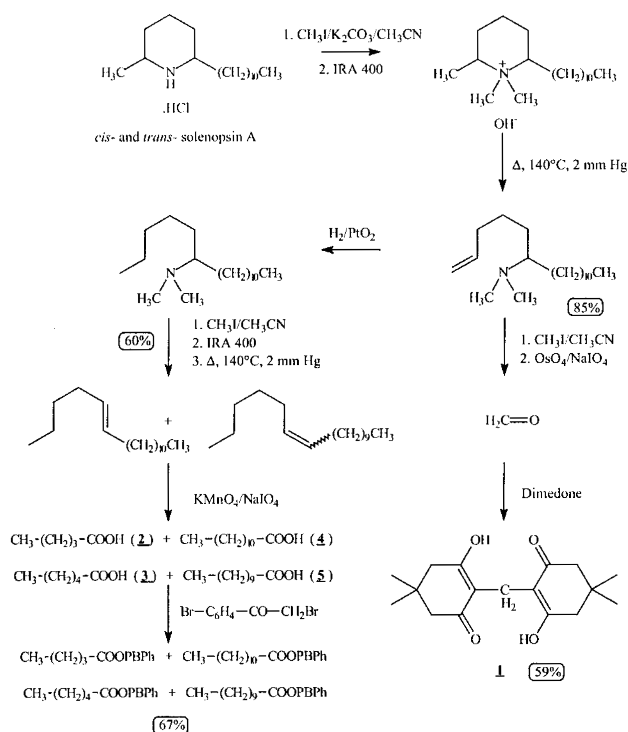

Figure 4. Degradation scheme of solenopsin A.

Table 2. Observed and expected distribution of label within the degradation products of solenopsin A after feeding with Na[$1-^{14}C$] and Na[$2-^{14}C$] acetate.

Product	Na[$1-^{14}C$] acetate		Na[$2-^{14}C$] acetate	
	Activity observed (%)	Activity expected (%)	Activity observed (%)	Activity expected (%)
Solenopsin A	100	100	100	100
1	0	0	10	11
2	26	25	30	33
3	38	37	31	33
4	77	75	64	67
5	62	62	65	67

ogy of the sting apparatus[11]. The modified sting is not a suitable injection device, but is well-adapted for the deposition of liquid on an enemy. Gas chromatographic analysis of the venom showed that it contains eight related tricyclic derivatives for which the names tetraponerine 1 to 8 have been coined. The structure of the major derivative (+)-tetraponerine-8 (T8) was established by X-ray diffraction analysis[11]. Subsequently, detailed spectroscopic studies combined with the total syntheses demonstrated that the tetraponerines can be distributed into two groups, depending on the structure of the tricyclic skeleton, which is either a decahydropyrido[1,2-c] pyrrolo[1',2'-a]pyrimidine or a decahydrodipyrrolo[1,2-a:1',2'-c]pyrimidine[12,13]. In each group, the alkaloids differ by the length of the alkyl chain and/or the stereochemistry at the carbon atom bearing the alkyl chain (Fig. 5).

Such skeletons are new among natural products. The biosynthetic pathway to the tetraponerines is therefore a matter of particular interest. The administration of sodium [1-^{14}C]acetate, sodium[2-^{14}C]acetate, L-[U-^{14}C]glutamic acid, γ-amino[U-^{14}C]butyric acid, L-[U-^{14}C]ornithine.HCl, and [1,4-^{14}C]putrescine.2HCl to *Tetraponera sp.* ants resulted in the formation, for each feeding experiment, of labelled T8(14). To evaluate the distribution of label in T8, the sequence of reactions shown in Fig. 6 was used. This resulted in the isolation of compounds **6** (containing C-5 to C-8), **7** (containing C-1 to C-4 + C-9 to C-16), **8** (containing C-1 to C-4 + C-10 and C-11), and **9** (containing C-9 + C-12 to C-16). The labelling patterns that were observed after the incorporation of each of the above precursors are reported in Table 3[14].

The patterns of the incorporation of the activity from acetate, glutamic acid and γ-aminobutyric acid into T8 (feeding experiments 1 to 4) suggest that these precursors enter both parts of the molecule by two independent pathways, their incorporation into the pyrrolidine ring (C-4 to C-8) being more efficient than into the side chain and the two hexacyclic rings. Moreover, within experimental error, the activity of olefin **7** is equally divided between the pipecolic acid moiety (**8**) and the caproic acid moiety (**9**),

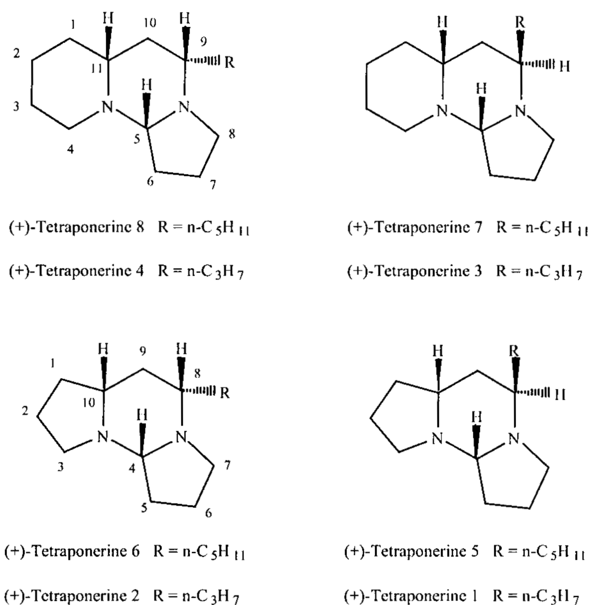

Figure 5. Structures of the tetraponerines.

Table 3. Distribution of label within T8 and its degradation products (%).

Experiment no.	Compound administred	ARI* (%)	Distribution of label within T8 and its degradation products (%)				
			T8	6	7	8	9
1	Na[1-^{14}C] acetate	0.05	100	54	35	15	15
2	Na[2-^{14}C] acetate	0.31	100	53	43	19	23
3	γ-amino[U-^{14}C]butyric acid	0.14	100	53	46	21	22
4	L-[U-^{14}C]glutamic acid	0.13	100	80	25	14	15
5	L-[U-^{14}C]ornithine.HCl	1.31	100	80	15	9	9
6	[1,4-^{14}C]putrescine.2HCl	2.02	100	96	0	0	0

*ARI: Absolute Rate of Incorporation.

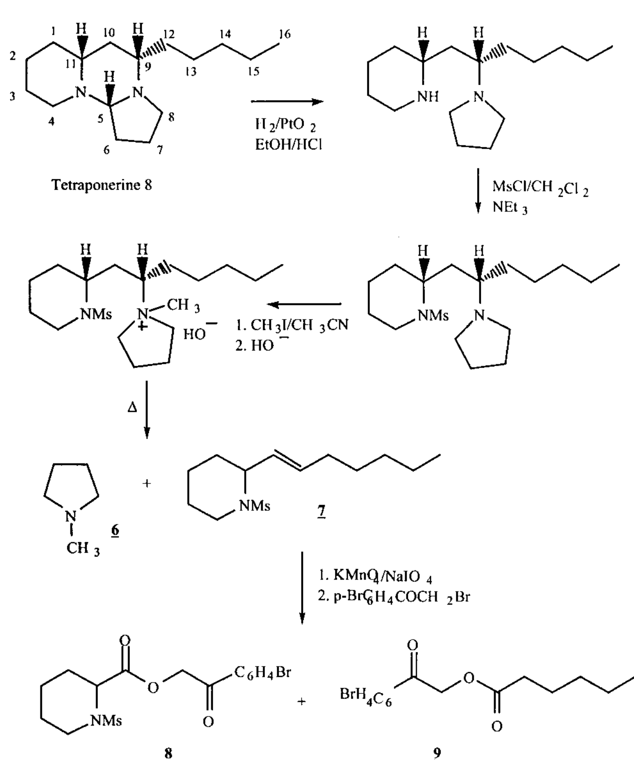

Figure 6. Degradation scheme of tetraponerine **8**.

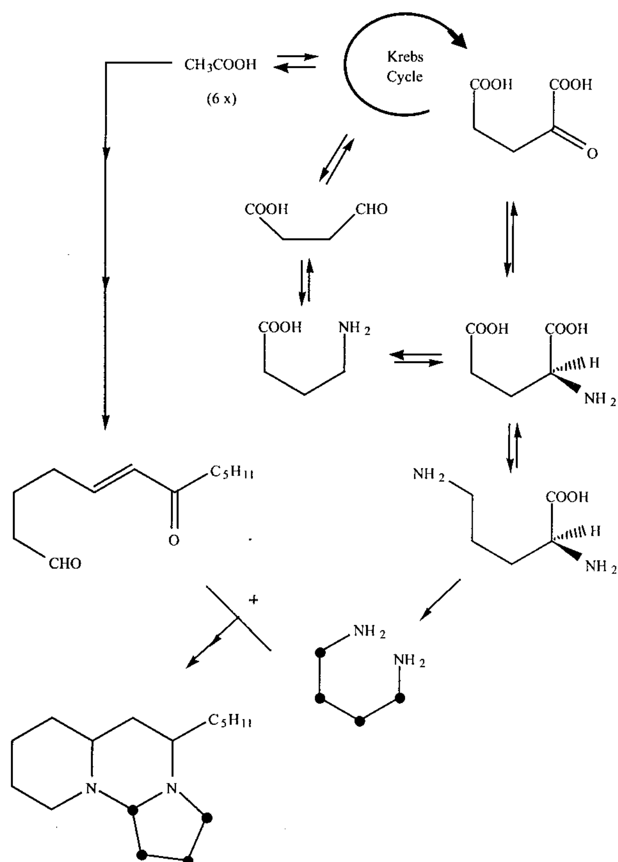

Figure 7. Hypothetical biosynthetic scheme of tetraponerine **8**.

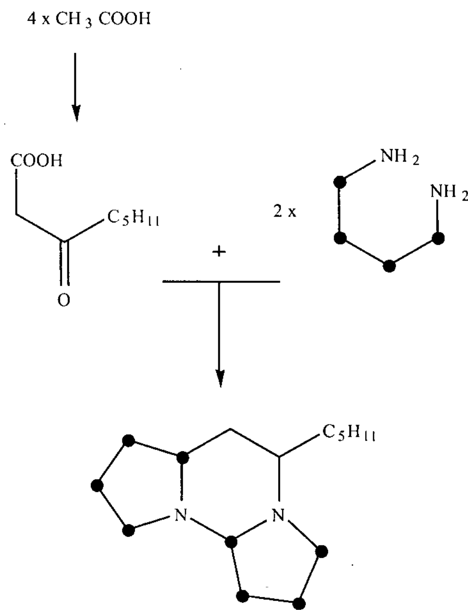

Figure 8. Hypothetical biosynthetic scheme of tetraponerine **6**.

suggesting that the 12-carbon linear chain of T8 might be formed by a linear combination of acetate units.

Ornithine and putrescine (feeding experiments 5 and 6) not only enter the T8 skeleton with higher rates of incorporation, but the pyrrolidine moiety also contained most of the labelling of intact T8. Such a distribution led to the inference that these two metabolites serve as specific precursors for the pyrrolidine ring of T8. All these results support the biosynthetic scheme (Fig. 7) proposed for T8[14]. This hypothesis of biosynthesis can be extrapolated to all of the tetraponerines pertaining to the decahydropyrido[1,2-c]pyrrolo[1',2'-a]pyrimidine ring system.

Turning to the biosynthesis of T6, a tetraponerine structurally related to the decahydro- dipyrrolo[1,2-a:1',2'-c]pyrimidine ring system, we have observed that the pattern of the incorporation of the activity of putrescine into T6 suggested that both pyrrolidine rings originate from this diamine[15]. Indeed, following the application of an experimental procedure similar to that used for T8, it was observed that the activity of T6 is equally distributed between the carbon atoms C-4 to C-7 and C-1 to C-3 + C-8 to C-15. Moreover, the activity supported by the second group of carbon atoms is restricted to C-1 to C-3 + C-9 + C-10. This indicated that, in contrast to T8, two molecules of putrescine are needed to build the skeleton of T6. We thus propose the hypothetical biosynthetic scheme depicted in Fig. 8 for T6 and the related tetraponerines.

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