Naturwissenschaften 83, 222–225 (1996)

Biosynthesis of the Solenopsins, Venom Alkaloids of the Fire Ants

S. Leclercq, J.C. Braekman, D. Daloze

Laboratory of Bio-Organic Chemistry (CP 160/7), Faculty of Sciences, University of Brussels, B-1050 Brussels, Belgium

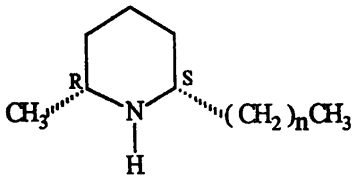
J.M. Pasteels

Laboratory of Animal and Cellular Biology, Faculty of Sciences, University of Brussels, B-1050 Brussels, Belgium

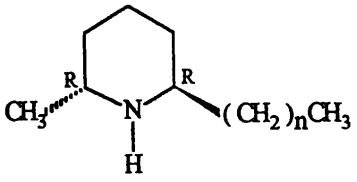
R.K. Van der Meer

Laboratory of Medical and Veterinary Entomology Research, US Department of Agriculture, Gainesville, Florida 32604, USA

Ants of the genus *Solenopsis* (Myrmicinae) secrete a venom consisting of a mixture of 2-methyl-6-alkylpiperidines accompanied in some cases by N-methylated, imino, or side-chain-unsaturated derivatives [1–4]. These piperidine alkaloids have been assigned the trivial name solenopsins by MacConnell et al. [5]. The solenopsins (Fig. 1) differ from each other by the relative configuration of their substituents, the length, and unsaturation of the alkyl chain. The absolute configuration of the *trans* alkaloids is always (2*R*,6*R*) while that of the *cis* alkaloids is (2*R*,6*S*) (see *1* to *6* for examples [6]).



1: *n* = 10,*cis-solenopsin A*
2: *n* = 12,*cis*-solenopsin B
3: *n* = 14,*cis*-solenopsin C



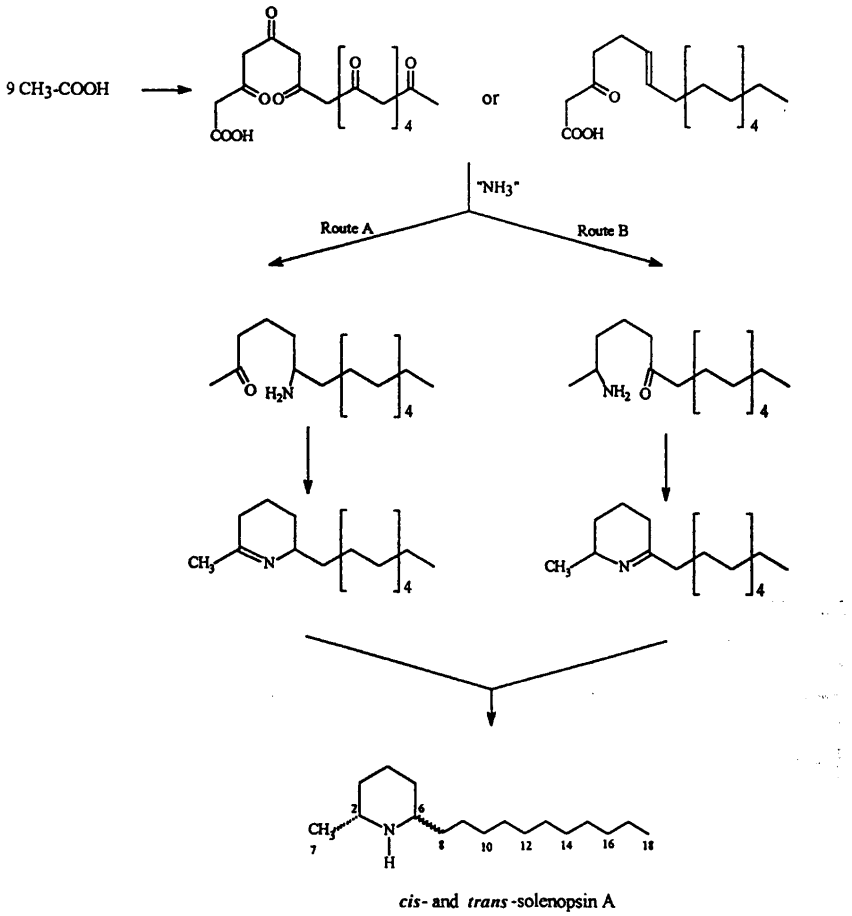
4: *n* = 10,*trans*-solenopsin A
5: *n* = 12,*trans*-solenopsin B
6: *n* = 14,*trans*-solenopsin C

Fig. 1. Structure of natural solenopsins

Although these alkaloids have been the subject of numerous synthetic [4, 7] and biological [8] studies, their biosynthesis remains, however, unknown. The aim of this paper is to report our first results on this topic, which clearly demonstrate that *cis*- and *trans*-solenopsin A (*1* and *4*) are acetate-derived.

Based on our previous results on the biosynthesis of insect alkaloids (tetraponerine-8 [9], coccinelline [10]), we hypothesized that the solenopsins (*1* to *6*) are formed by the linear combination of 9, 10, or 11 acetate units. Loss of the carboxyl group from the resulting long-chain acid, followed by introduction of an amino group, intramolecular cyclization, and reduction of the imino group thus obtained, could afford the *cis*- and *trans*-solenopsins. The intermediate long-chain acid can be either a suitably functionalized fatty acid or a polyketo acid. Figure 2 illustrates this hypothesis for *cis*- and *trans*-solenopsin A. This biosynthetic pathway would therefore be similar to the well-established biosynthesis of the hemlock alkaloid coniine, which is formed by the linear combination of four acetate units [11, 12].

To test the acetate origin of the *cis*- and *trans*-solenopsin A, tracer experiments



cis- and *trans*-solenopsin A

Fig. 2. Biosynthetic hypothesis for *cis*- and *trans*-solenopsin A

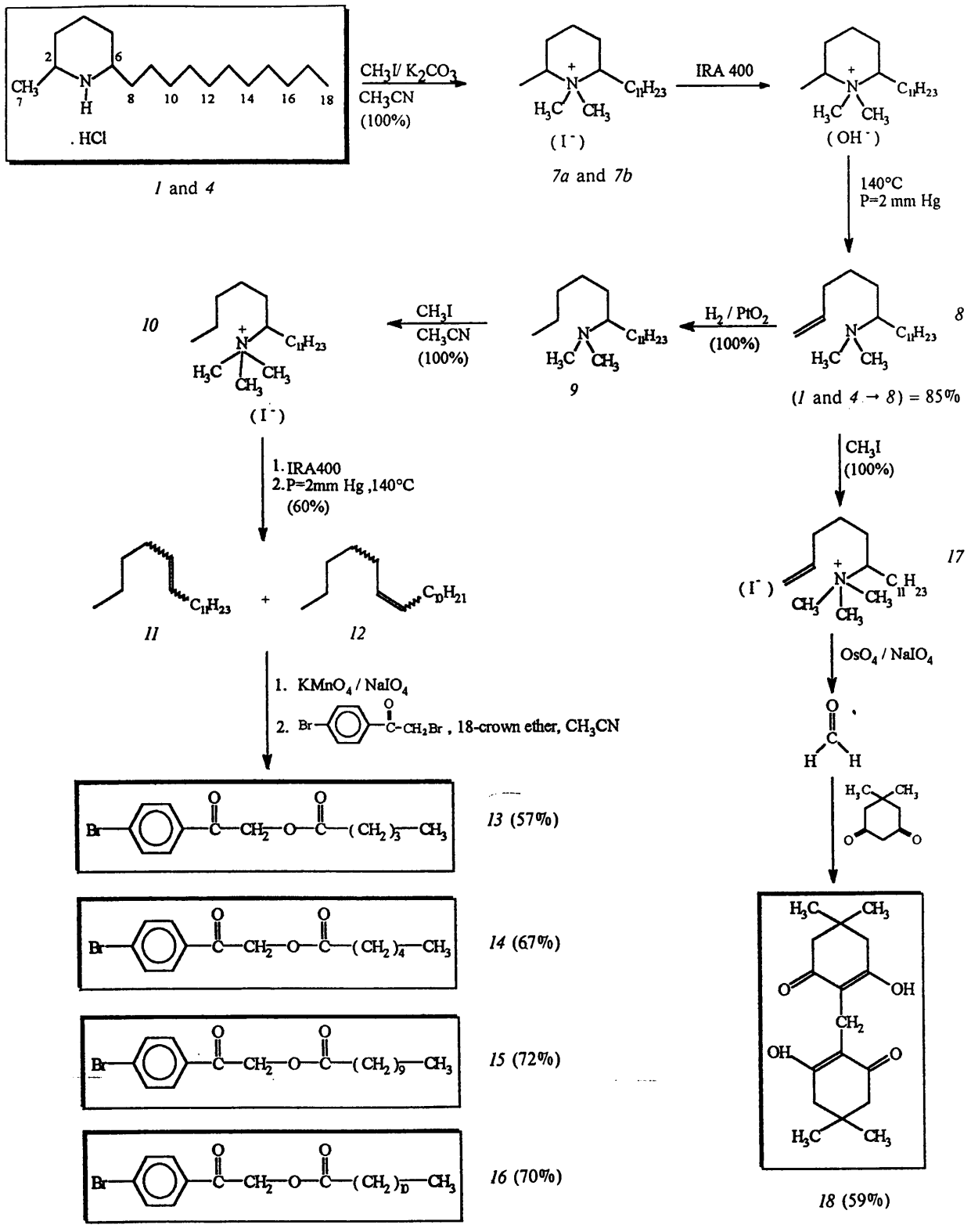


Fig. 3. Degradation of radioactive *cis*- and *trans*-solenopsin A

Table 1. Incorporation of labeled compounds into solenopsin A

Exp. no.	Precursor fed	No. of ants fed	Duration of feeding [h]	Isolated amount of *1* and *4*. HCl [mg]	Specific activity of *1* and *4*. HCl [mCi/mmol]	Incorporation rate [%]
1	Sodium [1-^{14}C]acetate (250 μCi, 60 mCi/mmol)	3 to 4×10^3	60	37.05	6.1×10^{-3}	0.010
2	Sodium [2-^{14}C]acetate (125 μCi, 59 mCi/mmol)	3 to 4×10^3	60	12.28	1.58×10^{-2}	0.027
3	[2-^{14}C]-malonic acid along with inactive sodium acetate (100 μCi, 56.7 mCi/mmol)	3 to 4×10^3	60	11.90	3.73×10^{-3}	0.0066

were performed by feeding *Solenopsis geminata* ants with aqueous solutions of sodium [1-^{14}C]- and [2-^{14}C]acetate. This ant species was chosen because its venom is essentially constituted of *cis*- and *trans*-solenopsin A, in contrast with other *Solenopsis* species, whose venoms are much more complex [14]. The specific activity of the mixture of the *cis*- and *trans*-solenopsin A hydrochlorides isolated from the tracer experiments and its specific incorporation rate are summarized in Table 1. Each experiment yielded a radioactive mixture of *1* and *4* (about 10 mg), which was diluted with racemic synthetic material as carrier (total amount 150 mg) and degraded, as illustrated in Fig. 3, to determine the distribution of radioactivity in these molecules. This scheme allowed us to isolate compounds *18* (containing C-7), *13* (C-7, C-2 to C-5), *14* (C-7, C-2 to C-6), *15* (C-8 to C-18), and *16* (C-6 and C-8 to C-18).

Thus, the labeled samples of *cis*- and *trans*-solenopsin A obtained from each feeding experiment were chromatographed on a column of silica gel and converted into their crystalline hydrochlorides. Then each sample was degraded as shown in Fig. 3. *Cis*- and *trans*-solenopsin A hydrochlorides were refluxed with methyl iodide in the presence of potassium carbonate in acetonitrile, yielding *cis*- and *trans*-solenopsin A methiodides *7a* and *7b*. The corresponding tetraalkylammonium hydroxides were obtained from *7a* and *7b* by treatment with a strongly basic anion-exchange resin (IRA-400). A Hofmann degradation afforded 6-dimethylamino-1-heptadecene (*8*) (in an 85% yield from *cis*- and *trans*-solenopsin A), some of which was catalytically reduced and treated with methyl iodide to afford 6-dimethylamino-1-heptadecane methiodide (*10*). After treatment with IRA-400, as previously described, pyrolysis of the tetraalkylammonium hydroxide gave a mixture of 5-heptadecene (*11*) and 6-heptadecene (*12*), which were oxidized with potassium permanganate and sodium metaperiodate. The four resulting acids – pentanoic, hexanoic, undecanoic, and dodecanoic acid – were converted to their *p*-bromophenacyl esters *13*, *14*, *15*, and *16*, respectively. The latter were separated by HPLC on a C-18 reversed-phase column. Methylation of 6-dimethylamino-1-heptadecene (*8*) by methyl iodide afforded needles of the corresponding ammonium salt *17*. This salt was converted to the corresponding 1,2-diol with osmium tetroxide and cleaved with sodium metaperiodate, yielding formaldehyde (corresponding to carbon atom C-7), which was trapped as its dimedone derivative *18*. The latter was repeatedly crystallized from aqueous methanol to constant specific activity.

The specific activities of the degradation products obtained after feeding the ants with sodium [1-^{14}C]- and [2-^{14}C] acetate are reported in Tables 2 and 3, respectively. Comparison between the observed distribution of activity and that expected for the biogenetic path-

Table 2. Observed and expected distribution of label within the degradation products of *1* and *4* after feeding with sodium [1-^{14}C]acetate

Products	Specific activity [mCi/mmol]	C atoms of solenopsin A	Activity (observed) [%]	Activity (expected) [%]
8	1.46×10^{-3}	All	–	–
9	1.45×10^{-3}	All	–	–
18	3.65×10^{-6}	7	0.25	0
11+12	1.43×10^{-3}	All	100	100
13	3.71×10^{-4}	7, 2, 3, 4, 5	26	25
14	5.47×10^{-4}	7, 2, 3, 4, 5, 6	38	37.5
15	8.88×10^{-4}	8 – 18	62	62.5
16	1.11×10^{-3}	6 and 8 – 18	77	75

Table 3. Observed and expected distribution of label within the degradation products of *1* and *4* after feeding with sodium [2-^{14}C]acetate

Products	Specific activity [mCi/mmol]	C atoms of solenopsin A	Activity (observed) [%]	Activity (expected) [%]
8	1.00×10^{-3}	All	–	–
9	1.02×10^{-3}	All	–	–
18	9.34×10^{-5}	7	10	11
11+12	9.45×10^{-4}	All	100	100
13	2.84×10^{-4}	7, 2, 3, 4, 5	30	33.3
14	2.95×10^{-4}	7, 2, 3, 4, 5, 6	31	33.3
15	5.99×10^{-4}	8 – 18	64	66.6
16	6.14×10^{-4}	6 and 8 – 18	65	66.6

Table 4. Observed distribution of label within the degradation products of *1* and *4* after feeding with sodium [2-^{14}C]malonic acid along with inactive acetate

Products	Specific activity [mCi/mmol]	C atoms of solenopsin A [%]	Activity (observed) [%]
8	2.81×10^{-4}	All	–
9	2.88×10^{-4}	All	–
18	2.51×10^{-5}	7	9
11 + *12*	2.95×10^{-4}	All	100
13	8.78×10^{-5}	7, 2, 3, 4, 5	30
14	9.51×10^{-5}	7, 2, 3, 4, 5, 6	32
15	1.84×10^{-4}	8 – 18	62
16	1.90×10^{-4}	6 and 8 – 18	64

way depicted in Fig. 1 supports, within experimental errors, the polyacetate origin of the *cis*- and *trans*-solenopsin A. In the insect kingdom, only three other alkaloids, coccinelline [10], tetraponerine-8 [9], and epilachnene [13], have so far been reported to be, at least in part, acetate-derived. It is presumed that *cis*- and *trans*-solenopsin A are formed from an 18-carbon polyacetate chain produced by the condensation of acetyl-coenzyme A with subsequent units of malonyl-coenzyme A. At this stage of the work, it was not possible to distinguish between a cyclization mode where C-18 is derived from the starter of the hypothetical 18-carbon polyacetate chain and an alternative scheme in which C-7 would be part of the starter of the 18-carbon precursor. To settle this question, *Solenopsis geminata* ants were fed with [2-^{14}C]malonic acid along with inactive acetate (Table 1), an experiment devised by Leete to solve the same problem in the case of pinidine [14]. The labeling pattern observed in *cis*- and *trans*-solenopsin A coming from this experiment was almost identical to that obtained with sodium [2-^{14}C]acetate (Table 4). This may be explained by the fast decarboxylation of [2-^{14}C]malonic acid into [2-^{14}C]acetate by the *Solenopsis* metabolism. We could therefore not identify which of C-18 or C-7 is derived from the acetate starter.

The hypothetical 18-carbon polyacetate chain mentioned above could yield *cis*- and *trans*-solenopsin A by two alternative pathways. In route A (Fig. 2), $\Delta^{1,(2)}$ solenopsin A is a plausible intermediate, whereas in route B the $\Delta^{1,(6)}$-unsaturated derivative is suggested. Further feeding experiments are in progress to refine the biosynthetic pathway outlined in Fig. 2.

This work was supported by grants from the Belgian Fund for Basic Research (Grant 2.4513.90-96) and the French Community of Belgium (ARC 93/98-137). One of us (S. L.) thanks the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA) for financial support. We also thank Dr. R. Ottinger for the NMR spectra and C. Moulard for the mass spectra.

Received November 30, 1995

1. Wilson, E.O.: The Insect Societies. Cambridge: Belknap 1971
2. Blum, M.S., Hermann, H.R., in: Arthropod Venoms, p. 801 (S. Bettini, ed.). Berlin: Springer 1978
3. Blum, M.S.: Chemical Defenses of Arthropods. New York: Academic Press 1981
4. Braekman, J.C., Daloze, D., in: Chemical defense in ants. Studies in Natural Products Chemistry, vol. 6, p. 421 (Atta-ur-Rahman, ed.). New York: Elsevier 1990
5. MacConnell, J.G., Blum, M.S., Fales, H.M.: Tetrahedron *26*, 1129 (1971)
6. Leclercq, S., Thirionet, I., Broeders, F., Daloze, D., Van der Meer, R., Braekman, J.C.: ibid. *50*, 8465 (1994)
7. Oppolzer, W., Bochet, G., Merifield, E.: Tetrahedron Lett. *35*, 7015 (1994) and references cited therein
8. Lofgren, C.S., Banks, W.A., Glancey, B.M.: Ann. Rev. Entomol. *20*, 1 (1975)
9. Renson, B., Merlin, P., Daloze, D., Braekman, J.C.: Can. J. Chem. *22*, 105 (1994)
10. Tursch, B., Daloze, D., Braekman, J.C., Hootelé, C., Pasteels, J.M.: Tetrahedron *31*, 1541 (1975)
11. Henery-Logan, K.R., Rodricks, J.V.: J. Am. Chem. Soc. *85*, 3523 (1963)
12. Leete, E., Olson, J.O.: ibid. *94*, 5472 (1972)
13. Attygalle, A.B., Blankespoor, C.L., Eisner, T., Meinwald, J.: Proc. Nat. Acad. Sci. USA *91*, 12790 (1994)
14. Leete, E., Lechleiter, J.C., Carver, R.A.: Tetrahedron Lett. 3779 (1975)