Tetraponerine-8, an Alkaloidal Contact Poison in a Neoguinean Pseudomyrmecine Ant, *Tetraponera* sp.*

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The sting of the Neoguinean pseudomyrmecine ant, *Tetraponera* sp. (group *punctulata*) is modified in a structure resembling a drawing pen. These ants do not sting, but smear upon enemies a contact poison. The venom which originates from the poison gland is a mixture of eight alkaloids with an original tricyclic ring system. The structure of the major component, tetraponerine-8, has been determined by X-ray diffraction analysis. The poison of *Tetraponera* is highly efficient in deterring aggressive sympatric ants and tetraponerine-8 shows toxic properties against *Myrmica rubra* when applied topically, with a LD_{50} of 5×10^{-4} mg/mg of ants.

Introduction

Pseudomyrmecine ants are well known for their powerful defensive and offensive sting. There are many vivid accounts in the literature of unforgettable experiences lived through by natives or naturalists stung by members of the four genera which constitute this subfamily [1–3]. Their aggressiveness towards both insects and mammals is considered to be a characteristic feature predisposing these ants to enter mutualistic relations with plants [2, 4]. The venom of *Pseudomyrmex pallidus,* the only species of the subfamily chemically studied, is proteinaceous [5] which is the rule for stinging *Hymenoptera* [6].

We report here on a completely different defensive mechanism in a yet undetermined Neoguinean *Tetraponera.* This species does not sting, but smears on enemies an alkaloidal venom with strong contact insecticidal activity. Although the ability to sting has been lost in other subfamilies and contact alkaloidal poisons are known in some specialized members of the more advanced Myrmicinae [7, 8], chemical defense in this *Tetraponera* species is unique both in the morphology of its sting apparatus and in the structure of the alkaloids of the venom.

Material and Methods

The ants were collected near Bogia along the North Coast of Papua-New Guinea. B. Bolton and C. Baroni Urbani have identified the species as a *Tetraponera* member of the *punctulata*-group, but not *punctulata* itself. The present state of knowledge of the taxonomy of this genus precludes further identification. Voucher specimens are deposited at the University of Brussels (Collectif de Bio-écologie).

The venom of 250 workers of *Tetraponera* sp. was collected from their sting on bits of filter paper and stored in hexane. Thin layer chromatographic analyses were performed on Al_2O_3 plates (eluent: hexane/ethyl-acetate 8:2), visualized with Dragendorff reagent.

The GC/MS analyses were performed at 170 °C on a Finnigan 9500 gas chromatograph equipped with a 25 m OV-1 capillary column, coupled to a Finnigan 3000 D mass spectrometer. The preparative gas chromatographic separations were realized on a Hewlett-Packard 402 equipped with a 1.80 m column packed with 2% SE-30 on Chromosorb W, at 150 °C. The EI.HRMS was performed on a Micromass 7070 F mass spectrometer. The IR spectrum was ob-

tained from a neat liquid film with a Perkin-Elmer 237 infrared spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WM 400 at 400 MHz and 100 MHz respectively. Chemical shifts are quoted in δ values downfield from TMS as internal standard.

X-Ray diffraction analysis on a crystal of **1**: intensities of 1388 independent reflections were collected on a Syntex P2$_1$ diffractometer using MOK$_\alpha$ radiation. The structure was solved using the MULTAN 80 program [9] and the refinements were realized using the SHELX-76 program [10]. The final R value is 0.058 for 412 observed reflections. The atomic coordinates will be deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 IEW, U.K.

Serial solutions (logarithmic scale) were tested on batches of 20 ants which each received 0.1 μl applied on the abdomen with a microsyringe. Mortality was observed after 24 h. After this delay, no ants ever recovered from paralysis. LD$_{50}$ was determined by extrapolation of the results of two successive dilutions (differing at most by a factor of 3). Control experiments demonstrated that 0.1 μl of pure solvent, either methanol of hexane, has no effect on the ants. The scanning electron microscopy observations were made on a DS 130 ISI microscope. The specimens were critical point dried before being coated with gold.

Results

Defensive behaviour and sting structure

This rather small *Tetraponera* (about 5 mm long) nests in hollow stems of lianas and feed on extrafloral nectaries of malvaceous bushes. They often run on twigs together with the much larger and highly aggressive *Oecophylla* ants without being attacked. Both species, however, feed separately on the nectaries. When disturbed, *Tetraponera* quickly bends its gaster at right angle in a horizontal plane, still running in this typical alarmed position. Fights between ants were observed by placing some *Tetraponera* workers close to a foraging trail of *Anoplolepis longipes*. Although *A. longipes* is a large and aggressive ant, *Tetraponera* safely escaped all attacks. As soon as *A. longipes* was touched by the abdominal tip of *Tetraponera*, it lost its hold and ran away with jerky and uncoordinated movements suggesting nervous poisoning. The ants recovered later on.

When a *Tetraponera* is seized with forceps, a large droplet of secretion is observed at the end of its sting. SEM photographs (Fig. 1) illustrate how the sting is modified, being no more a penetrating structure but well adapted to deposit a liquid on a surface. The lancets go beyond the tip of the sting shaft even when the sting is retracted in the abdomen and diverge from each other much like a drawing pen when the sting is extruded. The sting apparatus retains otherwise the general structure found in the subfamily [8] including the barbs at the tip of the lancets.

Preliminary comparisons with stinging pseudomyrmecine *Pachysina aethiops* and *Pseudomyrmex ferriginea* have confirmed the originality of the sting structure in *Tetraponera* sp. In these stinging species, the tip of the lancets is much sharper and the shaft and lancets have about the same length. The lancets are somewhat curved in *P. ferriginea* but never to the extent found in *Tetraponera* sp. Furthermore the tips of lancets in *Tetraponera* sp. are flexible, reinforcing the idea that they are not able to penetrate the skin of an enemy, arthropod or vertebrate.
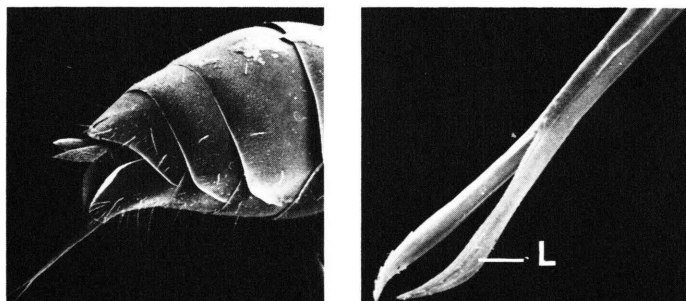

Fig. 1. The sting of *Tetraponera* is no more a penetrating organ. The lancets (L) diverge from each other like a drawing-pen well adapted to smear a contact poison on enemies. Left: ×42; right: ×350.

Chemical identification

Capillary GC indicates that the hexane extract of the venom contains eight closely related derivatives.

Rapid filtration of the hexane solution on alumina and evaporation of the solvent afforded 16 mg of crude extract. Combined capillary gas chromatography − mass spectrometry (GC-MS) indicates that this extract, homogneous by TLC, corresponds in fact to a mixture of eight closely related derivatives. The same mixture (57 mg) could also be obtained from the CH_2Cl_2 extract of whole ants (1250) after acid/base partition. Part of this mixture was submitted to preparative gas chromatography affording 7 mg of one of the main constituents (28%): tetraponerine-8 (**1**), which could be induced to crystallize on standing [m.p.: 40 °C; $[\alpha]_{579}$ + 102° (c, 0.15 g/100 ml in $CHCl_3$)].

Whereas the infrared spectrum of tetraponerine-8 does not show absorption bands characteristic of carbonyl, hydroxyl or NH groups, intense Bohlmann bands at 2720 and 2800 cm^{-1} suggest the presence of a *trans*-quinolizidine or *trans*-indolizidine ring system [11]. High resolution mass spectrometry measurements on the molecular ion yielded the molecular formula $C_{16}H_{30}N_2$ (measured 250.239; calculated 250.241). The ^1H NMR [0.88 (3H; tr; H-16), 2.04 (1H; ddd, 9, 9, 9; H-8ax), 2.25 (1H; dd, 6, 9; H-5), 2.87 (1H; ddd, 2, 2, 11; H-4eq), 3.18 (1H; ddd, 9, 9, 2; H-8eq). The other signals are superimposed between 2.00 and 1.00 ppm] and the ^{13}C NMR spectra [BBD and SEFT pulse sequence: 85.4 (d, C-5), 62.4 (d, C-9*), 61.6 (d, C-11*), 51.1 (t, C-4), 49.5 (t, C-8), 38.0 (t, C-10), 34.1 (t, C-1), 32.4 (t, C-12§), 32.3 (t, C-14§), 29.2 (t, C-6), 25.6 (t, C-3), 24.8 (t, C-2°), 24.6 (t, C-13°), 22.7 (t, C-15), 19.6 (t, C-7) and 14.0 (q, C-16). *, §, ° Interchangeable signals] show the absence of sp^2 carbon atoms, tetraponerine-8 must thus be tricyclic. The data obtained from the ^{13}C NMR spectrum together with two-dimensional (2 D) NMR correlated spectra ($^1H/^1H$ and $^1H/^{13}C$) at 400 MHz suggested the presence of structural block A, an ethyl group and 6 methylenes. Moreover, a comparison of the ^{13}C NMR spectrum of **1** with those of 2-methylquinolizidine [12] and indolizidine [13] suggest that analogous moieties may be present in the molecule.
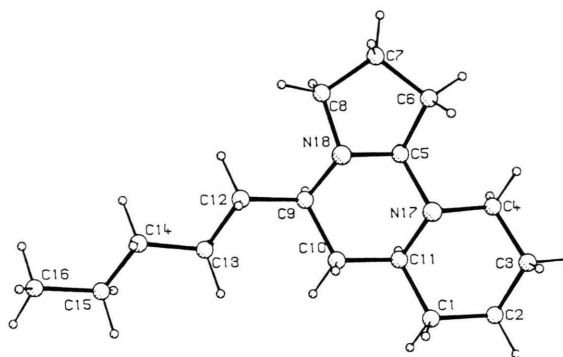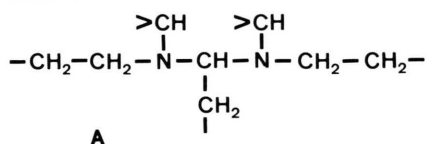
A

Fig. 2. Computer-generated structure of tetraponerine-8.

Finally, the complete structure of **1** was determined by X-ray diffraction analysis. The crystals of **1** belong to the orthorhombic system, space group $P2_12_12_1$, with a = 4.56 (1), b = 14.27 (4), c = 23.81 (6) Å, Z = 4. A computer-generated drawing of **1** showing the relative configuration is depicted in Fig. 2. To our knowledge, the parent ring system of tetraponerine-8 has never been found before in nature. Its structure is related to those of the defensive alkaloids (2,6-dialkylpiperidine [14, 15], 2,5-dialkylpyrrolidine [16−18], indolizidine [19, 20], and/or pyrrolizidine [20] derivatives) found in the venom of ants of the genera *Solenopsis* and *Monomorium*. Although these ants are taxonomically very different from *Tetraponera*, this analogy may reflect the existence of a basic biogenetic scheme common to all these derivatives. Comparative TLC analysis of extracts of dissected Dufour's and poison glands shows that the alkaloid mixture is synthesized in the poison gland only.

1
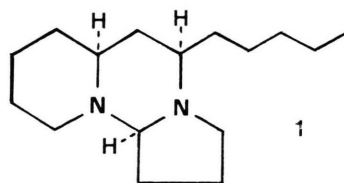
Toxicity of tetraponerine-8

Tetraponerine-8 is toxic to the ant *Myrmica rubra* on topical application in methanolic solution (Table I). Its toxicity is high compared to a natural alkaloidal insecticide like nicotine, but cannot quite match the toxicity of DDT. Sublethal doses induce

Table I. Toxicity of tetraponerine-8, nicotine and DDT when applied topically on the ant *Myrmica rubra*.

Substance	Solvent	mg/ant mg	LD_{50} mol/ant mg
Tetraponerine-8	methanol	5×10^{-4}	2.0×10^{-9}
Nicotine	methanol	4×10^{-3}	2.5×10^{-8}
DDT	hexane	1×10^{-5}	2.8×10^{-11}

paralysis which may last several hours. As little as 3×10^{-5} mg/ant may cause paralysis in 12 out of the 20 tested *M. rubra*, of which all but 3 ants recovered.

The replacement of stinging by various modes of chemical defense has occurred many times independently during the evolution of ants, but was considered to be characteristic of highly advanced species. Maschwitz [21] has convincingly argued that stinging is rather inefficient in fighting other ants which are quite mobile and hard sclerotized (see also [22]). This study demonstrates that such evolution has occurred even in ants considered to be primitive on other grounds, including in the structure of their sting apparatus [8]. Since the main enemies of ants are often other ants, independent evolution towards the replacement of stinging ability by more efficient chemical defenses is expected. Interestingly, both stinging [3] and non-stinging species are found in the same genus *Tetraponera*, indicating that venom chemistry and sting structure are evolutionary labile and strongly dependent on specific selective pressures which remain to be disclosed in more detail. The synthesis of tetraponerine-8 as well as the structures of the seven other alkaloids present in the venom of *Tetraponera* sp. are currently under investigation.

[1] W. M. Wheeler, Bull. Am. Nat. Hist. **45,** 39 (1922).
[2] E. O. Wilson, The Insect Societies, Belknap, Cambridge 1971.
[3] G. A. J. Rothney, Trans. Ent. Soc. London **1889,** 347.
[4] D. H. Janzen, Evolution **20,** 249 (1968).
[5] M. S. Blum and P. S. Callahan, Psyche **70,** 69 (1963).
[6] S. Bettini (ed.), Arthopod venoms, Springer Verlag, Berlin 1978.
[7] U. W. J. Maschwitz and W. Kloft, in: Venomous Animals and Their Venoms, **Vol. 3** (W. Bückler and E. E. Buclkey, eds.), Academic Press, New York 1971.
[8] M. S. Blum and H. R. Hermann, in [6].
[9] P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, M. M. Woolfson, G. Germain, and J. P. Declercq, Multan 80 Program Universities of York and Louvain-la-Neuve (1980).
[10] G. M. Sheldrick, Shelx-76 (1976), University of Cambridge.
[11] T. A. Crabb, R. F. Newton, D. Jackson, Chem. Rev. **71,** 109 (1971).
[12] D. Tourwe and G. Vanbinst, Heterocycles **9,** 507 (1978).
[13] P. E. Sonnet, D. A. Netzel, and R. Mendoza, J. Het. Chem. **16,** 1041 (1979).
[14] J. G. MacConnel, M. S. Blum, and H. M. Fales, Tetrahedron **26,** 1129 (1971).
[15] T. H. Jones, M. S. Blum, and H. M. Fales, Tetrahedron **38,** 1949 (1982).
[16] D. J. Pedder, H. M. Fales, T. Jaouni, M. S. Blum, J. G. MacConnel, and R. M. Crewe, Tetrahedron **32,** 2275 (1976).
[17] T. H. Jones, M. S. Blum, R. W. Howard, C. A. McDaniel, H. M. Fales, M. B. Dubois, and J. Torres, J. Chem. Ecol. **8,** 285 (1982).
[18] F. J. Ritter, I. E. M. Bruggemann-Rotgans, P. E. J. Verwiel, E. Talman, F. Stein, J. Labrijn, and C. J. Persoons, Proc. 8th Int. Congr. IUSSI, p. 41, Pudoc, Wageningen 1977.
[19] T. H. Jones, R. J. Highet, M. S. Blum, and H. M. Fales, J. Chem. Ecol. **10,** 1233 (1984).
[20] T. H. Jones, M. S. Blum, H. M. Fales, and C. R. Thompson, J. Org. Chem. **45,** 4778 (1980).
[21] U. Maschwitz, in: Pheromones and Defensive Secretion in Social Insects (Ch. Noirot, P. E. Howse, and O. Le Masne, eds.), p. 41, Université de Dijon, Dijon 1975.
[22] A. Buschinger, U. Maschwitz, in: Defensive Mechanisms in Social Insects (H. R. Hermann, ed.), Praeger, New York 1984.