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Ammonia chemical ionization tandem mass spectrometry in structural determination of alkaloids. II. Tetraponerines from pseudomyrmecine ants

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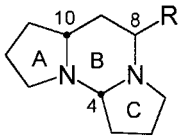
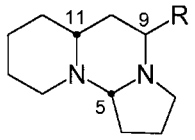
Chemical ionization tandem mass spectrometry (CI-MS/MS) of alkaloids with ammonia reagent gas and collision-activated dissociation as well as EI-MS/MS were applied to the tetraponerine alkaloids in extracts from six pseudomyrmecine ants of the genus *Tetraponera*. The MS/MS techniques along with gas chromatography Fourier transform infrared (GC/FTIR) spectra allowed identification in two extracts of seven of the eight known tetraponerines. The EI-MS/MS fragmentations proved diagnostic for the ring system and the CI-MS/MS patterns for the C-8 or C-9 substitution, while the Bohlmann bands in FTIR spectra were diagnostic for the C-8 or C-9 configurations. An Indian ant (*T. allaborans*) had T–2, T–4 and T–8, while a Chinese ant (*T. binghami*) had T–5, T–6, T–7 and T–8. Four other ants, *T. rufonigra* (India), *T. penzigi* (Africa), *T. clypeata* (Africa) and *T. sp. cf. emeryi* (Africa), had no tetraponerines. Copyright © 2001 John Wiley & Sons, Ltd.

Tandem mass spectrometry following chemical ionization with ammonia (CI (NH_3)-MS/MS) has been applied to the structural determination of mono- and bicyclic alkaloids with results complementary to electron-impact mass spectrometry (EI-MS).[1] The markedly different fragmentation patterns resulting from CI-MS/MS provide additional structural information. As a continuation of our studies on CI-MS/MS of alkaloids, we provide here a comparison of the EI-MS, EI-MS/MS, and CI (NH_3)-MS/MS data from tetraponerines to demonstrate the useful structural information that can be obtained by combining these techniques.

Tetraponerines are alkaloids with two unprecedented tricyclic structures originally detected in the venom of a pseudomyrmecine ant, *Tetraponera* sp., from Papua New Guinea.[2] Ants of this genus are found only in Africa, Asia and the South Pacific. The eight tetraponerines from this species of ant have been characterized and named tetraponerine-1 (**T–1**) to tetraponerine-8 (**T–8**). Four have a C-8 *n*-propyl or *n*-pentyl substituent and a 5-6-5 fused ring system; four have a C-9 *n*-propyl or *n*-pentyl substituent and a 6-6-5 system. Despite the side-chain substituted carbon being adjacent to a nitrogen atom, α-cleavage on EI-MS is not significant but rather major ethyl and butyl cleavages are seen from the two side chains. These unexpected fragmentations were misleading in the initial assignment of structures.[2] To our knowledge, no further tetraponerines have been reported.



(5-6-5) system



(6-6-5) system

R = —n-C_3H_7 (*R*) **T–1**
R = ····n-C_3H_7 (*S*) **T–2**
R = —n-C_5H_{11} (*R*) **T–5**
R = ····n-C_5H_{11} (*S*) **T–6**

R = —n-C_3H_7 (*R*) **T–3**
R = ····n-C_3H_7 (*S*) **T–4**
R = —n-C_5H_{11} (*R*) **T–7**
R = ····n-C_5H_{11} (*S*) **T–8**

Recent work by Daloze and Pasteels' group,[3] based chiefly on ^{1}H-and ^{13}C-NMR spectroscopy, revised four of the structures originally proposed[2] for tetraponerines **T–3**, **T–5**, **T–6** and **T–7** to those shown above, and proposed for the first time structures for **T–1** and **T–2**. Absolute stereochemistries were proposed for **T–3**, **T–4**, **T–7** and **T–8** based upon CD spectra[3,4] and enantioselective syntheses of (+)- and (−)-**T–7** and **T–8**.[4] Syntheses by Jones of racemic **T–3**, **T–4**[5] and **T–8**[6] and by the Belgian group of racemic **T–5** and **T–6**[3] and **T–8**[7]

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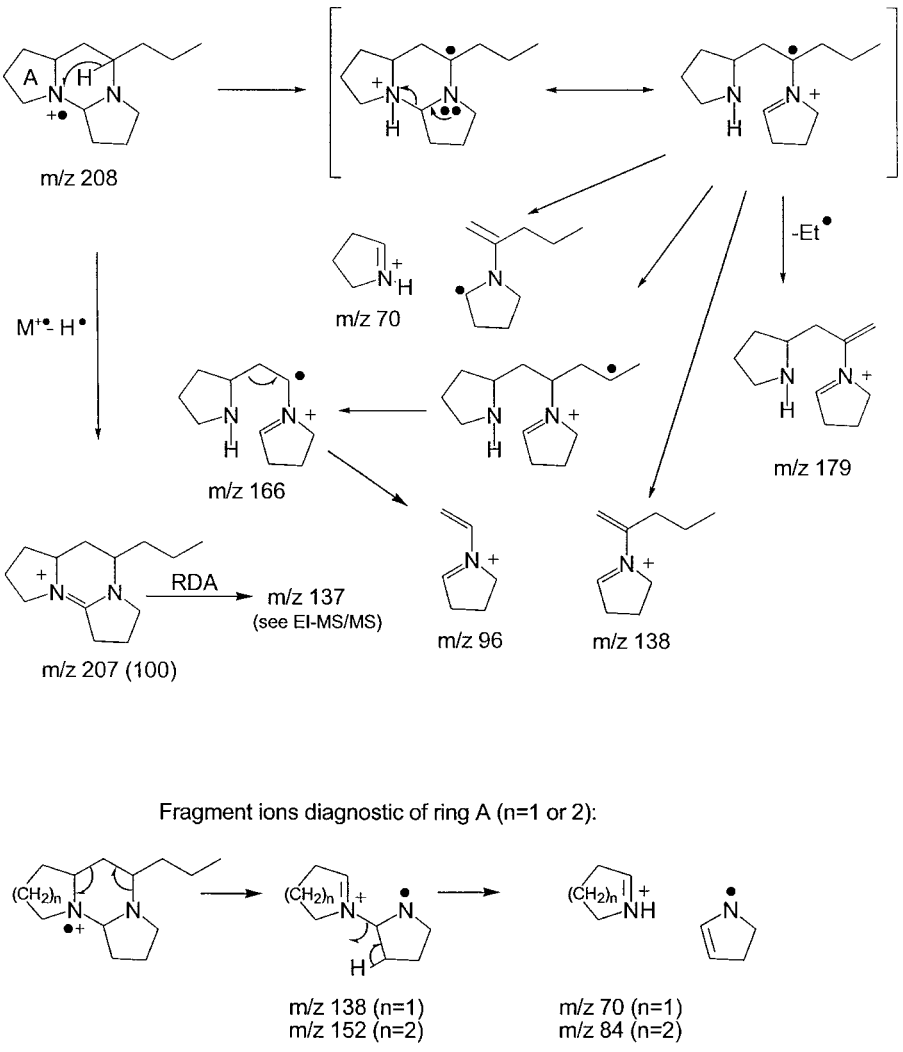
and by Husson's group of all eight (+)-tetraponerines[8] by an enantioselective synthesis have confirmed the structures and absolute stereochemistries. A simple three-step synthesis of a known key intermediate to the 5-6-5 system has recently been reported[9] as well as a three-step synthesis of (±)-**T–8**[10] and another enantioselective synthesis of (+)-**T–3**, **T–4**, **T–7** and **T–8**.[11] In the present study on the tetraponerine alkaloids in ants of the genus *Tetraponera*, the extracts (typically 20–30 ants though sometimes as few as a dozen) contained amounts far too small for isolation and NMR analysis. Accurate mass measurements (see Appendix) on three alkaloid GC peaks from one such extract of a then incompletely identified *Tetraponera* ant from India indicated molecular formulae with two nitrogen atoms and three double-bond equivalents and confirmed we were dealing with tetraponerine alkaloids. Consequently, we have attempted to develop methodology based exclusively on MS techniques and GC/FTIR spectroscopy that would permit the structural elucidation of these or other tetraponerines. The following mass spectrometry experiments provide useful inferences on tetraponerines. We also document Bohlmann band pattern differences between the C-8 epimers of the 5-6-5 and C-9 epimers of the 6-6-5 systems, which can be used to determine their relative stereochemistry and also to distinguish the ring systems. Bohlmann bands are C-H stretching vibrations observed in an infrared (IR) spectrum (typically 2800–2700 cm^{-1}) due to the interaction of a lone-pair of electrons from an adjacent nitrogen atom where at least two of these C-H bonds are aligned with the lone pair in a *trans*-anti-parallel orientation (cf. Ref. 14a). In the absence of NMR data, a combination of mass spectral techniques and IR data can unambiguously assign structures to any of the known tetraponerines in μg amounts without the necessity for reference materials.

Extensive EI-MS data has been available for some time for **T–1** through **T–8**,[2,3] which can be used on an unidentified tetraponerine to easily distinguish the four pairs of C-8 or C-9 epimers by their molecular ions alone; thus, **T–1** and **T–2** have *m/z* 208; **T–3** and **T–4** have *m/z* 222, etc. Although not as unambiguous and possibly instrument-dependent, the 6-6-5 tricycles can be distinguished from the 5-6-5 by the more significant ions at *m/z* 152 and 84 in the former and *m/z* 138 and 70 in the latter. See Scheme 1 for our proposals for those four fragment ion structures. The intensity ratios of *m/z* 152/138 are ca. 1.6–4.2 for the 6-6-5 and ca. 0.5 for the 5-6-5 systems and the *m/z* 84/70 ratios are ca. 1.6 for the 6-6-5 and 0.2–0.5 for the 5-6-5 systems. Thus a careful comparison of the EI-MS data alone of isomeric *pairs* of tetraponerines may,



Scheme 1. EI-MS fragmentation of tetraponerines (**T–2**, MW 208, used as an example).

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in principle, be sufficient to deduce their gross structures.[2] However, the major ions produced when the C-8 or C-9 side chain undergoes fragmentation are the result of a radical-ion rearrangement (see Scheme 1 for one possibility), which might be sensitive to the design of the mass spectrometer, e.g. it might be enhanced in ion-trap designs where longer ion lifetimes are encountered.

We have discovered that GC/EI-MS/MS spectra of tetraponerines, where $[M-H]^+$ ions are sequestered and fragmented, are diagnostic for the 6-6-5 or the 5-6-5 systems in that a simple, characteristic and rational fragmentation pattern is observed. On the other hand, GC/CI (NH_3)-MS/MS on the $[M+H]^+$ ion of tetraponerines provides spectra dominated by one ion that retains the side chain, easily permitting identification of the side chain and indicating whether the A-ring is five- or six-membered simply by mass difference from the $[M+H]^+$ ion. HPLC/MS provides fragmentation patterns similar to those of GC/CI (NH_3)-MS/MS, since both processes involve protonation of the molecule followed by a facile fragmentation. This behavior is seen even with ammonia CI-MS although less abundant ions also are observed.

EXPERIMENTAL

A Finnigan (San Jose, CA, USA) GCQ mass spectrometer and chromatograph fitted with an RTX-5MS (Restek) column (30 m × 0.25 mm i.d.) programmed from 100–280 °C at 10 °C/min was used with NH_3 in the CI mode to generate CI-MS and collision-activated CI-MS/MS spectra of alkaloids, and in the EI mode to generate EI-MS and EI-MS/MS spectra (instrument 1). Helium carrier gas was used as the collision gas. A Hewlett-Packard model 5890 gas chromatograph fitted with an HP-5 column (25 m × 0.32 mm i.d.), programmed as above, interfaced with a Hewlett-Packard model 5965B FTIR detector (narrow band, 4000–750 cm^{-1}, resolution 8 cm^{-1}) and an HP 5971 Series mass selective detector with a 59970 IRD ChemStation was used to record FTIR and some EI-MS spectra (instrument 2). High-resolution mass spectrometry (HRMS) was performed with a JEOL SX 102 instrument fitted with a 30 m × 0.20 mm i.d. DB-1 column operated with a program of 60–280 °C at 10 °C/min. The scan range and speed was *m/z* 40–400/s, the calibrant was perfluorokerosene and the resolution was 5000. The maximum error in six different accurate mass measurements (see Appendix) was 3.4 ppm. A Finnigan LCQ mass spectrometer was used in the atmospheric pressure chemical ionization (APCI) mode with a Hewlett-Packard model HP-1100 HPLC to generate HPLC/MS spectra. A Hewlett Packard Zorbax Eclipse XDB-C18 reversed-phase column (4.6 mm × 250 mm) was used with a flow rate of 0.5 mL/min and solvent composition 90% A/10% B→50% A/50% B over 40 min, where A = 0.1% HOAc/H_2O and B = 0.1% HOAc/CH_3CN. The order of elution from this column is the same as observed on an OV-1 GC column,[2] i.e. **T–2** < **T–3** < **T–4** < **T–5** < **T–6** < **T–7** < **T–8** (**T–1** not available).

Tetraponera allaborans (Walker) ants from two nearby colonies were collected by T.M.M.A. in Bangalore, Karanataka Province, India, and placed into vials with CH_2Cl_2. The CH_2Cl_2 extract was injected directly for GC/MS and in methanol for HPLC/MS analysis of alkaloids. These ants were collected on 24 Nov. 1994 and again on 10 Sept. 1997. *Tetraponera rufonigra* (Jerdon) were collected also by T.M.M.A. in the same area on 13 Sept. 1997. Voucher specimens for both are deposited in the museum of the University of Agricultural Sciences, G. K. V. K. campus, Bangalore, India. *Tetraponera binghami* (Forel) was collected by R.R.S. from a bamboo sprout in Hong Kong N. T. campus of the Chinese University of Hong Kong on 9 June 1996 (position 22.38°N, 114.18°E, ca. 20 m elev.) and handled as above. Voucher specimens are deposited in the Los Angeles County Museum of Natural History. *Tetraponera penzigi* (Mayr) were collected by L.I. at Segera Ranch, Laikipia, Kenya, on 11 Aug. 2000 from three colonies found on swollen thorns of *Acacia drepanolobium* trees. Small and large ants were collected in methanol. Four nest samples of *Tetraponera clypeata* (Emery) were collected by H.R in the Kleinmond Nature Reserve, W. Cape, South Africa, on 27–30 Sept. 2000 at mesic Mt. Fynbos from nests in hollow dead stems of *Watsonia* (two collections), *Aristea* (one collection) or an unidentified hollow stem (one collection). Three populations of *Tetraponera* sp. *cf. emeryi* (Forel) were collected by H.R. in the same reserve as above at Feetjiesbos in an indigenous evergreen forest and found in nests on hollow dead tree twigs.

RESULTS AND DISCUSSION

The tetraponerines exhibit a major fragment ion in EI-MS corresponding not to α-cleavage of the side chain as would be anticipated, but rather to a loss of one methylene less than those present in the side chain at C-8 or C-9. We propose a simple explanation of this perplexing phenomenon by invoking a transannular hydrogen radical transfer to the initially produced nitrogen radical ion in the γ-position of the side-chain-substituted central perhydropyrimidine ring (see Scheme 1). The resulting carbon radical then cleaves by a simple elimination mechanism, the driving force being conjugation. This initially produced nitrogen radical ion can also be stabilized by loss of a hydrogen atom from the position α to both nitrogens and is, we assume, responsible for the production of the intense $[M-H]^+$ ion, which is often the base peak in EI-MS. An alternative process for the ethyl and butyl loss invoking the loss of ethylene or butylene from the $[M-H]^+$ ion is not seen in MS/MS spectra from that ion. Interestingly, the ratio of the $[M-H]^+$ ion to the major side-chain fragmentation ions, $[M-Et]^+$ or $[M-Bu]^+$, appears to reflect the stereochemistry at C-8 or C-9, being greater for the *R* (1.15–1.58) than the *S* (0.63–1.04) configuration. (It also appears that the *m/z* 96 ion may reflect the C-8 or C-9 stereochemistry, evidently being more significant in the 8*R* or 9*R* configurations (**T–odd** numbers) than the 8*S* or 9*S* configuration (**T–even**); ratio *m/z* 96 ions (**T–odd**/**T–even**) ≈ 1.2–2.0.)

The EI-MS/MS and CI-MS/MS techniques provide complementary data that quickly allow the assignment of the tricyclic system and the pyrimidine ring's (B) C-8 or C-9 substituent, respectively (see Schemes 2 and 3). In the former, a major ion at *m/z* 137 (cf. ion ***I***) is seen directly by a retro-Diels-Alder process from the $[M-H]^+$ ion of the 5-6-5

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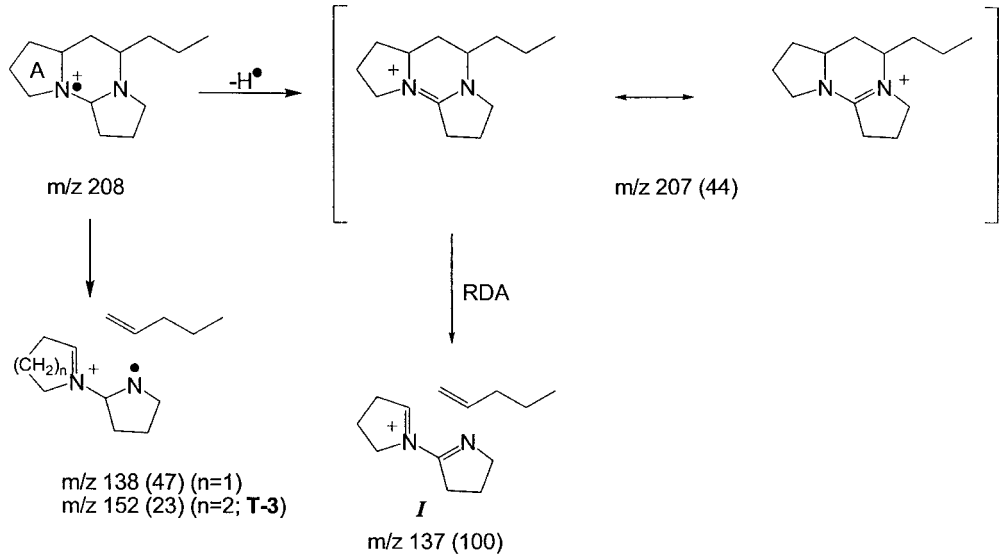
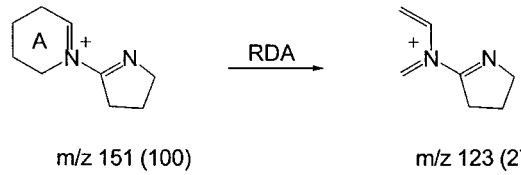
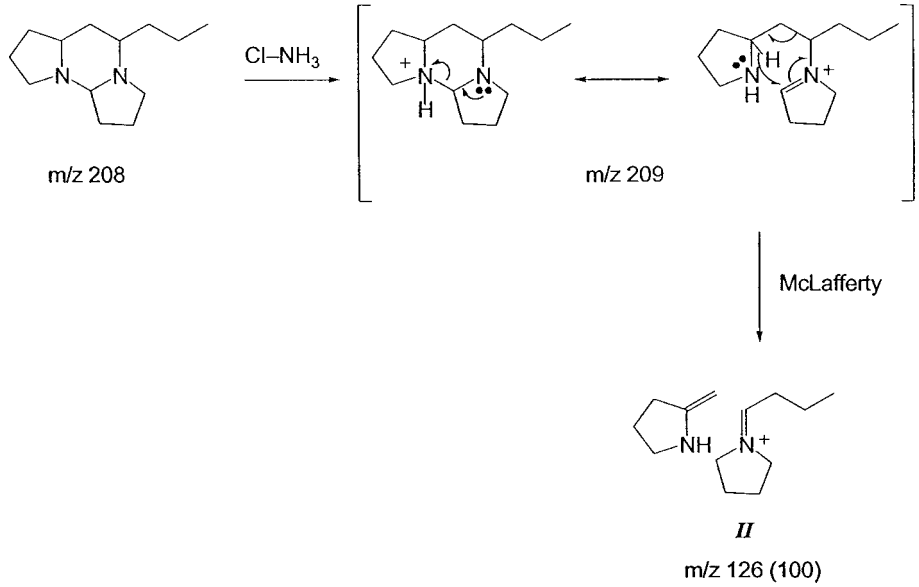


For Tetraponerines **T-3** and **T-4** the ions corresponding to $[M\text{-}H]^+$ and ***I*** are 221 and 151; 151 yields 123 by another RDA process, only possible with 6-membered rings.



Scheme 2. EI-MS/MS fragmentation of tetraponerines (**T–2**, MW 208, used as an example; MS/MS applied to M^+, *m/z* 208, and to the base peak in EI-MS, $[M - H]^+$, *m/z* 207).

system, while *m/z* 151 is the major ion from the 6-6-5 system. A second retro-Diels-Alder process, possible only with the 6-6-5 system, on the *m/z* 151 ion gives *m/z* 123. The CI-MS/MS spectra of the two tetraponerine tricycles can directly distinguish the ring systems as they both fragment to the same base peak ion involving the common five-membered (C) ring; however, that ion (cf. ***II***) does include the side chain (see Scheme 3). By difference, this fragment allows identification of the first ring (A) as either five- or six-membered. Thus, the $[M + H]^+$ ions from **T–1**/**T–2**, *m/z* 209, give a base peak at *m/z* 126 as proposed by heterolytic cleavage and a McLafferty-type rearrangement as do the *m/z*



Scheme 3. CI-MS or CI-MS/MS fragmentation of tetraponerines (**T–2**, MW 208, used as an example).

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223 ions from **T–3**/**T–4**. The $[M + H]^+$ ions, *m/z* 237 from **T–5**/**T–6** and *m/z* 251 from **T–7**/**T–8**, yield an *m/z* 154 ion as the base peak by the same postulated process. Butenyl- or hexenyl-pyrrolidinium ion structures (***II***) are proposed to accommodate these observations and indicate side-chain propyl or pentyl substituents in the tetraponerines. HPLC/MS in the APCI mode (where only the loss of neutral moieties is seen from the $[M + H]^+$ ion upon fragmentation) afforded with the tetraponerines of *Tetraponera binghami* the same ions as were observed from GC/CI (NH_3)-MS/MS, i.e. the $[M + H]^+$ ion and a significant ion at *m/z* 154 from **T–5**, **T–6**, **T–7** and **T–8**, but with an important difference; in **T–5** and **T–6** a less abundant fragment ion is also seen at *m/z* 84 reflecting the 5-6-5 system while in **T–7** and **T–8**, a less abundant fragment ion is seen at *m/z* 98 reflecting the 6-6-5 system. HPLC/MS like CI-MS/MS thus may provide an alternative one-step method to define both the substituent and ring system of an unknown tetraponerine.

GC-FTIR Bohlmann band frequencies and intensities provide data complementary to the mass spectral fragmentation patterns that allow for the distinguishing of side-chain configurations at positions adjacent to nitrogen;[14] this latter information necessary to unambigously assign the structure to an unidentified tetraponerine. Liquid-phase IR spectra were used in some of the original structural assignments.[2] The 8*S* or 9*S* isomers in either ring system (**T–even numbers**) have more intense Bohlmann bands at ca. 2790 cm^{-1} (as might be expected in that they have three *trans*-anti-parallel hydrogens to the two nitrogen atom lone-pairs), while the 8*R* or 9*R* isomers (**T–odd**) have weaker bands at slightly higher frequency (ca. 2805 cm^{-1}). The range of values of the percentage of the Bohmann band intensity to that of the C-H stretching frequency at 2940 cm^{-1} for **T–even** is 28–57 and 16–39 for **T–odd**.

The FTIR spectra of synthetic **T–5** and natural **T–6**, **T–7** and **T–8** are presented in Fig. 1. The FTIR spectrum of synthetic **T–3** was identical to natural **T–3**. These data are not shown since the propyl-substituted tetraponerines examined (**T–2**, **T–3** and **T–4**) had FTIR spectra virtually identical to their pentyl-substituted analogs (**T–6**, **T–7** and **T–8**) of Fig. 1. **T–3** had a weak Bohlmann band pattern, very similar to **T–7** of Fig. 1, as it has only two hydrogens (H-5 and H-11) *trans*-anti-parallel to the two N lone-pairs and not three as in the case of **T–2**, **T–4**, **T–6** and **T–8**. Tetraponerines **T–2** (not shown) and **T–6** have the strongest Bohlmann band patterns; they may be considered to be composites of two aza-indolizidine-type spectra where H-4, H-8 and H-10 are *trans*-anti-parallel to N lone-pairs. The 6-6-5 system can be viewed as a composite of one aza-indolizidine and one aza-quinolizidine. We have observed that indolizidines have stronger and sharper Bohlmann bands than the corresponding quinolizidines with the same number of *trans*-anti-parallel hydrogens.[14b] Thus, the overall appearance of the Bohlmann band region is characteristic of the ring systems, being much more intense with the 5-6-5 than the 6-6-5 system for the same relative stereochemistry of the side chain.

The extract from the Indian ant *Tetraponera allaborans* collected in 1994 had three main tetraponerines, **T–2**, **T–4** and **T–8** in a ratio of 3:15:1. The configuration at C-8 or C-9 is assumed to be *S* in each of these cases analogous to the

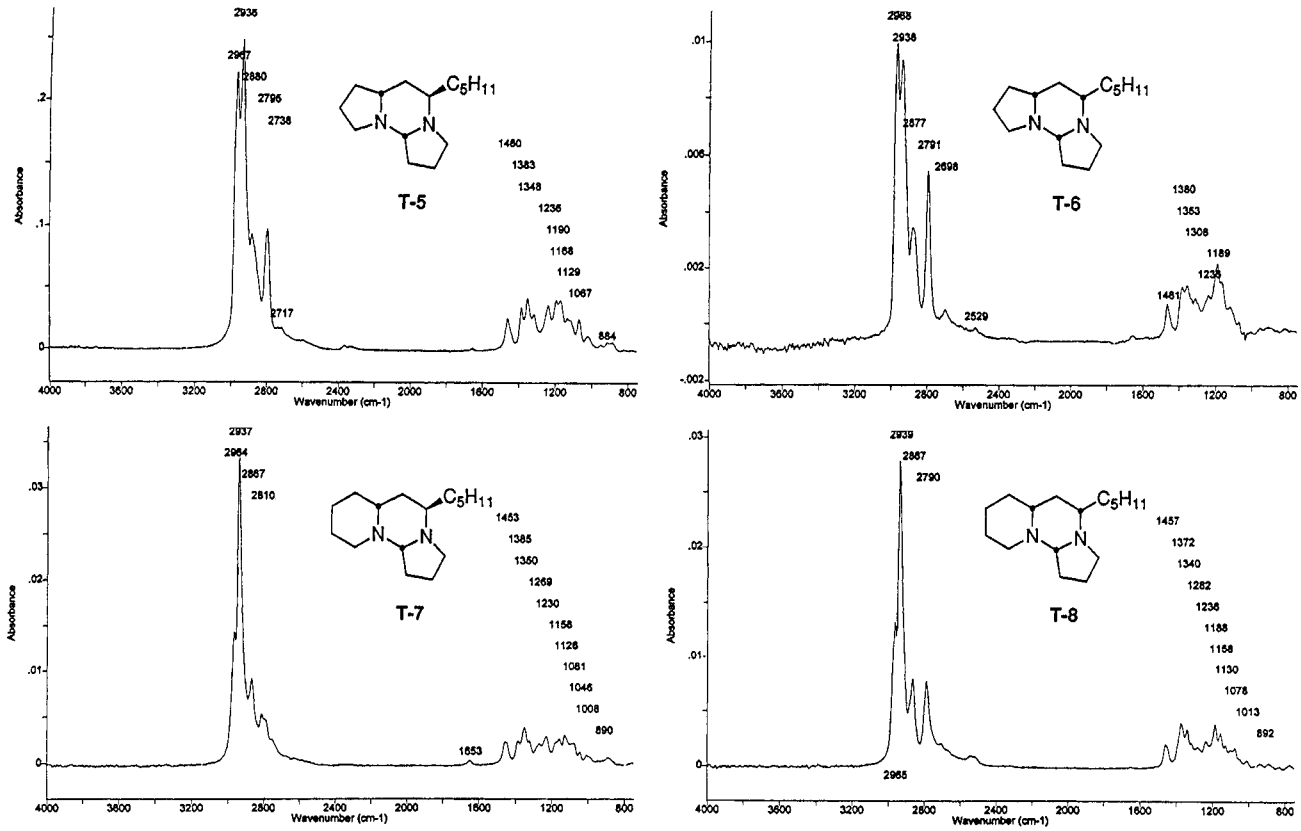

Figure 1. FTIR spectra of synthetic **T–5** and natural **T–6**, **T–7** and **T–8**.

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absolute configuration reported for the original tetraponerines from a New Guinean ant.[3,4,8] In addition, trace amounts of **T–3** and **T–6** were detected. The tetraponerines were identified by GC/MS techniques and GC/FTIR analyses with the identities of three of the tetraponerines being confirmed by additional comparison with synthetic samples of (±)-**T–3**, **T–4** and **T–8**.[5,6] It should be noted that as a consequence of the revision of the original structure for **T–3**, that Jones' epimer of **T–4** (originally referred to by Jones as $\mathbf{T}_x$[5]) is identical to **T–3**. The extract from the Chinese ant *T. binghami* had **T–6**, **T–7** and **T–8** in a ratio of 1:2.3:1.5 with minor amounts of **T–5**; thus this extract contained only the four pentyl-substituted tetraponerines.

Surprisingly, the extract from the 1997 collection of *T. allaborans* was devoid of alkaloids as was the extract from a 1997 collection of another Indian ant *T. rufonigra*. Extracts of the African *Tetraponera* ants we examined (*T. penzigi, T. clypeata, T.* sp. *cf. emeryi*) also had no detectible tetraponerines. Certainly we would have expected to find the same alkaloids in the *T. allaborans* collected at the same locale only 3 years after the first collection. Such variation within a species would be surprising if encountered in the more commonly studied ants of another subfamily, Myrmicinae, where the profile of alkaloids is considered characteristic of species, although it differs by caste. Recently, a careful study has revealed variations in both composition and amounts of alkaloids in the venom of myrmicine fire ants (*Solenopsis invicta*) with worker age and size[15] indicating that chemical taxonomic markers may have more variation than originally supposed. The solenopsins (2-alkyl or-alkenyl 6-methylpiperidines), main components of the venom, were more abundant for the younger or older workers but at lower levels in the middle-aged workers; total amounts differed by as much as ten-fold. The composition of the solenopsins, in particular the ratio of saturated to unsaturated, likewise varied with age and size.

At present it would appear that the presence or absence of tetraponerine alkaloids can not be considered a taxonomic marker for the genus until the genetic or environmental factors responsible for their production are uncovered and understood. *Tetraponera* ant morphology was considered a possible factor as, for example, whether the venoms are dabbed or injected. The ant *Tetraponera* sp. of Papua New Guinea, which contains all eight tetraponerines,[2] is known to be a dabber; however *T. binghami* is a stinger (R.R.S.). Biosynthetic studies on **T–6**[12] and **T–8**[13] indicate that they arise in *Tetraponera* sp. from polyacetate-glutamic acid pathways. At present only propyl and pentyl substituents are known in tetraponerines but the involvement of a polyacetate precursor suggests that a methyl substituent or a side chain two carbons higher than pentyl might be detected in Nature.

SUMMARY

The tandem MS/MS techniques provide unambiguous structural information on the tetraponerines in that no unexpected fragmentation pathway is encountered in the C-8 or C-9 side chain, at least for the major ions observed. CI (NH_3)-MS/MS on the $[M + H]^+$ ion segregates **T–1** to **T–8** into two groups (**T–1** to **T–4** and **T–5** to **T–8**) depending on whether C-8 or C-9 is *n*-propyl or *n*-pentyl. In the former case the principal fragment ion is at *m/z* 126; in the latter case, *m/z* 154 (Scheme 3, ion ***II***). The difference in mass with $[M + H]^+$ provides a simple, rapid identification of any of the eight tetraponerines. As additional confirmation, within each of the above group of four, an additional segregation by ring type is achieved by EI-MS/MS on the $[M - H]^+$ ion where a retro-Diels-Alder (RDA) cleavage provides solely ion ***I*** (Scheme 2), which is either *m/z* 137 or 151 depending on whether the tricycle is a 5-6-5 or a 6-6-5 system, respectively. These tandem methods should prove useful in assigning structures to a single tetraponerine or to mixtures and perhaps will find application in uncovering new substituents of these two ring systems. (Kovats indices have been published[2] for **T–3** to **T–8** and provide an alternative method of identification of these tetraponerines by GC retention times on an OV-1 column if mass spectrometry is not available.) Finally, the vapor phase spectra of the tetraponerines are fundamental in determining the side-chain stereochemistry and can be used to confirm the ring system of tetraponerines.

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APPENDIX

Characterization of tetraponerines by mass spectrometry

Data are reported for natural tetraponerines unless noted

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otherwise. EI-MS data are reported for instruments 1 or 2 (in parentheses; see Experimental section). Unless indicated otherwise, they are reported for instrument 1.

T–1: not available

T–2: EI-MS (instr. 2): *m/z* 208 (49), 207 (100), 193 (7), 179 (63), 167 (13), 166 (12), 152 (4), 151 (5), 138 (71), 124 (36), 123 (9), 96 (61), 82 (8), 70 (51). EI-MS/MS $[M - H]^+$: *m/z* 207 (44), 164 (4), 137 (100), 120 (8). CIMS: *m/z* 209 (74), 179 (7), 166 (5), 138 (5), 126 (100). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 126 (100), 84 (3). HRMS: calcd for $C_{13}H_{24}N_2$, 208.1939; observed 208.1934; calcd for $C_{13}H_{23}N_2$ $[M - H]^+$, 207.1861; observed, 207.1860.

T–3: (synthetic) EI-MS (instr. 2): *m/z* 222 (40), 221 (63), 207 (6), 193 (100), 180 (9), 179 (13), 165 (4), 152 (45), 151 (14), 138 (21), 137 (13), 124 (12), 123 (8), 110 (16), 96 (54), 84 (22), 70 (10). EI-MS/MS $[M - H]^+$: *m/z* 221 (67), 179 (7), 151 (100), 123 (27). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 152 (4), 126 (100), 96 (2), 84 (1). HPLC/MS: *m/z* 223 (85), 221 (32), 126 (100), 98 (15).

T–4: EI-MS (instr. 2): *m/z* 222 (51), 221 (96), 207 (6), 194 (15), 193 (100), 180 (15), 179 (15), 165 (6), 152 (63), 151 (23), 138 (26), 137 (16), 124 (19), 123 (8), 110 (19), 97 (12), 96 (57), 84 (34), 70 (16). EI-MS/MS $[M - H]^+$: *m/z* 221 (76), 179(6), 151 (100), 134 (2), 123 (23). CIMS: *m/z* 223 (86), 179 (7), 166 (5), 126 (100), 123 (27). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 126 (100), 84(2). HPLC-MS: (synthetic **T–4**) *m/z* 223 (100), 221 (40), 126 (100), 98 (10). HRMS: calcd for $C_{14}H_{26}N_2$, 222.2096; observed 222.2094; calcd for $C_{14}H_{25}N_2$ $[M - H]^+$, 221.2018; observed, 221.2023.

T–5: (synthetic **T–5**: EI-MS: *m/z* 236 (33), 235 (100), 193 (10), 180 (10), 179 (68), 166 (32), 165 (10), 152 (8), 151 (4), 138 (25), 137 (27), 124 (11), 110 (6), 96 (44), 70 (15). EI-MS/MS $[M - H]^+$: *m/z* 235 (100), 137 (27), 120 (3). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 208 (14), 166 (6), 154 (100), 95 (7). HPLC/MS: *m/z* 237 (100), 154 (24), 84 (2).

T–6: EI-MS: *m/z* 236 (60), 235 (100), 193 (8), 179 (64), 166 (43), 165 (15), 152 (5), 151 (10), 138 (25), 137 (30), 124 (16), 110 (10), 96 (15), 70 (10). EI-MS/MS $[M - H]^+$: *m/z* 235 (100), 137 (10), 120 (2). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 179 (2), 166 (2), 154 (100), 84(2). HPLC/MS: 238(15), 237 (100), 154 (22), 84 (10).

T–7: EMS: *m/z* 250 (70), 249 (78), 207 (14), 193 (100), 180 (54), 179 (12), 165 (12), 152 (26), 151 (23), 138 (20), 137 (14), 123 (10), 110 (10), 96 (14), 84 (12). EI-MS/MS $[M - H]^+$: *m/z* 249 (100), 151 (15). CIMS: *m/z* 251 (66), 250 (46), 249 (28), 193 (34), 180 (16), 154 (100), 138 (5), 96 (3). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 222 (2), 194 (5), 181 (5), 180 (5), 154 (100), 138 (2), 84 (2). HPLC-MS: 252(16), 251 (100), 154 (26), 98 (20).

T–8: EI-MS: *m/z* 250 (62), 249 (100), 221 (4), 208 (5), 207 (15), 193 (95), 180(50), 165 (12), 152 (26), 151 (25), 138 (22), 137 (12), 110(8), 96 (10), 84 (8). EI-MS/MS $[M - H]^+$: *m/z* 249 (100), 207 (6), 163 (4), 151 (77), 123 (28). CI-MS: *m/z* 251 (100), 250 (35), 249 (35), 193 (20), 180 (20), 154 (100), 152 (5), 138 (5), 110(3). CI (NH_3)-MS/MS $[M + H]^+$: *m/z* 194 (2), 181 (2), 179 (2), 154 (100), 98 (1). HPLC-MS: 252(15), 251 (100), 154 (34), 98 (14). HRMS calcd for $C_{16}H_{30}N_2$, 250.2409; observed 250.2403; calcd for $C_{16}H_{29}N_2$ $[M - H]^+$, 249.2331; observed, 249.2339.

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