3-Methyl-4-phenylpyrrole from the Ants *Anochetus kempfi* and *Anochetus mayri*

T. H. Jones,*,† R. C. Flournoy,† J. A. Torres,‡ R. R. Snelling,§ T. F. Spande,⊥ and H. M. Garraffo⊥

Department of Chemistry, Virginia Military Institute, Lexington, Virginia 24450, Department of Biology, CUTB, #170 Carr. 174 Parque Industrial Minillas, Bayamón, PR 00959 and IITF, Forest Service, P.O. Box 25000, Río Piedras, PR 00928, Los Angeles County Museum of Natural History, 900 Exposition Blvd, Los Angeles, California 90007, Laboratory of Bioorganic Chemistry, NIDDK,NIH, Bethesda, Maryland 20892-0820

Received May 24, 1999

The cephalic extracts of the ant *Anochetus kempfi* were found to contain 2,5-dimethyl-3-isoamylpyrazine (**1**) and 3-methyl-4-phenylpyrrole (**2**). The structures of these compounds were established from their spectral data and by comparison with synthetic samples. This is the first report of a phenylpyrrole found in an insect and only the third report of a pyrrole from ants.

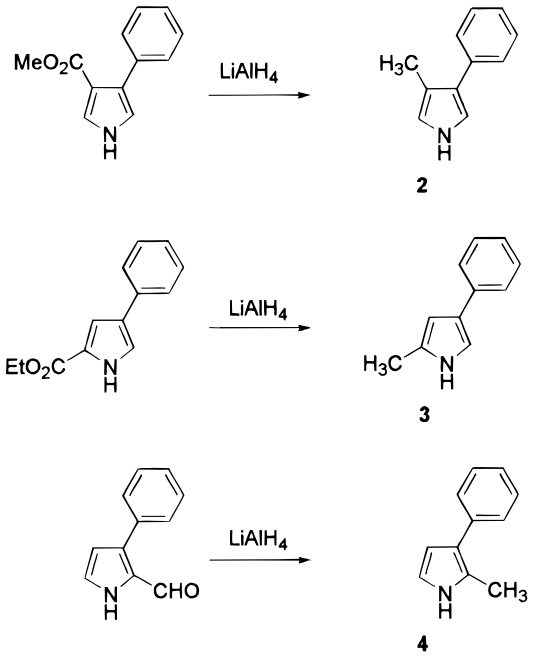
As part of an ongoing investigation of the ant fauna of Puerto Rico, GC−MS examination of the whole-body extracts of the nocturnal Ponerine ant *Anochetus kempfi* Brown (Formicidae) revealed the presence of two nitrogen heterocycles in a 1:9 ratio. The minor component was identified as 2,5-dimethyl-3-isoamylpyrazine (**1**) from its mass spectrum and by comparison of its gas chromatographic retention time with those of synthetic samples.[1]

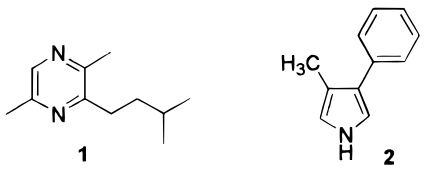
The major component had an EIMS with intense ions at *m*/*z* 157 [M^+], 156, and 77, and the molecular ion was confirmed by its CIMS (NH_3) [$M+H^+$, 158]. HRMS established a formula of $C_{11}H_{11}N$ for the *m*/*z* 157 ion. These data, along with a strong N−H absorption at 3522 cm^{-1} in the GC−FTIR of this component and a positive spot test for pyrroles,[2] suggested that this compound might be a pyrrole, substituted with methyl and phenyl groups. The CIMS (ND_3) corroborated the exchangeable hydrogen but only under forcing conditions (see Experimental Section). The presence of a methyl group was established by CIMS (NH_3) followed by collision-induced dissociation of the protonated molecular ion (*m*/*z* 158), which showed a major fragment for the loss of methyl [*m*/*z* 143 (100%)] and little else.[3] Additionally, collision-induced dissociation of the *m*/*z* 156 ion showed only a fragment at *m*/*z* 128, an ion also appearing in the EIMS spectrum of the compound, indicating that the fragmentation pathway producing *m*/*z* 128 is an H radical cleavage from the molecular radical ion followed by loss of ethylene and not an ethyl cleavage from the molecular ion.

All of the possible methyl α-phenylpyrroles are known, and samples were prepared by the zinc−acetic acid-catalyzed ring contraction of the appropriate methyl-2-phenylpyrimidines.[4] Although the methyl α-phenylpyrroles had mass spectra that were similar but not identical to that of the major component, direct comparison and co-injection showed that the gas chromatographic retention times of all the methyl α-phenylpyrroles were different.

The methyl *β*-phenylpyrroles were all prepared by lithium aluminum hydride reduction of an appropriate *β*-phenylpyrrole carbonyl compound.[5] In this way, 3-methyl-4-phenylpyrrole (**2**) was prepared from 3-(methoxycarbonyl)-4-phenylpyrrole,[6] 2-methyl-4-phenylpyrrole (**3**) was prepared from 2-(ethoxycarbonyl)-4-phenylpyrrole, [7] and 2-methyl-3-phenylpyrrole (**4**) was prepared from 3-phenylpyrrole-2-carboxaldehyde[8] (Scheme 1). The gas chromatographic retention time and mass spectrum of **2** matched those of the major component from *A. kempfi*.

Scheme 1. Lithium Aluminum Hydride Reductions of Known *β*-Phenylpyrrole Carbonyl Compounds





Additionally, the assignment of **2** as the major component from *A. kempfi* is confirmed by its GC−FTIR spectrum, which matched that obtained from the natural material. The GC−FTIR spectra of **3** and **4** are clearly different from that of **2** (Figure 1).

The occurrence of pyrazines, in particular **1**, in the mandibular glands of Ponerine ants is well documented.[9]

* To whom correspondence should be addressed. Tel.: (540) 464-7422. Fax: (540) 464-7261. E-mail: jonesth@vmi.edu.
† Department of Chemistry, Virginia Military Institute, Lexington, VA 24450.
‡ Department of Biology, CUTB, #170 Carr. 174 Parque Industrial Minillas, Bayamón, PR 00959 and IITF, Forest Service, P.O. Box 25000, Río Piedras, PR 00928.
§ Los Angeles County Museum of Natural History, 900 Exposition Blvd, Los Angeles, CA 90007.
⊥ Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892-0820.

10.1021/np990245t CCC: $18.00
Published on Web 08/27/1999

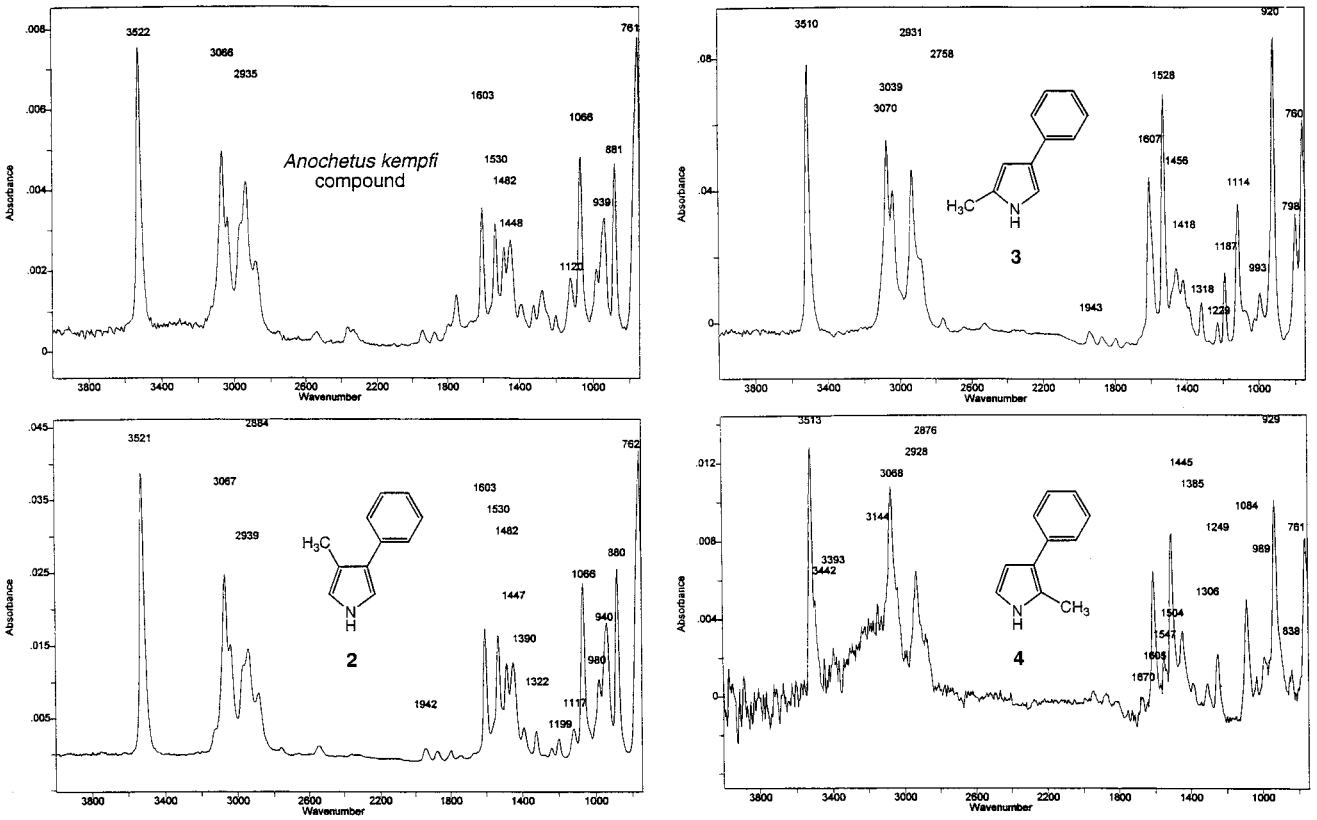


Figure 1. Comparison of the GC−FTIR spectra of the three methyl β-phenylpyrroles with the GC−FTIR spectrum obtained from the cephalic extracts of *A. kempfi*.

Analysis of trisected *A. kempfi* revealed the presence of heterocycles **1** and **2** only in the heads of these ants. Subsequent analysis of the heads of a small sample of *Anochetus mayri* revealed that heterocycles **1** and **2** are also present in the heads of this species, while neither compound could be detected in a sample of *Anochetus cato*. These compounds most likely are mandibular gland products and have a pheromonal role.[9]

The structure elucidation of 3-methyl-4-phenylpyrrole (**2**) in these *Anochetus* species is the first report of a phenylpyrrole found in an insect, and only the third report of a pyrrole from ants. It is clearly unrelated to the 2-carbomethoxy-4-methylpyrrole described as the trail pheromone of the leaf-cutting ant *Atta texana*,[10] or myrmicarin 217, a pentasubstituted pyrrole from the poison glands of *Myrmicaria* species.[11]

The failure to detect **1** and **2** in the sample of the Melanesian species *Anochetus cato* from Indonesia should not be surprising because it is a member of the *A. isolatus* group, one of the most "primitive" groups in the genus, while the Caribbean ants *A. kempfi* and *A. mayri* are in the *A. haytianus* and *A. mayri* complexes, respectively. The behavior and ecology of *Anochetus kempfi* have been studied extensively and will be reported elsewhere.[12]

Experimental Section

General Experimental Procedures. FTIR spectra were obtained using a Hewlett−Packard model 5965B detector interfaced with a Hewlett−Packard 5890 gas chromatograph equipped with a Hewlett−Packard HP-5 column, 30 m × 0.25 mm i.d. MS were obtained in the EI mode using a Shimadzu QP-5000 GC-MS equipped with an Rtx-5 column, 30 m × 0.25 mm i.d., or a Finnigan ion-trap model 800 equipped with a HP-5 column, 30 m × 0.32 mm i.d. The latter instrument with NH_3 or ND_3 reagent gases was used for CIMS.

Animal Material. One collection of workers of *A. kempfi* from Loiza, Puerto Rico, was made in July 1995, and three collections from Fajardo, Puerto Rico, were made from 1997 through 1998. The ants were placed in vials containing CH_2Cl_2 or MeOH immediately upon collection. The ants of one of the collections from Fajardo (1998) were trisected before they were placed in vials, and heads, mesosomae, and gasters were analyzed separately. Voucher specimens of all collections were deposited in the collection of the Los Angeles County Museum of Natural History, Los Angeles, CA. Each collection containing 25−50 workers of *A. kempfi* represented a different colony. A collection of seven specimens of *A. mayri* was made from Guaynabo, Puerto Rico, in February 1999. The heads of these ants were placed in a small vial containing MeOH. A sample of *A. cato* (Forel) was collected at Siewa Camp, along the Wapoga River, Irian Jaya (03.04° S, 136.38° E), Indonesia.

Analytical Data for 1 and 2. Initial GC−MS analysis of the *A. kempfi* extracts revealed the presence of compounds **1** and **2** in a 1:9 ratio, respectively, comprising nearly 95% of the detectable components in the extracts of workers. These compounds were not detected in the queens. Trisection showed that they occurred only in the heads of the ants. Analysis of the collection of *A. mayri* showed the presence of compounds **1** and **2** in a 4:1 ratio, respectively, although these compounds were not detected in the collection of *A. cato*.

The mass spectrum of **1** was identical to that reported for 2,5-dimethyl-3-isoamylpyrazine,[13] and its gas chromatographic retention time matched that of a synthetic sample. For **2**: MS *m*/*z* 157 (81 [M]$^+$), 156 (100), 129 (16), 128 (20), 127 (11), 115 (5), 102 (4), 89 (2), 80 (6), 78 (13), 77 (50), 65 (29), 63 (10), and 51(11); EIMS−MS on *m*/*z* 156 showed only *m*/*z* 128; CIMS (NH_3) gave *m*/*z* 158; CIMS−MS (NH_3) on *m*/*z* 158 showed only *m*/*z* 143 (100%); CIMS (ND_3) *m*/*z* 160/159 = 0.65 at 120 mTorr (pyrazine **1** did not exchange under these conditions); HRMS *m*/*z*, calcd for $C_{11}H_{11}N$, 157.0891; obsd, 157.0885. The GC−FTIR spectrum showed significant absorptions at 3522, 3066, 2935, 1603, 1530, 1482, 1448, 1120, 1066, 939, 881, and 761 cm^{-1} (Figure 1). A small sample gave a bright violet color on

a porcelain spot plate when treated with acidic *p*-dimethylaminobenzaldehyde (Ehrlich's reagent).

Pyrrole Syntheses: 3-Methyl-4-phenylpyrrole (2). A solution containing 0.40 g of 3-carboethoxy-4-phenyl pyrrole [6] in 10 mL of anhydrous Et_2O was added dropwise to 20 mL of ether containing $LiAlH_4$ (2.50 g) under an argon atmosphere. The resulting mixture was stirred for 30 min and quenched with EtOAc and H_2O. The resulting ether layer was separated, dried ($MgSO_4$), filtered, and the solvent removed to yield 0.28 g of crude 3-methyl-4-phenylpyrrole (**2**), the major volatile product: MS *m*/*z* 157 (78), 156 (100), 128 (21), 77 (49), 65 (27), 51 (12); HRMS *m*/*z* calcd for $C_{11}H_{11}N$, 157.0891, observed 157.0885; GC−FTIR spectrum of **2**, Figure 1.

2-Methyl-4-phenylpyrrole (3). A sample of 2-methyl-4-phenylpyrrole (**3**) was prepared in a similar manner as pyrrole **2** from 2-(ethoxycarbonyl)-4-phenylpyrrole:[7] MS *m*/*z* 157 (93), 156 (100), 128 (23), 115 (9), 77 (18), 65 (7), 51 (7); HRMS *m*/*z* calcd for $C_{11}H_{11}N$, 157.0891, obsd 157.0910; GC−FTIR spectrum of **3**, Figure 1.

2-Methyl-3-phenylpyrrole (4). A sample of 2-methyl-3-phenylpyrrole (**4**) was prepared in a similar manner as pyrrole **2** from 3-phenylpyrrole-2-carboxaldehyde:[8] MS *m*/*z* 157 (87), 156 (10), 129 (22), 128 (16), 127 (9), 115 (10), 80 (14), 79 (9), 78 (15), 77 (30), 65 (20), 63 (14), 51 (18); HRMS *m*/*z* calcd for $C_{11}H_{11}N$, 157.0891, observed 157.0901; GC−FTIR spectrum of **4**, Figure 1.

Acknowledgment. We thank Dr. Lewis Pannell and coworkers, L.B.C. and L.M.C., NIDDK, NIH, for the HRMS measurements. T.H. J. gratefully acknowledges the support of the Jeffress Memorial Trust and Virginia Military Institute Chemistry Department. We would like to thank J. Coll, F. Silva, and The Conservation Trust of Puerto Rico for allowing us to work in the Cabezas de San Juan Reserve.

References and Notes

(1) Houminer, Y.; Southwick, E. W.; Williams, D. L. *J. Heterocyclic Chem.* **1986**, *23*, 497−500.
(2) Feigl, F. *Spot Tests in Organic Analysis*, 7th ed.; Elsevier: New York, 1966; p 381.
(3) Garraffo, M. H.; Spande T. F.; Jones, T. H.; Daly J. W. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1−11.
(4) Thompson, T. W. *Chem. Commun.* **1968**, 532−533.
(5) Jones, A. R.; Bean, G. P. *The Chemistry of Pyrroles*; Academic Press: New York, 1977; pp 292−293.
(6) Pavri, N. P.; Trudell, M. L. *J. Org. Chem.* **1997**, *62*, 2649−2650.
(7) Gupton, J.; Keertikar, K.; Krumpe, K.; B. Burnham, B.; Dwornik, K.; Petrich, S.; Du, K.; Bruce, M.; Vu, P.; Vargas, M.; Hosein, K.; Sikorski, J. *Tetrahedron* **1998**, *54*, 5075−5082.
(8) Cue, B. W., Jr.; Chamberlain, N. *J. Heterocyclic Chem.* **1981**, *18*, 667−670.
(9) Morgan, E. D.; Do Nascimento, R. R.; Keegans, S. J.; Billen, J. *J. Chem. Ecol.* **1999**, *25*, 1395−1409.
(10) Tumlinson, J. H.; Moser, J. C.; Silverstein, R. M.; Brownlee, R. G.; Ruth, J. M. *J. Insect Physiol.* **1972**, *18*, 809−814.
(11) Schroder, F.; Francke, W. *Tetrahedron* **1998**, *54*, 5259−5264.
(12) Torres, J. A.; Snelling R. R. Unpublished results.
(13) *NIST/EPA/NIH Mass Spectral Database*; Version 4.0; National Institute of Standards and Technology: Gaithersburg, MD, 1992.

NP990245T