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A reinvestigation of the chemistry of the Dufours gland of the formicine ant, *Anoplolepis custodiens*

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Abstract. The components of individual Dufours glands excised from *Anoplolepis custodiens* workers were analysed by GC-MS. In addition to the *n*-alkanes and *n*-alkenes previously reported[2] in these glands, primary alcohols (C_{19}–C_{22}), secondary alcohols (C_{20}–C_{23}), 2-ketones (C_{20}–C_{23}) and possibly carboxylate ethyl esters (C_{19} and C_{21}) were identified as components of these glands. It seems possible that these high-boiling compounds are used by the workers in laying trails on the hot sandy surfaces of their characteristic habitat and in lining of the inner walls of nests, but no standard compounds have been available to us for any behavioral studies.
Key words. Anoplolepis custodiens; Dufours gland; *n*-alkanes; *n*-alkenes; 1-alcohols; 2-alcohols; 2-ketones.

A positive correlation between the presence of the formicine ant, *Anoplolepis custodiens*, in citrus fields and the incidence of red scale has long been observed[1]. This ant is thus of considerable economic importance in citrus farming in South Africa and its control could solve much of the problem of red scale in citrus. Methods for the biological control of this ant remain to be developed and it is hoped that control measures using pheromones might be possible if certain behavioral compounds of this pugnacious ant are characterised and tested.

The alarm-defense system of this ant has been investigated previously and it was reported that the Dufours gland contains only hydrocarbons[2]. In these early investigations, packed GC columns were used and eluting compounds were trapped on graphite for MS analysis. The possibility that additional components exist in this gland was considered and the increased sensitivity, resolution, and high temperature capability of present day capillary GC columns and the ready availability of GC-MS, prompted our reinvestigation of the chemistry of this glandular secretion. In addition, the solventless injection procedure used in this work minimised the physical and chemical processing of the samples prior to GC or GC-MS analyses. This is an important point since the components of the Dufours glands are present in nanogram quantities only.

Worker ants were collected on the University of Fort Hare farm (26°50′E, 332°47′S) and kept in the laboratory at room temperature and fed dead insects and sucrose solution. Dissected Dufours glands were placed in the cup of a glass capillary for solventless introduction in a gas chromatograph[3]. For $NaBH_4$ reduction, a Dufours gland was sealed in a glass capillary containing finely powdered $NaBH_4$ (1 mg or less) and the tube crushed in the GC inlet as described by Morgan[4] for solventless sample introduction. For BSTFA derivatisation, a Dufours gland was placed in the cup of the capillary tube containing 1 µl of BSTFA (Regis Chemical Company, IL, USA) and introduced into the GC inlet[3]. GC-MS analyses were run on a Hewlett Packard 5890 GC and 5970 MSD. A splitless injection with purge off time of 0.5 min was used with a HP-Ultra 1 column (50 m × 0.2 mm i.d. × 0.3 µm film thickness) held at 35 °C for 3 min, then temperature programmed to 300 °C at 5 °C min^{-1}, and finally held at 300 °C for 10 min.

Helium (25 cm s^{-1}) was used as the carrier gas. The mass selective detector monitored m/z 26 to 450 using 70 eV ionisation and the power supply to the electron multiplier was 2000 volts.

Chromatograms of single Dufours glands of workers indicated the presence of at least 39 components. The identities and evidence for the identities of these components are summarised in the table. The majority of the components were readily identified as *n*-alkanes and *n*-alkenes. Standards of *n*-alkanes were available for comparison of retention time and mass spectrum, whereas standard 1-alkenes only were available. The identified alkanes and alkenes confirmed previously reported results[2] except the additional presence of two dodecenes and three tridecenes are reported here. The difference between the two dodecenes (and the three tridecenes) is in the position of double bond within the molecule. An alkene with the unsaturation point closer to the centre of the molecule elutes earlier than the one with the unsaturation towards either end of the molecule. However, the position of the double bond in each alkene has not been determined.

The primary alcohols, 2-alcohols, 2-ketones and the components tentatively identified as carboxylate ethyl esters have not been reported as components of this gland before. Together, these components contribute an average of 26% to the total volatiles of this gland. Each

Identities and evidence for the identifications of components of single Dufours glands from *A. custodiens* workers

Peak No.	Kovats index	Area %	Component identity	Evidence for identity*
1	1000	2.57	*n*-Decane	GC, MS
2	1087	0.96	*n*-Undec-?-ene	MS
3	1100	27.88	*n*-Undecane	GC, MS
4	1173	0.08	3-methyl undecane	MS
5	1191	0.06	*n*-Dodoc-?-ene	MS
6	1195	0.09	*n*-Dodec-?-ene	MS
7	1200	0.63	*n*-Dodecane	GC, MS
8	1284	0.75	*n*-Tridec-?-ene	MS
9	1286	0.29	*n*-Tridec-?-ene	MS
10	1294	3.67	*n*-Tridec-?-ene	MS
11	1300	4.59	*n*-Tridecane	GC, MS
12	1400	0.05	*n*-Tetradecane	GC, MS
13	1488	tr	*n*-Pentadec-?-ene	MS
14	1500	0.41	*n*-Pentadecane	GC, MS
15	1600	tr	*n*-Hexadecane	GC, MS
16	1678	tr	*n*-Heptadec-?-ene	MS
17	1700	0.10	*n*-Heptadecane	GC, MS
18	1790	tr	*n*-Octadec-?-ene	MS
19	1800	1.00	*n*-Octadecane	GC, MS
20	1881	0.50	*n*-Nonadec-?-ene	MS
21	1900	14.06	*n*-Nonadecane	GC, MS
22	1986	0.12	*n*-Eicos-?-ene	MS
23	2000	3.16	*n*-Eicosane	MS
24	2086	0.78	*n*-Heneicos-?-ene	MS
25	2100	10.16	*n*-Heneicosane	MS
26	2168	0.35	Nonadecan-1-ol	MS, BSTFA
27	2188	0.26	Eicosan-2-one	MS, $NaBH_4$
28	2197	0.46	Eicosan-2-ol	MS, $NaBH_4$, BSTFA
29	2269	0.48	Eicosan-1-ol	MS, BSTFA
30	2287	5.52	Heneicosan-2-one	MS, $NaBH_4$
31	2295	5.77	Heneicosan-2-ol	MS, $NaBH_4$, BSTFA
32	2370	1.96	Heneicosan-1-ol	MS, BSTFA
33	2387	0.33	Docosan-2-one	MS, $NaBH_4$
34	2393	0.14	Docosan-2-ol	MS, $NaBH_4$, BSTFA
35	2408	2.39	Nonadecanoate ethyl ester?	
36	2481	0.34	Docosan-1-ol	MS, BSTFA
37	2487	2.80	Tricosan-2-one	MS, $NaBH_4$
38	2491	0.74	Tricosan-2-ol	MS, $NaBH_4$, BSTFA
39	2509	4.19	Heneicosanoate ethyl ester?	MS

*BSTFA, identification was based on the mass spectrum of the BSTFA derivative.
$NaBH_4$, evidence for the identity was obtained from increase/decrease in peak size after incubation of the gland with sodium borohydride.

of these compounds was identified by interpretation of the mass spectrum obtained, as well as by comparison with those in the NBS library of mass spectra available in the data base of the Hewlett Packard 5970 MSD. Support for the identities of 2-ketones and 2-alcohols was derived from the observation that peaks tentatively identified as those of 2-ketones were reduced in size, and those tentatively identified as the corresponding 2-alcohols, were increased in size when glands were treated with $NaBH_4$.

Further evidence for the identities of 1-alcohols and 2-alcohols was obtained from the mass spectra of the products of bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) reaction with these compounds. Peaks of m/z 75, 103 and M-15 (base peak) were quite discernible in the mass spectra of the primary alcohols, whereas for the 2-alcohols, peaks of m/z 73 and 75 were equally abundant (ca 33% of base peak). The base peak in each mass spectrum of the 2-alcohols was m/z 117 and the M-15 peak contributed about 15% of the base peak in each mass spectrum.

The mass spectra of components 35 and 39 were each characterised by a base peak of m/z 43, an abundant M-45 peak (about 45% of base peak) and a rather uncommon m/z 88 peak. The M-45 and m/z 88 ions are indicative of ethyl esters[5] and components 35 and 39 are thus tentatively identified as nonadecanoate ethyl ester and heneicosanoate ethyl ester, respectively.

These more polar compounds of rather low volatility (1-alcohols, 2-alcohols, 2-ketones and possible carboxylate ethyl esters) were detected readily in glands of all workers analysed. Alate females, in contrast, showed evidence only on some chromatograms of possible trace amounts of certain of these compounds (no functional queens were available for analysis). As workers of *A. custodiens* follow long trails on bare ground, which is

often extremely hot, one of us (JMB) has long been of the opinion that the non-polar compounds reported previously[2] are not the most likely compounds responsible for trail marking. Alate females are not expected to lay trails and it is considered possible that these more polar compounds reported here may be employed in trail marking by workers, and even in lining the inner walls of nests.

It has recently been reported that pseudomyrmicine ants living in tropical environments have higher melting substances in their Dufours glands than ants living in colder climates[6] and relatively long-chain hydrocarbons have been identified as components of Dufours glands from *Atta laevigata* where they were also identified on foraging trails in the field as components of the territorial odour[7].

As most of these long chain compounds occurring in the Dufours gland of *A. custodiens* are not commercially available (to our knowledge), we appeal to anyone who could supply us with standards for behavioral studies to communicate with us.

1 Steyn, J. J., Mem. ent. Soc. sth. Afr. *3* (1954) 1.
2 Schreuder, G. D., and Brand, J. M., J. Ga ent. Soc. *7* (1972) 188.
3 Burger, B. V., Munro, Z., Smit, D., Schmidt, U., Wu, C.-L., and Tien, F.-C., J. Chromat. *518* (1990) 207.
4 Morgan, E. D., Analyt. chim. Acta *236* (1990) 227.
5 Silverstein, R. M., Bassler, G. C., and Morrill, T. C., Spectrometric Identification of Organic Compounds, 5th ed., p. 3. John Wiley & Sons, Inc., New York 1991.
6 Attygalle, A. B., Billen, J. P. J., Jackson, B. D., and Morgan, E. D., Z. Naturforsch. *45* (1990) 691.
7 Salzemann, A., Nagnan, P., Tellier, F., and Jaffe, K., J. chem. Ecol. *18* (1992) 183.