Belg. J. Zool. — Volume 122 (1992) — issue 1 — pages 69-74 — Brussels 1992

(Manuscript received on 18 April 1991)

ALKANOLS IN THE MANDIBULAR GLAND SECRETION OF THE ANT *TETRAMORIUM CAESPITUM*

by

E. DAVID MORGAN[1] (*), BRIAN D. JACKSON[1], SARAH J. KEEGANS[1], DEBORAH J. NICHOLLS[1], MAHMOUD FADL ALI[1] and ROGER CAMMAERTS[2]

[1]Department of Chemistry, University of Keele, Staffordshire, ST5 5BG, England

[2]Laboratoire de Biologie animale et cellulaire, Université Libre de Bruxelles, B-1050 Brussels, Belgium

SUMMARY

The madibular glands of workers of *T. caespitum* contain 4-methyl-3-hexanone and 4-methyl-3-hexanol as major substances, and nonanal, 2-pentanone, 4-methyl-3-heptanol, 4-methyl-3-octanol and 4,6-dimethyl-3-octanol as minor substances. The last three have been newly identified by mass spectrometry and their identification confirmed by synthesis. The probable biosynthetic origins of all these compounds are discussed.

Key words : Hymenoptera, Formicidae, *Tetramorium caespitum*, mandibular glands, alcohols, 4-methyl-3-hexanol.

INTRODUCTION

Many, but by no means all, species of Formicidae contain volatile chemicals in their mandibular glands. The release of these volatile chemicals has often been shown to induce alarm, aggression or attraction in nestmates of the releasers. Although some of these substances, particularly ketones and alcohols (ATTYGALLE and MORGAN, 1984a) may be found in several species in more than one subfamily, they tend to form a specific blend for each species. The correct identification of this pheromonally active secretion can at times be a useful tool in recognizing a species.

MASCHWITZ (1964) observed that crushed heads and mandibular glands of *Tetramorium caespitum* (L., 1758) released « alarm » behaviour in conspecifics. LONGHURST *et al.* (1980) examined the mandibular glands of six species of *Tetramorium* and in three of them, including *T. caespitum*, found 3-octanone as the major substance. In *T. caespitum* it was accompanied by five minor components

(*) Author for correspondence.

and one major one, all unidentified. PASTEELS *et al.* (1980) reported that heads of *T. caespitum* contained 4-methyl-3-hexanol and that heads of sexuals contained 4-methyl-3-hexanone additionally.

T. caespitum is almost indistinguishable morphologically from *T. impurum* (FOERSTER, 1850) though they are pheromonally distinguishable (ATTYGALLE and MORGAN, 1984b; BILLEN *et al.*, 1986; MORGAN and OLLETT, 1987). PASTEELS *et al.* (1981) corrected their first report when they found they had examined *T. impurum* and not *T. caespitum*. They went on to show that *T. caespitum* also contained 4-methyl-3-hexanol and 4-methyl-3-hexanone (ROISIN, unpublished data ; CAMMAERTS *et al.*, 1985). To see if the difference between the observations of LONGHURST *et al.* (1980) and CAMMAERTS *et al.* (1985) was due to possible racial differences, we examined workers from seventeen colonies of *T. caespitum* from various sites in Western Europe and found in all cases that 4-methyl-3-hexanol was usually the major substance, with small amounts of 4-methyl-3-hexanone, 2-pentanone, (as claimed there) decanal and three unidentified compounds (ALI *et al.*, 1987). In some individuals (from the same nests) there was a large amount of 4-methyl-3-hexanone, but there was no evidence of different races within the species. At the time, equipment to identify the other minor components was not available.

The mandibular glands of *T. caespitum* have now been re-examined by linked gas chromatography-mass spectrometry (GC-MS) and the minor components identified by comparison with synthetic compounds.

MATERIALS AND METHODS

Ants for the present work were collected in Dorset (from where the ants used by LONGHURST *et al.* (1980) were collected) and maintained in artificial colonies in the laboratory as described by ALI *et al.* (1987).

Samples of individual heads of workers were sealed in glass capillaries as described by MORGAN (1990) and subjected to gas chromatography-mass spectrometry using the solventless solid injection technique of MORGAN and WADHAMS (1972), with a Hewlett-Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation. Chromatography was carried out on a fused silica capillary column (12 m × 0.2 mm) coated with methyl silicone gum (equivalent to OV-1) of 0.33 m film thickness connected through a 10 m length of deactivated silica tubing (i.d. 0.32 mm) to the source of the mass spectrometer. The capillary tubes were heated in the injector for 2-3 minutes at 140°C before crushing. The injection splitter was closed for the injection and opened 30 seconds later. The carrier gas was helium at 10 psi column head pressure. The oven temperature was at 30°C for 2 min and then increased at 8°C min^{-1} to 250°C. The Mass Selective Detector was set to monitor m/z 35-350 in the scan mode (about 1.5 scans S^{-1}) under Autotune conditions using 70 eV ionization.

4-Methyl-3-heptanol, 4-methyl-3-octanol, and 4-methyl-3-nonanol were synthesized from propanal and 2-bromopentane, 2-bromohexane and 2-bromononane respectively via a Grignard reaction. 4,6-Dimethyl-3-octanol was synthesized via a

crossed aldol condensation between 2-methylbutanal and 3-pentanone (FALES *et al.*, 1980), followed by dehydration to give 4,6-dimethy-4-octen-3-one which was hydrogenated to 4,6-dimethyloctan-3-one and reduced ($NaBH_4$) to 4,6-dimethyloctan-3-ol.

RESULTS AND DISCUSSION

A typical gas chromatogram of a head of *T. caespitum* is shown in Fig. 1. The mass spectra of the three unknown compounds were all similar to that of 4-methyl-3-hexanol and had base peaks at m/z 59 (Table 1), indicating that they were 3-alkanols. On possible biosynthetic grounds, they were thought to be 4-methyl-3-heptanol, 4-methyl-3-octanol and 4-methyl-3-nonanol. The retention times and mass spectra of authentic specimens of the first two confirmed these identifications, but the third compound eluted earlier than 4-methyl-3-nonanol and the two mass spectra did not correspond. Presuming these compounds to be produced from

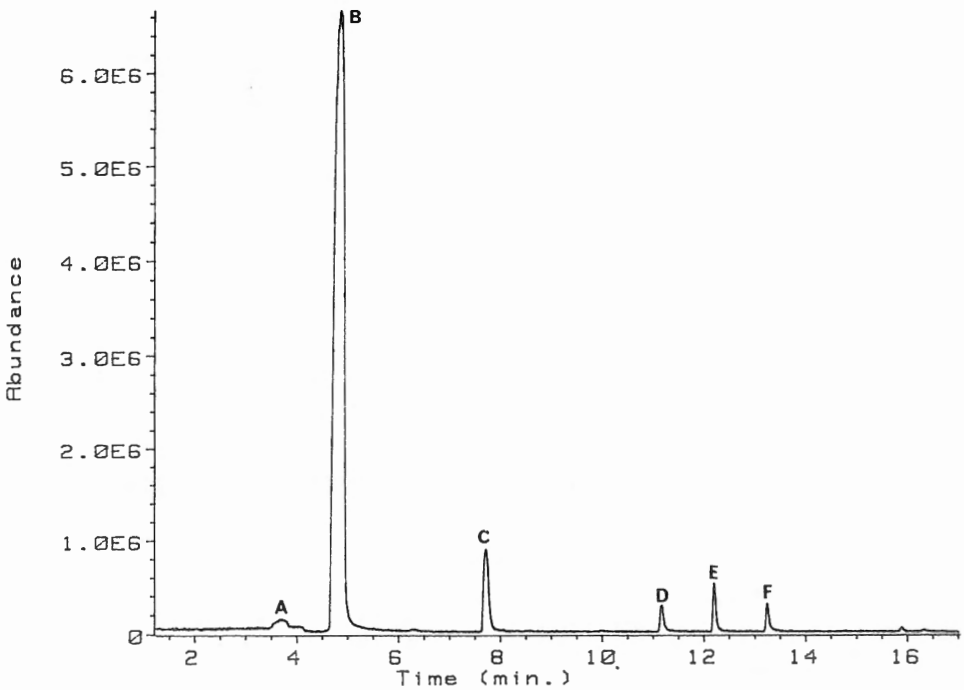

Fig. 1. — Gas chromatogram obtained from four heads of workers of *T. caespitum* without use of solvent, by the solid sampling technique. 2-Pentanone elutes under these conditions with the initial air and water peak (<2 min). The identified peaks are A, 4-methyl-3-hexanone; B, 4-methyl-3-hexanol; C, 4-methyl-3-heptanol; D, 4-methyl-3-octanol; E, nonanal; F, 4,6-dimethyl-3-octanol.

polyketides formed by mixed condensations between malonate (or acetate) and methylmalonate (or propionate) groups, the structure was now presumed to be 4,6-dimethyl-3-octanol. This too was prepared synthetically and shown to be identical in retention and mass spectrum to the mandibular compound. In our earlier paper we had claimed to identify the third minor component as decanal, but we now believe this was wrong and have confirmed, with a synthetic specimen, that it is nonanal.

The mandibular glands of *Tetramorium caespitum* from Western Europe therefore contain, in increasing molecular size, the compounds 2-pentanone, 4-methyl-3-hexanone, 4-methyl-3-hexanol, 4-methyl-3-heptanol, 4-methyl-3-octanol, nonanal and 4,6-dimethyl-3-octanol. Only 4-methyl-3-hexanone and 4-methyl-3-hexanol are found in more than minor amounts. Usually the alcohol is by far the major component, but in occasional individuals from the same nests, there is as much or more of 4-methyl-3-hexanone (ALI *et al.*, 1987).

The carbon skeletons of these compounds are familiar products of ants, other insects and their close relatives. For example, 4-methyl-3-hexanone, 4-methyl-4-hexen-3-one, 4-methyl-4-hepten-3-one, 4,6-dimethyl-4-octen-3-one and 4,6-dimethyl-4-nonen-3-one are defensive secretions of opilionids (EISNER *et al.*, 1978). Manicone, or [(4*E*)-4,6-dimethyloct-4-en-3-one] and its homologues are compounds from the mandibular gland of *Manica rubida* (BESTMANN *et al.*, 1988), and 4-methyl-3-heptanol, is the trail pheromone of the ant *Leptogenys diminuta* (ATTYGALLE *et al.*, 1988). It is also interesting to note that the absolute configuration of the compound in the mandibular glands of *T. impurum* is (3R,4S)-4-methyl-3-hexanol (PASTEELS *et al.*, 1981) and the *L. diminuta* trail pheromone is (3R,4S)-4-methyl-3-heptanol. We have reported the isolation of a series of 3-alkanones, and 3-alkanols from the mandibular glands of *Myrmica* ants (CAMMAERTS *et al.*, 1983 and earlier papers cited therein). These 3-alkanones form a regular series from 3-hexanone to 3-dodecanone, but include 6-methyl-3-octanone.

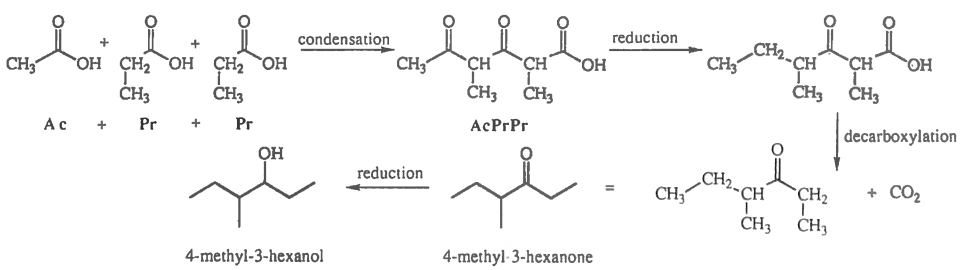

Fig. 2. — The proposed biosynthetic route to the mandibular gland compounds, illustrated for the examples of 4-methyl-3-hexanone and 4-methyl-3-hexanol. For all the compounds found in *T. caespitum* except 2-pentanone, the two right hand units (PrPr) are fixed and variation of the left hand unit (Here Ac), to Pr, AcAc and AcPr gives the other compounds identified.

No biosynthetic studies have been carried out on any of these compounds. It is difficult to see how any of them can arise by simple polyketide synthesis from acetate, but it is possible to propose routes to all of them by simple biosynthetic steps from a combination of acetate (Ac) and propionate (Pr) units (or malonate and methylmalonate), as illustrated in figure 2. The presumed polyketide intermediates can give 2− or 3−alkanones by β-ketoacid decarboxylation. The *T. caespitum* mandibular compounds may be formed in the following ways : 2-pentanone by head-to-tail condensation of three acetate units (AcAcAc) followed by reduction and decarboxylation, 4-methyl-3-hexanol arises from AcPrPr, 4-methyl-3-heptanol from PrPrPr, 4-methyl-3-octanol from AcAcPrPr, and 4,6-dimethyl-3-octanol from AcPrPrPr. Except for 2-pentanone, the final two units of each of these *Tetramorium* compounds are both Pr. The *Myrmica* mandibular gland compounds are postulated to be formed by the following combinations; AcAcPr, PrAcPr, AcAcAcPr, AcPrAcPr, PrAcAcPr, AcAcAcAcPr, PrAcAcAcPr, and AcAcAcAcAcPr. In this group the final two units are always Ac and Pr, once these are fixed, the remainder of the compound appears to be made up of a random mixture of one, two or three acetate or propionate units, to give the various ketones or alcohols.

ACKNOWLEDGEMENTS

We thank P.J. Wright for collecting colonies of ants, the Egyptian Government for a studentship to M.F. Ali and Sience and Engineering Research Council for studentships to B.D. Jackson and S.J. Keegans. The GC-MS equipment was purchased with a grant from the Sience and Engineering Research Council to E.D. Morgan.

REFERENCES

ALI M.F., R. CAMMAERTS, R.P. EVERSHED and E.D. MORGAN (1987) — A re-examination of the mandibular gland contents of *Tetramorium caespitum* (Hymenoptera : Formicidae). *Insect Biochem.*, **17** : 237-241.

ATTYGALLE A.B. and E.D. MORGAN (1984a) — Chemicals from the glands of ants. *Chem. Soc. Revs.*, **13** : 245-278.

ATTYGALLE A.B. and E.D. MORGAN (1984b) — Identification of trail pheromone of the ant *Tetramorium caespitum* L. (Hymenoptera : Myrmicinae). *J. Chem. Ecol.*, **10** : 1453-1468.

ATTYGALLE A.B., O. VOSTROWSKY, H.J. BESTMANN, S. STEGHAUS-KOVAC and U. MASCHWITZ (1988) — (3R,4S)-4-methyl-3-heptanol, the trail pheromone of the ant *Leptogenys diminuta*. *Naturwiss.*, **75** : 315-317.

BESTMANN H.J., A.B. ATTYGALLE, J. GLASBRENNER, R. RIEMER, O. VOSTROWSKY, M.G. CONSTANTINO, G. MELIKIAN and E.D. MORGAN (1988) — Identification of the mandibular gland secretion of the ant *Manica rubida* : structure elucidation, synthesis and absolute configuration of manicone. *Liebigs Annalen* : 55-60.

BILLEN J.P.J., R.P. EVERSHED, A.B. ATTYGALLE, E.D. MORGAN and D.G. OLLETT (1986) — The contents of the Dufour glands of workers of three species of *Tetramorium* (Hymenoptera : Formicidae). *J. Chem. Ecol.*, **12** : 669-685.

CAMMAERTS M.C., R.P. EVERSHED and E.D. MORGAN (1983) — The volatile components of the mandibular gland secretion of workers of the ants *Myrmica lobicornis* and *Myrmica sulcinodis*, *J. Insect Physiol.*, **29** : 659-664.

CAMMAERTS R., J.M. PASTEELS and Y. ROISIN (1985) — Identification et distribution de *Tetramorium caespitum* (L.) et *T. impurum* (Foerster) en Belgique (Hymenoptera : Formicidae). *Actes Coll. Insectes Soc.*, **2** : 109-118.

EISNER T., D. ALSOP and J. MEINWALD (1978) — Secretions of opilionids, whip scorpions and pseudoscorpions, in *Arthropod Venoms* (S. BETTINI, Ed.) p. 87-99 Springer, Berlin.

FALES H.M., T.M. JAOUNI, J.O. SCHMIDT and M.S. BLUM (1980) — Mandibular gland allomones of *Dasymutilla occidentalis* and other mutillid wasps. *J. Chem. Ecol.*, **6** : 895-903.

LONGHURST C., R. BAKER and P.E. HOWSE (1980) — A comparative analysis of mandibular gland secretions in the ant tribe Tetramorini. *Insect Biochem.*,**10** : 107-112.

MASCHWITZ U. (1964) — Alarm substances and alarm behaviour in social Hymenoptera *Nature* (London), **204** : 324-327.

MORGAN E.D. (1990) — Preparation of small scale samples from insects for chromatography. *Anal. Chim. Acta*, **236** : 227-235.

MORGAN E.D. and D.G. OLLETT (1987) — Methyl 6-methylsalicylate, trail pheromone of the ant *Tetramorium impurum*. *Naturwissen.*, **74** : 596-597.

MORGAN E.D. and L.J. WADHAMS (1972) — Gas chromatography of volatile compounds in small samples of biological materials. *J. Chromatogr. Sci.*, **10** : 528-529.

PASTEELS J.M., J.C. VERHAEGHE, J.C. BRAEKMAN, D. DALOZE and B. TURSCH (1980) — Caste-dependent pheromones in the head of the ant *Tetramorium caespitum.*, *J. Chem. Ecol.*, **6** : 467-472.

PASTEELS J.M., J.C. VERHAEGHE, R. OTTINGER, J.C. BRAEKMAN and D. DALOZE (1981) — Absolute configuration of (3R,4S)-4-methyl-3-hexanol — a pheromone from the head of the ant *Tetramorium impurum* Foerster. *Insect. Biochem.*, **11** : 675-678.