COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Biosynthesis of the insect pheromone (*S*)-4-methyl-3-heptanone

Andrew P. Jarvis,[a] Jürgen Liebig,[b] Bert Hölldobler[b] and Neil J. Oldham*[ac]

[a] *Max-Planck-Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll-Straße 8, D-07745 Jena, Germany*

[b] *Lehrstuhl Verhaltensphysiologie und Soziobiologie (Zoologie II), Biozentrum, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany*

[c] *Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, UK OX1 3TA. E-mail: neil.oldham@chem.ox.ac.uk*

Received (in Cambridge, UK) 3rd March 2004, Accepted 22nd March 2004
First published as an Advance Article on the web 14th April 2004

Using stable isotope-labelled probes and mass spectrometry, the insect pheromone (*S*)-4-methyl-3-heptanone is shown to be biosynthesised from three propionate units following a polyketide/fatty acid-type metabolic route.

Simple 3-ketones (Fig. 1) are common secondary metabolites in insects and other arthropods, where they serve a range of communicatory and ecological functions.[1] Ketones **1**–**5** have all been identified in various exocrine glands of ants, with (*S*)-4-methyl-3-heptanone (**1**) exhibiting particularly widespread taxonomic distribution. Ketone **1** is usually located in the mandibular glands and serves as an alarm pheromone, but, in at least one species (*Aphaenogaster albisetosus*), it is stored in the poison gland and is used to coordinate nestmate recruitment to food sources.[2] In addition to its role as a pheromone, ketone **1** is employed by opilionids (Arachnida) as a defensive allomone against ants.[3] Moreover, in the interaction between the ant *Paraponera clavata* and its parasite *Apocephalus paraponerae* (Diptera), there is evidence that **1** functions as a kairomone (a semiochemical that disfavours the emitter and benefits another organism).[4]

In comparison with plants and microorganisms, very little is known about the biosynthesis of secondary metabolites in insects. The route to 3-ketone **1** and its relatives, for example, has never been investigated. It has been proposed that these simple alkanones are aceto/propiogenins.[5] Indeed, it is easy to see how all the structures in Fig. 1 can be assembled from the condensation of acetate and/or propionate units. A potential route to 4-methyl-3-heptanone (**1**) is shown in Scheme 1. A starter unit of propionyl–SEnz is condensed with methylmalonate to yield diketide **6**. Following total reduction of the β-keto group (by the action of a putative ketoreductase, dehydratase and hydrogenase), a second methylmalonate is incorporated to give triketide **8**. Hydrolysis of the thioester and decarboxylation then results in the production of methyl ketone **1**. Thus, although **1** has only eight carbons, it is assembled from three C_3 units.

Here, we report a study on the biosynthesis of **1** in the ant *Harpegnathos saltator*[6] using stable isotope-labelled probes together with mass spectrometric (MS)-based detection. [$^2H_3$]Methylmalonic acid and [$^2H_3$]methyl[1,3-$^{13}C_2$]malonic acid were synthesised and introduced into the diet of *H. saltator*.† GC/MS analysis of the mandibular gland contents from individual treated ants revealed clear incorporation of labelling. Combining MS scans over the entire GC peak of 4-methyl-3-heptanone resulted in a spectrum containing ions from a number of isotopomers (Fig. 2). A maximum of nine ^2H atoms, in multiples of three, were incorporated into ketone **1**, a result consistent with the biogenetic origin outlined in Scheme 1. As a consequence of the higher volatility of [$^2H_9$]-**1** over isotopomers bearing fewer deuterium atoms, it was possible to achieve partial GC resolution of this species, such that

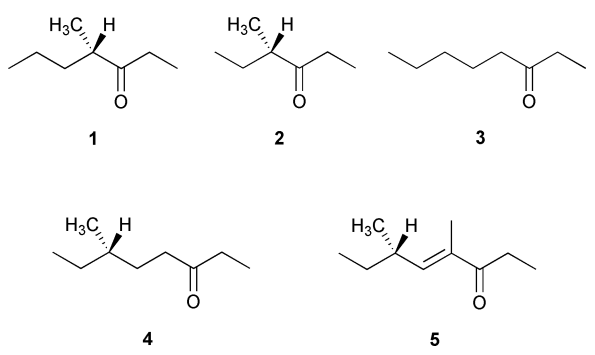
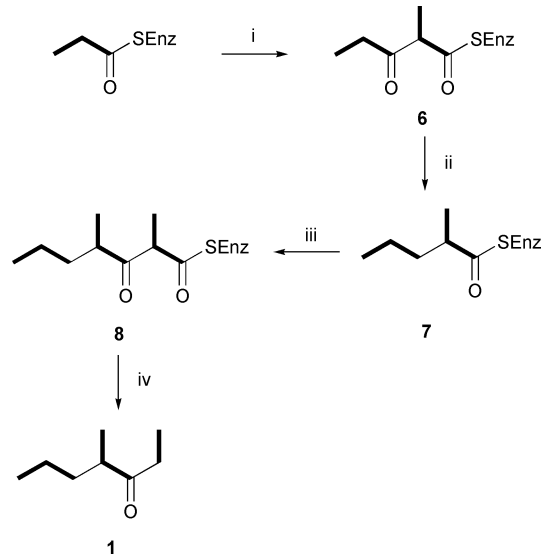


Fig. 1 Examples of 3-ketones found in insects and other arthropods.



Scheme 1 Proposed biosynthetic route to **1**: (i) methylmalonate, $-CO_2$; (ii) reduction, dehydration, reduction; (iii) methylmalonate, $-CO_2$; (iv) thioester hydrolysis, $-CO_2$.

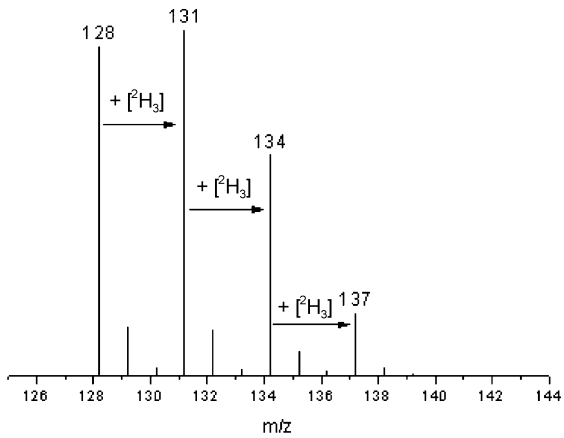


Fig. 2 Molecular ion region of the mass spectrum of ketone **1** from *H. saltator* following exposure to [$^2H_3$]methylmalonic acid. $M^{\cdot+}$ for unlabelled **1** (*m*/*z* 128) is accompanied by signals due to incorporation of one, two and three [$^2H_3$]methyl groups.

DOI: 10.1039/b403186e

a pure spectrum of [$^2H_9$]-**1** could be obtained. Similarly, upon treatment of the ants with [$^2H_3$]methyl[1,3-$^{13}C_2$]malonic acid, a clean spectrum of [$^2H_9$][$^{13}C_2$]-**1** was recorded (Fig. 3). Comparison of the *m*/*z* values for fragment ions from unlabelled [Fig. 3(A)] and labelled [Fig. 3(B) and (C)] **1** revealed a deuterium labelling pattern consistent with the structures in Fig. 3. Moreover, α-cleavage either side of the C=O group uniquely identified C3 as the site of one of the ^{13}C labels in Fig. 3(C). Strictly, the second ^{13}C could possibly have resided at C5 or C6, as the fragmentation of ketone **1** did not distinguish between these positions. Only C5, however, exhibits a 1,3-relationship with two [$^2H_3$] groups, retaining the relative position of labels seen in the malonate precursor. Thus, it is highly probable that the second ^{13}C was located at C5.

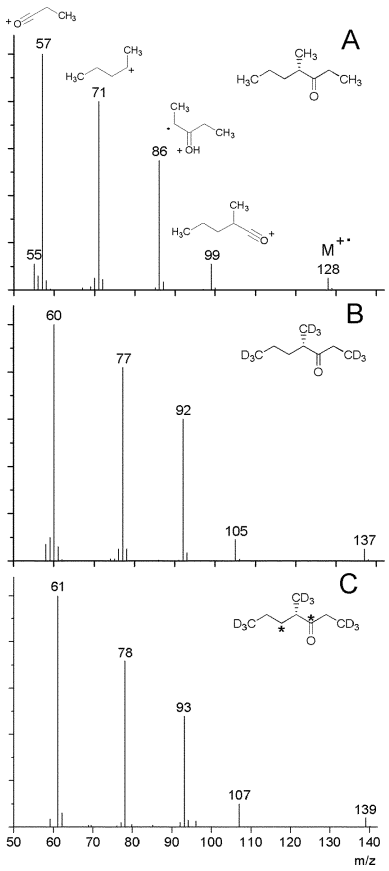


Fig. 3 Mass spectra of ketone **1** from *H. saltator*: (A) without treatment; (B) following exposure to [$^2H_3$]methylmalonic acid; (C) following exposure to [$^2H_3$]methyl[1,3-$^{13}C_2$]malonic acid. The asterisks on the structure indicate ^{13}C.

The labelling patterns observed in **1** (Fig. 3) demonstrate that this ketone is produced from three propionate building blocks, with loss of C1 from one C_3 unit. These results provide the first evidence to support the proposed biosynthetic route shown in Scheme 1 and demonstrate that 4-methyl-3-heptanone is a product of polyketide/fatty acid-type metabolism. The notion that related ketones are also produced by this general route is supported by the observation that labelling from [$^2H_3$]methylmalonic acid was incorporated into 4-methyl-3-hexanone (**2**), a trace component in the mandibular glands of *H. saltator*. In this case, the Me branch and C1 were labelled with [$^2H_3$], but the C6 Me group remained unlabelled (data not shown). This result is consistent with a mixed acetate/propionate origin for **2**, where C5 and C6 stem from acetate and the remaining carbons are propionate-derived.

In summary, we have shown that simple ketones (Fig. 1) can be synthesised by insects, using the polyketide/fatty acid pathway, and stored in exocrine glands for use as semiochemicals.

We gratefully acknowledge the technical support of Janine Rattke and funding from the Max-Planck-Gesellschaft.

Notes and references

† Labelled methylmalonic acids were synthesised from [1,3-$^{13}C_2$]- or unlabelled dimethyl malonate and [$^2H_3$]iodomethane in NaOMe/MeOH, followed by saponification. Aqueous solutions of the labelled probes (5 μl at 0.1 g ml^{-1}) were injected into crickets (pre-paralysed by *H. saltator* venom) and the ant colonies fed on a diet of three treated crickets per week. After three weeks, callow worker ants were dissected and their mandibular glands extracted individually in dichloromethane (10 μl) before GC/MS analysis. Approximately 10% of samples revealed incorporation into **1**.

1 M. S. Blum, *Chemical Defenses of Arthropods*, Academic Press, New York, 1981, p. 138.
2 B. Hölldobler, N. J. Oldham, E. D. Morgan and W. A. König, *J. Insect Physiol.*, 1995, **41**, 739.
3 T. Eisner, D. Alsop and J. Meinwald, in *Handbook of Experimental Pharmacology*, ed. S. Bettini, Springer-Verlag, Berlin, 1978, **vol. 48**, p. 89.
4 D. H. Feener, L. F. Jacobs and J. O. Schmidt, *Anim. Behav.*, 1996, **51**, 61.
5 E. D. Morgan, B. D. Jackson, S. J. Keegans, D. J. Nicholls, M. F. Ali and R. Cammaerts, *Belg. J. Zool.*, 1992, **122**, 69; W. Francke and S. Schulz, in *Comprehensive Natural Products Chemistry*, ed. D. Barton, K. Nakanishi and O. Meth-Cohn, Pergamon, Oxford, 1999, **vol. 8**, p. 197.
6 R. R. Do Nascimento, J. Billen and E. D. Morgan, *Comp. Biochem. Physiol., B*, 1993, **104**, 505.