Biogenesis of sex pheromones in the female olive fruit-fly

Natasha L. Hungerford,[a] Basilis E. Mazomenos,[b] Maria A. Konstantopoulou,[b] Fragoulis D. Krokos,[b] George E. Haniotakis,[b] Achim Hübener,[a] Mary T. Fletcher,[a] Christopher J. Moore,[c] James J. DeVoss[a] and William Kitching*[a]†

[a] *Department of Chemistry, The University of Queensland, Brisbane, Qld. 4072 Australia*
[b] *Biology Department, NCSR 'Demokritos', Aghia Paraskeri, Attikis, Greece*
[c] *Department of Primary Industries, Yeerongpilly, Qld. 4105, Australia*

A likely pathway to the sex pheromones of *Bactrocera oleae* (olive fruit-fly) is presented, based mainly on feeding experiments with deuterium labelled precursors.

There is little knowledge of the chemistry, enzymology or molecular biology of the biosynthesis of fruit-fly pheromones which could be linked with the known chemistry[1,2] of these species to facilitate species-specific monitoring and control. We now describe experiments with the pestilent species, *Bactrocera oleae* (olive fruit-fly),[1,3] that permit a proposal for the biogenesis of the (female) pheromone, which is essentially one component.[4] This suggests the operation of a single, major biosynthetic pathway for study, and possible disruption.

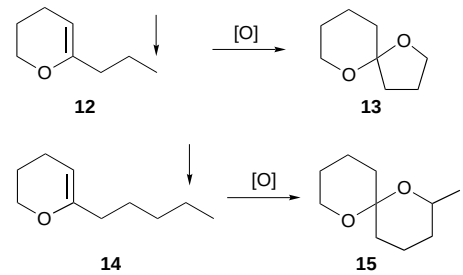
The major component of the pheromone is racemic 1,7-dioxaspiro[5.5]undecane **1**[5] which is accompanied by low levels (*ca.* 3%) of hydroxy derivatives **2**–**4**.[6] We selected 6-*n*-butyl-3,4-dihydro-2*H*-pyran **5**[7] for investigation as an advanced precursor of **1**[8] for the following reasons. Although **5** has not been identified in the olive fruit-fly, it co-occurs with **1** in *B. cacuminata*,[9] in which keto alcohol **6a** also occurs; dehydration of the corresponding hemiketal **6b** would afford **5**. Secondly, 2-methyl-6-*n*-pentyl-3,4-dihydro-2*H*-pyran **8**[10] and keto alcohol **9** accompany isomers of 2,8-dimethy-1,7-dioxaspiro[5.5]undecane **10** in *B. halfordiae* and *B. kraussii*,[2] so that the nexus that applies to **5** and **1** also applies to **8** and **10**. Appropriate side-chain hydroxylation of these dihydropyrans (**5** and **8**) followed by cyclisation would yield the corresponding spiroacetals **1** and **10**.

A [$^2H_3$]-labelled analogue of **5**, *viz* **11**, was prepared from [$^2H_6$]-propanone,[11] and essentially complete deuteration at the indicated positions in **11** was confirmed by ^{1}H and ^{13}C NMR and mass spectral analyses.‡ Examination by GC–MS of the rectal glandular contents of female olive flies, subsequent to the receipt of diet-administered **11**, established that **1** was now significantly ^{2}H-enriched, as were the accompanying hydroxyspiroacetals **2**–**4**, and the fragmentation patterns confirmed that deuterium was located at the anticipated positions α to the spiro centre. In this and in other cases described here, variation is isotopic composition for **1** was observed by progressive mass spectral examination from the foreside to the centre of the GC peak. More highly deuterated species elute earlier.[11] These examinations showed at least 40% of **1** incorporated deuterium. It is possible that *in vivo* hydration of **5** to form the hydroxy ketone **6a** (or the corresponding cyclic hemiketal **6b**) provided the real substrate for methyl oxidation. Thus, **7** (a deuterium analogue of **6**) was synthesised and administered and resulted in efficient formation of labelled **1**, indicating that **6** is also a possible penultimate precursor of **1**.

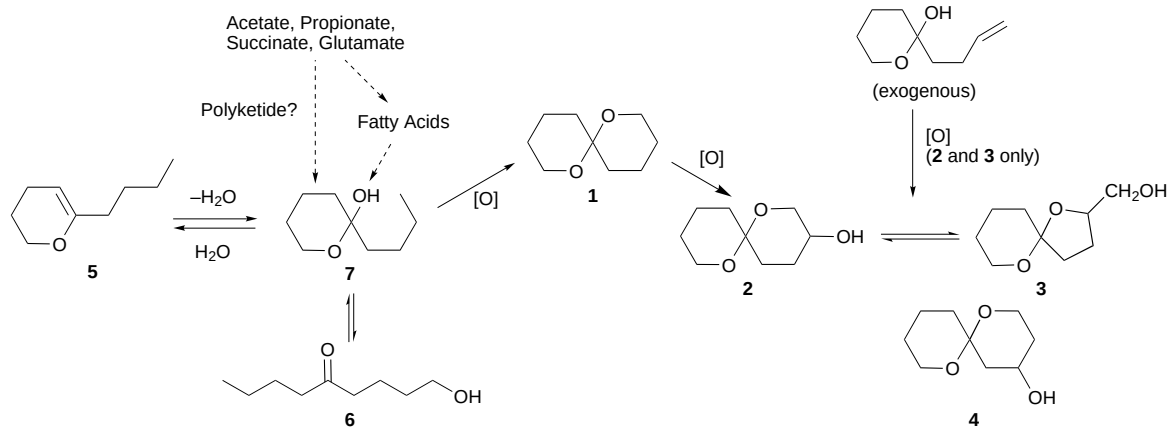
To explore the nature and specificity of the presumed side-chain oxidation, dihydropyrans **12** and **14** were also administered to female olive flies and inefficient oxidation (compared with **11**) occurred, to provide low levels of the known spiroacetals **13** and **15**,[2] respectively (Scheme 1). To the best of our knowledge these have never been detected in olive fruit-flies under natural conditions. Labelled alkenes **16** and **17**, which correspond to possible precursors of the hydroxyspiroacetals **2** and **3**, were also administered to whole female *B. oleae*. Analyses confirmed that deuterium from **16** or, more effectively, **17** was specifically incorporated into **2** and **3**, but not **4** or the parent spiroacetal **1**. This outcome is shown in Scheme 2.

The proportion of labelled **2** and **3** relative to **1** and **4** increased to a level twenty times higher when **17**, but not **16**, was administered. This supports the view that the penultimate step in the biosynthesis of **1** in *B. oleae* is ω-oxidation of **6**, followed by cyclisation. Significantly, the results for **12**, **14**, **16** and **17** demonstrate that exogenous compounds can access the enzymes of the biosynthetic sequence, a crucial feature if enzyme inhibitors[12] are to be devised for pest control.

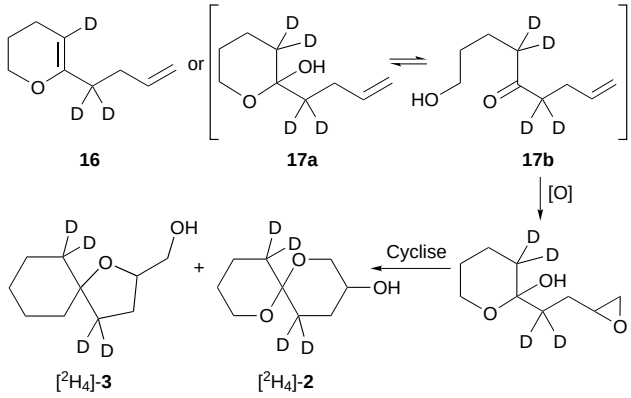
With respect to the origin of the minor co-occurring hydroxyspiroacetals **2**–**4**, we considered these might arise from hydroxylation of intact, initially formed spiroacetal **1**. To test this proposal, [5,5,11,11-$^2H_4$]-**1** was synthesised[13] and administered, and GC–MS analysis confirmed the efficient formation



Scheme 1



Scheme 3



Scheme 2

of labelled **2**–**4**. The enantiomeric compositions of the hydroxy spiroacetals from this trial group closely matched those from the control group.[13,14] In contrast to this, the enantiomeric profile of the hydroxy derivatives resulting from administration of **16** or **17** was quite different. For example, **3** from the control group was predominantly (2*R*,5*R*) and (2*R*,5*S*) (20% ee), but was predominantly (2*S*,5*S*) and (2*S*,5*R*) (60% ee) for those derived from **17**.[13] This indicates that epoxidation of an unsaturated precursor is an unlikely natural route to **2** and **3**. We believe that **2**–**4** are hydroxylation products of intact **1** (or a chemically equivalent species).[15]

In summary, the above results are consistent with ω-hydroxylation of **6** (or possibly **5**) followed by dehydrative spirocyclisation to yield **1**. The remarkable, highly regioselective oxidation (compare results from **5**, **12** and **14**) of a remote Me or CH_2 group is reminiscent of similar oxidations mediated by cytochrome P450s in other eucaryotic systems.[16] This class of enzymes is known to occur in insects, mediating important transformations such as pesticide detoxification.[17]

With this framework established (Scheme 3), we are now directing attention to the origin of **6**, the likely precursor of **1**, and to establishing the generality of the biosynthetic relationship between keto alcohols and the corresponding spiro acetals, *e.g.* **9** and **10**. Keto alcohols **6** and **9** could conceivably arise by either a fatty acid or polyketide pathway and experiments to differentiate these are being conducted. In this context, it is of interest that labelled nonanoic acid and 5-oxononanoic acid were not incorporated into **1** in *B. oleae*. The isolation and characterisation of the likely P450 enzyme in *B. oleae* and *B. cacuminata* are also being pursued.

We are grateful to the Australian Research Council for financial support.

Notes and References

† E-mail: kitching@chemistry.uq.edu.au

‡ All new compounds exhibited appropriate ^{1}H and ^{13}C NMR spectra and high resolution mass spectra.

1 H. Kuba, *Proc. Internat. Symp. Biological Control of Fruit Flies*, ed. K. Kawasaki, O. Iwahashi and K. Y. Kaneshiro, Okinawa, Japan, 1991, p. 214. See also *Pheromone Biochemistry*, ed. G. D. Prestwich and G. J. Blomquist, Academic Press, Orlando, 1987.
2 M. T. Fletcher and W. Kitching, *Chem. Rev.*, 1995, **95**, 789.
3 B. E. Mazomenos, *World Crop Pests: Fruit Flies. Their Biology, Natural Enemier and Control*, ed. A. S. Robinson and G. H. S. Cooper, Elsevier, Amsterdam, 1989.
4 For a summary, see ref. 2.
5 R. Baker, R. Herbert, P. E. Howse, O. T. Jones and W. Francke, *J. Chem. Soc., Chem. Commun.*, 1980, 52.
6 R. Baker and R. H. Herbert, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1123.
7 W. Francke, W. Mackenroth, W. Schröder, S. Schulz, J. Tengö, E. Engels, W. Engels, R. Kittmann and D. Schneider, *Z. Naturforsch. Teil C*, 1985, **40**, 145.
8 Previously, Mazomenos had reported that the sodium salts of [2-^{14}C]-acetate, [2-^{14}C]malonate, [2-^{14}C]propionate and [2,3-^{14}C]succinate and L-[U-^{14}C]glutamate were incorporated into **1** from *B. oleae*, and a biosynthetic proposal based on 5-oxo-1,9-nonanedioic acid was presented. See ref. 3 and J. G. Pomonis and B. E. Mazomenos, *Int. J. Invert. Reprod. Devel.*, 1986, **10**, 169.
9 S. Krohn, M. T. Fletcher, W. Kitching, R. A. I. Drew, C. J. Moore and W. Francke, *J. Chem. Ecol.*, 1991, **17**, 485.
10 M. V. Perkins, M. F. Jacobs, W. Kitching, P. J. Cassidy, J. A. Lewis and R. A. I. Drew, *J. Org. Chem.*, 1992, **57**, 365; M. T. Fletcher, J. A. Wells, M. F. Jacobs, S. Krohn, W. Kitching, R. A. I. Drew, C. J. Moore and W. Francke, *J. Chem. Soc., Perkins Trans. 1*, 1992, 2827.
11 A. B. Attygalle, J. Meinwald and T. Eisner, *Tetrahedron Lett.*, 1991, **37**, 4849.
12 Terminal alkynes are mechanism-based irreversible inhibitors of cytochrome P-450s (see P. R. Ortiz de Montellano and M. Correia, *Inhibition of Cytochrome P450 Enzymes*, in *Cytochrome P450*, ed. P. R. Ortiz de Montellano, Plenum, New York, 1995, ch. 9, p. 305 and experiments with the alk-1-yne anologue of **17** are being planned.
13 For a discussion, see N. L. Hungerford, PhD Thesis, The University of Queensland, 1997.
14 M. T. Fletcher, M. F. Jacobs, W. Kitching, S. Krohn, R. A. I. Drew, G. E. Haniotakis and W. Francke, *J. Chem. Soc., Chem. Commun.*, 1992, 1457.
15 G. D. Prestwich, *Pure Appl. Chem.*, 1989, **61**, 551; W. Francke, F. Schröder, P. Philipp, H. Meyer, V. Sinwell and G. Gries, *Bioorg. Med. Chem.*, 1996, **4**, 363.
16 R. Feyeresien, *Cytochrome P450*, ed. J. B. Schenkmna and H. Greim, Springer, Heidelberg, 1993, pp. 311–314.
17 E. Hodgson, *Microsomal Mono-Oxygenases*, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, ed. G. A. Kerkut and L. I. Gilbert, 1985, vol. 11, p. 225; P. F. Dowd, C. M. Smith and T. C. Sparks, *Insect Biochem.*, 1983, **13**, 453.

Received in Cambridge, UK, 26th January 1998; 8/00691A