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CHEMISTRY AS AN AID IN THE STUDY OF SOCIAL INSECTS

par

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Resumé: La recherche chimique en tant qu'aide dans l'étude des insects sociaux.

Une collaboration efficace entre la recherche biologique et la recherche chimique peut améliorer et faire progresser nos connaissances sur l'écologie et l'éthologie des insectes sociaux. La caractérisation d'importantes substances actives biologiquement peut-être facilitée par une récolte adéquate des substances en s'assurant qu'elles ne sont ni diluées ni contaminées par des solvants. Des exemples sont donnés grâce auxquels l'étude des substances chimiques peut stimuler de nouvelles recherches biologiques et permette des études chimiques appropriées a' l'aide de tests biologiques. La valeur d'une bonne collaboration entre chimistes et biologistes est rehaussée par le succès de leur travail d'équipe.

Mots-clés: *Hymenoptera, échantillonage chimique, sécrétions glandulaires, test biologique, collaboration.*

Summary: The pursuit of knowledge of the ecology and ethology of social insects can be improved and advanced by good collaboration between Biology and Chemistry. The careful collection of chemicals and the avoidance of dilution and contamination with solvents can make easier the identification of important biologically active substances. Examples are given whereby chemical studies can

stimulate new biological investigations and where a biological assay can stimulate appropriate chemical studies. The value of good collaboration between chemist and biologist is emphasized for the successful conclusion of their work together.

Key words: *Hymenoptera, chemical sampling, glandular secretions, bioassay, collaborative research.*

We are at a period of great advances in science, but it is in the little-known territories between the traditional divisions of science, that the most rapid advances are taking place. It is through the interaction of Chemistry and Biology, or Physics and Biology that exciting discoveries are being made (Fig. 1).

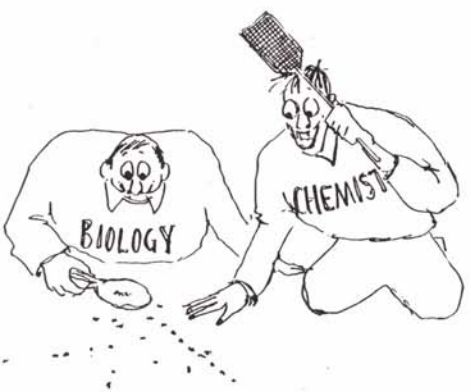


Fig. 1. Chemistry helping Biology

For example, the techniques and instruments of Chemistry for identification of very small amounts of organic substances have advanced far in recent years. Social insect communication and defense for the greater part rely on chemical secretions of various glands. Chemistry is therefore now well placed to aid the study of social insects.

We may identify three areas to illustrate this interaction between Chemistry and Biology that will aid our understanding of the ecology and ethology of this fascinating group of insects. First, to show how the collection of biological material for chemical examination can be more efficient and more informative. Secondly, to look at some examples where a chemical viewpoint can illuminate biology, and

thirdly to consider how biological discoveries can stimulate new chemical investigations.

The traditional method of obtaining samples for chemical examination is by dissolving or extracting biological material with a solvent. It has many disadvantages. It dilutes the sample, introduces impurities, masks components and usually, as a consequence requires rather large amounts of material to be extracted. Various investigators have described methods to avoid use of solvents, some of them are described by Golub and Weatherstone (1984). We have used for over 15 years our own invention of a solid injector, (Morgan and Wadhams 1972). A gland or piece of tissue is dissected, placed inside a small glass capillary tube and sealed in a flame. The sample can then be safely kept or transported by post and analysed when convenient. For analysis, the capillary tube is placed inside a stainless steel device which is fitted into the injection area of a gas chromatograph. It is heated until the contents of the capillary have been raised to 140 to 240°C (depending upon the stability and volatility of the substances being studied), a plunger is depressed which crushes the glass tube, causes the tissue to disintegrate through formation of steam and all volatile materials are flushed onto the chromatograph and separated in the usual way (for further description, see Attygalle and Morgan, 1988).

Numerous examples of the use of this method with ant glands can be found in our publications (c.f. Ali *et al.*, 1987), but the method has also been used for sex pheromone glands from lepidopterans (Attygalle *et al.*, 1987). Advantages of this method include being able to collect material in the field, remote from the laboratory. All that is required is a supply of glass capillary tubing cut into short lengths, a microscope for dissection and a small flame to seal the glass capillaries. In addition, it can be advantageous to send the samples to a suitably equipped laboratory anywhere in the world, and to store them there until the analyses can be performed. The contents of a single gland can be analysed (in practice, we find

the limit for detection and identification is about 1 ng), and by using a still finer capillary tube, samples of secretion, such as cornicle secretions of aphids, can be collected as they are produced, or a glandular reservoir can be pierced with the capillary, and the contents collected without any contamination with tissue, or secretion can be drawn from an intersegmental gland.

Because single glands or individual insects can be examined in this way, far less insect material is required, the variation from one individual to another within a species or colony can be judged, and a greater variety of studies can be conducted, than when large numbers of insects are required.

Micro-chemical methods can also be used for sampling the air in a nest or near a nest. In some early (unpublished) work we were surprised to find quite large quantities of 3-octanol and 3-octanone (the chief substances from the mandibular glands) in the air over laboratory colonies of *Myrmica scabrinodis*. We still do not know what this means in terms of behaviour. One can also collect material laid on the substrate, as has been done recently, in the identification of the trail pheromone of tent caterpillars (Crump *et al.*, 1987). Flower extracts, faecal material, extracts of prey, larvae, eggs and dead congeners can all be collected in studies to learn more about the interactions of insects with all these sources of odour.

With these collected materials, the chemist performs gas chromatography to separate the components, to find how many substances there are and how much of each one, and by mass spectrometry and other micro-chemical methods (Attygalle and Morgan, 1988) to identify each one. The chemist can then report back to the biologist on which substances, their amounts and proportions are present in the gland, tissue, exudate or surface. Then, sometimes with the aid of a colleague expert in methods of synthesis, the chemist hopes to provide the biologist with these substances in a pure state, and in mixtures in the correct proportions and concentrations as they are found in nature, for behavioural studies.

Chirality or 'handedness' is a property important in many pheromones. When four different atoms or groups are attached to one carbon atom in a molecule, that carbon atom is said to be chiral, and the compound can exist in two chiral forms. These two chiral forms can be considered like left and right hands. They are similar in form and identical in most properties, but one form is the mirror image of the other. Frequently, when pheromone molecules are chiral, we find only one form in nature, and the other chiral form may be inactive, or may reduce or cancel the behavioural effect of the natural one (Silverstein, 1985). It is therefore important to know about this property before biological tests. Such chiral forms are identical in almost all their chemical properties. There are no simple ways of detecting chirality in compounds, other than measuring the way the compound rotates the plane of polarized light in a polarimeter. However, for most pheromones we never have sufficient material to make this measurement. Therefore indirect methods must be devised. To extend the analogy, although a left hand and right hand are very similar, if we combine each of these with another chiral object, namely a right-handed glove, then we produce two things which look quite different; a left hand in a right-handed glove and a right hand in a right-handed glove. These are not related like mirror images and are separable. Nature is an abundant source of chiral substances in which only one of the two possible forms is produced. We can use these substances as reagents to combine with a pheromone, using only minute amounts to make new derivatives, which can be separated by chromatography and so the chirality of the natural pheromone can be deduced. Our work on the chirality of 3-octanol of *Myrmica* ants (Attygalle *et al.*, 1983; Cammaerts *et al.*, 1985) and that of Cammaerts *et al.* (this volume; also Bestmann *et al.*, 1987) on manicone from *Manica rubida* provide examples.

Next we should look at some examples that show how this work of chemical analysis and identification is of real help to the biologist.

A simple example is as an aid in taxonomy. There frequently occur two species which are so similar that it is difficult to tell them apart morphologically, but the species may have different pheromone blends. All ants so far studied (some 50 species) have oily chemicals in their Dufour glands. These Dufour gland chemicals are characteristic for that species in almost every case. *Formica cunicularia* and *F. rufibarbis* are a pair of species of very similar morphology which can be distinguished by their Dufour gland chemicals (Billen *et al.*, 1983), as are *Formica fusca* and *F. lemani* (Ali *et al.*, 1987). The myrmicines *Tetramorium caespitum* and *T. impurum* are so similar, they can only be distinguished by their male genitalia, but are easily distinguished by their Dufour glands (Billen *et al.*, 1986) and their trail pheromones (Morgan and Ollett, 1987). Bagneres (this volume) gives an example of colony and species specificity of cuticular hydrocarbons and Everaerts (this volume) similarly gives examples of chemical taxonomy in termites.

Chemistry may also be of use in the wider question of systematics. Billen (this volume) describes recent work on the primitive Australian ant *Nothomyrmecia macrops*, which stands as the solitary member of the subfamily Nothomyrmeciinae, separated from its possible relatives the Myrmeciinae. Chemistry can at least add its evidence to any debate over classification.

Chemistry may independently turn up new facts that stimulate biological examination. We have recently found that the major and minor subcastes of workers of the desert ant *Camponotus aegyptiacus* have distinctly different Dufour gland compositions (Ali and Morgan, unpublished results). We hope this can provide the nucleus of some study on polyethism in the species.

The slave-making species *Harpagoxenus sublaevis* has very large worker Dufour glands, which are used in some way in the enslaving process. We have found that this gland contains (E)-β-farnesene (Ollett *et al.*, 1987) a substance well-known as an aphid alarm pheromone, but not previously encountered among

ants. The evidence points strongly towards this substance being involved in slave-raiding and awaits behavioural examination.

With the very small number of insects required with the solvent-less methods, it has been possible to carry out the first study on the filling of the Dufour gland in very young adult workers of *F. sanguinea*, which has shown how slowly the gland, empty at emergence, fills with secretion. This study has also suggested a connection between glandular contents and slave-raiding by the oldest workers (Ali *et al.*, 1988).

Perhaps the greatest help that biology can provide for chemistry is the discovery or perfecting of good bioassays. When a simple and reproducible bioassay is available that gives a clear result, then chemistry has the stimulus to make a worthwhile discovery. A good example of such a bioassay is the trail following test of Pasteels and Verhaeghe (1974) which we have found reproducible and quantitative. Use of this bioassay has enabled us to identify the trail pheromones of a number of species of ants (Attygalle and Morgan, 1985).

Chemical methods are available now (Attygalle and Morgan, 1984) for the recovery of the secretion from a single insect after chromatography or from several individuals, or a selected part of the secretion, or individual components of the secretion for testing in a bioassay, as well as for chemical examination. If there is a biological test available, then a pure component can be isolated and the small amount produced can be checked for behavioural activity with that test. We have used such methods in locating and identifying each of the trail pheromones we have reported. However, such assaying is not always simple, for we found in the case of the ant *Pheidole pallidula* that more than one substance is required to both initiate and sustain trail-following behaviour (Ali *et al.*, 1988).

The combination of neurophysiological techniques and chemistry are much used in the study of lepidopteron pheromones, but there has been little done in electroantennography or recording from single sensillae in Hymenoptera, largely

because of the smaller neurone potentials experienced and because of the extremely tough exoskeleton to be pierced with electrodes. However, the technical difficulties may now be overcome and we may see more use of this combined technique among social insects.

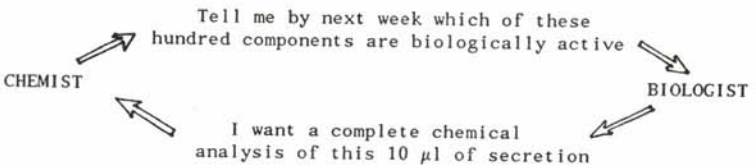
Still another difficult area is the study of primer pheromones. The original work of Butler *et al.* (1961) on queen substance of honeybees was a splendid example of a primer pheromone study. But there was a simple and rapid bioassay available – the stabilizing or destabilizing of the colony. More bioassays of this type need to be perfected so that chemical studies of colony stability, queen control, or brood care can begin.

Little has been done on the effects of insect hormones in social insects, particularly on the effects of juvenile hormone and moulting hormone. Almost all hormone studies are on non-social insects, yet the chemicals are available, and also in radio-labelled form and the chemical assays are there for their identification and quantification.

Fig. 2. Collaboration is a little like marriage

It is easy to say that biologists and chemists should work together, and that they can accomplish far more working together than they can alone, but such collaboration is not easy to achieve. Good collaboration is a little like marriage (Fig. 2) not to be entered upon lightly, and requiring some limitations upon the freedom of action of both sides. In collaboration, each science must recognize the limitations of the other's techniques and attempt to achieve what is possible together, not to attempt to make the other do what would be interesting to oneself. First of all, each must be able

to understand a little of the others subject as well as being an expert in his own. Often each expect too much of each other. Albone (1984) gives a humorous illustration of how collaboration is not achieved:



I have enjoyed some excellent collaboration in my work on insect chemicals. Perhaps I have been lucky with my partners, several of whom also contribute to this volume. It has been very interesting for me working with them, and I am grateful to them for what they have taught me and I hope that the work we have done together will be of use to fellow scientists. I must also thank my research students in this field over a number of years, particularly R.P. Evershed, A.B. Attygalle, M.F. Ali and B.D. Jackson, their dedication and enthusiasm has been essential to our progress. I wish to thank also the UIEIS Section Française for inviting me to contribute to this colloquium.

REFERENCES

Albone, E.S., 1984.–Mammalian Semiochemistry. John Wiley and Sons, Chichester, 1984, p. 8.

Ali, M.F., Attygalle, A.B., Billen, J.P.J., Jackson, B.D., Morgan, E.D., 1988.–Change of Dufour gland contents with age of workers of *Formica sanguinea* (Hymenoptera:Formicidae). *Physiol. Entomol.*, **13** (in press).

Ali, M.F., Attygalle, A.B., Morgan, E.D., Billen, J.P.J., 1987.–The Dufour gland substances of the workers of *Formica fusca* and *Formica lemani*. *Comp. Biochem. Physiol.*, **88B**, 59–63.

Attygalle, A.B., Morgan, E.D., 1984.–Chemical reactions with nanogram quantities of compounds collected from GC effluent. *J. Chromatogr.*, **290**, 321–330.

Attygalle, A.B., Morgan, E.D., 1985.–Ant trail pheromones. *Adv. Insect Physiol.*, **18**, 1–30.

Attygalle, A.B., Morgan, E.D., 1988.–Microchemical methods linked to gas chromatography in the structure determination of nanogram quantities of pheromones. *Angew. Chem. Internat. Ed. English*, (in press).

Attygalle, A.B., Evershed, R.P., Morgan, E.D., Rowland, S.J., 1983.–Comparison of three derivatives for the enantiomeric separation of chiral alcohols and the absolute configuration of *Myrmica* ant 3–octanol. *J. Chromatogr.*, **260**, 411–417.

Attygalle, A.B., Herrig, M., Vostrowsky, O., Bestmann, H.J., 1987.–Technique for injecting intact glands for analysis of sex pheromones of Lepidoptera by capillary gass chromatography. *J. Chem. Ecol.*, **13**, 1299–1311.

Bestmann, H–J., Attygalle, A.B., Glasbrenner, J., Riemer, R., Vostrowsky, O., Constantino, M.G., Melikian, G., Morgan, E.D., 1987.–Identification of the volatile components of the mandibular gland secretion of the ant *Manica rubida*: Structure elucidation, synthesis, and absolute configuration of manicone. *Liebigs Annalen.*, **87**, (in press).

Billen, J.P.J., van Boven, J.K.A., Evershed, R.P., Morgan, E.D., 1983.–The chemical composition of the Dufour gland contents of workers of the ant *Formica cunicularia*, a simple test for recognition of the species. *Annal. Soc. Roy. Belg. Zool.*, **113**, 283–289.

Billen, J.P.J., Evershed, R.P., Attygalle, A.B., Morgan, E.D., Ollett, D.G., 1986.–The contents of the Dufour glands of workers of three species of *Tetramorium*. *J. Chem. Ecol.* **12**, 669–685.

Butler, C.G., Callow, R.K., Johnston, N.C., 1961.–The isolation and synthesis of queen substance, 9–oxodec–*trans*–2–enoic acid, a honey bee pheromone. *Proc. Roy. Soc. B*, **155**, 417–432.

Cammaerts, M.C., Attygalle, A.B., Evershed, R.P., Morgan, E.D., 1985.–The pheromonal activity of chiral 3–octanol for *Myrmica* ants. *Physiol. Entomol.* **10**, 33–36.

Crump, D., Silverstein, R.M., Williams, H.J., Fitzgerald, T.D., 1987.–Identification of the trail pheromone of the eastern tent caterpillar *Malacosoma americanum* (Lepidoptera:Lasiocampidae). *J. Chem. Ecol.*, **13**, 397–402.

Golub, M.A., Weatherstone, I., 1984.–Techniques for extracting and collecting sex pheromones from live insects and from artificial sources. *In* Techniques of Pheromone Research (H.E. Hummel and T.A. Miller, Eds.). Springer–Verlag, Berlin, p. 223–285.

Morgan, E.D., Ollett, D.G., 1987.–Methyl 6–methylsalicylate, trail pheromone of the ant *Tetramorium impurum* (Formicidae:Myrmicine). *Naturwissen.* (in press).

Morgan, E.D., Wadhams, L.J., 1972.–Gas chromatography of volatile compounds in small samples of biological materials. *J. Chromatogr. Sci.*, **10**, 528–529.

Ollett, D.G., Morgan, E.D., Attygalle, A.B., Billen, J.P.J., 1987.–The contents of the Dufour gland of the ant *Harpagoxenus sublaevis* Nyl. *Z. Naturforsch.*, **42C**, 10–15.

Pasteels, J.M., Verhaeghe, J.C., 1974.–Dosage biologique de la phèromone de piste chez les fourrageures et les reines de *Myrmica rubra*. *Insectes Soc.*, **21**, 167–180.

Silverstein, R.M., 1985.–Chiral semiochemicals. *In* "Semiochemicals, flavors and pheromones T.E. Acree and D.M. Soderlund Eds., Walter de Gruyter, Berlin, and N.Y., p. 121–140.