Hymenoptera from the primitive-growing ants, *Mycocepurus goeldii*[4].

Electroantennograph (EAG) measurements on the 3 isomers were obtained using whole insect preparations. The body of the insect was immobilized on a block of plasticine and the antennae secured with microstaples. Glass electrodes drawn out of capillary tubing (Medde GC-100 F-4) were filled with insect ringer solution and connected to a high impedance ($10^{12}$ Ω) differential amplifier via chloridized silver wires inserted down the center of each electrode. The EAG response was recorded on a digital transient recorder (DTR Type DL901). A test substance in solution was applied to the inside of a pasteur pipette and any solvent allowed to evaporate; the substance was then puffed over the antennae by hand. The pipette tip was 10–20 mm from the insect antenna. The mean response of the male antennae to 1 µg of o-aap was 2.4 mV, to m-aap 0.63 mV and *p*-aap 0.30 mV. Live females elicited a 1.8 mV response, while female extracts a 0.8 mV response compared to 0.25 mV for the solvent control. The EAG responses to *o*-aap, as well as those of live females and female extracts, had a sharp leading edge characteristic of genuine pheromone responses[5]. This sharp edge was lacking in the responses from other isomers.

Laboratory observations of the behavioral responses of male *C. lariciphila* to females and experimental compounds were carried out in a plastic wind tunnel[6] of 1.1 m diameter. The behavioral responses leading to close range courtship and mating can be classified into the following sequence[7]: 1. Movement of the antennae (including searching of the substrate), accompanied by extrusion of the genitalia and opening of the mandibles. 2. Vertical flexing of the abdomen from the horizontal and 'flitting of the wings'. 3. Short flights (< 100 mm). 4. Upwind flight. 5. Close range courtship and mating.

Only calling virgin females elicited this full behavioral sequence (table 1); extracts of females on rubber sleeve stoppers did elicit upwind flight. but males did not attempt to perform close range courtship displays or mate with the pheromone source. The *ortho*-aminoacetophenone released the first 3 sequences of the behavioral repertoire, but did not elicit upwind flight.

Field testing was carried out in larch plantations at Wopley Hill (Mortimer Forest) in 1980 and 1981. Horizontal plywood traps (200×200 mm), with removable sticky surfaces (190×210 mm), were mounted on stakes 1 m above the ground. A 5×5 latin square experimental design was utilized to compare the responses of males to virgin female *C. lariciphila* (in plastic chambers[2]), female extracts (0.5 female equivalents/trap) and the 3 isomers of aap at 1 µl/trap. The aminoacetophenones were dissolved in dichloromethane and applied to 7×20 mm rubber sleeve stoppers (West Pharma Rubber).

When deployed on open horizontal board traps the *ortho*-isomer caught significantly more males of *C. lariciphila* than the other isomers (table 2, experiment 1) and as many as the female extract. In 1981 an attempt was made to repeat this experiment using plastic delta traps (Wolfson Unit, Southampton University; 190×210×120 mm high). In this experiment (table 2, experiment 2) no significant differences could be seen between the different isomers of aap and control traps. Previous experiments[7] had demonstrated that delta traps baited with live females perform as well as horizontal board traps; it was, therefore, concluded that the differences between experiments 1 and 2 were due to trap design interacting with the pheromone. A comparison was made between delta traps and board traps (table 2, experiment 3) which showed that board traps baited with *o*-aap caught significantly more males than enclosed delta traps baited with the same compound.

These data, from laboratory and field studies, are interpreted as indicating a role for *o*-aap in a multicomponent sex pheromone. It is apparent that the aap does not release attraction of males to the pheromone source but only activates the early stages of the courtship sequence. The behavior released by the *o*-aap leads to an increased activity in the vicinity of the trap in the form of non-orientated random flights. This leads to an increased catch of male sawflies on the exposed sticky surface near the source of the activation stimulus. In contrast, to be captured in a delta trap, a male sawfly must make an orientated flight through the trap entrance. Laboratory studies (table 1) also indicate the role of *o*-aap in increasing flight activity. The significantly larger numbers of males found in delta traps baited with females (table 2, experiment 3) or female extracts (table 2, experiment 2), together with laboratory data from females and their extracts, indicate that other behavioral releasing chemicals, such as an orientation stimulant, are present in the complete pheromone system.

Our results also indicate the unsuitability of unenclosed traps for pheromone research as they do not distinguish orientated responses from unorientated action responses when the density of flying insects is high, as in this sawfly infestation[2].

1 Acknowledgments. C.L. was supported by N.E.R.C. and S.E.R.C. Fellowships. D.S. was supported by Cyanamid Ltd, Gosport, England.

2 Borden, J.H., Billany, D.J., Bradshaw, J.W.S., Edwards, M., Baker, R., and Evans, D., Ecol. Ent. *3* (1978) 13.
3 Jewett, D.M., Matsumara, F., and Coppel, H.C., J. chem. Ecol. *4* (1978) 277.
4 Blum, M.S., Brand, J.M., and Amante, E., Experientia *37* (1981) 816.
5 Roelofs, W.L., in: Biochemistry of insects, p.427. Ed. M. Rockstein. Academic Press, New York 1978.
6 Jones, O.T., Howse, P.E., and Lomer, R.A., Physiol. Ent. *6* (1981) 175.
7 Longhurst, C., unpublished data.

0014-4754/83/090993-02$1.50 + 0.20/0


## Condensation of an alkyl chain on 1,7,7-trimethylbicycloheptane: A model for the effect of camphor on lipid membranes

G.J. Smith[1]

*Physics and Engineering Laboratory, DSIR, Private Bag, Lower Hutt (New Zealand), January 18, 1983*

*Summary.* Enthalpies of mixing of the hydrocarbon portion of camphor with n-octane show that it exerts a condensation interaction on adjacent alkyl chains. This is a similar interaction to that displayed by cholesterol in cell membranes which results in an alteration of the membrane fluidity.

A number of membrane related cellular functions such as respiration[2,3], radiation repair[4], bacterial growth and division are affected by the presence of camphor. The variety of these functions suggests that camphor may alter the activities of the membrane bound enzymes involved in each function by changing the fluidity of their environment rather than by direction interaction with the enzymes[5]. It is therefore of interest to examine the nature of the physicochemical, molecular interactions which would be expected to occur when camphor is in a lipid membrane environment. Consideration of some of the molecular properties of camphor suggest that it may be able to exert a condensation interaction on the alkyl chains of phospholipid molecules which would result in a decrease in the fluidity of phospholipid membranes[5-9]. The theoretical work of Scott[10,11] and Cherng[11] has highlighted the important structural feature of molecules like cholesterol which, when embedded in lipid membranes, decreases the fluidity of membranes above their phase transition temperatures. Such molecules have rigid structures which inhibit the trans-gauche isomerism in the hydrocarbon chains of adjacent lipid molecules and this results in a 'condensation' of the normally flexible hydrocarbon chains on the rigid molecule. This type of 'condensation' interaction has been observed experimentally by enthalpy of mixing measurements of long chain n-alkanes with rigid nonane isomers[12,13]. A model system was chosen for thermodynamic study, to see if this interaction could occur with camphor in a lipid membrane.

The camphor molecule consists of a large and almost spherical hydrocarbon portion, 1,7,7-trimethylbicycloheptane, with a carbonyl oxygen at the 3 position. The shape and size of the camphor molecule is therefore virtually the same as the trimethylbicycloheptane portion. If camphor enters a cell membrane, it is expected that it will adopt a position such that the polar carbonyl group is associated with the polar head groups of the lipid molecules and the trimethylbicycloheptane portion is surrounded by the alkyl chains of the lipid molecules. The trimethylbicycloheptane moiety is almost rigid and therefore is a structure likely to exert the 'condensation' interaction described above.

The molar excess enthalpy of mixing, $H^E$, in a model system of 1,7,7-trimethylbicycloheptane (camphane) in n-octane was compared with published $H^E$ for several nonane isomers in n-octane. In mixtures of n-octane with alkanes of similar size, $H^E$ may be expressed as the sum of three contributing enthalpies, i.e.

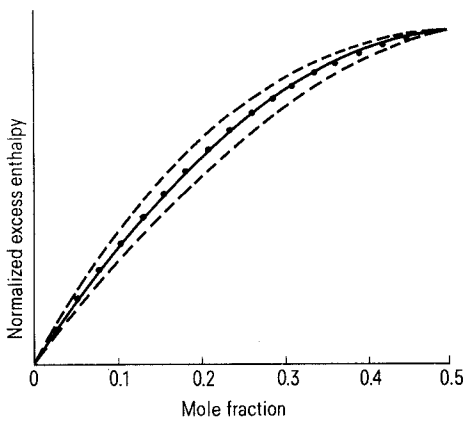
$$H^E = h_o + h_d - h_c \quad (1)$$

where $h_o$ is associated with a decrease in the short range orientational order which exists between n-octane molecules, and $h_d$ arises from differences in the magnitude of the dispersion forces associated with the pure components and the mixture. If a condensation interaction occurs between n-octane and the other component there will also be an exothermic (negative) term, $h_c$ in equation (1)[13]. In these mixtures, the effects of changes in free volume in mixing on $H^E$ are very small[14].

$H^E$ as a function of mixture composition, for a 0.50-mole fraction solution of camphane (a saturated solution) in n-octane mixed with n-octane was measured at 25 °C relative to the enthalpies of pure octane and the 0.50-mole fraction mixture using the microcalorimeter described by Stokes and Marsh[15]. $H^E$ measured relative to the enthalpies of the pure components for n-octane mixed with several different nonane isomers of similar size to camphane have been reported[16]. Although this data was fitted to a more complex function, we found that the equation

$$H^E = C\,X\,(1-X)\,[1+0.06\,(2X-1)] \quad (2)$$

fitted the data quite well over the composition range from pure octane to $X=0.5$ where X is the mole fraction of nonane and C is a constant for a given mixture which normalizes $H^E$ for all mixtures to the same value at $X=0.5$ (i.e. functions with the same shape though different magnitudes). This is illustrated in the figure. In order to compare $H^E$ reported for the n-octane/nonane isomer mixtures[16] with that measured for the n-octane/camphane mixture, it is necessary to relate $H^E$ for the latter mixture to the molar enthalpies of its pure components. This was done by assuming that the $H^E$-composition data for the camphane/octane mixture relative to the molar enthalpies of the pure components is also fitted by equation (2) over this composition range and a value for the difference between the molar enthalpies of the 0.5-mole fraction mixture and pure camphane was then found which satisfied this condition. The value of $H^E$ obtained following this procedure for the n-octane/camphane mixture at 0.5-mole fraction is 30 J · mole$^{-1}$. If $H^E$ for the n-octane/camphane mixture are made instead to fit the normalized $H^E$ of the mixtures from Romain[16] which deviate the most from equation (2) (i.e. those shown by the broken curves in the figures) the change in $H^E$ at $X=0.5$ for the octane/camphane mixture only amounts to $\pm 5$ J · mole$^{-1}$. The values of $H^E$ at $X=0.5$ reported for 2,2,4,4-tetramethylpentane and 2,2,5-trimethylhexane mixed with n-octane are 39 J · mole$^{-1}$ and 95 J · mole$^{-1}$ respectively[16]. By virtue of its molecular shape and size, camphane is expected to decrease the short range order present in pure n-octane at least as much as 2,2,4,4-tetramethylpentane or 2,2,5-trimethylhexane, i.e. $h_o$(camphane) $\geqslant h_o$(nonanes). Patterson et al. have concluded that $h_o \gg h_d$ in most of the alkane/n-alkane mixtures[13,14,17,18] they studied. Therefore, since $H^E$ for the camphane/n-octane mixture is less than those of the relatively flexible 2,2,5-trimethylhexane molecule and the rather more sterically hindered 2,2,4,4-tetramethylpentane molecule mixed with n-octane, it is concluded that there is a sizeable negative 'condensation' interaction, $h_c$, between n-octane and camphane. The most widely studied chemical which affects cell membrane fluidity by exerting a condensation interaction on the lipid, alkyl chains is cholesterol[5-8]. Cholesterol is virtually insoluble in water and consequently must be transported from its site of synthesis to the membrane by a special protein[19,20]. By contrast, camphor has a relatively high solubility in water ($4\times10^{-3}$ M) despite




Excess enthalpies of mixing (normalized so the mixtures all have the same enthalpy at $X=0.5$) plotted against mole fraction. The solid line is the function given by equation 2. The broken curves are those obtained from the two mixtures studied by Romain[16] which deviate the most from equation 2. The points are those obtained for the n-octane/camphane mixture if $H^E$ at $X=0.5$ is 30 J · mole$^{-1}$.

its predominantly hydrocarbon structure. If this feature of camphor is coupled with a general ability to decrease the membrane fluidity of cell plasma membranes it could be a useful agent for the modification of membrane related cellular functions in vivo. This kind of effect has been sought by membrane biophysicists[21,22] and it is hoped that the work described here may stimulate an investigation of the effect of camphor on the fluidity of cell membranes.

1 Acknowledgments. I thank Professor K. Marsh for his hospitality and assistance in the use of his microcalorimeter.
2 Guilland-Cumming, D.F., and Smith, G.J., Experientia *38* (1982) 236.
3 Guilland-Cumming, D.F., and Smith, G.J., Experientia *35* (1979) 659.
4 Roberts, P.B., and Smith, G.J., Int. J. Radiat. Biol. *32*, (1977) 589.
5 Chapman, D., Q. Rev. Biophys. *8* (1975) 186.
6 Demel, R.A., and De Kruyff, P., Biochim. biophys. Acta *457* (1976) 109.
7 Marsh, D., and Smith, I.C.P., Biochim. biophys. Acta *298* (1973) 133.
8 Tajima, K., and Gershfeld, N.L., Biophys. J. *22* (1978) 489.
9 Hubbell, W.L., Accts, chem. Res. *8* (1975) 85.
10 Scott, H.L., Biochim. biophys. Acta *469* (1977) 264.
11 Scott, H.L., and Cherng, S-L, Biochim. biophys. Acta (1978).
12 Mathot, V., Bull. Soc. chim. Belg. *59* (1950) 111.
13 Tancrede, P., Bothorel, P., de St.Romain, P., and Patterson, D., J.C.S. Faraday Trans. *73* (1977) 15.
14 de St.Romain, P., Van, T.H., and Patterson, D., J.C.S. Faraday Trans. *75* (1979) 1700.
15 Costigan, M.J., Hodges, L.J., Marsh, K.N., Stokes, R.H., and Tuxford, C.W., Aust. J. Chem. *33* (1980) 2103.
16 de St.Romain, P., thesis, McGill University, 1977.
17 Tancrede, P., Patterson, D., and Botherel, P., J.C.S. Faraday Trans. *73* (1977) 29.
18 Lam, V.T., Picker, P., Patterson, D., and Tancrede, P., J.C.S. Faraday (1974) 1465.
19 Bell, F.P., Artery *3* (1977) 98.
20 Madden, R.D., Vigo, C., Bruckdorfer, K.R., and Chapman, D., Biochim. biophys. Acta *599* (1980) 528.
21 Chapman, D., and Quinn, P.J., Chem. phys. Lipid *17* (1976) 363.
22 Chapman, D., Peel, W.E., and Quinn, P.J., Ann. N.Y. Acad. Sci. *308* (1978) 67, and references cited therein.

0014-4754/83/090994-03$1.50 + 0.20/0


## Ocular responses evoked by capsaicin and prostaglandin $E_2$ are inhibited by a substance P antagonist[1]

G. Bynke, R. Håkanson[2] and J. Hörig

*Departments of Experimental Ophthalmology and Pharmacology, University of Lund, S-22362 Lund (Sweden), and Ferring Arzneimittel GmbH, D-2300 Kiel (Federal Republic of Germany), January 31, 1983*

*Summary.* Injection of capsaicin or prostaglandin $E_2$ into the vitreous chamber of the rabbit eye resulted in miosis and breakdown of the blood-aqueous barrier, manifested in aqueous flare. Pretreatment with the neuronal blocker tetrodotoxin or the substance P antagonist (D-Pro$^2$, D-Trp$^{7,9}$)-$SP_{1-11}$ greatly reduced the ocular responses to capsaicin and prostaglandin $E_2$. The results suggest a role for neuronal substance P in the ocular response to injury.

The response to ocular injury in the rabbit eye is characterized by miosis, vasodilatation and a breakdown of the blood-aqueous barrier. Among stimuli known to elicit one or more of these responses are chemical irritants, such as nitrogen mustard[3] and capsaicin[3,4], endogenous chemicals, such as bradykinin[5], prostaglandins[6] and substance P (SP)[7-9] and ocular trauma, induced, for instance, by IR-irradiation of the iris[10]. Following sensory denervation of the eye by diathermic destruction of the trigeminal nerve the miotic and hypertensive response to laser irradiation[11], capsaicin, bradykinin and prostaglandins is prevented[7,12,13] whereas that to SP remains[7,13]. It has therefore been proposed that all chemical irritants, except SP, act to release a mediator from sensory nerve endings[13]. It has also been suggested that the mediator is related to SP[13]. Recently, it was shown that the ocular response to IR-irradiation and to bradykinin can be prevented by pretreatment of the eye with a newly developed SP antagonist[14,15]. The present study is concerned with the mechanism behind the ocular injury evoked by capsaicin and prostaglandin $E_2$ ($PGE_2$).

*Methods.* Adult pigmented rabbits (1.5-3 kg) of mixed strain were anesthetized with methohexital sodium (Brietal®, Lilly; 5 mg/kg) when given intravitreal or retrobulbar injections. No anesthesia was administered during the rest of the experiments. The time course of the barrier damage was followed by photoelectric measurement[16] of the aqueous flare response (AFR). This response is a Tyndall phenomenon in the anterior chamber, reflecting protein leakage across the blood-aqueous barrier. A correlation between the AFR and the protein concentration has been established[17]. The method has the advantage of being atraumatic, permitting continuous recording of the AFR during the experiment. Furthermore, the method detects changes in AFR that cannot be observed by conventional focal illumination The results are expressed in arbitrary units with reference to a standard[17]. Unless otherwise indicated, the AFR and pupillary diameter were measured every 30 min. A potent blocker of nervous conduction, tetrodotoxin (TTX)[18], (10 µg in 20 µl bidest $H_2O$) was injected into the vitreous chamber of the left eye by means of a Hamilton precision syringe, 3-4 mm posterior to the limbus. The control (right) eye received 20 µl $H_2O$. These injections were made 4 h before the administration of either capsaicin or $PGE_2$ to both eyes. The SP antagonist (D-Pro$^2$, D-Trp$^{7,9}$)-$SP_{1-11}$ was applied topically in a volume of 50 µl to the left eye. The SP antagonist was given twice, 1 h and 30 min, respectively, before capsaicin or $PGE_2$. The right eye (control eye) received instead 0.9% saline topically. Capsaicin (150 µl of a 0.33% solution) was administered to both eyes by retrobulbar injection using a thin needle (0.4 mm diameter, 20 mm length), introduced through the center of the lower eyelid, near the lid margin, and around the interior aspect of the globe to a depth of 10 mm. Aspiration was invariably performed to make sure that the injection was not into the venous sinus. $PGE_2$ (0.85 µg in 5 µl) was applied topically onto both eyes. The doses of capsaicin and $PGE_2$ were selected because they produced small but consistent effects in the eye.

Capsaicin and $PGE_2$ were purchased from Sigma, St.Louis, Mo, USA. 50 mg of capsaicin was dissolved in 75 µl 99.5%