Isolation and Identification of the Sex Pheromone of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied)

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The volatile compounds emitted by sexually mature male Mediterranean fruit flies (*Ceratitis capitata*) have been identified and the key component involved in the sexual attraction of virgin female flies to males demonstrated to be the novel sex pheromone 3,4-dihydro-2*H*-pyrrole (**1**).

The Mediterranean fruit fly *Ceratitis capitata* (Wied) is a serious pest of many deciduous and subtropical fruits, and occurs throughout S. Europe, the near East, Africa, S.W. Australia, Hawaii, and C. and S. America.[1] Féron[2] reported that sexually mature male *C. capitata* release a volatile chemical substance from their erect anal ampoules which sexually excites and attracts virgin females. Two compounds have been isolated by aeration–cold-trapping of the volatile substances produced by male *C. capitata*, *E*-non-6-en-1-ol and methyl *E*-non-6-enoate,[3] and the latter has been shown to be a male attractant in the field.[4] A biologically inactive compound has recently been identified as (−)-β-fenchol (1*S*-*exo*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol).[5] We have now demonstrated that the mixture of volatile substances produced by male *C. capitata* consists of nine components and that one of these, 3,4-dihydro-2*H*-pyrrole (Δ^1-pyrroline) (**1**) appears to be the key constituent in relation to the biological activity of the volatile emissions of the male fly.

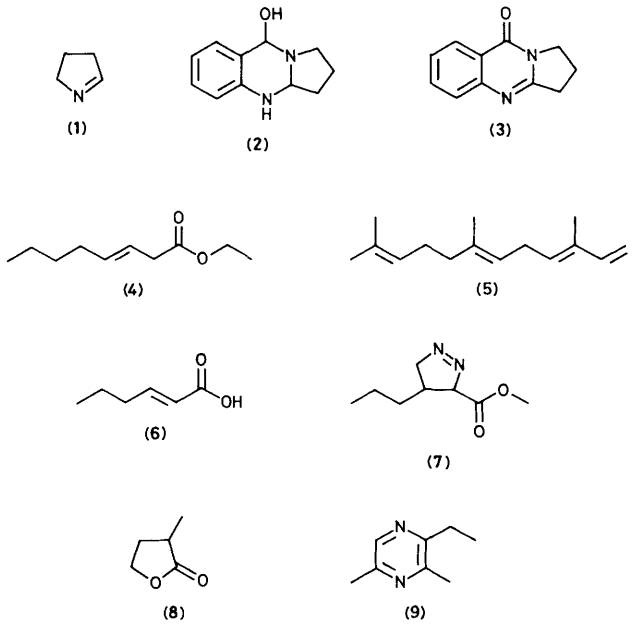
The volatile emission from ~40 male flies (5 days old, fed on sucrose solution to minimise the complications of dietary-linked artefacts), was collected by aeration over a period of 4—5 days, using Porapak Q or activated charcoal as the adsorbent. It was estimated that *ca.* 50—100 μg of volatile material was obtained. In a second approach the analysis of the secretion contained in the anal ampoule of 4 or 5 male flies was achieved by extirpation of the ampoules followed by solid-sample gas chromatography–mass spectrometry (s.s. g.c.–m.s.).[6]

The cyclic imine (**1**) was initially identified by thermal desorption from 'aerated' Porapak Q obtained from male flies, trapping (acetone–liquid N_2) on a Bergstrom g.c. column[7] (Carbowax C20M 5% + KOH 4%, 3 m × 3 mm) followed by gas chromatography coupled with mass spectrometry; *m*/*z*(%) 69(M^+,85), 68(40), 42(23), 41(100), 40(18), and 39(22). Unambiguous identification was provided by elution of the 'aerated' Porapak Q with water, and treatment of the aqueous solution with freshly prepared *o*-aminobenzaldehyde,[8] resulting in the formation of the highly coloured adduct (**2**).[9] Oxidation of (**2**) with CrO_3–dilute H_2SO_4 led quantitatively to 2,3-trimethylene-4-quinazolone (**3**);[10] *m*/*z*(%) 186(M^+,99) and 185(100). It was estimated that *ca.* 10 μg of the cyclic imine (**1**) was isolated, but the actual rate of emission was certainly higher, as its quantitative determination was complicated by its chemical instability. The mass spectral and gas chromatographic properties of synthetic Δ^1-pyrroline, prepared by treatment of ornithine hydrochloride with *N*-bromosuccinimide,[11] and its derivative (**3**) were identical to those of the natural product.

The three major components were obtained by elution of the 'aerated' Porapak Q or activated charcoal filter with methylene chloride and purified by preparative gas chromatography collecting in spectroscopic grade CCl_4. Deuteriated benzene (10% v/v) was added and the 400 MHz ^{1}H n.m.r. spectra were recorded, in conjunction with any necessary decoupling experiments. These compounds were identified as ethyl *E*-oct-3-enoate (**4**), *E*,*E*-α-farnesene (**5**), and geranyl acetate. The farnesene derivative was demonstrated (s.s.g.c.–m.s.) to be the major volatile component (~200 ng/insect) in the anal ampoule of the male flies.

The remaining components were not isolated but were identified utilising g.c., g.c.–m.s., and derivatisation techniques. A further substantial component was found to be *E*-hex-2-enoic acid (*ca.* 40 μg) (**6**). Treatment of an aeration extract with *N*,*O*-bis(trimethylsilyl)acetamide led to the formation of a product whose mass spectrum was consistent with that of a silyl hexenoate. Similarly, treatment with diazomethane afforded a single new compound with a mass spectrum indicative of a methyl hexenoate. Interestingly, reaction with excess of diazomethane for 48 h led to a different product (**7**); [*m*/*z*(%) 170(M^+,26), 139(20), 128(24), 127(20), 111(43), 95(100), 69(22), 59(21), 55(11), 42(10), and 41(12)] resulting from the 1,3-dipolar addition of diazomethane to the α,β-unsaturated methyl ester,[12] thus establishing that the natural product is a hex-2-enoic acid. Reaction of diazomethane with synthetic *Z*-hex-2-enoic acid afforded methyl *Z*-hex-2-enoate, whose mass spectral and gas chromatographic properties were distinctly different from those of methyl *E*-hex-2-enoate and the methyl ester of the natural product. Four other minor components, dihydro-3-methylfuran-2(3*H*)-one (**8**), 2-ethyl-3,5-dimethylpyrazine (**9**), linalo-ol, and ethyl acetate were also identified.

It should be emphasised that the studies to this stage



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involved only male flies. It is highly significant that analysis of aeration products from female *C. capitata* failed to show any evidence of the nine components isolated from the male flies. The biological activity of these nine male specific components was studied using a double tube olfactometer.[13,14] An aqueous solution of the cyclic imine (**1**), prepared as described previously,[11] was found to be highly attractive to virgin female *C. capitata*, and compared favourably with the response of females to live males. No other compound was active singly, although several of the other components displayed activity in mixtures approximating to the ratios found in aeration experiments. Further biological studies are in progress and will be reported more fully elsewhere.

Several of the components isolated from the male-produced volatile substances of *C. capitata* have been identified in other insects. The cyclic imine (**1**) has been isolated, amongst other amines, from colonies of male desert locusts, *Schistocerca gregaria*, but the function of this compound was not described.[15] Ethyl *E*-oct-3-enoate (**4**) and the furanone (**8**) have not previously been identified from insects. None of the compounds previously identified from male *C. capitata*[5,7] has been found in this study, even though the flies used were obtained from three different populations. The major conclusion from the present work, that the cyclic imine (**1**) is produced by the male fly and is highly attractive to virgin females, is particularly important for the development of an effective pheromone-based control system for *C. capitata*.

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