Chemical components of the rectal gland secretions of male *Dacus cucurbitae*, the melon fly

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Summary. The volatile constituents of the rectal gland secretion of male *Dacus cucurbitae* have been shown to contain 2-methoxy-N-3-methylbutyl acetamide together with 2 other amides, 3 pyrazine derivatives and 2-ethoxybenzoic acid. Excised male rectal glands have been demonstrated to elicit strong responses from female melon flies.

The melon fly, *Dacus cucurbitae* Coquillet, is distributed throughout E. Africa, India, S.E. Asia and Hawaii[2], and is a serious pest of melon and other cucurbits. Control is mainly by the use of insecticides. A synthetic attractant for male *D. cucurbitae*, 4-(p-acetoxyphenyl)-2-butanone (cuelure), is used for monitoring populations[3]. It has been shown that male *D. cucurbitae* produce a sex pheromone which is attractive to conspecific females, and secreted from a glandular structure associated with the posterior ventral region of the rectum[4].

The structures of the volatile components present in dissected rectal glands of sexually mature male *D. cucurbitae* were investigated by utilizing a solid sampling technique[5], in conjunction with either gas chromatography (GC) or gas chromatography-mass spectrometry (GCMS). Typically, 5 male rectal glands were analyzed by GC (5% Carbowax 20 M on 100–200 Diatomite AAW-DMCS, 5% OV101 on 100–120 Diatomite CLQ; solid sample heater 140 °C, 5 min), and subsequently by GCMS (Kratos MS30, EI 70eV). Several pyrazines were identified by comparison of their mass spectra with those of authentic samples[6–9]. The major pyrazine component was tetramethylpyrazine (**1**), (0.25 µg per insect); methylpyrazine (**2**) and 2,3,6-trimethylpyrazine (**3**) were present as minor components.

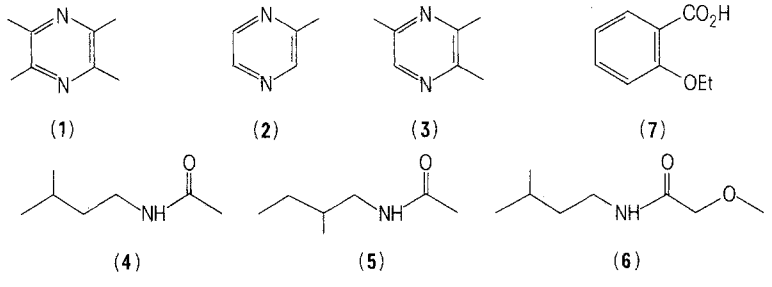
3 components were identified as amides. Two of these were shown to be N-3-methylbutylacetamide (**4**) (~ 3 µg per insect), and N-2-methylbutylacetamide (**5**). The mass spectra[10] and chromatographic properties were found to be identical to authentic samples, prepared by unambiguous synthesis. The 3rd amide component was initially assigned, from mass spectral data, as 2-methoxy-N-3-methylbutylacetamide (**6**), (0.35 µg per insect). This derivative has not previously been reported either as a component of an insect secretion or, indeed, as a synthesized derivative; molecular ion, m/e 159.1251 corresponding to $C_8H_{17}NO_2$, m/e 159(8%), 144(8), 129(61), 116(12), 103(34), 102(33), 90(11), 71(87), 45(100). The ion m/e 129.1155 indicated the loss of a CH_2O fragment by a McLafferty rearrangement. This observation, together with the loss of a fragment of 45 mass units from the molecular ion, and ions m/e 102 and m/e 90, suggested a 2-methoxyacetamide. In contrast, the corresponding ions were observed at m/e 72 and m/e 60, both 30 mass units lower, for the 2 acetamides (**4,5**). 2-Methoxy-N-3-methylbutylacetamide (**6**) was synthesized from 2-chloro-N-3-methylbutylacetamide (4.9 g), (prepared from N-3-methylbutylamine and 2-chloroacetyl chloride), which was added dropwise in dry diethyl ether (50 cm^3) to a solution of sodium (1.4 g) in dry ethanol (50 cm^3) and the mixture stirred at room temperature for 48 h. Work-up and distillation gave (**6**), 3.5 g (76%), b.p. 78–80 °C, 0.2 mm Hg; ^1H NMR ($CDCl_3$) δ: 0.95(d,3H), 1.45(m,3H), 3.3(m,2H) 3.4(s,3H), 3.9(s,2H), 6.5(bs,1H); ν_{max} (thin film): 1530, 1650, 3300 cm^{-1}. The gas chromatographic and mass spectral properties of the natural product and synthetic compound were found to be identical. N-3-methylbutylacetamide (**4**) and 2-methoxy-N-3-methylbutylacetamide (**6**) were readily separated by gas chromatography using 5% DEGS or 5% OV17 liquid phases.

A further major component was identified as 2-ethoxy benzoic acid (**7**) (~ 4 µg per insect), identified by comparison of mass spectral data of the natural product and an authentic sample. 2-Ethoxy benzoic acid (**7**) is also a novel component in fruit fly secretions, and bears a structural resemblance to the to the synthetic attractants in current use.

Finally, a bishomologous series of ethyl esters of C_{10}, C_{12}, C_{14}, C_{16}, C_{18} saturated, C_{16}, C_{20} monounsaturated, C_{18} diunsaturated fatty acids was identified, with a characteristic ion m/e 101 and an ion m/e 88 from the McLafferty rearrangement. The loss of a fragments of 45 mass units from the molecular ion served to distinguish the ethyl esters from the corresponding isomeric α-methyl methyl esters.

Both N-3-methylbutylacetamide (**4**) and N-2-methylbutylacetamide (**5**) have been reported as components of the rectal gland secretion of the males of 2 related species, *Dacus tryoni* and *D. neohumeralis*[10], in a similar ratio to that found in *D. cucurbitae*. It is intriguing that the 2-methoxy derivative (**6**) has now been shown to be present in *D. cucurbitae*. It has been suggested that the N-3-methylbutyl and N-2-methylbutyl amine moieties originate from leucine and isoleucine, respectively[10]. The 2-methoxyacetamide component (**6**) may arise from the sequential hydroxylation and methylation of the acetamide (**4**), or via a route involving glycolic acid.

The gland complex of the rectal sac of male *Dacus cucurbitae* has been reported to be only weakly attractive to female *D. cucurbitae*[11]. In contrast, using a new wind tunnel design[12], bioassays of virgin female *D. cucurbitae* demonstrated that both live males and excised male rectal glands elicited a zig-zag flight response upwind towards the odour source. N-3-methylbutylacetamide has been shown to elicit activation and increased flight activity of female *D. cucurbitae* but the exact role of the other components remains to be defined.



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Methoxymercuration-demercuration and mass spectrometry in the identification of the sex pheromones of *Panolis flammea*, the pine beauty moth

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Summary. The major components of the sex pheromone system of *Panolis flammea*, pine beauty moth, have been identified as (Z)-9-tetradecenyl acetate, (Z)-11-hexadecenyl acetate and (Z)-11-tetradecenyl acetate in the ratio 100:5:1; the double bond position of these derivatives was established by microscale application of a methoxymercuration-demercuration technique and GC-MS followed by multiple ion monitoring.

The pine beauty moth *Panolis flammea* (Lepidoptera: Noctuidae) has recently become a serious pest of Lodgepole Pine *(Pinus contorta)* in Scotland and Northern England, although its normal host tree is Scots Pine *(Pinus sylvestris)*[2]. The use of chemical attractants (pheromones) in sticky traps to monitor populations of insects, particularly moths, is now well-established[3], and such a monitoring system was urgently needed for the large, inaccessible areas of forest at risk from attack by this species. We wish to report the identification of the major components of the sex pheromone system of *P. flammea*. In this work we have demonstrated that the technique of methoxy-mercuration-demercuration[4] can be applied on microscale to a complex mixture of unsaturated methyl esters and acetates to define the position of the double bonds in the components of interest.

An extract of approximately 3000 female abdomen tips was prepared in redistilled methylene chloride (30 ml). The females were killed at the estimated optimum time of pheromone production (3rd day of emergence, between 20.00 and 24.00 h) by rapid freezing and the excised tips placed directly into solvent. The extract was partially purified by filtration through a small column of Florisil, eluted with methylene chloride, and subsequently concentrated in a stream of nitrogen (about 700 µl). Analysis of the concentrated extract by GC and GC-MS[5] yielded the partial identification of at least 12 components (table). Capillary GC-MS and single ion monitoring (m/z 194, the pseudo-molecular ion for tetradecenyl acetates) of the GC peak corresponding to tetradecenyl acetate indicated the presence of 2 monosaturated isomers. The major problem for the identification of acetates by MS is their tendency for elimination of acetic acid to yield the corresponding olefin with subsequent migration of the radical sites along the chain. Thus, total identification of monounsaturated acetates by MS alone is not possible and an additional technique is required to determine the double bond position of these compounds which were anticipated to be the important pheromonal components.

A number of techniques[6] such as oxidation, ozonolysis and epoxidation have been used to determine the position of the double bond in unsaturated derivatives but a disadvantage of all these methods is that some separation of the compounds is required prior to analysis. For the current work a procedure involving methoxymercuration was employed. About 500 µl of the extract obtained from *P. flammea* was concentrated in a stream of nitrogen to remove the methylene chloride. Addition of methanol (3 ml) afforded only partial solubility with the formation of a yellow globule on the base of the flask. Mercuric acetate (150 mg; 75-fold molar excess[7]) was added to the suspension which was shaken overnight in the dark. The yellow globule was removed from the methanolic solution and was thought to contain saturated alkanes and other cuticular material. Excess sodium borohydride (100 mg) was added to the cooled (0 °C) methanolic solution followed by acetic acid (300 µl) to destroy the excess borohydride. The methanol was removed in a stream of nitrogen and the concentrated reaction mixture was partitioned between distilled water (3 ml) and ether (3 × 1.5 ml). The resulting pale yellow ether solution was concentrated to approximately 300 µl and analyzed by GC and GC-MS.

Analysis of the methoxylated extract unambiguously identified the monounsaturated acetate components present in

Compounds identified from GC-MS of original extract	Ions observed on GC-MS of methoxylated extract[a] (3)	(4)	(5)	(6)	Double bond position determined
Tetradecenyl acetate	215	115	229	101	9-Tetradecenyl
	243	87	257	73	11-Tetradecenyl
Hexadecenyl acetate	243	115	257	101	11-Hexadecenyl
	Ions derived from (7) and (8)				
Methyl hexadecenoate	201	143	215	129	9-Hexadecenoate
Methyl octadecenoate	201	171	215	157	9-Octadecenoate
Methyl tetradecanoate					
Methyl hexadecanoate					
Methyl octadecanoate					
Methyl octadecadienoate					
Methyl octadecatrienoate					
Tricosane					
Pentacosane					

[a] Accurate mass data was obtained for all the observed ions.