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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.

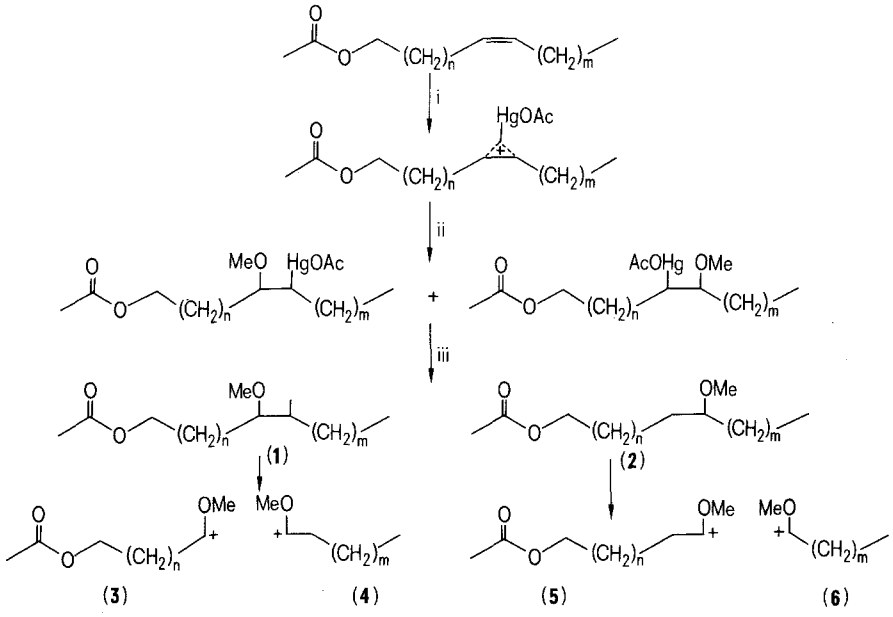
An extract of approximately 3000 female abdomen tips was prepared in redistilled methylene chloride (30 ml). The females were killed at the estimated optimum time of pheromone production (3rd day of emergence, between 20.00 and 24.00 h) by rapid freezing and the excised tips placed directly into solvent. 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.

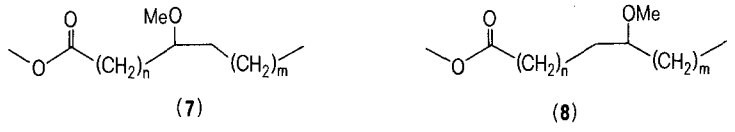
Analysis of the methoxylated extract unambiguously identified the monounsaturated acetate components present in

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.



Scheme Reagents:-(i) $Hg(OAc)_2$; (ii) MeOH; (iii) $NaBH_4$, AcOH.



the original mixture to be 9-tetradecenyl acetate, 11-hexadecenyl acetate and 11-tetradecenyl acetate. The assignment of these structures is based upon the mass spectral fragmentations of the methoxylated derivatives. For each monounsaturated derivative an approximately equal amount of the isomers (**1**) and (**2**) (scheme) would be formed and these are readily apparent in the GC trace of the reaction mixture (small amounts of unreacted olefinic derivatives are also observed). GC-MS[8] analysis of the reaction mixture indicated the formation of the required ions (**3**)-(**6**) and multiple ion monitoring for these ions afforded the identification of (**1**) and (**2**) for each of the acetates under study. For 9- and 10-methoxytetradecanyl acetates, obtained from 9-tetradecenyl acetate, the ions at m/z 215, 115, 229, 101 correspond to the fragments (**3**)-(**6**) respectively (table). Single ion monitoring of these ions and those at m/z 243, 87, 257, 73 corresponding to 11- and 12-methoxytetradecanyl acetates indicated the presence of derivatives with a double bond at the 9- and 11-positions in the original mixture. A similar procedure was adopted for the identification of 11- and 12-methoxyhexadecanyl acetates yielding the ions at m/z 243, 115, 257, 101. For the monounsaturated methyl esters the methoxylated derivatives obtained would be (**7**) and (**8**) and the ion fragments obtained from these are recorded (table). The validity of the conclusions drawn from this procedure have been fully confirmed by analogous treatment of a synthetic mixture of the unsaturated components in the extract, in the natural ratio, from which comparable spectral data were obtained. This technique was not extended to the di- and tri-unsaturated derivatives in this complex mixture.

Based upon the GC-MS of the original solvent extract the estimated ratio of 9-tetradecenyl acetate: 11-hexadecenyl acetate: 11-tetradecenyl acetate was 100:5:1. Each female moth was estimated to possess approximately 50 ng of the major component at the time of extraction and thus 2.5 ng and 0.5 ng of the minor components.

Assignment of the geometry of the double bonds was not possible by chemical means, due to the small amounts of compound available. Electrophysiological evidence[9,10] indicates that males of *P. flammea* are more sensitive to (Z)- than to (E)-isomers of a range of alkenyl acetates, including the 3 compounds identified from females.

A mixture of the (Z)-isomers of these 3 compounds, in the ratio found in the females, elicits a complete sequence of sexual behaviour in males, consisting of upwind flight, landing near to the source of chemicals, approach, eversion of genitalia, and protracted attempts to copulate with the source. None of the individual compounds, or the 3 possible 2-component mixtures, elicits all of these elements of behaviour. We conclude that a) the acetates found in females are likely to be the (Z)-isomers and b) all 3 compounds are essential components of the sex pheromone system of *Panolis flammea*.

1 We thank the Science Research Council and Forestry Commission for financial support. We are grateful for the collaboration of Messrs Stoakley and Bevan, Dr C. Longhurst and Mrs J. Edwards.

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