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CHARACTERIZATION OF FULGORID WAXES (HOMOPTERA: FULGORIDAE: INSECTA)

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Abstract—A series of waxes produced by three fulgorid species are shown to be mixtures of homologs identical to those previously found in the unrelated cochineal insect *Dactylopius confusus*. Using direct insertion probe mass spectrometry, the intact wax was found to give characteristic spectra obviating the need for extensive hydrolysis. The utility of these compounds as taxonomic characters in Fulgoridae is discussed.

Key Word Index: Fulgoridae, Homoptera, wax, mass spectrometry, *Cerogenes auricoma*, McLafferty rearrangement, lantern bugs

INTRODUCTION

The family Fulgoridae is one of the 20 families in the superfamily Fulgoroidea, many representatives of which are characterized by production of copious amounts of wax in nymphs and adults (O'Brien and Wilson, 1985) which is often extruded as fine skeins of considerable complexity (Pope, 1985). One species, *Cerogenes auricoma* (Burmeister) produces wax in both sexes, mainly from wax-producing plates usually on the sixth through eighth abdominal tergites. One of the apparent functions of these waxy, plume-like tails is protection against predators and parasites, as in the case for the waxy investitures of scale insects and aphids (Eisner *et al.*, 1978; Pope, 1983, 1985). Although several investigations have been made on the waxes produced by other Homoptera (Tamaki, 1970; Meinwald *et al.*, 1975; Calderon *et al.*, 1978), no information is available on the chemistry of fulgorid waxes.

In the present report, the chemical compositions of the waxy skeins produced by three fulgorid species are described and shown to be a mixture of homologs *1a–b*, identical to that found by Meinwald *et al.* (1975) in the unrelated cochineal insect *Dactylopius confusus* (Cockerell). These investigators also reported the closely related compound *2* in both the cochineal insect *Dactylopius coccus* (Costa) and the wooly alder aphid *Prociphilous tessalatus* (Fitch).

METHODS AND MATERIALS

Wax samples were obtained from adult males and females of *C. auricoma* and two related fulgorid species, *Fulgora lampetis* (Burmeister) and *F. castresii* (Guerín-Ménville).

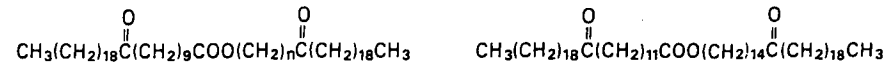
Mass spectra were obtained on an LKB 2091 spectrometer operating at 70 eV and 20 μA ionizing current; the ion source was maintained at 270°C. The gas chromatograph was run at 8°C/min using a 30 m 0.32 mm i.d. SE-54 capillary column. NMR spectra were taken on a Varian 200 MHz spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Infrared spectra were obtained with a Perkin–Elmer model 3600 spectrometer using a nujol mull. Combustion analyses were conducted by Galbraith Laboratories Inc., Knoxville, Tenn.

Protein determinations were obtained by the method of Lowry *et al.* (1951) using albumin as a standard.

RESULTS AND DISCUSSION

The wax from *C. auricoma* presented the same appearance as that illustrated by Pope (1985), i.e. a tangle of irregular fine threads intermingled with larger cylinders. The crude material melted at 106–112°C and showed an analysis of C, 76.02; H, 12.26; N, 0.80; and 34.72% ash. Because of the large amount of ash, the wax was extracted continuously in a Soxhlet extractor with tetrahydrofuran, a 110 mg sample yielding 92.4 mg of soluble residue. The remainder in the Soxhlet cup was then extracted with water and evaporated leaving 16 mg of residue, presumably salts. The wax, now a white crystalline solid, melted at 101–104°C. The NMR ($CDCl_3$) showed the presence of two methyl groups (0.88, t; J = 7 Hz), *c.* 40–50 isolated methylene groups (1.28–1.30, br. s.), six methylenes beta to oxygen (1.55, br. s.), four methylenes adjacent to ketones (2.38, t; J = 8 Hz), one methylene adjacent to an ester oxygen (4.05, t; J = 7 Hz) and one methylene adjacent to an ester carbonyl (2.28, t; J = 8 Hz). An infrared spectrum (nujol mull) showed the presence of ketone and ester carbonyl groups at 1705 and 1740 cm^{-1}, respectively, but showed no hydroxyl absorption in the 2000–3200 cm^{-1} region.

These properties suggested that the wax was similar to that described by Meinwald *et al.* (1975). To establish the carbon chain length, a sample was



1a n=14
1b n=16

2

hydrolyzed by prolonged refluxing with KOH in methanol according to the procedure used by these authors, giving an insoluble precipitate. Trimethylsilylation of this residue and gas chromatography revealed three peaks eluting at 2.25, 7.0 and 12 min in the ratio 4.7:1:2.6, respectively, for the keto acid and the two keto alcohols derived from *1a* and *1b*. Although the latter values suggest a 1:2.6 molar ratio for *1a* and *1b*, the fact that the acid is 4.7 instead of 3.6 shows that either the molar responses of these trimethylsilyl derivatives are not uniform, or that the hydrolysis and derivitization was incomplete. The following characteristic ions were observed: (keto acid [*m/z* (rel. intensity)]: 538 (M, 4), 523 (M-CH_3, 41), 382 [$CH_3(CH_2)_{18}COTMSCH_2$, 35], 286 [$CH_2COH(CH_2)_9COOTMS$, 53], 229 [$(CH_2)_8COOTMS$, 100], keto alcohol from *1b* [*m/z* (intensity)]: 607 (M–H, 27), 593 (M–CH_3, 100), 356 (M–$C_{18}H_{36}$, 46), 341 (356-CH_3, 16), 313 (356-CH_3CO, 38), 299 (356-CH_2COHCH_2, 20). The keto alcohol from *1b* showed an analogous spectrum.

Direct precipitation of the free acid from the basic hydrolysis medium is unusual and likely due to its extreme insolubility. We also note that the ion at *m/z* 382 in the mass spectrum of the keto acid implies a McLafferty type of rearrangement where the proton from the gamma position has been replaced by a TMS unit from the omega position. This point is under further investigation.

In sum, this analysis shows that the wax is exactly the same as that found by Meinwald *et al.* (1975) even though the insect is very distantly related. However, since we wished to chemically analyze samples of the wax from other insects and even from different locations within one insect, we sought to avoid the need for the tedious hydrolysis step used above. In spite of its very high mass, we have found that samples of the wax give very satisfactory mass spectra when examined by direct insertion probe. Thus, the mass spectrum of *1a*, with ions from the higher homolog removed, is shown in Fig. 1. A molecular ion appears at *m/z* 957 (the large number of hydrogens increases the molecular weight by one unit at this mass) while the fragment peaks suggest the processes on the next page.

Compounds *1a* and *1b*, with both acyl ends the same length as shown by the single acyl peak at *m/z* 295, have simple analyses. Thus, an ion at *m/z* 449 establishes the total carbon number of the acid moiety and the structure follows merely by subtraction from the molecular ion. Generally, in fact, one could use the presence of peaks homologous with this mass to establish the nature of the acid moiety. The peak at *m/z* 491 representing loss of the acyloxy ion, as occurs in glycerides (Ryhage and Stenhagen, 1960) gives the total keto alcohol carbon number (this ion is shown in its cyclic form here but there is no direct evidence for this structure). Rearrangement

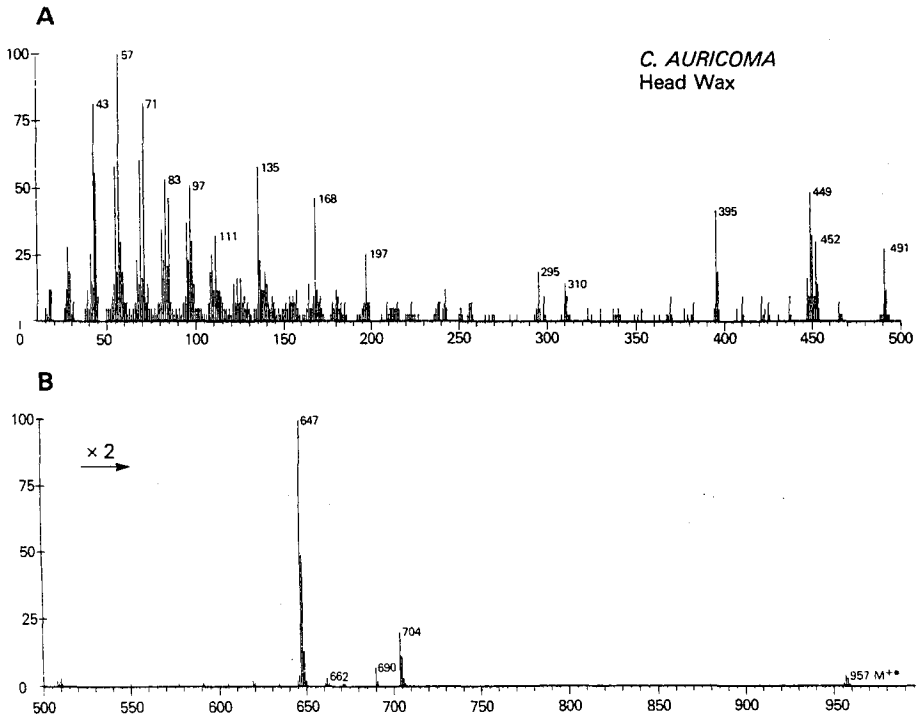
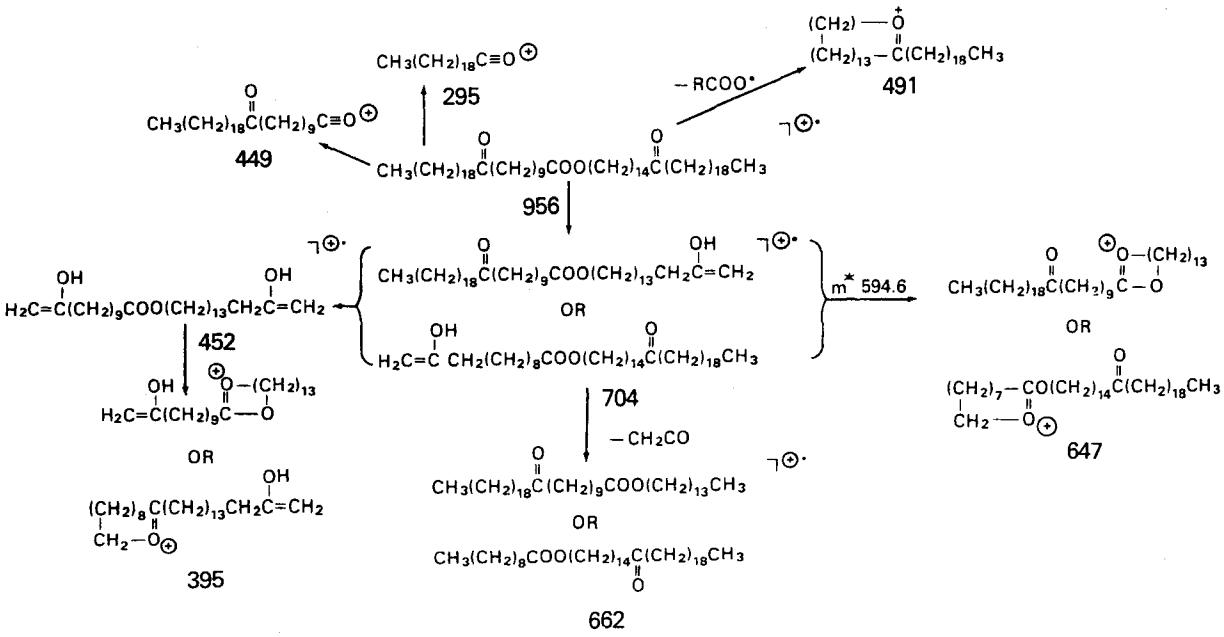


Fig. 1. Direct insertion probe mass spectrum of head wax from *Cerogenes auricoma*.



ions homologous with m/z 704 and then m/z 662, 647, 452 and 395 give no further information since they can be, and probably are, derived from both ends of the molecule. If the chain lengths of both keto functions were in fact different, it seems likely that the masses of the resulting acyl ions (m/z 295 in this case) could be correlated with the corresponding acid and alcohol components.

Using this technique, specimens from filaments of the head region of *C. auricoma* were found to contain over 96% of the lower homolog. Two related fulgorid species were also examined. The first, *F. lampetes* showed the same series of keto waxes with 74% of the higher homolog *Ib*, but in addition contained straight chain C_{44}–C_{54} normal carbon chain wax esters. The second species, *F. castresii* showed almost the same mixture as *C. auricoma*. A third compound found in this species as well as some samples of the abdominal skeins of *C. auricoma*, showed a molecular ion at m/z 998 whose abundance was *c.* 27% of that at m/z 984, the molecular ion of *Ib*. It was associated with an abundant fragment ion at m/z 689 but its structure is not immediately apparent. No evidence was obtained for the presence of 2 as found by Meinwald *et al.* (1975) in *D. coccus* and *P. tessalatus*.

The waxy filaments of *C. auricoma* were also demonstrated to contain proteins when analyzed by the method of Lowry *et al.* (1951). Using albumin as a standard, it appeared that proteins constituted minor constituents in the extended filaments and this likely accounts for the 0.80% nitrogen found on combustion analysis.

The production of keto ester waxes by these three fulgorid species considerably extends the distribution of these compounds in the Homoptera. Keto esters have previously been identified in the waxes of two species of Coccidae and one species of Aphididae (Chibnall *et al.*, 1934; Meinwald *et al.*, 1975). Their occurrence in fulgorid secretions suggests that they may be characteristic products of Homoptera and is not unexpected. However, finding the same compound in both generalized and specialized insects *was* unexpected. Evans (1963), in his phylogeny of the Homoptera, places Fulgoroidea as one of the most primitive superfamilies and Aphidioidea and Coccoidea among the most advanced superfamilies and places the latter two in a different evolutionary line. We hope to test this soon by checking for compound *1* through mass spectrometry in as many Homopteran superfamilies as possible.

Morphological comparisons may help explain the diversity in Coccoidea. The waxes are extruded in Fulgoroidea through multilocular pores with 6 openings (Pope, 1985) in the one case known. Within *Ceroplastes* and other scales, waxes are extruded through several types of multilocular pores with from 1 to 10 pores (Gimpel *et al.*, 1974) and quite different internal anatomy (Waku and Foldi, 1984). Furthermore, there are three types of waxes in scale insects: a wet, pastelike wax, a dry filamentous wax, and a dry granular wax (Gimpel *et al.*, 1974) which are mixed together in the wax cover. This may explain the diversity of compounds and composition found in species of scales and the higher consistency in Fulgoridae, in which only filamentous wax was analyzed.

Whether the presence of these waxes is a synapotypy for Homoptera or for one or more of its suborders, or varies independently with species can only be determined by analysis of more Homopterous taxa.

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