# Cuticular lipids of insects. VI. Cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii*

**C. L. Soliday, G. J. Blomquist, and L. L. Jackson**

Department of Chemistry, Montana State University, Bozeman, Montana 59715

**Abstract** The cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii* contain 60 and 68% alkanes and 28 and 18% secondary alcohol wax esters, respectively, with lesser amounts of normal and sterol wax esters, triglycerides, alcohols, sterols, and free fatty acids. All the hydrocarbons are saturated, and four types of alkanes are present: *n*-alkanes, 3-methylalkanes, internally branched monomethylalkanes, and internally branched dimethylalkanes. The principal *n*-alkanes in both insects are $C_{29}$ and $C_{27}$, with a range from $C_{21}$ to $C_{33}$. Trace amounts of 3-methylalkanes of 28, 30, and 32 total carbons are present. The principal internally branched monomethylalkanes are $C_{32}$ and $C_{34}$, whereas the main dimethylalkane contains 35 carbons. The *n*-alkanes do not correspond in chain length to the secondary alcohols. The primary alcohols range from $C_{22}$ to $C_{32}$ in both insects, with $C_{24}$ and $C_{26}$ predominating. The fatty acids in the triglyceride and free fatty acid fractions range from $C_{12}$ to $C_{24}$ in *M. sanguinipes* and from $C_{12}$ to $C_{18}$ in *M. packardii*.

**Supplementary key words** methyl-branched alkanes · secondary alcohols

Aliphatic secondary alcohols in the form of wax esters were recently reported in the cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii* (1). This was the first report of the presence in insects of secondary alcohols with the hydroxyl group located near the center of the chain, although such secondary alcohols are commonly found in plant cuticular lipids (2). In plants, the secondary alcohols usually correspond in chain length to the *n*-alkanes (3–7) and Kolattukudy (8) has shown in broccoli and peas that *n*-alkanes are converted to the corresponding secondary alcohols. Labeled *n*-alkanes fed to *M. sanguinipes* are incorporated into the cuticular lipids, and significant amounts of $C_{23}$ and $C_{25}$ *n*-alkanes appear as the corresponding secondary alcohols (9). Similarly, labeled *n*-alkanes administered to the surface of *M. sanguinipes* are hydroxylated and esterified (10). Thus, it became of interest to look at the structural relationship between the secondary alcohols and *n*-alkanes of *M. sanguinipes* and *M. packardii*.

Many insects have simple or complex mixtures of methyl-branched alkanes in addition to *n*-alkanes. The presence of 3-methylalkanes has been reported in the cuticular lipids of seven cockroaches (11–13) and the big stonefly (14). 2-Methylalkanes are in the common house cricket (15) and 2- or 3-methylalkanes or both have been reported in the honey bee (16). In a number of insects, internally branched monomethylalkanes are reported associated with the 3- or 2-methylalkanes (11–16). Dimethylalkanes have been recently reported in insects (17–19), and Nelson et al. (17, 18) have reported trimethylalkanes with internal methyl branches. Martin and MacConnell (20) found 3,7,11- and 4,8,12-trimethylalkanes in ants. In the present paper we report the characterization of 3-methylalkanes, internally branched monomethylalkanes, and internally branched dimethylalkanes in the cuticular hydrocarbons of *M. sanguinipes* and *M. packardii*.

Kolattukudy, Buckner, and Brown (21) have shown that the *n*-alkanes of plants can be formed by decarboxylation of the fatty acid one carbon longer than the resulting *n*-alkane. Primary alcohols of insects have been shown to be formed by reduction of the fatty acid of corresponding chain length (22). The fatty acids and primary alcohols from the cuticular lipid extracts of *M. sanguinipes* and *M. packardii* were investigated for indications of possible biosynthetic relationships between these cuticular lipid components.

## MATERIALS AND METHODS

Adult *M. sanguinipes* and *M. packardii* were collected from wild populations in southwestern Montana. The cuticular lipids were extracted and column chromatography was performed with an eight-fraction step-wise elution as described earlier (1). Further purification using prepara-

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; EGS, ethylene glycol succinate.

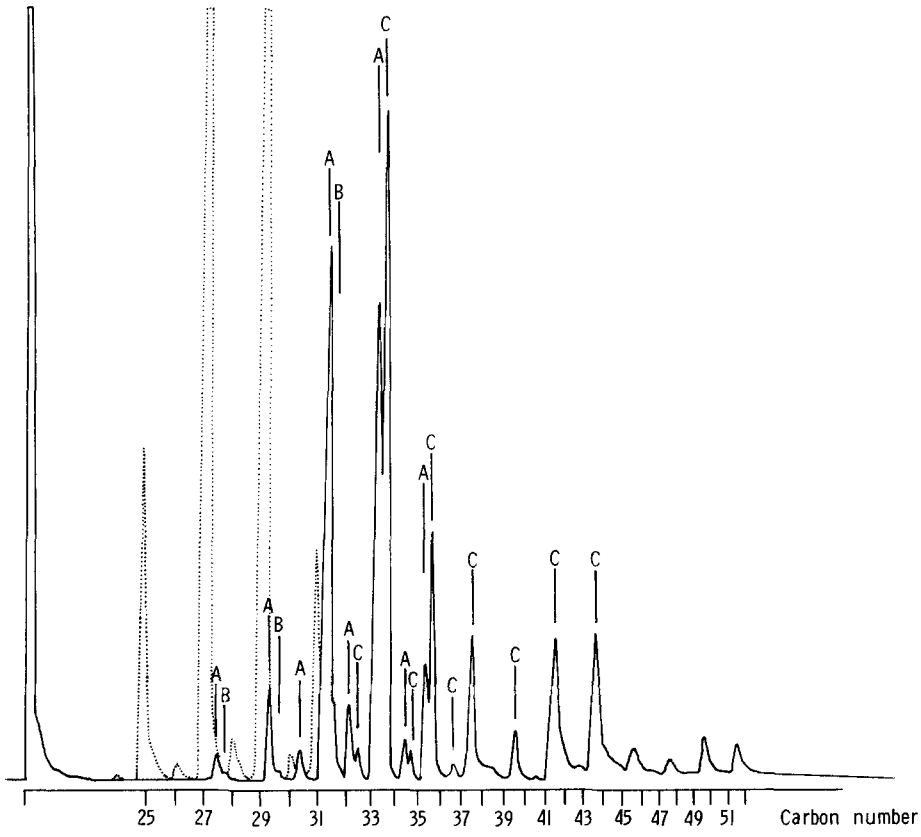


**Fig. 1.** GLC analysis of *n*-alkanes (dotted lines) and branched alkanes (solid line) from *M. packardii* on a 5 ft × ⅛ inch column of Gas-Chrom Q coated with 2% SE-54, temperature programmed from 150 to 300°C. The vertical lines with the capital letters above them indicate the type of branched alkane eluting at that position: *A*, internally branched monomethylalkanes; *B*, 3-methylalkanes; and *C*, internally branched dimethylalkanes. Ordinate, relative intensity.

tive TLC on silica gel plates developed in hexane–chloroform 50:50 (v/v) (solvent I) yielded the following fractions: alkanes, secondary alcohol wax esters, normal and sterol wax esters, triglycerides, aliphatic alcohols, sterols, and free fatty acids. The plates were visualized with rhodamine 6G under UV light. Preparative TLC was repeated until each fraction was shown to be pure by analytical TLC in solvent I. Quantitation of the fractions was obtained by weighing. Quantitation of the individual components in each fraction was obtained by GLC (disc integration). Analytical TLC plates were visualized by spraying with concentrated sulfuric acid and charring.

### Alkanes

A silver nitrate-impregnated silica gel TLC plate developed in hexane was used to check for unsaturation. GLC was carried out on a 5 ft × ⅛ inch column containing 2% SE-54 on Gas-Chrom Q and programmed from 150 to 300°C in 15 min. Retention times were compared with those of standards. *n*-Alkanes were removed from the alkane mixture by inclusion in molecular sieve 5A suspended in a solution of 2,2,4-trimethylpentane (23). The branched alkanes were separated on a 6 ft × ¼ inch column containing 3% OV-17 on Gas-Chrom Q. The column was operated isothermally at either 265 or 300°C, and the branched alkanes were collected in glass capillary tubes. Mass spectra were obtained with a Varian CH-5 instrument operating at ionizing voltage of 70 eV and collector current of 100 μA. The samples were inserted into the ion source from gold crucibles.

### Triglyceride fatty acids and free fatty acids

The triglycerides were transesterified by the method of Morgan, Hanahan, and Ekholm (24). The free fatty acids were esterified by the method of Schlenk and Gellerman (25). The resulting fatty acid methyl esters were analyzed by GLC on both a 6 ft × ¼ inch, 15% EGS, column operated isothermally at 170°C and a 9 ft × ⅛ inch, 3% OV-101 on Gas-Chrom Q, column programmed from 150 to 250°C in 10 min. Retention times were compared with those of standards.

### Aliphatic alcohols and sterols

The primary alcohols and sterols were acetylated by the method of Johnston, Gautschi, and Bloch (26). The resulting primary alcohol acetates were analyzed by GLC on the 3% OV-101 column operated isothermally at 250°C. The retention times were compared with those of standards.

TABLE 1. Comparison of the alkanes of *M. sanguinipes* and *M. packardii* (percentage of total alkanes by number of carbons)

| Number of Carbons | Normal | | 3-Methyl | | Internal Methyl | | Dimethyl | |
|---|---|---|---|---|---|---|---|---|
| | *M.s.*[a] | *M.p.*[b] | *M.s.* | *M.p.* | *M.s.* | *M.p.* | *M.s.* | *M.p.* |
| 21 | tr | | | | | | | |
| 23 | tr | tr | | | | | | |
| 24 | | tr | | | | | | |
| 25 | 1 | 6 | | | | | | |
| 26 | tr | 1 | | | | | | |
| 27 | 16 | 21 | | | | | | |
| 28 | tr | 1 | tr | tr | 1 | tr | | |
| 29 | 38 | 20 | | | | | | |
| 30 | tr | 2 | tr | tr | 2 | 2 | | |
| 31 | 6 | 4 | | | 1 | tr | | |
| 32 | | | tr | tr | 9 | 10 | | |
| 33 | tr | | | | 1 | 1 | | |
| 34 | | | | | 8 | 8 | | |
| 35 | | | | | tr | tr | 6 | 13 |
| 36 | | | | | 1 | 1 | tr | tr |
| 37 | | | | | | | 2 | 2 |
| 38 | | | | | tr | tr | tr | tr |
| 39 | | | | | | | 1 | 1 |
| 41 | | | | | | | tr | tr |
| 43 | | | | | | | 2 | 1 |
| 45 | | | | | | | 2 | 1 |
| 47, 49, 51, 53 | | | | | | | tr | tr |
| TOTAL | 61+ | 55+ | tr | tr | 23+ | 22+ | 13+ | 18+ |

[a] *M.s.* indicates *M. sanguinipes*.
[b] *M.p.* indicates *M. packardii*.

Analytical GLC of the secondary alcohol fraction was carried out in the same manner as the primary alcohols and compared with standards.

## RESULTS

Approximately 0.5 mg of cuticular lipids per insect was extracted from *M. sanguinipes* and *M. packardii*. The compositions of the cuticular lipids were similar: 60 and 68% alkanes and 28 and 18% secondary alcohol wax esters from *M. sanguinipes* and *M. packardii*, respectively, and 1–5% each of wax esters, sterol esters, triglycerides, primary alcohols, sterols, and free fatty acids in the remaining cuticular lipids.

### Hydrocarbons

*M. sanguinipes* and *M. packardii* have similar hydrocarbon compositions. Over 30 components, all saturated and ranging in chain length from 21 to 53 carbons, were found in the total hydrocarbon fraction by GLC analysis (**Fig. 1**). The normal alkanes (dotted lines, Fig. 1) account for more than 61% and 55% of the hydrocarbons of *M. sanguinipes* and *M. packardii*, respectively. They consisted primarily of odd carbon chain lengths from 21 to 33 carbons, with a predominance of *n*-nonacosane and *n*-heptacosane (**Table 1**). *n*-Alkanes with an even number of carbon atoms, ranging from 24 to 30 carbons, were present only in minor quantities.

The solid line tracing in Fig. 1 is the chromatogram of the branched-chain alkanes after removal of the *n*-alkanes by molecular sieving. Three homologous series of branched alkanes were identified by GLC and mass spectral analysis: (A) internally branched monomethylalkanes, (B) 3-methylalkanes, and (C) internally branched dimethylalkanes.

The peaks in the gas chromatogram labeled A in Fig. 1 are the internally branched monomethylalkanes, which account for 23% and 22% of the total alkanes from *M. sanguinipes* and *M. packardii*, respectively. Each of the alkanes in this series appears 0.6 to 0.7 carbon unit in front of *n*-alkanes of the same number of carbons. The majority of the monomethylalkanes in both insects are the $C_{32}$ and $C_{34}$ internally branched alkanes; the remainder of the series ranges from 28 to 38 carbons, and compounds with an even number of carbons predominate.

After separation by preparative GLC, mass spectral analysis of each isolated alkane in series A provided mass spectra characteristic of internally branched monomethylalkanes. Fragmentation on either side of the branch point with partial loss of a hydrogen atom produces two major odd mass peaks ($C_nH_{2n+1}$) and two major even mass peaks ($C_nH_{2n}$). An example of the mass spectra from these alkanes is shown in **Fig. 2**, *A*, which is the spectrum of the $C_{32}$ monomethylalkanes from peak 31-A of the gas chromatogram (Fig. 1). This mass spectrum has major peaks at 309 (308) and 169 (168) due to an 11-methyl isomer and

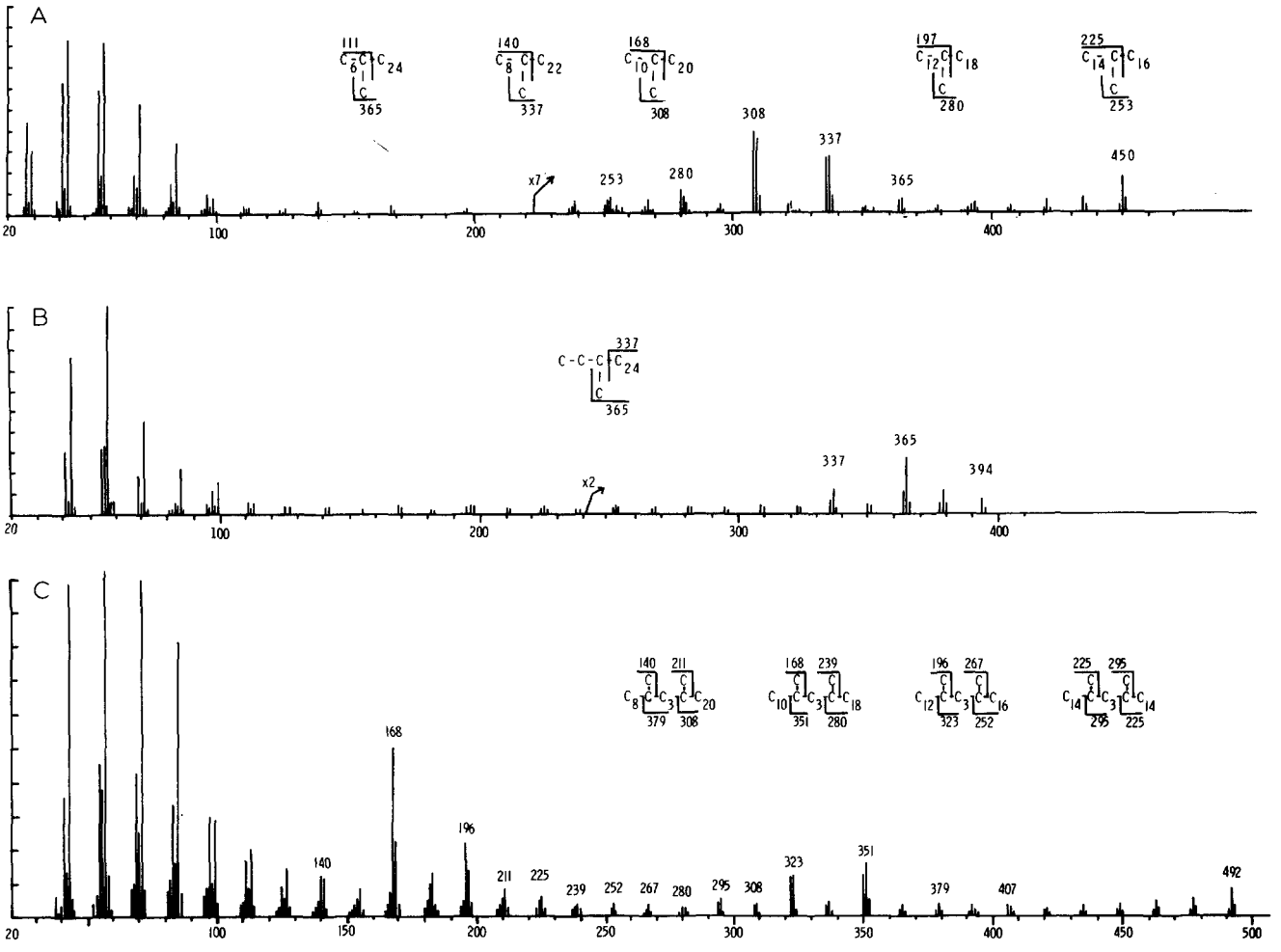


**Fig. 2.** Mass spectra of branched hydrocarbons collected from the gas chromatograph and run on a Varian CH-5 mass spectrometer: *A*, GLC peak 31-A from *M. packardii* hydrocarbons interpreted as 5-, 7-, 9-, 11-, 13-, and 15-methylhentriacontane; *B*, GLC peak 27-B from *M. sanguinipes* hydrocarbons interpreted as 3-methylheptacosane; *C*, GLC peak 33-C from *M. packardii* hydrocarbons interpreted as 9,13-, 11,15-, 13,17-, and 15,19-dimethyltritriacontane. Ordinate, relative intensity; abscissa, *m/e*.

major peaks at 337 (336) and 141 (140) due to a 9-methyl isomer. 11-Methylhentriacontane and 9-methylhentriacontane are the two major isomers present in the $C_{32}$ monomethylalkane fractions of both insects. Other isomers are present in smaller quantities as determined by the presence of mass peaks at 365 (364) and 111 (110), 281 (280) and 197 (196), and 253 (252) and 226 (225) arising from cleavage on either side of the 7-, 13-, and 15-methyl isomers. A 5-methyl isomer may also be present, as indicated by slightly increased peaks at 393 (392). The mass spectra from the $C_{28}$, $C_{30}$, $C_{31}$, $C_{33}$, and $C_{34}$ internally branched monomethylalkanes were interpreted in the same manner, and their respective isomers are summarized in **Table 2.** The $C_{35}$, $C_{36}$, $C_{37}$, and $C_{38}$ monomethylalkanes were identified by GLC retention times and from mass spectral data. There were insufficient quantities for complete characterization of these peaks.

Trace amounts of 3-methylalkanes of 28, 30, and 32 total carbons were found on both insects. This series of alkanes, labeled B in Fig. 1, elutes 0.3 carbon unit in front of *n*-alkanes of the same total carbons. Mass spectral analysis of these individual alkane fractions gave characteristic 3-methylalkane spectra with a large M − 29 and a smaller M − 57 peak, as shown in Fig. 2, *B*. This series of branched alkanes also chromatographed identically with 3-methylalkanes in a mixture of alkanes from Kusner lemon given to us by H. E. Nordby (27).

The dimethylalkanes account for more than 13% of the total hydrocarbons of *M. sanguinipes* and more than 18% of the total hydrocarbons of *M. packardii*. This series of alkanes, labeled C in Fig. 1, elutes 1.3 to 1.4 carbon units in front of *n*-alkanes of the same total carbons. This difference of 1.4 carbon units is indicative of the presence of two methyl branches towards the center of the molecule, with each methyl branch decreasing the GLC retention time by the equivalent of 0.7 carbon unit (17, 18). Most of the dimethylalkanes have an odd number of total carbons, with the major dimethylalkane having 35.

TABLE 2. Internally branched mono- and dimethylalkanes of *M. sanguinipes* and *M. packardii* according to position of the methyl group(s) in order of decreasing quantities

| Total Carbons | *M. sanguinipes* | *M. packardii* | Alkane |
|---|---|---|---|
| | | Monomethylalkanes | |
| 28 | 11-, 13-, 9-, 15-, 5-, 7- | 13-, 11- 9-, 5-, 7- | methylheptacosane |
| 30 | 11-, 9-, 7-, 5-, 13- | 9-, 11-, 7-, 13-, 15- | methylnonacosane |
| 31 | 9-, 8-, 10-, 11- | 9-, 10-, 11-, 8-, 12-, 13- | methyltriacontane |
| 32 | 11-, 9-, 13-, 7-, 15- | 11-, 9-, 13-, 15-, 7-, 5- | methylhentriacontane |
| 33 | 11-, 10-, 9-, 12- | 11-, 10-, 12-, 9-, 13- | methyldotriacontane |
| 34 | 11-, 13- | 11-, 13-, 15- | methyltritriacontane |
| | | Dimethylalkanes | |
| 35 | 11,15-, 13,17-, 9,13-, 15,19- | 11,15-, 13,17-, 15,19-, 9,13- | dimethyltritriacontane |
| 37 | 11,15-, 13,17-, 15,19-, 9,13- | 11,15-, 13,17-, 15,19- | dimethylpentatriacontane |
| 39 | 13,17-, 15,19-, 11,15-, 17,21- | 13,17-, 15,19-, 11,15-, 17,21- | dimethylheptatriacontane |
| 41 | | 13,17-, 15,19-, 11,15-, 17,21- | dimethylnonatriacontane |
| 43 | 11,15-, 13,17-, 15,19-, 17,21-, 19,23- | 13,17-, 11,15-, 19,23-, 15,19-, 17,21- | dimethylhentetracontane |

The mass spectra from isolated alkanes in series C are characteristic of dimethylalkanes. Internally branched dimethylalkanes fragment on both sides of each methyl group when subjected to mass spectrometry. Cleavage internal to a methyl group results in a secondary ion fragment with the even mass predominating. Cleavage external to a methyl group results in a secondary ion fragment containing a second methyl group with the odd mass predominating (18).

The GLC peak at 33-C (Fig. 1) gave a mass spectrum of the $C_{35}$ dimethylalkanes (Fig. 2, *C*). The major even and odd mass peaks show that the $C_{35}$ dimethylalkanes are a mixture of 11,15-, 13,17-, 9,13-, and 15,19-dimethyltritriacontane. The peaks at 168, 351, 280, and 239 arise from cleavage on each side of the two methyl branches of 11,15-dimethyltritriacontane with loss or retention of the hydrogen atom. When the charged species contain a second methyl group, the odd mass peak predominates. Cleavage on the inside of the two methyl groups results in a secondary ion fragment in which the even mass peak predominates. The peaks at 196, 323, 252, and 267 arise from cleavage on each side of the two methyl branches of 13,17-dimethyltritriacontane. The peaks at 140, 379, 211, and 308 arise from cleavage on each side of the two methyl branches of 9,13-dimethyltritriacontane. The peaks at 225 (224) and 295 arise from cleavage of the symmetrical 15,19-dimethyltritriacontane. The mass spectra of the $C_{37}$, $C_{39}$, $C_{41}$, and $C_{43}$ homologs were interpreted in a similar manner, and the isomers are summarized in Table 2. Parent peaks for $C_{36}$, $C_{38}$, and $C_{45}$ alkanes were observed in mass spectral data obtained from preparative GLC samples isolated from peaks corresponding to their respective dimethylalkane retention times. The branched alkanes beyond the $C_{45}$ dimethylalkane (43-C in Fig. 1) were of insufficient quantity and too poorly resolved for mass spectral analysis; however, from GLC retention times they appear to be $C_{47}$, $C_{49}$, $C_{51}$, and $C_{53}$ dimethylalkanes.

TABLE 3. Fatty acid composition of free fatty acid and triglyceride fractions from cuticular lipids of *M. sanguinipes* and *M. packardii* (percentage by weight)

| | Free Fatty Acids | | Triglyceride Fatty Acids | |
|---|---|---|---|---|
| Fatty Acid[a] | *M.s.*[b] | *M.p.*[b] | *M.s.* | *M.p.* |
| 12:0 | 2 | 2 | 3 | |
| 14:0 | 2 | 1 | 2 | 4 |
| 15:0 | | | tr | 2 |
| 16:0 | 13 | 10 | 27 | 22 |
| 16:1 | 3 | 4 | 5 | 4 |
| 17:0 | | | tr | |
| 18:0 | 6 | 5 | 6 | 5 |
| 18:1 | 21 | 23 | 23 | 44 |
| 18:2 | 24 | 26 | 14 | 11 |
| 18:3 | 28 | 28 | 19 | 8 |
| 20:0 | tr | | tr | |
| 22:0 | tr | | tr | |
| 24:0 | | | tr | |

[a] Number of carbon atoms:number of double bonds.
[b] *M.s.* indicates *M. sanguinipes*.
[c] *M.p.* indicates *M. packardii*.

### Wax esters

The secondary alcohol wax esters have been reported earlier (1). In both insects, sterol and primary alcohol wax esters are also observed; however, due to insufficient quantity of the fraction and complexity of the mixture, they were not characterized.

### Triglyceride fatty acids and free fatty acids

Triglyceride fatty acids of both insects are quite similar (**Table 3**). *M. sanguinipes* has 3% 12:0 and traces of 20:0, 22:0, and 24:0, which are not observed in *M. packardii*. The major triglyceride fatty acids in *M. sanguinipes* are 16:0 > 18:1 > 18:3 > 18:2, whereas those of *M. packardii* are 18:1 > 16:0 > 18:2 > 18:3. The free fatty acids range from $C_{12}$ to $C_{22}$ in *M. sanguinipes* and from $C_{12}$ to $C_{18}$ in *M. packardii*. The major free fatty acids in both insects are 18:3, 18:2, and 18:1.

TABLE 4. Cuticular primary alcohols and sterols of *M. sanguinipes* and *M. packardii* (percentage by weight in each fraction)

| | *M. sanguinipes* | *M. packardii* |
|---|---|---|
| Number of carbons in primary alcohol | | |
| 22 | 3 | 1 |
| 24 | 34 | 48 |
| 25 | tr | |
| 26 | 36 | 36 |
| 27 | tr | tr |
| 28 | 9 | 4 |
| 29 | 5 | 2 |
| 30 | 12 | 5 |
| 32 | tr | tr |
| Sterol | | |
| Cholesterol | 96 | 96 |
| Stigmasterol | 3 | 3 |
| Unidentified sterol | 1 | 1 |

### Aliphatic alcohols and sterols

The major free primary alcohols in both *M. sanguinipes* and *M. packardii* are $C_{24}$ and $C_{26}$, with a range from $C_{22}$ to $C_{32}$ **(Table 4).** Free secondary alcohols were observed in both grasshoppers in trace amounts and were found in the same composition as the secondary alcohols found in the secondary alcohol wax ester fraction (1).

The major sterol in both insects was cholesterol, which was 96% of the sterol fraction. 3% stigmasterol and 1% unidentified sterol were also present (Table 4).

## DISCUSSION

Unlike the situation observed in most plants (2), the secondary alcohols of these grasshoppers do not correspond in chain length to their major *n*-alkanes. In the cuticular lipids of *M. sanguinipes* and *M. packardii,* the major secondary alcohols are $C_{25}$ and shorter, and the *n*-alkanes are predominantly $C_{27}$ and longer. This can be at least partly explained by the fact that the alkane hydroxylating system is specific for alkanes of $C_{25}$ and shorter (9, 10). Likewise, the plant alkanes that would be dietary to the grasshoppers usually range from $C_{25}$ to $C_{35}$ (2). It therefore appears that the carbon chains for the secondary alcohols are predominantly biosynthesized by the insect, whereas the diet can supply some of the cuticular lipid alkanes.

The primary alcohols and *n*-alkanes of *M. sanguinipes* and *M. packardii* have a similar carbon chain length range of from $C_{22}$ to $C_{32}$, suggesting the possibility of a similar precursor. Kolattukudy et al. (21) have demonstrated in *Brassica oleracea* the direct decarboxylation of triacontanoic acid to *n*-nonacosane. Lambremont (23) has demonstrated the reduction of fatty acids to fatty alcohols and the oxidation of fatty alcohols to fatty acids in the tobacco budworm. If a decarboxylation of fatty acids to alkanes and reduction of fatty acids to alcohols is operating in *M. sanguinipes* and *M. packardii,* the very long chain fatty acids must either be very efficiently decarboxylated to alkanes or reduced to the corresponding alcohols because only trace amounts of fatty acids 20:0, 22:0, and 24:0 are present in *M. sanguinipes* and no fatty acids longer than 24:0 are observed. Further work is being carried out in this laboratory to determine the metabolic relationship between very long chain fatty acids and fatty alcohols.

The chemistry and metabolism of the branched alkanes of grasshoppers, and insects in general, have not been extensively studied. The comparisons of the branched alkanes of the grasshoppers *M. sanguinipes* and *M. packardii* presented in Tables 1 and 2 show that although there are discernible quantitative differences in various chain lengths (Table 1) and in isomer composition (Table 2), the overall compositions are very similar. Most of the monomethylalkanes have an even number of total carbons with the methyl branch on an odd-numbered carbon atom. However, minor quantities of odd-numbered total carbon monomethylalkanes were observed with the methyl branch on an even- or odd-numbered carbon atom. Three other insects have been reported to have monomethylalkanes with methyl branches on an even-numbered carbon atom (14, 19, 28). Most of the dimethylalkanes have an odd number of total carbons with the methyl branches on odd-numbered carbon atoms.

Although the biosynthetic relationship between *n*-alkanes and secondary alcohols has been established and the possibility of a precursor–product relationship between fatty acids to primary alcohols and fatty acids to alkanes is suggested, information on the origin of a principal component of the insect cuticular lipids, the methyl-branched alkanes, is lacking. Why do insects biosynthesize branched hydrocarbons when they can incorporate normal hydrocarbons from their diet into cuticular lipids (9)? Why are one-third to one-half of the hydrocarbons branched? What particular role do branched hydrocarbons play, and what particular biosynthetic pathways are utilized for the biosynthesis of branched hydrocarbons? The answers to these and other questions will help tremendously in our understanding of the chemistry and biochemistry of insect cuticular lipids.

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