Novel Surface Lipids of Diapausing Manduca sexta Pupae

LONG CHAIN OXOALCOHOL ESTERS OF ACETOACETIC, HYDROXYBUTYRIC, AND ACETIC ACIDS

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Ester components in the surface wax from diapausing tobacco hornworm pupae, Manduca sexta L., were separated by thin layer chromatography and gas-liquid chromatography, and characterized by infrared spectroscopy and gas-liquid chromatography-mass spectrometry. Three groups of esters were identified as natural derivatives of acetic acid, acetoacetic acid, and 3-hydroxybutyric acid. The major ester fraction was identified as a mixture of $C_{26}$ (10%), $C_{27}$ (5%), and $C_{28}$ (85%) oxoalcohol esters of acetic acid. The major homolog consisted of equal amounts of 11-o xoocotacosanyl 3-oxobutanate and 12-o xoocotacosanyl 3-oxobutanate. Lesser amounts of 11- and 12-oxoocotacosanyl and n-octacosanyl esters of acetic and 3-hydroxybutyric acids were also identified. The chain length distributions of these $C_{26}$, $C_{27}$, and $C_{28}$ oxoalcohols and n- primary alcohols ester moieties, as well as the isomeric ratios for the 11- and 12-oxoalcohol isomers, were similar to the aldehydeoxyis and unesterified oxoalcohols previously identified by Buckner et al (Buckner, J. S., Nelson, D. R., Haak, H., and Pomonis, J. G. (1984) J. Biol. Chem. 259, 8452-8470) as lipid components of the surface wax of M. sexta pupae.

The major constituents of the surface lipids of the tobacco hornworm, Manduca sexta, have been identified as long chain oxoaldehydes and oxoalcohols (1). These aldehydes and alcohols consisted of a homologous series of $C_{m}$, $C_{n}$, and $C_{p}$ components, with the major component ($C_{28}$, 75-85%) consisting of nearly equal amounts of the 11-oxo and 12-oxo isomers. Alkaline hydrolysis of the surface lipids indicated that the majority of the oxoalcohols existed as esters of unknown acids. These acids appeared to be short chain, volatile compounds that were not the usual fatty acids.

To our knowledge, long chain oxoalcohols esterified to short chain acids have not been reported from insects. However, esters consisting of long chain oxoalcohols and long chain oxoacids have been reported as major components of the surface wax of several insects. A $C_{6}$ wax ester, 15-o xo tet ratriacontanyl 13-oxodotriacontanoate, was produced by the cochineal insect, Coccus cacti (2), and the wooly alder aphid, Prociphilus tesselatus (3). Nearly 100% of the surface wax of another cochineal insect, Dactylopius confluens, was the $C_{6}$ oxo ester, 15-o xo tet ratriacontanyl 11-oxotriacontanoate (3). The rhizomes of the plant Cryptocoryne spiralis contained two oxo acid esters, ethyl 14-o xo tetraoxocanoate and 15-oxo cetanoyl 14-oxoheptadecanoyl (4).

In this paper, we present evidence for the composition and identification of novel esters as major components of the surface lipids of M. sexta pupae in diapause. Structures of intact ester components as well as their alcohol and acid moieties were determined by TLC, GLC, IR spectroscopy and GLC-MS.

MATERIALS AND METHODS AND RESULTS

The surface lipids of M. sexta were separated by TLC into nine fractions, designated Fractions I-IX (1). Fraction I was identified as hydrocarbons (3). Fractions V and VIII were identified as $C_{27}-C_{28}$ oxoaldehydes and $C_{27}-C_{28}$ oxoalcohols, respectively. The other major lipids, Fractions IV, VI, VII, and IX, were not identified, but they were shown to contain $C_{27}-C_{28}$ oxoalcohols and n-primary alcohols esterified to unknown short chain acids.

Identification of Fraction IV: Acetate Esters

Fraction IV migrated just ahead of the oxoaldehydes (Fraction V) when chromatographed on silica gel plates developed with solvent system A (Fig. 1). GLC analysis of Fraction IV showed three components designated as peaks 1-3 (Fig. 2). The major component of Fraction IV (peak 3) had an equivalent chain length of 33.7. In comparison, the $C_{28}$ oxoaldehyde in Fraction V had a value of 31.5. The column retention times of GLC peaks 1-3 relative to the retention times of alkane standards suggested that Fraction IV was a homologous series of components.

The major component of Fraction IV (Fig. 2, peak 3) was obtained pure by trapping from the gas chromatograph. Analysis by IR spectroscopy indicated two intense absorption bands at 1700 and 1740 cm$^{-1}$, indicative of the carbonyl for aliphatic ketones and esters, respectively (Fig. 3A). This compound is also included in the microfilm edition of the Journal that is available from Waverly Press.
component of Fraction IV was analyzed also by EI- and CI-mass spectrometry and was concluded to be a mixture of the acetate esters of 11- and 12-oxooctacosanol (Fig. 4). The CI mass spectrum (Fig. 4B) indicated a molecular weight of 466 as a result of an M + 1 ion at m/z 467 (base peak) with isobutane as ionizing gas. The ion at m/z 407 corresponds to the loss of acetate. α-cleavage at the 12-oxo position was indicated in the CI mass spectrum as well as the EI mass spectrum (Fig. 4A) by a fragment ion at m/z 253 and McLafferty rearrangement ions (6) at m/z 268 and 256. β-cleavage at the 12-oxo position was indicated by the fragment ion at m/z 199. Another source of the ion at m/z 199 may be α-cleavage of the 12-oxoalcohol moiety followed by dehydration to yield the ion at m/z 181 (see Fig. 6 for fragmentation of the 11- and 12-oxoalcohols). The ion at m/z 214 corresponds to a McLafferty rearrangement of the 12-oxoalcohol moiety (Fig. 6). Rearrangement fragment ions at m/z 242 and 282 indicated the presence of the 11-oxo isomer. A characteristic pair of ions observed in all mass spectra of esters of 12-oxoalcohol occurred at m/z 138:139 (see Figs. 4, A and B, 7, A and B, and 9C).

Alkaline hydrolysis of Fraction IV yielded the same homologous series of C_{26}, C_{27}, and C_{28} oxoalcohols that were previously found in Fraction VIII (1). Also, the GLC-EI mass spectrum observed for the major component of Fraction IV (Fig. 4A), was the same as the mass spectrum obtained for the acetate derivative of the C_{28} oxoalcohol derived from acetylation of Fraction VIII. In addition to the acetate ester of the C_{28} oxoalcohol, the minor peaks 1 and 2 (Fig. 2) were identified from their GLC-EI mass spectra as the acetate esters of C_{26} and C_{27} oxoalcohols (data not shown). The C_{26} homolog was primarily the 11-oxo isomer, whereas the C_{27} homolog was a mixture of 11-oxo and 12-oxo isomers.

**Identification of Fraction VI: Acetoacetate Esters**

Characterization of Fraction VI and Its Hydrolysis Products—Alkaline hydrolysis of surface lipid extracts from M. sexta pupae indicated that Fraction VI contained esters of oxoalcohols (1). This observation was confirmed by TLC separation of Fraction VI and subsequent hydrolysis (60 °C for 2 h in sealed ampules with 0.2 M KOH in 80% isopropanol) to yield oxoalcohols. Me_{2}Si derivatives of the oxoalcohols were identified by GLC-MS as being the same homologous series of compounds that had been previously identified for the oxoalcohols and for the partially reduced oxoaldehydes, namely, C_{26} (5–10%), C_{27} (2–5%), and C_{28} (85–90%). The C_{28} oxoalcohols of Fraction VI contained mainly the 12-oxo isomer with a lesser amount of the 11-oxo isomer. Consistent with earlier findings for the oxoalcohols and oxoaldehydes, the 11-oxo isomer was predominant for the C_{26} homolog.

IR spectroscopic analysis of purified Fraction VI also indicated an ester; a characteristic absorption band at 1740 cm⁻¹ (Fig. 3C) that had been observed for the acetate ester of oxoalcohols (Fig. 3A) and the band at 1700 cm⁻¹ characteristic of aliphatic ketones. The acetate esters of oxoalcohols (Fraction VI) had a greater R_f on TLC plates than that of Fraction VI. Hence, the acid moiety of Fraction VI must be more polar than acetate.

Fraction VI possessed poor chromatographic properties when analyzed by GLC (Fig. 5A). Only one major peak was observed having a broad asymmetrical shape. Mass spectral scans taken across this peak showed a molecular ion at m/z 424, a fragment ion at m/z 253, and rearrangement ions at m/z 214 and 268 and indicated that the peak was mainly 12-
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Fig. 4. EI mass spectrum (A) and CI mass spectrum (B) of 11- and 12-oxo-octacosanyl acetate (Fraction IV, GLC peak 3 in Fig. 2). Values in parentheses are the masses of ions formed by McLafferty rearrangements. The base peak in the CI spectrum was at m/z 467.

oxoalcohol (Fig. 6). Fragment ions in the mass spectrum also indicated the presence of lesser quantities of the 11-oxo isomer. Therefore, in contrast to the acetate esters, Fraction VI apparently underwent hydrolysis during GLC-MS analysis and only the o xoalcohol moieties were detected.

More useful mass spectra for Fraction VI were obtained from solid-sample-probe analysis in both the EI and CI modes. The ions at m/z 423 and 425 in the EI and CI mass spectra, respectively, indicated the presence of the C_{20} o xoalcohol (M, = 424) hydrolysis product (Fig. 7, A and B). The M + 1 ion at m/z 509 in the CI mass spectrum indicated a molecular weight of 508 for the intact ester of Fraction VI. Structures consistent with these EI and CI mass spectra were the 3-oxobutanoate (acetoacetate) esters of 11- and 12-oxo-octacosanol. The fragment ion at m/z 103 could arise from dehydration of the fragment ion at m/z 199. The ions at m/z 312 and 228 suggested a trace amount of a 13-oxo isomer may be present.

Esters of acetoacetic acid can exist as tautomers, both in the keto and enol form. The enol isomer reacts with ferric chloride to give a red or violet color (9). TLC plates spotted with either total lipid or purified Fraction VI and developed with either solvent system A or B were sprayed with a solution of ferric chloride. With mild heating and usually within 5 min, a reddish spot appeared at a position on TLC plates coincident with Fraction VI. A ferric chloride reaction was not detected with any of the other surface lipid components. However, the same coloration was observed with either ethyl or butyl acetoacetate standards spotted on TLC plates. Enolization of Fraction VI was also indicated from IR spectroscopy by a band at 1645 cm⁻¹, characteristic of a shift to a shorter carbonyl frequency due to enol tautomerization of β-oxo esters (10).

NaBH₄ Reduction of Fraction VI—If Fraction VI was a mixture of 11- and 12-oxoalcohol esters of acetoacetic acid, the 3-oxo of the acid moiety and the 11- and 12-oxo of the alcohol moieties should reduce in the presence of NaBH₄ to corresponding hydroxyl groups yielding diol ester structures. Purified Fraction VI, dissolved in isopropanol, was reacted with NaBH₄ at 40 °C for 30 min, at 60 °C for 60 min, and 82 °C for 120 min. Two reaction products were observed by TLC of the mildly reacted (40 °C) sample (Fig. 8, lane 2). Their positions on the TLC plate at Rᵢ 0.35 and 0.22 indicated more polar properties than unreacted Fraction VI (lane 1, Rᵢ 0.52). Under the stronger reaction conditions, the Rᵢ 0.22
material was the major product (Fig. 8, lanes 3 and 4).

The IR spectrum for the RF 0.35 material showed both ketone and ester carbonyl bands at 1700 and 1740 cm⁻¹, respectively, and a broad band at 3400 cm⁻¹ (Fig. 3D), characteristic of a hydroxyl function and similar to the band observed for the C₈₈ oxoalcohol (Fig. 3B). The more polar NaBH₄ reaction product (RF 0.22) showed the carbonyl function for an ester at 1740 cm⁻¹, but not that for the ketone (1700 cm⁻¹), and a hydroxyl function at 3400 cm⁻¹ (Fig. 3E). These data suggested that the RF 0.35 material was partially reduced and contained both a ketone and hydroxyl group and that the RF 0.22 material was the diol ester.

The compounds with the hydroxyl groups obtained by reduction were derivatized with TBDMS-Cl and the resulting derivatives were analyzed by GLC-MS. A typical ion profile of the derivatized RF 0.35 material showed three major peaks (Fig. 5B). Peak 3 gave a mass spectrum consistent for the t-BuMe₂Si derivatives of 3-hydroxybutanoate esters of 11- and 12-oxoocatcosanol (Fig. 9A). The molecular ion at m/z 624 was not detected, however, the M - 57 ion (representing loss of the t-butyl group) at m/z 567 was present. The base peak at m/z 161 represented the t-BuMe₂Si derivative of 3-hydroxybutanoic acid less the 57 mass units of the t-butyl group. The t-BuMe₂Si group on the penultimate hydroxyl of the acid moiety of the ester was also indicated by the presence of a fragment ion at m/z 159. The presence of the 12-oxo isomer of the alcohol moiety was indicated by the a-cleavage fragment ion at m/z 253 and the β-cleavage fragment ion at m/z 357. A corresponding fragment ion at m/z 343 indicated the presence of a lesser amount of the 11-oxo isomer.

Peak 1 of the RF 0.35 material was identified as the t-BuMe₂Si derivative of mainly 12-oxoocatcosanol (Fig. 9B). The mass spectrum for his derivative showed an intense ion (100%) for M - 57 at m/z 481 and a β-cleavage ion at m/z 271. A lesser amount of the 11-oxo isomer was indicated by m/z 257. Peak 2 gave a mass spectrum consistent with a structure derived from dehydration of the 3-hydroxybutanoate ester (Fig. 9C). This degradation product was characterized by a molecular ion at m/z 492, an RCOOH₂ fragment ion at m/z 87, and a base peak at m/z 69. Characteristic α- and β-cleavage fragmentation at the 12-oxo position supported the identification of this material as mainly 12-oxoocatcosanyl 2-butenoate. The ion at m/z 481 is believed to be from tailing of peak 1. Peaks 1 and 2 apparently resulted from partial degradation of the derivatized ester in the flash heater. All three mass spectra support the identification of Fraction VI as being oxoalcohol esters of acetocetic acid.

GLC-MS analysis of the t-BuMe₂Si derivatives of the more polar product (RF 0.22) following NaBH₄ reduction of Fraction VI showed other major components (Fig. 5C) and an elution profile similar to that of the RF 0.35 derivatives, but the three components possessed longer retention times. A mass spectrum of peak 3 of the RF 0.22 component confirmed that it was the t-BuMe₂Si derivative of the dihydroxy ester formed by reduction of the 3-oxoalcohol ester of the C₈₈ oxoalcohol (Fig. 10A). A molecular weight of 740 was indicated by the M - 57 fragment ion at m/z 683 and the presence of the 3-hydroxybutanoic acid moiety was indicated by the fragment ion at m/z 161 previously identified for the t-BuMe₂Si derivative of the C₈₈ oxoalcohol ester in Fig. 9A. The position of the NaBH₄ reduced carbonyl function on carbon number 12 of the alcohol moiety was clearly established by the fragment ions at m/z 369 and 515.

GLC peaks 1 and 2 were identified by their mass spectra as...
the t-BuMe2Si derivatives of 12-hydroxyoctacosanol (Fig. 10B) and 12-hydroxyoctacosanyl 2-butenoate (Fig. 10C), respectively, apparently formed by partial degradation of the ester in the flash heater. The hydroxycarboxylic acid derivative (Fig. 10B) had ions at m/z 75, 147, 149, 189, and 191 corresponding to (CH3)3-Si-OH+, (CH3)3-Si-O-Si-(CH3)2+, (CH3)3-Si-O-Si(OH)-(CH3)2+, (CH3)3-Si-O-Si(CH3)2-C(CH3)2+, and (CH3)3-Si-O-Si(OH)-C(CH3)2+, respectively (11, 12). The presence of a small amount of the 11-hydroxy isomer was indicated by the ions at m/z 383, 415, and 457. The ions at m/z 443 and 457 are unknown rearrangement ions believed to involve the C16 and C17 alkyl moieties, respectively. Also, analogous ions at m/z 401 and 415 were observed in mass spectra of the MeSi derivatives (1) and at m/z 303 for the t-BuMe2Si derivative of the 12-hydroxyoctadecanol (data not shown). The mass spectrum for the 12-hydroxyoctacosanly 2-butenoate t-BuMe2Si derivative (Fig. 10C) was characterized by an M–57 fragment ion at m/z 551 and α-cleavage ions for the 12-hydroxy position at m/z 369 and 383. The intense fragment ion at m/z 143 probably represents a rearrangement ion corresponding to the loss of 57 mass units (t-butyl) from the t-BuMe2Si derivative of 2-butenoic acid (CH3=CH=CH=C(O)–O–Si–(CH3)2). Again, all three mass spectra support the conclusion that Fraction VI was a mixture of oxoalcohol esters of acetoacetic acid.

The tendency for 3-hydroxybutanoate esters to undergo on-column hydrolysis and dehydration was confirmed by GLC-MS analysis of the authentic octadecyl 3-hydroxybutanoate t-BuMe2Si derivative (Fig. 11). In addition to the presence of peak 3 for the t-BuMe2Si derivative of the intact ester, the total ion plot showed the resolved peaks 1 and 2, the t-BuMe2Si derivative of the C18 alcohol and the 2-butenoic acid ester of the C18 alcohol, respectively (Fig. 5D). The mass spectrum for the t-BuMe2Si derivative of octadecyl 3-hydroxybutanoate (Fig. 11A) showed a fragmentation pattern similar to that shown previously for the t-BuMe2Si derivative of 12-oxyoctacosanly 3-hydroxybutanoate (Fig. 9A); a base peak at m/z 161, a less intense fragment ion at m/z 159 that was indicative of a t-BuMe2Si derivative of a penultimate hydroxyl group, and an M–57 fragment ion at m/z 413. The ion at m/z 327 was probably a rearrangement ion corresponding to the loss of 57 mass units from the t-BuMe2Si derivative of octadecanol. The mass spectrum of the 2-butenoic acid compound was characterized by a base peak at m/z 87 indicative of the RCOOH2+ fragment ion (Fig. 12B). An analogous base peak at m/z 89 has been reported for n-butenoic acid esters of fatty alcohols (13).

**Identification of Fractions IX and VII: 3-Hydroxybutyric Acid Esters**

Earlier studies of the surface lipids from *M. sexta* had indicated that Fraction IX was the most polar lipid constituent hydrolyzed by alcoholic KOH to yield oxoalcohols (1). Fraction IX was identified as a homologous series of C26, C27, and C28 oxoalcohol esters of 3-hydroxybutyric acid. These esters had been previously identified in the Rf 0.35 band following TLC separation (Fig. 9) of the NaBH4-reduced oxoalcohol esters of acetoacetic acid (Fraction VI).

Fraction IX showed the same IR spectrum as that of the Rf 0.35 band (Fig. 3D) and when silylated (TBDMSTFA), gave the same GLC-EI-MS elution profile and retention times (Fig. 5B) as did the RF 0.35 components. The mass spectra previously shown for the three C26 components in the RF 0.35 band, the intact ester (Fig. 10A), the oxoalcohol hydrolysis product (Fig. 10B), and the dehydrated butenoate derivative (Fig. 10C), were identical to those of the three GLC components derived from Fraction IX (data not shown).

GLC-EI-MS analysis of Fraction IX after treatment with BSA gave mass spectra for the C26 MeSi derivatives (Fig. 12) with a fragmentation pattern analogous to that observed for the t-BuMe2Si derivative. (It is interesting to note the suppressing effect that the t-butyl group has on the formation and/or intensity of some fragment ions.) The ions at m/z 340 and 130 correspond to rearrangements forming the fragments C18H37-C(O–Si–(CH3))2-C(CH3)=CH2 and (CH3)3-Si-O–CH=CH=C(O)O or (CH3)3-Si-O–CH=CH=CH2, respectively (1). The fragment ion at m/z 143 was previously postulated as the dimethylsilyl derivative of 2-butenoic acid (see Fig. 10C). As with the t-BuMe2Si derivatives, the MeSi derivatives rearranged to give the MeSi derivative of the oxoalcohol (data not shown). However, very little of the butenoate ester of the oxoalcohol was formed. Thus, all the data supported the conclusion that Fraction IX was a mixture of oxoalcohol esters of hydroxybutyric acid.

Fraction VII reacted with ethanolic KOH to give a product that migrated on thin layer plates coincident with n-primary alcohol standard and just ahead of Fraction VII. These apparent alcohols were analyzed by GLC-EI-MS as their t-BuMe2Si derivatives and were identified as a series of C26, C27, and C28 n-primary alcohols, with the C28 alcohol as the major component (85%). The mass spectrum for the t-BuMe2Si derivative of the C28 alcohol was identical to the mass spectrum shown previously for the t-BuMe2Si derivative of the C28 n-primary alcohol derived from hydrolysis of total lipid extracts (1).

The ester components in Fraction VII reacted with
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**Fig. 9.** GLC-EI mass spectra. A, t-BuMe₂Si derivative of 12-oxooctacosanyl 3-hydroxybutanoate (Fig. 5B, GLC peak 3); B, t-BuMe₂Si derivative of 12-oxooctacosanol (Fig. 5B, GLC peak 1); and C, 12-oxooctacosanyl butenolate (Fig. 5B, GLC peak 2). Base peaks in spectra A, B, and C were at m/z 161, 481, and 69, respectively. Values in parentheses are the masses of ions formed by McLafferty rearrangements.

TBDMSTFA and the t-BuMe₂Si derivatives were characterized by GLC-EI-MS as C₃₆₋C₃₈ n-primary alcohol esters of 3-hydroxybutyric acid. A typical mass spectrum of the t-BuMe₂Si derivative of the C₃₈ component showed a molecular ion at m/z 610, a M − 15 at m/z 595, and a more intense ion for M − 57 at m/z 553 (Fig. 13). The base peak at m/z 161 and the fragment ions at m/z 159, 203, and 219 are characteristic for the t-BuMe₂Si derivative of 3-hydroxybutyric acid (see Figs. 9A, 10A, and 11A). Thus, Fraction VIII was concluded to be a mixture of the long chain primary alcohol esters of 3-hydroxybutyric acid.

**DISCUSSION**

The major lipid constituents of the surface wax of diapausing M. sexta pupae were clearly established as long chain oxoaldehydes and corresponding oxoalcohols that existed as alcohol moieties of structures that were susceptible to alkaline hydrolysis (1). The data presented here established the major lipid component (Fraction VI) as oxoalcohol esters of acetocetic acid. Oxoalcohol esters of acetic acid (Fraction IV) and 3-hydroxybutyric acid (Fraction IX) were also found.

The relative composition of the various lipid components that comprised the surface wax was difficult to determine. Therefore, estimates on the composition of the surface lipids were based on the distribution of radioactivity on TLC plates from lipid extracted from the surface of pupae injected with [¹⁴C]malonate (1). The estimated composition was oxoalcohol esters of acetocetic acid (35-45%), oxoaldehydes (30-35%), oxoalcohol esters of hydroxybutyric acid (5-10%), unesterified oxoalcohols (5-10%), oxoalcohol esters of acetic acid (2-5%), hydrocarbons (2-5%), and unknown fractions including esters of n-primary alcohols (5-10%). The chain length distribution was much the same for oxoaldehydes, oxoalcohols, and n-primary alcohols; C₂₆ (50%), C₂₇ (2-5%), and C₃₈ (85-95%). The distribution of oxo isomers, estimated from mass spectral data, varied for the different lipid components. The C₂₆ oxoalcohol esters of acetocetic acid (from Fraction VI) showed a 12-oxo/11-oxo ratio of 2:1, whereas the ratio for the C₂₇ oxoaldehydes (from Fraction V) was 1:1. The C₂₈ components for all fractions were mainly the 11-oxo isomer and the C₃₈ components contained nearly equal amounts of the 11- and 12-oxo compounds.

Both the acetocetate and 3-hydroxybutyrate esters of oxoalcohols were susceptible to thermal degradation. The data
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Fig. 10. GLC-EI mass spectra. A, t-BuMe2Si derivative of 12-hydroxyoctacosanyl 3-hydroxybutanoate (Fig. 5C, GLC peak 3); B, the t-BuMe2Si derivative of 12-hydroxyoctacosanol (Fig. 5C, GLC peak 1); and C, the t-BuMe2Si derivative of 12-hydroxyoctacosanyl butenoate (Fig. 5C, GLC peak 2). Base peaks in all spectra were at m/z 75. The fragment ion peak at m/z 143 in spectrum C had a relative intensity of 85%.

Presented in Figs. 5A, 6, and 7 indicate that the acetoacetate esters (Fraction VI) underwent pyrolytic degradation during GLC, GLC-EI-MS, and solid sample probe EI- and CI-MS analyses. In addition to hydrolysis of the t-BuMe2Si derivatives of the 3-hydroxybutyrate esters during GLC and GLC-MS analyses, we observed the elimination of the penultimate hydroxyl group from the acyl moiety to form butenoate ester compounds (see Figs. 5B and 9). Since the acetate esters (Fraction IV) were stable to these procedures, the thermal instability of the acetoacetate and 3-hydroxybutyrate esters was apparently due to the presence of the oxo or hydroxyl function at a position β to the ester carbonyl.

Hydrolysis of the total surface lipid extracts followed by GLC-MS analyses had indicated the presence of C28-C38 primary alcohol moieties with the same chain length distribution as the oxoalcohols (1). Primary alcohol esters of 3-hydroxybutyric acid were identified in Fraction VII. GLC-MS analyses of fractions recovered from TLC of total lipid revealed the presence of small quantities of acetate esters of primary alcohols in the regions of the TLC plates between hydrocarbons (Fraction I) and acetate esters of oxoalcohols (Fraction IV). Acetoacetate esters of primary alcohols were not identified, however, alkaline hydrolysis of lipids from regions of TLC plates between Fractions V and VI yielded small quantities of primary alcohols. The Rf value of these alcohol esters was about the same as that for authentic octadecyl 3-oxobutanoate suggesting that the acyl moieties of the primary alcohols may have been acetoacetate.

To our knowledge, the natural occurrence of fatty alcohol esters of acetoacetic or 3-hydroxybutyric acids has not been reported. However, esters of n-butyric acid were identified as the major volatile constituents of skin secretion of a South American primate, the marmoset, Saguinus fuscicollis (13). The alcohol moieties were identified as a series of C16-C24 saturated and unsaturated fatty alcohols. Acetate esters of fatty alcohols have been found in insect surface lipids. Many of the female sex pheromones of Lepidoptera have been identified as acetate esters of C8-C16 unsaturated fatty alcohols (14, 15). Acetate esters of shorter chain alcohols C6-C10 have been identified as insect exocrine products (16). An acetate ester of a secondary alcohol, 8-heneicosanol, comprised 27% of the surface lipid extracted from the male little house fly, Fannia canicularis (17). cis-Vaccenyl acetate was present in cuticular lipid extracts from male fruit flies, Drosophila melanogaster (18).
Hydrocarbons comprise a majority of the cuticular lipids of many insects, and wax esters, sterol esters, fatty alcohols, and free fatty acids are also common components (19). The cuticular lipids of diapausing *M. sexta* have shown contain only 4% hydrocarbon (5). The composition of mainly o xoalcohol esters and o xoalcohol esters of acetoacetic acid and 3-hydroxybutyric acid represents a unique mixture of polar lipid compounds whose primary function is to prevent desiccation and provide protection from the environment during diapause. Previous studies have indicated that diapausing lepidopteran pupae possess thicker wax layers than nondiapausing pupae (20, 21). Scanning electron microscope studies of the epicuticular surfaces of diapausing and nondiapausing pupae of *M. sexta* shown that in nondiapausing pupae, the wax layer was smooth, thin, and transparent so as to reveal the underlying cuticulin layer (20). In diapausing pupae, the wax layer was rough, crusty and the underlying cuticulin layer was not visible, indicating a thick wax layer.

Preliminary TLC analysis of the surface lipids extracted from nondiapausing *M. sexta* pupae showed the same distribution of components as that shown for diapausing pupae (data not shown). Therefore, the main difference in the wax layer of diapausing and nondiapausing pupae is not in the quality of the wax but in the quantities of wax secreted on the cuticular surfaces of diapausing pupae. Furthermore, the utilization of increased quantities of acetoacetic and 3-hydroxybutyric acids as acyl moieties of major components of cuticular lipids may be of metabolic significance to the insect during diapause.

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Synthesis of Butyldimethylchlorosilane. Five grams of butyldimethylamine was placed into a 50 ml glass flask equipped with condenser, drying tube and dropping funnel. After heating to melting, 43 mg of sodium chloride was added while stirring, followed by dropwise addition of 6 ml of dimethylethanolamine to acetone, 11.6 ml water, 7.9 ml acetic acid, and 1.8 ml of 1.5 mol/L hydrochloric acid over a period of 1 h. The reaction was continued for 2 h at a temperature of 50°C. After the reaction was stopped, excess solvent was removed by distillation and the excess water was evaporated. The purified product was dried over anhydrous sodium sulfate. The purified product was distilled with the aid of a trap and the distillate was collected to give pure butyldimethylchlorosilane. The product was characterized as a meta-chlorophenylpolyoxyethylene glycol monoether, water, and methanol at 1470, 1700 and 1650 cm⁻¹, respectively. Structural identification for octoxane: 3-methanol was made by GC-MS mass spectral analysis of the butyldimethylchlorosilane (Fig. 6C, mass spectrum).
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Figure 11. GLC-MS mass spectra of A, the t-BWeS derivative of octadecyl 3-hydroxybutanoate (Fig. 10, GLC peak 3) and B, octadecyl butenoate (Fig. 10, GLC peak 2). Mass peaks in spectra A and B were at m/z 161 and 87, respectively.

Figure 12. GLC-MS mass spectrum of the t-BWeS derivative of 12-omoctacosanyl 3-hydroxybutanoate. Values in parentheses are the masses of ions formed by McLafferty rearrangements.

Figure 13. GLC-MS mass spectrum of the t-BWeS derivative of 3-omoctacosanyl 3-hydroxybutanoate. Values in parentheses are the masses of ions formed by McLafferty rearrangements.