HYDROXYLATION OF N-ALKANES TO SECONDARY ALCOHOLS AND THEIR ESTERIFICATION IN THE GRASSHOPPER MELANOPLUS SANGUINIPES

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Summary: Labeled n-alkanes administered to the grasshopper Melanoplus sanguinipes are hydroxylated at or near the middle of the carbon chain. The secondary alcohols formed are then esterified. Chain length specificity is evident in both the hydroxylation of n-alkanes and the esterification of secondary alcohols, with the shorter chain C_{23}, C_{21}, C_{19}, and C_{25} compounds converted to secondary alcohol wax esters more readily than the longer chain C_{27}, C_{29}, and C_{31} compounds. Secondary alcohols and ketones are not reduced to alkanes.

Hydrocarbons are common constituents of the cuticular lipids of plants and insects (1,2). In plants, ketones and/or secondary alcohols with the functional group located at or near the center of the carbon chain occur together with the corresponding hydrocarbons (1). The occurrence of nonacosan-15-one and n-nonacosane in Brassica oleracea led early workers (3,4) to propose a head to head condensation between two molecules of pentadecanoic acid to produce the ketone followed by reduction to the secondary alcohol and alkane. They later rejected this hypothesis (5) and more recently Kolattukudy et al. (6) have demonstrated an elongation-decarboxylation pathway for the biosynthesis of n-alkanes in B. oleracea. Kolattukudy and Liu (7) have also shown in B. oleracea that labeled n-nonacosane is converted to nonacosan-15-ol and that this secondary alcohol is oxidized to the corresponding ketone.

Aliphatic secondary alcohols and ketones are not commonly found in insect cuticular lipids. However, recently we characterized secondary alcohol wax esters from the grasshoppers Melanoplus sanguinipes and M. packardii (8) in which the hydroxyl group of the secondary alcohol is located at or near the middle of the carbon chain. The secondary alcohols of these insects do not correspond in chain length to the n-alkanes (9). The major n-alkanes are n-nonacosane and n-heptacosane, whereas the major secondary alcohols have

23,25, and 21 carbons. Studies with labeled dietary n-alkanes indicated that dietary n-alkanes are incorporated into the insect's cuticular lipid as both n-alkanes and secondary alcohol wax esters (10). This paper demonstrates that tritiated n-alkanes are hydroxylated and esterified and that secondary alcohols are esterified, with chain length specificity evident in both cases. Neither labeled secondary alcohols nor ketones are incorporated into the hydrocarbon fraction.

EXPERIMENTAL PROCEDURES

Labeled substrates were prepared as described earlier (10). The specific activities of the n-alkanes and secondary alcohols were adjusted to 0.1 mCi/mg by the addition of unlabeled compounds. Each substrate was repurified by thin layer chromatography just prior to use.

The grasshoppers were raised in wire cages at 27°C on a diet of rye shoots, lettuce leaves, and bran fed ad lib. The insects were used 1 to 4 weeks after the larval-adult ecdysis. Insects from the same population and of equal age were selected at random for the studies determining chain length specificities.

Between 10^4 and 10^5 counts/min were used in each experiment with similar amounts of each labeled substrate used where comparisons were being made. A comparison of the hydroxylation and esterification of n-tricosane-[R-^{3}H] and tricosan-12-ol-[R-^{3}H] showed that a similar metabolism occurred whether the substrate was injected beneath the cuticle at the third or fourth abdominal segment or layered on the surface of the abdomen. Because of the ease of administering the substrate on the surface of the abdomen, this method was used. The substrates were administered in diethyl ether at a concentration so that one ul per insect was used.

The insects were sacrificed at the times indicated and the lipids extracted, separated into classes by TLC, counted by liquid scintillation counting, and radio-GLC performed as described earlier (10). All data points are the average of 3 to 6 experiments and 3 insects were used in each experiment.

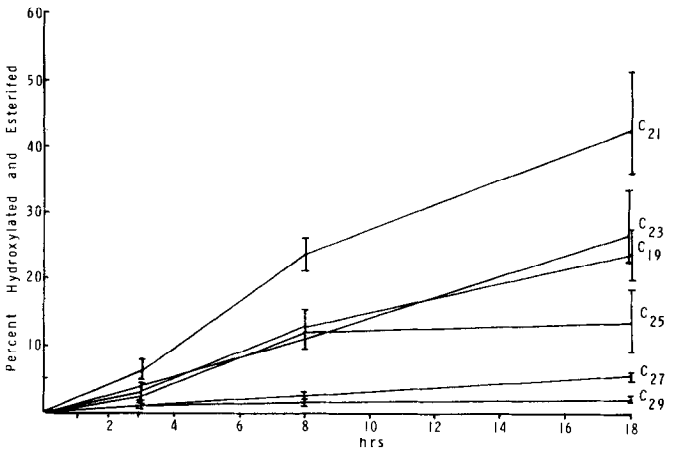

Figure 1. Hydroxylation and esterification of n-alkanes-[R-^{3}H] in Melanoplus sanguinipes.

RESULTS

Both labeled n-alkanes and secondary alcohols applied to the insect's surface are incorporated into the secondary alcohol wax ester fraction. Figure I shows the hydroxylation and esterification of n-alkanes by M. sanguinipes. n-Heneicosane-[R-^{3}H] is hydroxylated and esterified most rapidly followed by n-tricosane-[R-^{3}H], n-nonadecane-[R-^{3}H], and n-pentacosane-[R-^{3}H]; whereas the longer chain n-heptacosane-[R-^{3}H] and n-nonacosane-[R-^{3}H] are less efficiently incorporated. Saponification of the secondary alcohol wax esters shows that the radioactivity is in the secondary alcohol fraction. Radio-GLC of the secondary alcohols shows that the radioactivity is in the secondary alcohol of the same carbon chain length as that of the n-alkane-[R-^{3}H] administered.

Most of the hydroxylated n-alkanes appear as the secondary alcohol wax ester, however, a small portion remains as the free secondary alcohol. Only 2.2 $\pm$ 0.3% of n-nonadecane-[R-^{3}H], 4.9 $\pm$ 0.6% of n-heneicosane-[R-^{3}H], 6.8 $\pm$ 1.2% of n-tricosane-[R-^{3}H], 1.7 $\pm$ 0.5% of n-pentacosane-[R-^{3}H], 1.1 $\pm$ 0.1% n-heptacosane-[R-^{3}H], and 1.0 $\pm$ 0.3% of n-nonacosane-[R-^{3}H] appears in the polar lipid fraction as free secondary alcohol after 18 hours. The polar

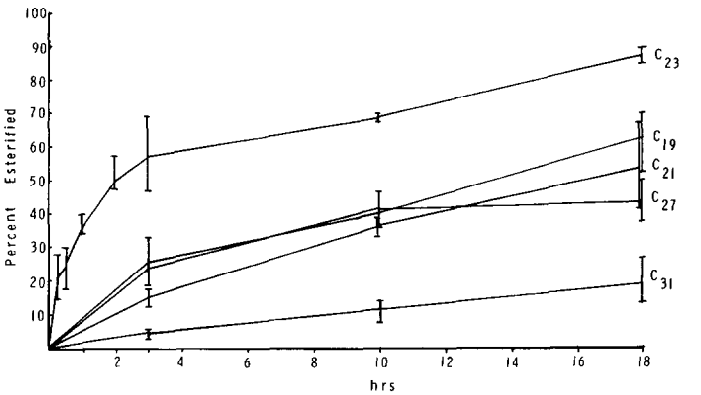

Figure 2. Esterification of secondary alcohols - [R-^{3}H] in Melanoplus sanguinipes.

lipid fraction contains all lipids more polar than ketones including primary and secondary alcohols, triglycerides, sterols and free fatty acids.

Figure II shows that the esterification of secondary alcohols-[R-^{3}H] occurs readily with chain length specificity evident. The 23 carbon tricosan-12-ol-[R-^{3}H] is most rapidly esterified followed by nonadecan-10-ol-[R-^{3}H], heneicosan-11-ol-[R-^{3}H], heptacosan-14-ol-[R-^{3}H], and hentriacontan-16-ol-[R-^{3}H].

Table I shows that labeled C_{27} secondary alcohol and ketone administered to M. sanguinipes are not incorporated into the hydrocarbon fraction and that the labeled C_{27} ketone is not reduced to a secondary alcohol. A similar metabolism of C_{19}, C_{21}, C_{23}, and C_{31} secondary alcohols and C_{23} and C_{31} ketones is observed. The secondary alcohols are found either in the free secondary alcohol fraction or in the wax ester fraction after 18 hours, whereas nearly all of the labeled ketones remain in the ketone fraction after 18 hours.

DISCUSSION

The results presented in this paper show that n-alkanes are hydroxylated to secondary alcohols and esterified, and that secondary alcohols and ketones are not converted to hydrocarbons. It appears that the biosynthesis of secondary alcohols in insects is similar to that shown by Kolattukudy (7) in plants: alkanes →secondary alcohols→ketones. However, no ketones were observed in M.

TABLE I

Percent of Recovered Radioactivity from Metabolism of Heptacosan -14-ol-[R-^{3}H] and Heptacosan-14-one-[R-^{3}H] by M. sanguinipes

Substrate	Heptacosan-14-ol-[R-^{3}H]		Heptacosan-14-one-[R-^{3}H]	
Fraction	0 hrs.	18 hrs.	0 hrs.	18 hrs.
Alkane	< 1	< 1	< 1	< 1
Secondary Alcohol Wax Ester	< 1	43 ± 8	< 1	< 1
Ketone	< 1	< 1	98	94 ± 2
Polar Lipids	99	57 ± 11	1	5 ± 1

sanguinipes cuticular lipids (9) nor as products from n-alkanes-[R-^{3}H] suggesting that the enzyme system responsible for oxidizing secondary alcohols to ketones is absent in this insect.

The very small incorporation of n-heptacosane-[R-^{3}H] and n-nonacosane-[R-^{3}H] into secondary alcohol wax esters demonstrates that chain length specificity is evident in the hydroxylating system and explains the distribution of secondary alcohols and alkanes in the cuticular lipids of M. sanguinipes (8). In contrast, in all plants studied, the secondary alcohols correspond in chain length to the major n-alkanes (1, 3, 11, 12, 13). Chain length specificity in n-alkane hydroxylation has not been studied in plants.

The direct demonstration of the metabolism of alkanes to secondary alcohols and the lack of conversion of the proposed intermediates in the condensation pathway (secondary alcohols and/or ketones) to alkanes indicate a condensation pathway for the biosynthesis of n-alkanes does not occur in M. sanguinipes. A similar nonconversion of ketones to secondary alcohols or hydrocarbons was observed in B. oleracea (14). It is possible that lack of accessibility of ketones and/or secondary alcohols to the site of hydrocarbon biosynthesis or their failure to equilibrate with enzyme bound intermediates could account for their lack of incorporation into hydrocarbons. However, a more likely pathway for hydrocarbon biosynthesis in insects is the elongation-

decarboxylation pathway, as was suggested for replacement hydrocarbon biosynthesis in Perplaneta americana (15).

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