

CHEMICAL POLYMORPHISM OF THE CUTICULAR LIPIDS OF THE CABBAGE WHITE *Pieris rapae*

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Abstract—The epicuticular composition of different body parts of the Cabbage White, *Pieris rapae* L., was investigated using GC and GC/MS. The major group of components, hydrocarbons, occurs in two distinct classes, which show different distributions on the cuticle of the insects. Unbranched shorter chain compounds (C₂₁ to C₃₁, linear group) dominate on body, head and wings, while longer chain, polymethyl-branched compounds (C₃₅ to C₃₉, branched group) are predominantly found on the antennae. Several other components like 1,3-pentacosadiene and oxygenated aliphatic compounds occur in minor amounts on the cuticle. The reason for this polymorphism is discussed.

Key Words—*Pieris rapae*, alkanes, alkenes, alkadienes, alcohols, alkanediols, cuticle, cuticular lipids, tetrahydrofurans.

INTRODUCTION

The epicuticular lipids of insects have been extensively studied by modern gas chromatographic and mass spectrometric methods, the predominant class of compounds being alkanes. Numerous reviews have been published dealing with the

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composition of the insect cuticle and its function as a barrier between the body and the environment or as a carrier of information (e.g., Nelson and Blomquist, 1995). Nevertheless, comparative analyses among different body parts have rarely been performed (see references cited in Howard, 1993; Buckner et al., 1994; Young et al., 2000), and antennal lipids have been neglected. During our studies on the mating system of Pierid butterflies, we found striking differences in the composition of the surface lipids of the Cabbage White, *Pieris rapae*, among different body regions.

METHODS AND MATERIALS

About 20 freshly eclosed butterflies of *P. rapae* L. (Lepidoptera: Pieridae) were killed by freezing, sexed, and dissected into wings, body (thorax, abdomen, and legs), head, and antennae. Extracts of the different body parts were obtained by a short rinse with pentane (Merck, Suprasolv). Care was taken to avoid contamination of the sample with hemolymph by not allowing contact of solvent and cut surfaces. The different extracts were subjected to GC and GC-MS analyses as previously described (Schulz, 2001). SPME analyses were performed by rubbing a 100 μm PDMS fiber, conditioned at 250°C, on all the respective body parts. This procedure was repeated twenty times to obtain enough material for analyses. All individual analyses were repeated three to five times. Derivatization with dimethyldisulfide (DMDS) was performed according to Francis and Veland (1981).

RESULTS AND DISCUSSION

The analyses revealed that hydrocarbons are the major class of lipids present on the epicuticle in all samples. They were characterized by well established methods based on the interpretation of mass spectra and gas chromatographic retention indices (Carlson et al., 1998; Schulz, 2001). Two different alkane groups could be distinguished: Straight chain alkanes ranging in chain length from C₂₁ to C₃₁ (linear group) and di-, tri-, and tetramethyl branched alkanes with longer chain length (C₃₅ to C₃₉, branched group). In the first group, small amounts of methyl branched alkanes and also straight chain 1-alkenes co-occurred. The double bond of the alkenes is predominately located at C-1, which was proven by DMDS derivatization {e.g., characteristic ions for 1-heneicosene $m/z = 327$ (100, M-61) and 61 (21)}. This position is rarely found in insect lipids (Howard, 1993; Nelson and Blomquist, 1995). The two alkane groups showed clear differences in their abundance among different body parts. The linear group predominates on the body and the head, while

it is markedly reduced on the antennae where the branched group predominates. This latter group is found in smaller amounts on the other body parts as well. On the wings, a modified linear group predominates, which is reduced in the amount of the shorter chain members (Figure 1). The two sexes did not differ in the distribution of hydrocarbons over the respective body parts, as proved by separate analyses. SPME analyses revealed that the identified compounds are present on the surface of the outer cuticle.

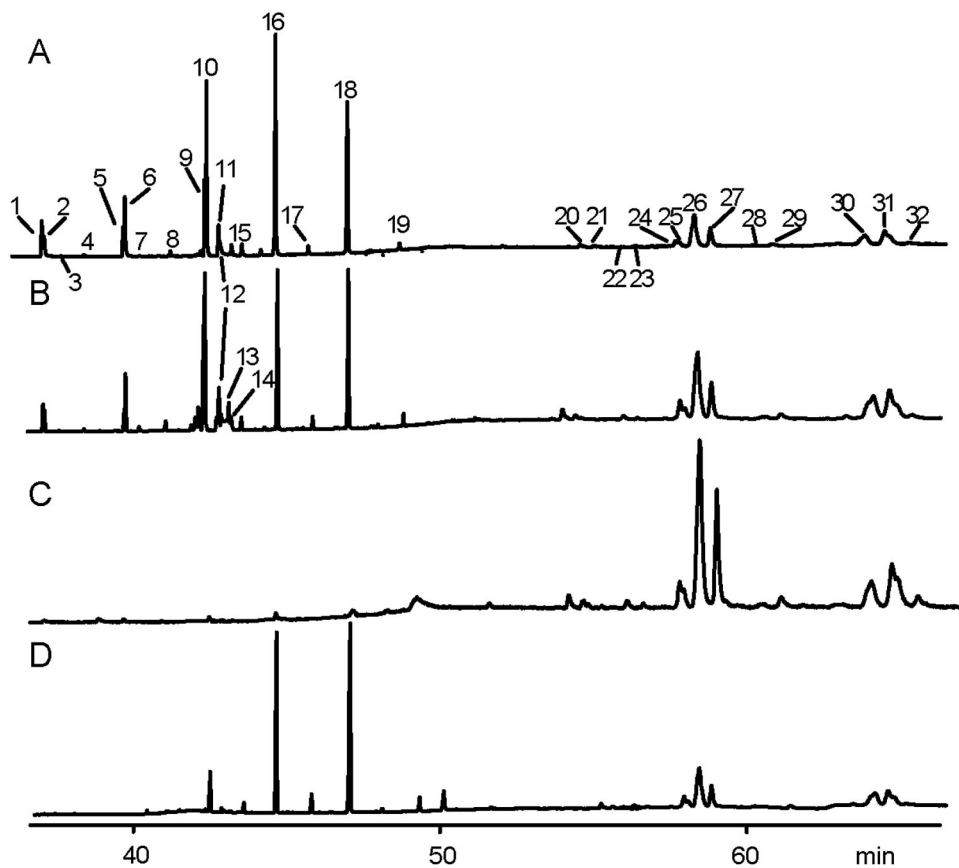


FIG. 1. Gas chromatograms of solvent extracts of cuticular lipids of *P. rapae*. A: body; B: head; C: antennae; D: wing. Numbers refer to Table 1.

TABLE 1. HYDROCARBONS IDENTIFIED IN THE EPICUTICLE OF *Pieris rapae*^a

No.	Compound	an	he	bo	wi
1	1-henicosenes	–	1.3	0.5	–
2	henicosane	0.03	1.0	0.4	0.5
3	9- and 11-methylhenicosane	–	0.1	0.02	–
4	docosane	0.01	0.1	0.03	0.1
5	1-tricosene	0.04	2.7	0.4	0.2
6	tricosane	0.2	3.7	1.3	0.9
7	9- and 11-methyltricosane	–	0.3	0.1	–
8	tetracosane	0.03	0.6	0.3	0.2
9	1-pentacosene	0.2	4.4	5.1	1.0
10	pentacosane	0.8	16.0	15.3	3.4
11	9-, 11- and 13-methylpentacosane	0.06	0.7	0.2	0.02
12	(<i>E</i>)- and (<i>Z</i>)-1,3-pentacosadiene	0.17	5.3	1.8	0.9
13	7,11-dimethylpentacosane	0.04	2.0	0.5	0.3
14	3-methylpentacosane	0.02	0.9	0.3	0.04
15	hexacosane	0.2	0.7	0.6	0.8
16	heptacosane	0.9	8.8	21.2	36.4
17	octacosane	0.1	0.6	0.8	1.2
18	nonacosane	0.8	8.5	16.0	21.3
19	hentriacontane	–	0.7	1.6	1.1
	sum linear group	3.6	58.4	66.4	68.4
20	11,15,19- and 13,17,21-trimethylpentatriacontane	1.7	1.3	0.7	0.9
21	11,15,19,23-tetramethylpentatriacontane	1.2	0.6	0.3	0.4
22	12,16,20-, 13,17,21- and 14,18,22-trimethylhexatriacontane	1.0	0.5	0.4	0.5
23	11,15,19,23-tetramethylhexatriacontane	0.5	0.1	0.2	0.2
24	13,17-, 15,19- and 17,21-dimethylheptatriacontane	4.4	2.4	1.3	1.8
25	11,15-dimethylheptatriacontane	2.5	1.3	1.2	1.8
26	11,15,19-, 13,17,21- and 15,19,23-trimethylheptatriacontane	34.2	11.4	9.7	9.5
27	11,15,19,23-tetramethylheptatriacontane	20.4	4.3	3.6	3.7
28	12,16-, 13,17-, 14,18-, 15,19- and 17,21-dimethyloctatriacontane	1.1	0.3	0.6	0.6
29	12,16,20- and 14,18,22-trimethyloctatriacontane	1.7	0.9	0.9	0.9
30	11,15-, 13,17-, 15,19- and 17,21-dimethylnonatriacontane	8.5	6.9	5.0	3.8
31	11,15,19-, 13,17,21- and 15,19,23-trimethylnonatriacontane	10.3	7.0	7.2	4.8
32	11,15,19,23-tetramethylnonatriacontane	4.4	1.0	0.8	0.2
	sum branched group	91.9	38.0	31.9	29.1

^ahe: head; an: antennae; bo: body; wi: wing. Numbers (%) are relative proportions within the sample. Where percentages do not add up to 100%, the remaining percentage is comprised of oxygen containing compounds.

Unique compounds are (*E*)- and (*Z*)-1,3-pentacosadiene, not previously reported from insects, which are most abundant on the head and not accompanied by any homologs, as found with the other hydrocarbons. Several long chain oxygenated compounds like C₂₉ 2,5-dialkyltetrahydrofurans (present only on the antennae), alkyl methyl ethers, secondary alcohols, and corresponding 1,2-, 1,3-, and 1,4-diols, like 6,9-nonacosanediol, were also identified. They often occur in enhanced proportions on the antennae or the head.

While qualitative differences in the composition of different body parts have been observed occasionally in insects, no such large differences as found in the present study have been reported. There might be several reasons for the observed chemical polymorphism. The water permeability of the epicuticle of insects seems to depend on the melting point of the lipids (Gibbs, 2002). Because methyl branchings have a much stronger influence on the melting temperature of hydrocarbons than chain length (Gibbs, 2002), it can be safely assumed that the branched group of hydrocarbons has a lower melting point than the linear group. This might result in higher water loss, higher fluidity, and, most importantly, also higher diffusion rates of dissolved molecules. The first event in the olfactory process is adsorption or solution of a signal molecule on the antennal cuticle. The molecule then has to travel by diffusion to pores in the sensillum surface, where it is caught by a binding protein (Kanaujia and Kaissling, 1985; Kaissling, 2001). This diffusion is certainly faster in materials with higher fluidity, a probable reason for the enhanced proportion of multi-branched hydrocarbons on the antennae. A fast transport of signal molecules on the antennal surface has been shown by Kanaujia and Kaissling (1985). Furthermore, the different patterns and singular components of the cuticular lipids may be used in chemical communication (Nelson and Blomquist, 1995), especially at close range and in the contact phase of courtship and mating behavior. This might be one reason for the presence of unique compounds on certain body surface areas. Most likely a distribution of lipids as in *P. rapae* may also be found in many other insects.

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