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CUTICULAR HYDROCARBONS OF THE DAMPWOOD TERMITE, *Zootermopsis nevadensis*: CASTE DIFFERENCES AND ROLE OF LIPOPHORIN IN TRANSPORT OF HYDROCARBONS AND HYDROCARBON METABOLITES

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Abstract—The epicuticular and internal hydrocarbons (HC) from different castes of the dampwood termite *Zootermopsis nevadensis* were analyzed by gas chromatography–mass spectrometry. The epicuticular HC profiles of workers and alates contained large proportions of *n*-heneicosane (*n*-C21), 5-methylheneicosane (5-MeC21), and 5,17- and 5,15-dimethylheneicosane (5,17-, 5,15-diMeC21). Sixty-three HC peaks were identified as normal-, monomethyl-, dimethyl-, and trimethylalkanes up to 41 carbons long. The HC content of the internal tissues was significantly greater than on the epicuticle in all castes examined (2.8-fold in female alates, 5.7-fold in male alates, and 13.7-fold in workers). The hemolymph of all castes, including workers, soldiers, nymphs, and female and male alates, contained large amounts of HC and all hemolymph samples had nearly identical HC profiles. However, the hemolymph profile was remarkably different from the cuticular profile. KBr equilibrium gradient ultracentrifugation of worker hemolymph showed that all HC were associated with a high-density lipophorin (density

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of 1.12 ± 0.005 g/ml) consisting of two subunits, apolipophorin-I (220 kDa) and apolipophorin-II (82 kDa). After topical application of radiolabeled 3,11-dimethylnonacosane, a HC that is closely related to a native HC, all the internalized HC and radiolabeled lipid metabolites that were recovered from the hemolymph were associated with lipophorin. These findings are consistent with the hypothesis that lipophorin transports HC and some HC metabolites through the hemolymph from the sites of synthesis to the integument and from the integument to metabolic and excretory tissues. In many social insects, different castes have different relative proportions of the same HC and lipophorin appears to play an important role in regulation of the externalization and internalization of HC and, therefore, in the attainment of caste-specific chemical profiles.

Key Words—Lipophorin, hydrocarbon, termite, Isoptera, castes, cuticle, metabolism.

INTRODUCTION

Hydrocarbons (HC) are major components of insect epicuticular lipids and are composed primarily of *n*-alkanes, alkenes, and methyl-branched components (Jackson and Blomquist, 1976; Nelson, 1978; Nelson and Blomquist, 1995). The primary function of cuticular HC is to protect insects from water loss (Noble-Nesbitt, 1991), but they also serve as chemical messengers within and between species. For example, epicuticular (*Z*)-9-tricosene serves as a sex pheromone in the house fly (Blomquist et al., 1987), modified HC serve as sex pheromone components in the German cockroach (Chase et al., 1992), and HC mediate species, caste, and colony recognition in social insects (Howard et al., 1982; Bagnères et al., 1991b; Howard, 1993; Lorenzi et al., 1996; Singer, 1998). Moreover, HC also mediate predator–prey (Howard, 1993), parasite–host (Lecuona et al., 1991; Dettner and Liepert, 1994; Bagnères et al., 1996; Bonavita-Cougourdan et al., 1996) and insect–plant interactions (Eigenbrode and Espelie, 1995).

Several studies indicate that HC are synthesized by oenocytes, whose location varies widely among insects (Romer, 1991). Nevertheless, even in insects whose oenocytes are located within the epidermis, it appears that HC are carried through the hemolymph to their epicuticular and other destinations, and lipophorin acts as the shuttle (see Chino, 1985; Schal et al., 1998b). In *Holomelina* tiger moths, for example, hemolymph lipophorin transports HC sex pheromone components to an internal pheromone gland (Schal et al., 1998a). In cockroaches, locusts, flies, and beetles lipophorin alone, and no other hemolymph proteins, contains HC and these are identical to hemolymph HC (Chino and Kitazawa, 1981; Katase and Chino, 1982, 1984; Katagiri and de Kort, 1991; Gu et al., 1995; Pho et al., 1996; Young et al., 1999).

In social insects, HC not only maintain water balance, but species-, colony-,

and caste-specific epicuticular HC profiles serve as recognition cues. Thus, social insects must closely regulate their epicuticular and exocrine secretions (Bagnères et al., 1996; Bonavita-Cougourdan et al., 1996; Howard, 1993; Clément and Bagnères, 1998; Singer, 1998). The mechanism of HC transport, however, has not been addressed in either the social Hymenoptera or the Isoptera. We report the characterization of cuticular, hemolymph, and lipophorin HC from the termite *Zootermopsis nevadensis* (Hagen) and show, for the first time in a social insect, that lipophorin transports HC through the hemolymph. Furthermore, we show that a radiolabeled HC, similar to a native HC, is internalized and metabolized by *Z. nevadensis* workers, and both HC and its metabolites are carried by lipophorin.

METHODS AND MATERIALS

Insects. Zootermopsis nevadensis was collected from Jeffrey pine, *Pinus jeffreyi*, stumps in the Toiyabe National Forest (T 20N, R 17E, S 23, Sierra County, California, approximately 6 km northeast of Verdi, Nevada). Colonies were maintained on decayed stumps of *P. jeffreyi* at 24 ± 1°C, approximately 60–80% relative humidity, and a 12L : 12D photoperiod. Large workers, nymphs (with wing pads), soldiers, and reproductives (alates) were separated from the colony and kept in small moist plastic boxes for at least a week prior to use in experiments.

Chemicals. All chemicals were purchased from Sigma (St. Louis, Missouri). Precast gels and molecular weight markers were obtained from Bio-Rad (Richmond, California). [11,12-^{3}H]3,11-Dimethylnonacosane was a gift from Dr. Glenn Prestwich (Salt Lake City, Utah).

Collection of Hemolymph. Hemolymph was obtained by puncturing the head with a fine needle or removing a leg and collecting the blood in a drop of cold buffered saline containing protease inhibitors [0.05 M sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, 10 mM ethylenediamine tetraacetic acid (EDTA), 5 mM glutathione, 2 mM phenylmethyl sulfonyl fluoride (PMSF), leupeptin (10 μg/ml), and pepstatin (10 μg/ml)]. Hemolymph was immediately collected into a chilled microcentrifuge tube and centrifuged at 13,000*g* for 10 min at 4°C to remove hemocytes; the supernatant was stored at −80°C for future use.

Fractionation of Hemolymph Proteins by KBr Density Gradient Ultracentrifugation. Serum proteins were fractionated by the method of Shapiro et al. (1984) with minor modifications (Sevala et al., 1997). Briefly, the plasma collected from 100 animals in saline was mixed with 2.57 g of KBr and adjusted to a final volume of 5.8 ml, transferred to a Beckman quick-seal tube, and overlaid with 0.9% NaCl. Tubes were sealed and centrifuged with slow accelera-

tion and deceleration at 285,000*g* in a Beckman L8-70M ultracentrifuge with a fixed angle rotor for 22 hr at 4°C. Fractions (400 μl each) were collected from the top of the tube and the absorbance at 280 and 455 nm was measured in a Beckman DV-64 spectrometer. The density of each fraction was determined gravimetrically and its purity was checked by SDS-PAGE on 4–15% gradient polyacrylamide slab gels as described by Laemmli (1970). Gels were stained with Coomassie brilliant blue G-250 to detect proteins.

Chemical Analysis. Epicuticular HC were extracted by rinsing three insects three times in hexane containing 1 μg *n*-eicosane as an internal standard (Young and Schal, 1997). After removal of surface HC, whole termites were cut with scissors into five to six parts and homogenized by ultrasonication for 30 sec (Kontes microultrasonic cell disrupter, Vineland, New Jersey) and internal HC extracted by a modification of the Bligh and Dyer (1959) procedure, replacing chloroform with hexane. Hemolymph and KBr fractions were similarly extracted.

The hexane phase, containing HC, was loaded onto silicic acid (particle size 100–200 mesh; Selecto Scientific, Norcross, Georgia) Pasteur pipet columns. HC were eluted with 7 ml hexane, and the solvent was evaporated with a slow stream of N_2. Samples were analyzed on an HP 5890II gas chromatograph equipped with a flame-ionization detector and interfaced with an HP 3365II Chemstation. Splitless injections were made into a 25-m × 0.32-mm × 1 μm HP-1 capillary column operated at 150°C for 2 min, then increased at 10°C/min to 200°C, then at 5°C/min to 310°C and held for 10 min. The injector and detector temperatures were 280°C and 300°C, respectively, and the carrier gas was helium at 40 cm/sec.

Gas chromatography–mass spectrometric (GC-MS) analyses were conducted on an HP5890 GC coupled to an HP5989 MS and controlled by an HPUX Chemstation. A Chrompack CPSiL 5CB fused silica column (25 m × 0.25 mm × 0.12 μm) was used. The source temperature was 240°C, the quadrupole was 100°C, and the interface was 300°C. The column temperature was programmed from 30°C to 200°C at 8°C/min and 3°C/min to 320°C (final hold 5 min); injector temperature was at 280°C. Scans were from m/z 35 to 600 at about 1 scan/sec in the electron impact mode (70 eV).

In Vivo Studies of Hydrocarbon Transport and Metabolism. The kinetics of HC internalization and metabolism were studied by topically treating each worker termite with 0.058 μCi [^{3}H]3,11-dimethylnonacosane in 1 μl hexane. After a specified duration the termites were surface extracted with hexane and then homogenized and extracted by the Bligh and Dyer (1959) procedure.

To determine which hemolymph proteins carried HC, each of 70 worker termites was topically treated with 0.45 μCi [^{3}H]3,11-dimethylnonacosane in 1 μl hexane, dispensed from a Hamilton repeating syringe, and hemolymph was collected 24 hr later. The hemolymph was fractionated by KBr gradient equilibrium

ultracentrifugation, lipids extracted as above, and HC eluted with hexane from a silicic acid column. More polar lipid metabolites of 3,11-dimethylnonacosane were eluted with 50 : 50 hexane–ether. The amounts of radioactive HC and its metabolites were measured with a Beckman 5801 liquid scintillation spectrometer (Beckman, Palo, Alto, California).

Statistical Analysis. Comparisons of the amounts of internal and epicuticular HC were conducted with the Wilcoxon test within the computer program StatView 5.0 (SAS Institute, 1998). The percentage abundance of the 12 HC peaks and the summed HC > *n*-C35, which are represented in Tables 2 and 3 below, were used in principal components analyses (StatView 5.0) to delineate differences among chromatograms.

RESULTS AND DISCUSSION

Epicuticular Hydrocarbons of Z. nevadensis. GC-MS analysis was performed on 63 peaks, two of which remain unidentified (Table 1, Figure 1). All castes had the same cuticular compounds, in different relative proportions. The epicuticular hydrocarbon profile was represented by repeating homologous series of *n*-alkanes, monomethyl and dimethyl alkanes, and dominated by 5,15- and 5,17-dimethylheneicosane (5,15- and 5,17-diMeC21; 24.1–27.1%) and, in decreasing amounts, *n*-heneicosane (*n*-C21; 18.0–20.4%), 5-methylheneicosane (5-MeC21; 13.0–20.0%), *n*-tricosane (*n*-C23), and *n*-pentacosane (*n*-C25) (Tables 2–4). Other, less abundant components extended to 41 carbons and included two trimethyl alkanes in low amounts. The homologous HC series starts with an odd-carbon number normal alkane (e.g., *n*-C21), followed by 7-, 5-, 3-monomethyl, and 5,17-dimethyl alkanes of the same chain length, and then the even-carbon-number normal alkane (e.g., *n*-C22). Other HC appeared in the series but in much lower proportions. The longer-chain HC were dominated by increasing proportions of the 5,17-dimethyl odd-carbon-number HC and declining amounts of the 7,11,15-trimethyl odd-carbon-number HC (Figure 1).

Based on an epicuticular analysis of workers, Haverty et al. (1988) defined four *Zootermopsis* HC phenotypes. Ninety-one percent of populations with phenotype I and 84% of populations with phenotype III were considered *Z. nevadensis*. The remaining 9% of populations with phenotype I, 100% of populations with phenotype II, and the remaining 16% of populations with phenotype III were considered *Z. angusticollis*. Thorne and Haverty (1989) correlated the HC phenotypes with morphological characters. Although most worker specimens with phenotypes I and III were determined to be *Z. nevadensis*, their HC profiles deviate substantially from those reported by Blomquist et al. (1979) and our present report. Our *Zootermopsis* worker epicuticular HC phenotype appears to be closest to phenotype I (collected from Eldorado National Forest, California)

TABLE 1. HYDROCARBONS IDENTIFIED FROM *Zootermopsis nevadensis* WORKERS

Peak number[a]	Haverty et al. (1988)[b]	Compound	ECL[c]
1	1	*n*-nonadecane	19.00
2	2	*n*-eicosane	20.00
3	3	5-methyleicosane	20.47
4a		4-methyleicosane	20.56
4b	4	2-methyleicosane	20.60
5	5	*n*-heneicosane	21.00
6	7	7-methylheneicosane	21.40
7	8	5-methylheneicosane	21.50
8	9	4- or 2-methylheneicosane	21.60
9	10	3-methylheneicosane	21.70
10	11	*n*-docosane	22.00
11	13	5,17- and 5,15-dimethylheneicosane	22.10
12	12	7,15-dimethylheneicosane	22.21
13		3,17-dimethylheneicosane	22.26
14		5,9,17-trimethylheneicosane	22.37
15		6-methyldocosane	22.47
16		5-methyldocosane	22.53
17	16–17	4- or 2-methyldocosane, and *n*-tricosene	22.66
18	19	*n*-tricosane	23.00
19a	21	11- and 9-methyltricosane	23.39
19b	22	7-methyltricosane	23.42
20	23	5-methyltricosane	23.51
21	24	3-methyltricosane	23.70
22	25	5,17- and 3,17-dimethyltricosane	23.92
23	28	*n*-tetracosane	24.00
24	29	*n*-pentacosane	25.00
25	30	13-, 11-, and 9-methylpentacosane	25.35
26	32	5-methylpentacosane	25.47
27	33	5,17-dimethylpentacosane	25.90
28	35	*n*-hexacosane	26.00
29	36	*n*-heptacosane	27.00
30		13- and 11-methylheptacosane	27.34
31	37	5-methylheptacosane	27.50
32	38	5,17-dimethylheptacosane	27.86
33	39	*n*-octacosane	28.00
34		8- and 6-methyloctacosane	28.48
35	41	*n*-nonacosane	29.00
36	42	15-, 13-, and 11-methylnonacosane	29.33
37	43	7-methylnonacosane	29.43
38	44	5-methylnonacosane	29.55
39		13,17-dimethylnonacosane	29.61
40		3-methylnonacosane	29.72
41	45	5,17-dimethylnonacosane	29.86

TABLE 1. (CONTINUED)

Peak number[a]	Haverty et al. (1988)[b]	Compound	ECL[c]
42	48	3,7-dimethylnonacosane	30.15
43		6-methyltriacontane	30.50
44		*n*-hentriacontane	31.00
45	54	7-methylhentriacontane	31.44
46	55	5-methylhentriacontane	31.54
47		5,17-dimethylhentriacontane	31.87
48		*n*-tritriacontane	33.00
49		7-methyltritriacontane	33.46
50		5-methyltritriacontane	33.54
51		7,11-dimethyltritriacontane	33.75
52	60	5,17-dimethyltritriacontane	33.86
53		unknown	34.69
54		9,13-dimethylpentatriacontane	35.50
55	61	5,17-dimethylpentatriacontane	35.70
56		unknown	37.10
57		9,13-dimethylheptatriacontane	37.48
58		5,17-dimethylheptatriacontane	37.60
50		7,11,15-trimethylheptatriacontane	37.72
60		unknown (and *n*-nonatriacontane)	39.00
61		5,17-dimethylnonatriacontane	39.49
62		7,11,15-trimethylnonatriacontane	39.60
63		5,17-dimethylhentetracontane	41.40

[a] Peak numbers refer to peaks identified in Figure 1.
[b] Numbers refer to peak numbers identified in Table 1 of Haverty et al. (1988).
[c] ECL = equivalent chain length.

(Haverty et al., 1988), but the latter contained threefold more *n*-C21 (39.5%) than 5-MeC21 (12.9%), whereas ours contained nearly equal percentages of *n*-C21 (20.4%) and 5-MeC21 (20.0%) (Table 2). Moreover, our workers contained 24.1% 5,17- and 5,15-diMeC21 compared with 7.6% in phenotype I of Haverty et al. (1988). Conversely, phenotype I workers contained more *n*-C23 than our workers (15.8% vs. 6.7%, respectively). The other three phenotypes corresponded even less with our workers.

The epicuticular HC of our *Zootermopsis* workers were most similar to those described for termites from Butte County, California (Blomquist et al., 1979). These 1979 collections, although originally identified as *Z. angusticollis*, are now recognized as *Z. nevadensis*. Voucher specimens from our current work, collected about 170 km southeast of the 1979 collection, were identified by Dr. Barbara L. Thorne (University of Maryland) as *Z. nevadensis*, but she notes that the mandibles are somewhat intermediate between *nevadensis* and *angusticollis*. We thus conclude that our *Z. nevadensis* appears most similar to that reported

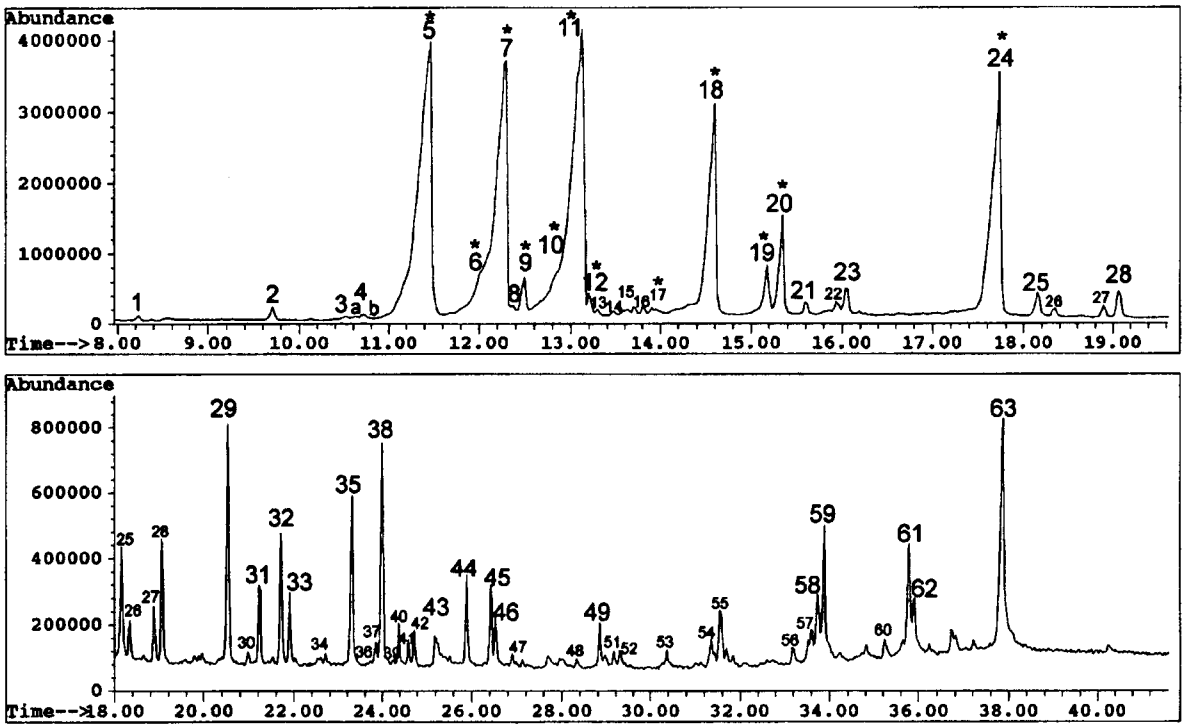

FIG. 1. Total ion chromatogram of the epicuticular hydrocarbons from *Z. nevadensis* workers. Numbers refer to peak numbers that appear in Table 1. The asterisks denote HC that were quantified in Tables 2, 3, and 4.

TABLE 2. SELECTED HYDROCARBONS FROM *Zootermopsis nevadensis* WORKERS

Peak No.[a]	Hydrocarbon[b]	Amount [%, mean (SEM)][c]			
		Epicuticle	Internal	Hemolymph	Lipophorin
5	*n*-C21	20.4 (0.35)	6.7 (0.90)	7.0 (0.35)	6.7 (0.18)
6	7-MeC21	2.1 (0.03)	1.1 (0.05)	0.9 (0.87)	1.7 (0.05)
7	5-MeC21	20.0 (0.51)	8.6 (0.32)	17.5 (0.83)	16.7 (0.46)
9	3-MeC21	1.4 (0.02)	1.1 (0.03)	1.3 (0.03)	1.4 (0.04)
10	*n*-C22	0.5 (0.02)	0.3 (0.01)	0.3 (0.28)	0.5 (0.03)
11	5,17-, 5,15-diMeC21	24.1 (0.35)	10.6 (0.57)	28.5 (0.95)	27.3 (0.76)
12	7,15-diMeC21	2.6 (0.02)	1.0 (0.07)	1.8 (0.10)	1.9 (0.07)
17	2- or 4-MeC22	0.3 (0.02)	0.1 (0.01)	0.0 (0.00)	0.1 (0.03)
18	*n*-C23	6.7 (0.11)	2.7 (0.31)	2.2 (0.08)	2.1 (0.05)
19	11-, 9-, and 7-MeC23	2.2 (0.36)	0.9 (0.06)	2.0 (0.07)	1.6 (0.04)
20	5-MeC23	3.2 (0.06)	1.7 (0.11)	3.9 (0.13)	3.8 (0.10)
24	*n*-C25	3.6 (0.07)	4.1 (0.38)	1.4 (0.08)	1.4 (0.04)
	>*n*-C35[d]	5.1 (0.75)	23.3 (1.21)	14.0 (0.28)	11.8 (1.31)

[a] Peak numbering is according to Table 1 and Figure 1.
[b] Carbon number is the number of carbons on the alkyl chain.
[c] $N = 5$ determinations of 3 workers each for epicuticle and internal; 2 for hemolymph (each representing hemolymph from >20 workers); 6 for lipophorin, representing 6 fractions from a single KBr gradient of hemolymph from >60 workers.
[d] Sum of all hydrocarbons that eluted after *n*-pentatriacontane.

by Blomquist et al. (1979) and is the closest to phenotype I of Haverty et al. (1988). It appears that morphological and HC-phenotype intermediates between *nevadensis* and *angusticollis* occur in the Nevada–California region, and more studies on species identification are warranted.

Epicuticular and Internal Hydrocarbons from Different Castes of Z. nevadensis. All castes of *Z. nevadensis* contained the same HC, but in different proportions, and Blomquist et al. (1979) suggested that these differences might mediate species and caste recognition (see also Bagnères et al., 1998; Clément and Bagnères, 1998). Likewise, based on analysis of variance, Haverty et al. (1988) deduced that the proportions of most epicuticular HC of *Z. nevadensis* were significantly different among soldiers, workers, nymphs, and alates. We, too, found differences among workers and male and female alates (Tables 2 and 3). However, the variation in epicuticular HC was much lower than between the epicuticle and other sites (internal, hemolymph lipophorin) within a caste.

In all three castes that we examined (workers, male and female alates), the internal HC, which ranged from 28.967 μg per worker to 61.179 μg per alate male, were significantly more abundant (Wilcoxon test, $Z = 2.023$, $P < 0.05$) than the respective epicuticular HC, which ranged from 2.218 μg per worker to 16.056 μg per alate female (Figure 2). Thus, female alates, male alates, and workers

TABLE 3. SELECTED HYDROCARBONS FROM *Zootermopsis nevadensis* MALE AND FEMALE ALATES

Peak No.[a]	Hydrocarbon[b]	Amount [%, mean (SE)][c]					
		Males			Females		
		Epicuticle	Internal	Hemolymph	Epicuticle	Internal	Hemolymph
5	*n*-C21	18.6 (0.36)	16.1 (0.38)	8.7	18.0 (0.31)	15.9 (1.68)	9.0
6	7-MeC21	2.0 (0.05)	1.6 (0.06)	1.6	1.7 (0.08)	1.2 (0.30)	1.9
7	5-MeC21	15.4 (0.41)	10.8 (0.40)	15.1	13.0 (0.67)	9.3 (0.67)	15.1
9	3-MeC21	1.2 (0.04)	1.1 (0.03)	1.4	1.1 (0.03)	1.0 (0.06)	1.5
10	*n*-C22	0.3 (0.01)	0.3 (0.02)	0.5	0.2 (0.06)	0.3 (0.01)	0.4
11	5,17-, 5,15-diMeC21	27.1 (0.92)	26.4 (0.62)	30.1	24.6 (1.25)	23.3 (1.58)	33.7
12	7,15-diMeC21	2.7 (0.03)	2.8 (0.06)	2.0	1.4 (0.67)	2.6 (0.15)	2.4
17	2- or 4-MeC22	0.2 (0.04)	0.2 (0.01)	0.2	0.2 (0.05)	0.2 (0.06)	0.2
18	*n*-C23	9.2 (0.43)	9.4 (0.29)	2.4	11.3 (0.52)	8.7 (0.27)	3.4
19	11-, 9-, and 7-MeC23	2.4 (0.14)	2.5 (0.11)	1.5	2.6 (0.12)	2.0 (0.51)	2.3
20	5-MeC23	3.7 (0.16)	4.8 (0.15)	3.2	4.1 (0.18)	4.7 (0.21)	4.2
24	*n*-C25	4.6 (0.38)	5.5 (0.26)	1.1	6.4 (0.40)	7.3 (0.76)	1.4
	>*n*-C35[d]	5.9 (0.38)	6.2 (0.46)	19.0	6.5 (1.00)	8.2 (1.18)	13.4

[a] Peak numbering is according to Table 1 and Figure 1.
[b] Carbon number is the number of carbons on the alkyl chain.
[c] $N = 5$ determinations of 3 alates each for epicuticle and internal; 1 each for hemolymph, representing hemolymph from >20 alates.
[d] Sum of all hydrocarbons that eluted after *n*-pentatricontane.

TABLE 4. SELECTED HYDROCARBONS FROM HEMOLYMPH OF *Zootermopsis nevadensis* NYMPHS AND SOLDIERS[a]

Peak No.[b]	Hydrocarbon[c]	Amount (%)	
		Nymph	Soldier
5	*n*-C21	10.4	13.1
6	7-MeC21	2.5	2.3
7	5-MeC21	19.3	19.8
9	3-MeC21	1.7	1.5
10	*n*-C22	0.5	0.7
11	5,17-, 5,15-diMeC21	39.9	34.9
12	7,15-diMeC21	2.7	2.6
17	2- or 4-MeC22	0.1	0.0
18	*n*-C23	3.1	2.5
19	11-, 9-, and 7-MeC23	2.2	1.5
20	5-MeC23	4.0	2.4
24	*n*-C25	1.0	1.1
	>*n*-C35[d]	2.8	8.7

[a] Single hemolymph samples representing hemolymph from >20 individuals.
[b] Peak numbering is according to Table 1 and Figure 1.
[c] Carbon number is the number of carbons on the alkyl chain.
[d] Sum of all hydrocarbons that eluted after *n*-pentatriacontane.

contained 2.8-fold, 5.7-fold, and 13.7-fold more internal than epicuticular HC, respectively ($N = 5$ each).

In male and female alates, the chromatographic profile of internal HC was similar to the epicuticle, with the most major change in 5-MeC21 (Figure 3, Table 3). In workers, however, *n*-C21, 5-MeC21, and 5,17- and 5,15-diMeC21 were significantly underrepresented in the internal tissues (3.0-, 2.3-, and 2.3-fold less, respectively), and later eluting HC (>*n*-C35) increased in abundance 4.6-fold in the internal tissues (Figure 4, Table 2).

Regardless of the patterns of internal HC, the hemolymph HC profile was nearly identical in all three castes, represented by low proportions of *n*-C21 (7.0–9.0%), greater amounts of 5-MeC21 (15.1–17.5%) and 5,17- and 5,15-diMeC21 (28.6–33.7%), and unusually high amounts of HC that elute after *n*-C35 (13.4–19.0%) (Tables 2 and 3). The hemolymph HC of soldiers and nymphs exhibited a similar pattern, but the late-eluting compounds were relatively less abundant (Figure 5, Table 4).

These patterns are especially clear in a principal components analysis (Figure 6) that compares the epicuticular, internal, and hemolymph HC of workers and male and female alates. Whereas in workers the three body sites separate into three discernible HC profiles (see principal component 2 vs. principal component

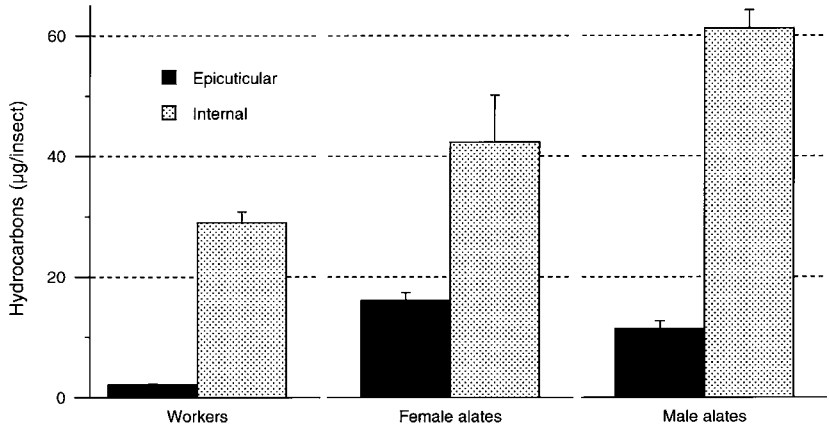

FIG. 2. Mass of epicuticular and internal hydrocarbons of *Z. nevadensis* workers and female and male alates, as determined by capillary gas chromatography. Data are means + SEM ($N = 5$).

3), in alates, the epicuticle and internal HC profiles are similar and hemolymph HC exhibit a different profile. It is worth noting that the great disparity we found between the epicuticular and hemolymph HC suggests that if internal HC are accidentally extracted during surface washes, the hybrid epicuticular/internal HC profile might confound chemotaxonomic studies and possibly lead to erroneous conclusions.

Why then are epicuticular HC different from hemolymph HC in *Z. nevadensis*? Clearly, postdeposition processes, including grooming, selective abrasion, and selective resorption (internalization) of certain HC must be considered. However, this disparity also suggests a selective externalization (deposition) of hemolymph HC. According to this hypothesis, all castes would produce (internally) the same mix of HC, represented by the hemolymph profile, which then forms unique cuticular profiles through selective externalization. The internal HC profile of alates, however, matches their exterior but not the hemolymph HC profile. A possible explanation for this is that the "internal" HC profile of allates is dominated not by hemolymph HC, as in workers, but by internal HC deposition sites. In social insects, exocrine glands might selectively sequester HC from the hemolymph, confounding a clear interpretation of how much of the internal HC is hemolymph HC. The fat body, gonads, and accessory reproductive tissues also contain HC in some insects (Schal et al., 1994, 1998b; Gu et al., 1995; Young et al., 1999), and it is reasonable to speculate that the male and female allates are preparing for their imaginal roles. This also might explain why alates have more HC than workers. They are significantly larger, need to

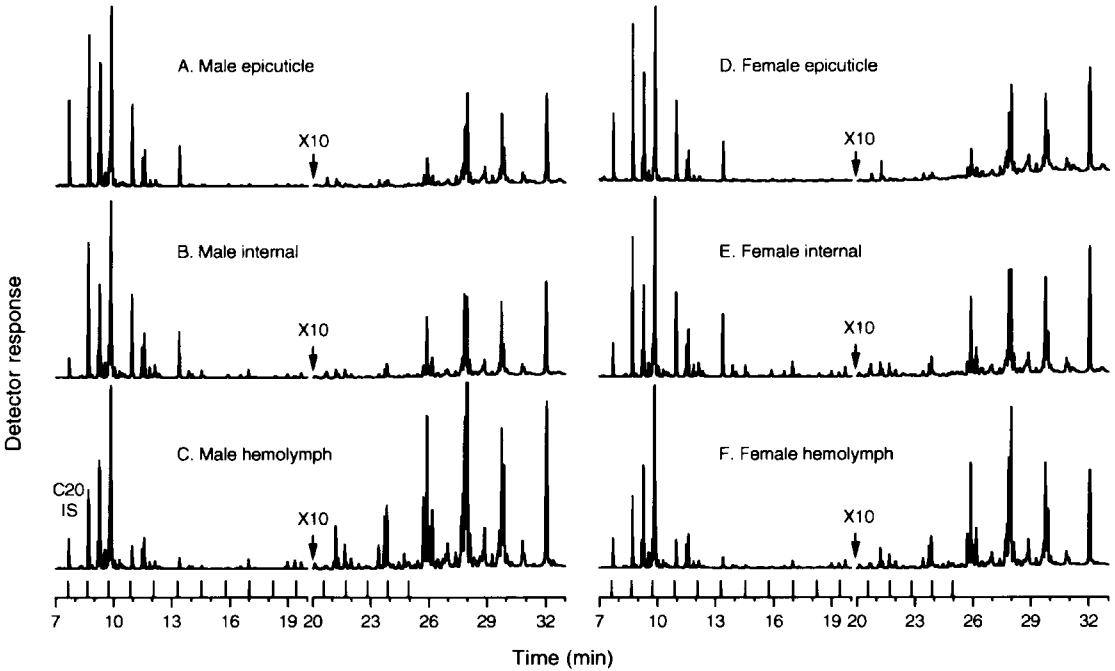

FIG. 3. Partial gas chromatograms of hydrocarbons extracted from the epicuticle, internal tissues, and hemolymph of *Z. nevadensis* male and female alates. The vertical lines above the *x* axis denote retention times of *n*-alkanes between C_{20} and C_{35}, from left to right. After 20 min the detector response is shown at 10-fold greater sensitivity.

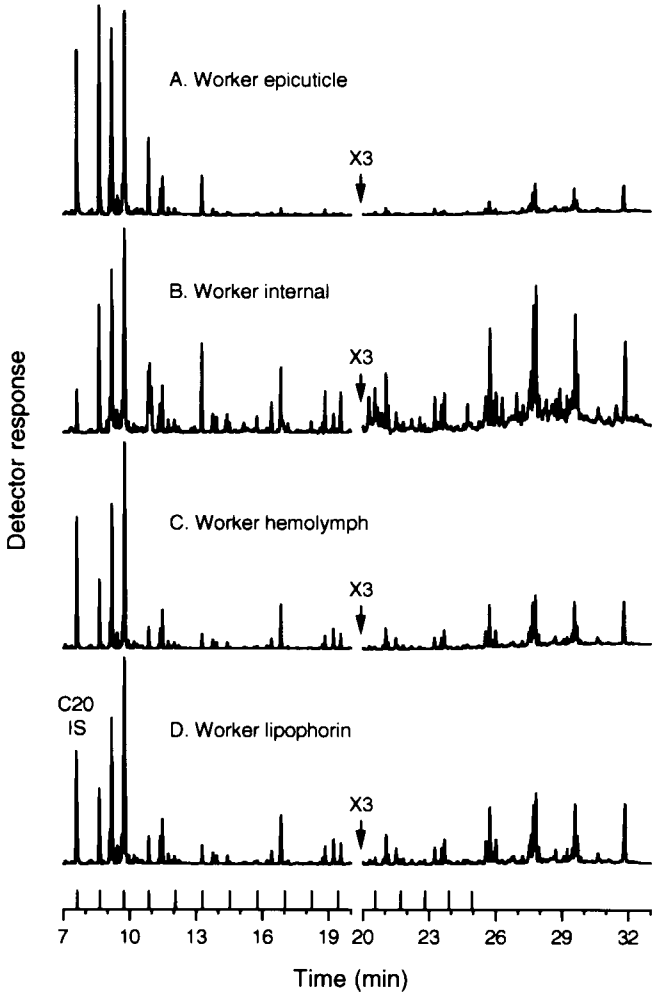

FIG. 4. Partial gas chromatograms of hydrocarbons extracted from the epicuticle, internal tissues, hemolymph, and purified lipophorin of *Z. nevadensis* workers. The vertical lines above the *x* axis denote retention times of *n*-alkanes between C_{20} and C_{35}, from left to right. After 20 min the detector response is shown at threefold greater sensitivity.

provision their wings with HC, and spend more time in the harsh environment outside the nest.

A clear example of spatial (tissue) dissociation of unique HC is in the tiger moth, *H. aurantiaca*. The adult female simultaneously synthesizes short-

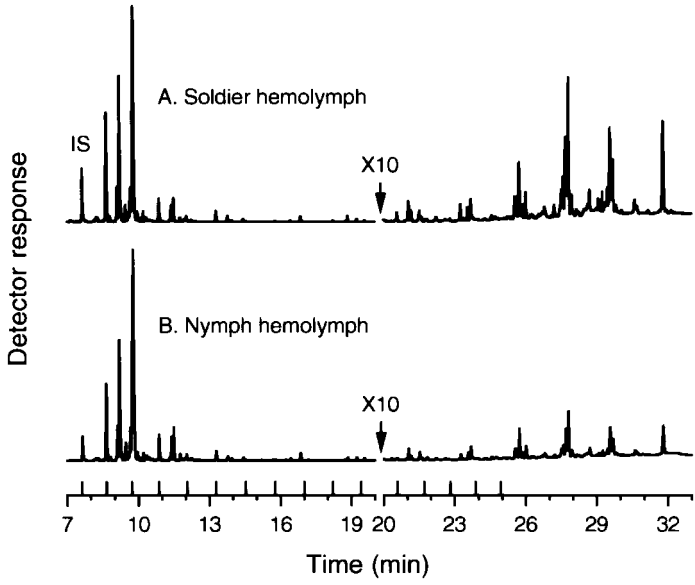

FIG. 5. Partial gas chromatograms of hydrocarbons extracted from the hemolymph of *Z. nevadensis* soldiers and nymphs. The vertical lines above the *x* axis denote retention times of *n*-alkanes between C_{20} and C_{35}, from left to right. After 20 min the detector response is shown at 10-fold greater sensitivity.

chain HC that serve as volatile sex pheromone components and long-chain HC that are deposited on the epicuticle (Schal et al., 1998a). All HC are loaded onto a hemolymph high-density lipophorin. 2-Methylheptadecane and related pheromone homologs of similar chain length are specifically deposited by lipophorin into tubular pheromone glands that in turn open and emit the pheromone near the ovipositor. Long-chain HC, on the other hand, appear on the epicuticular surface. In this insect, the profile of internal HC is diffrent from hemolymph HC because specific HC are sequestered (enriched) in an internal pheromone gland. This, however, is vastly different from most reports, which show that specific types of HC may be found at particular locations on the body (see Howard, 1993; Nelson and Blomquist, 1995), but internal and epicuticular HC are relatively similar (Schal et al., 1998b).

Lipophorin Carries Hydrocarbons and Their Metabolites in Social Insects. How are HC transported through the hemolymph? A preponderance of the evidence implicates lipophorin in the transport of HC in both hemimetabolous and holometabolous insects, but not in any social insect (see Chino, 1985; Kanost et al., 1990; Soulages and Wells, 1994; Schal et al., 1998b). We analyzed

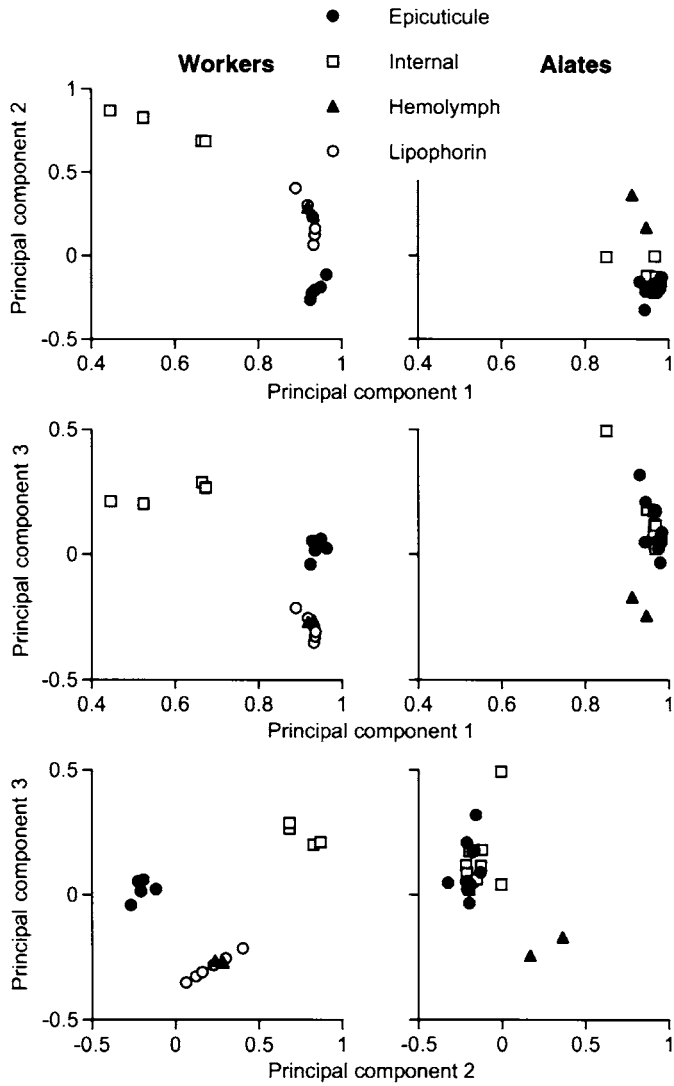

FIG. 6. Principal components analyses of worker (left) and male and female alates (right). The relative peak areas of 12 HC peaks and the summed HC > *n*-C35 for the epicuticle, internal tissues, hemolymph, and lipophorin were subjected to principal components analysis.

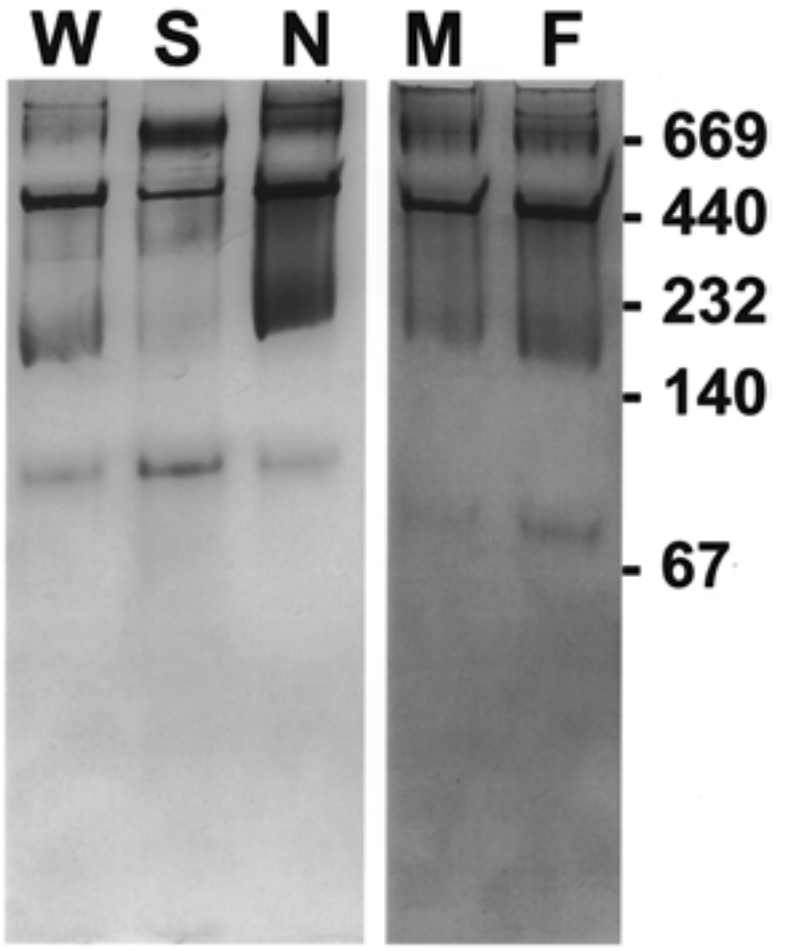

FIG. 7. Native polyacrylamide gel electrophoretic analysis of the hemolymph proteins from different *Z. nevadensis* castes. W, workers; S, soldiers; N, nymphs; M, males; F, females. The positions of the molecular weight markers (kDa) are indicated on the right.

the hemolymph of workers, soldiers, nymphs, and male and female alates by both native and SDS–polyacrylamide gel electrophoresis (PAGE). Three major hemolymph proteins were resolved by native PAGE, with relative molecular weights of about 710, 470 and 110 kDa (Figure 7).

The hemolymph protein profiles under SDS-PAGE indicated that all castes of *Zootermopsis* have two to four similar major hemolymph polypeptides with apparent molecular sizes near 220 and 82 kDa (Figure 8). The 82 kDa band was

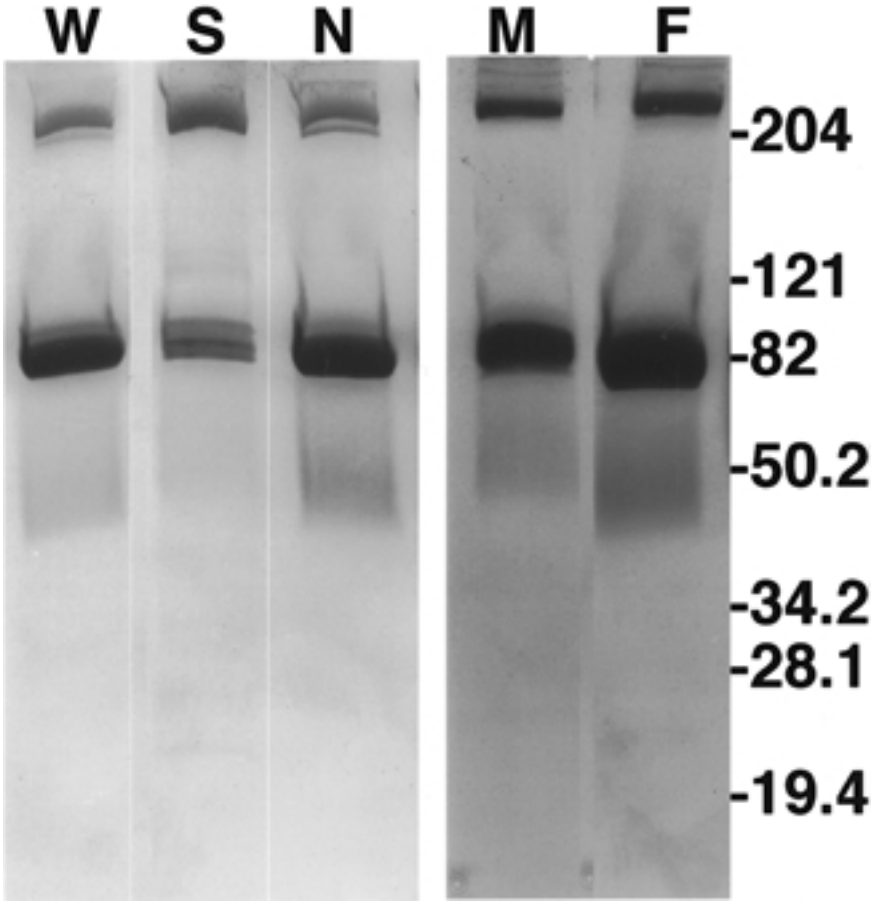

FIG. 8. SDS-PAGE analysis of the hemolymph proteins from different *Z. nevadensis* castes. W, workers; S, soldiers; N, nymphs; M, males; F, females. The positions of the molecular weight markers (kDa) are indicated on the right.

highly abundant, and appeared to consist of several distinct polypeptides. This suggests that it includes the apoproteins of more than one hemolymph protein. Nonetheless, 220 and 82 kDa polypeptides were indicative of the apoproteins of lipophorin (Shapiro et al., 1984; Chino, 1985; Kanost et al., 1990; Soulages and Wells, 1994).

To determine which hemolymph proteins carry HC, worker plasma was subjected to KBr gradient ultracentrifugation. The absorbance of each KBr fraction at 280 nm and at 455 nm showed that most of the hemolymph proteins were

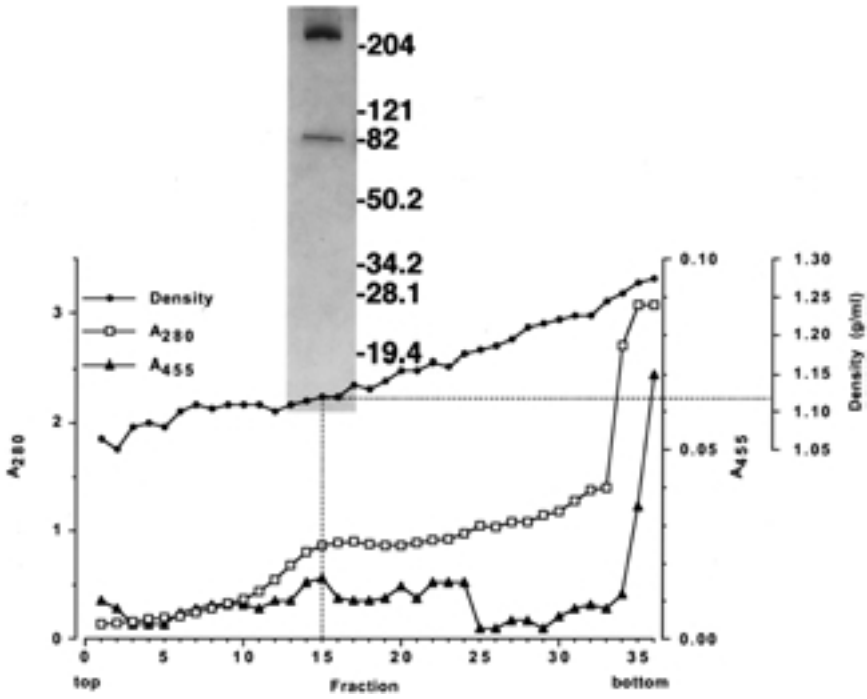

FIG. 9. Fractionation of worker *Z. nevadensis* hemolymph proteins by KBr gradient ultracentrifugation. KBr gradient fractions (400 μl) were collected from the top to the bottom of the tube. Absorbances at 280 and 455 nm are measures of protein and carotenoids, respectively. The density of each fraction was determined gravimetrically. Inset: SDS-gradient polyacrylamide gel electrophoresis (4–15%) of KBr fraction 15, showing purity of the two constituent apoproteins of high-density lipophorin. The positions of the molecular weight markers (kDa) are indicated on the right.

of high density and carried carotenoids (Figure 9). Fraction 15, purified from the KBr gradient, represented lipophorin and was composed of two apoproteins, apolipophorin-I and apolipophorin-II, with relative molecular weights of 220 and 82 kDa (Figure 9 inset) and a density of 1.12 ± 0.005 g/ml. The lipophorin-containing fractions (fractions 11–19) appeared to contain less protein than fractions at the bottom of the gradient that contained denser proteins. Nevertheless, all the hemolymph HC associated with the high density lipophorin-containing fractions and only trace amounts with proteins of higher density (Figure 10). Moreover, the HC profile of worker lipophorin was nearly identical to hemolymph HC (Figure 4, Table 2), as also revealed by principal components analysis (Figure 6).

These results with *Z. nevadensis* implicate high-density lipophorin in HC

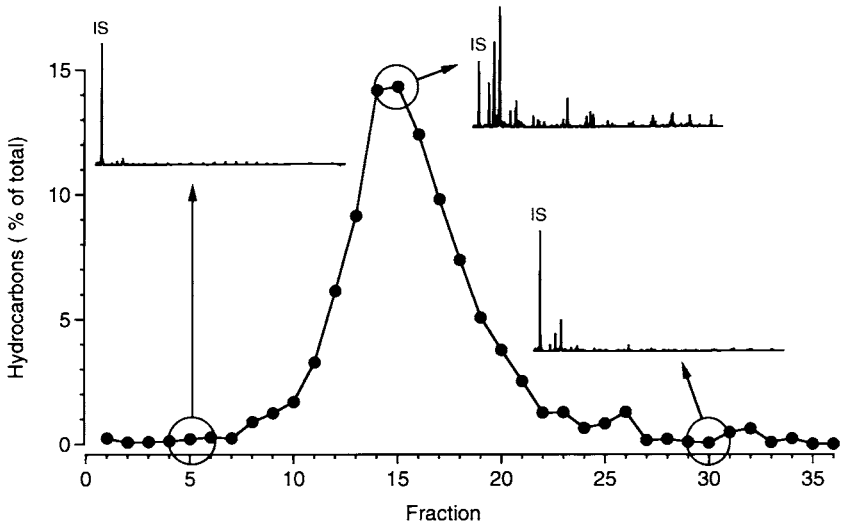

FIG. 10. Hydrocarbon content of each hemolymph KBr fraction as a percentage of the total HC. Hemolymph was collected from *Z. nevadensis* workers and the plasma subjected to KBr fractionation. Lipids were extracted from each fraction and the HC were determined by capillary gas chromatography. Insets show the HC gas chromatograms of fractions 5, 15, and 30.

transport. The lipophorins of termites have specific, high-affinity binding sites for juvenile hormone-III (Okot-Kotber and Prestwich, 1991), and as in cockroaches and beetles, it appears that termite lipophorin plays multiple roles in development and reproduction, as do HC.

To gain further insight into the transport of HC by lipophorin, radiolabeled HC was topically applied to workers and the kinetics of HC internalization and metabolism were studied. Within the first 48 hr, a large fraction of the ^{3}H-labeled HC was lost from the epicuticle and ^{3}H-labeled lipids appeared in internal tissues (Figure 11). Some ^{3}H-labeled HC was metabolized and ^{3}H appeared in the aqueous methanolic fraction of the Bligh and Dyer (1959) extraction.

Radiolabeled HC was also topically applied to workers, and after 24 hr hemolymph was collected and fractionated by KBr gradient centrifugation. Almost all of the radiolabeled HC and its semipolar lipid metabolites were associated with the lipophorin fractions and only trace amounts were found in association with other proteins (Figure 12). In contrast, the methanolic aqueous phase of the Bligh and Dyer (1959) extraction of hemolymph fractions contained radiolabeled compounds whose abundance increased linearly with protein density (Figure 12). This suggests that highly polar products of HC metabolism become

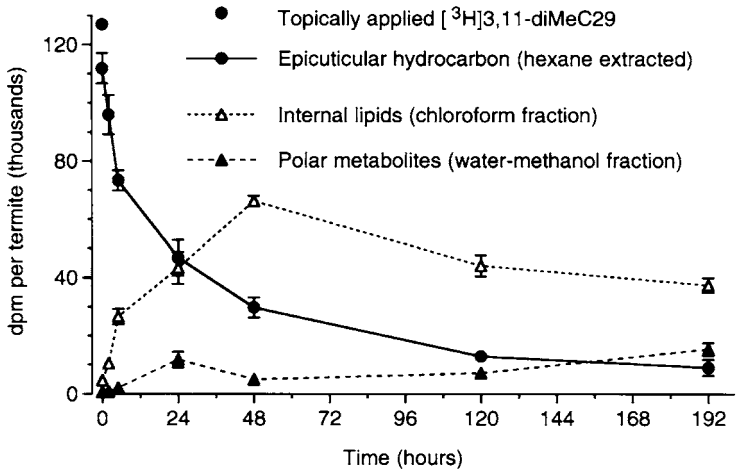

FIG. 11. In vivo uptake and metabolism of [11,12-^{3}H]dimethylnonacosane in *Z. nevadensis* workers. [^{3}H]HC (0.058 μCi in 1 μl hexane) was topically applied to workers, which were subsequently surface and internally extracted in groups of 3. The chloroform phase of the Bligh and Dyer (1959) extraction represents lipids, whereas the water–methanol phase represents polar metabolites of HC (N = 3).

soluble in the hemolymph and/or are cleared by various proteins. Alternatively, HC catabolites might have been incorporated into native hemolymph proteins. Our results suggest that HC are internalized, carried by lipophorin, metabolized, and some metabolites are cleared by lipophorin. Because of the high specific activity of 3,11-diMeC29 (>39 Ci/mmol) only nanogram amounts were applied to each termite. Its close similarity to 3,7-diMeC29, a native HC, suggests that, aside from the delivery in hexane, the transport and metabolism of 3,11-diMeC29 shown in Figures 11 and 12 represent the native behavior of epicuticular HC.

Our results from topical application of HC differ from work with the honeybee. Neither HC from pollen nor individual HC added to ingested pollen are incorporated appreciably in hemolymph HC by the honeybee (Francis et al., 1989). While the mode of entry of the foreign HC, and other procedures, differed in the two studies, it is also possible that species differences account for these observations.

The internalization, metabolism, and lipophorin transport of both the parent HC and its metabolites are particularly relevant to the interpretation of studies of artificially mixed colonies of social insects. Such procedures result in quantitative and qualitative changes and the emergence of artificial hybrid HC profiles in both ants and termites (Bonavita-Cougourdan et al., 1989, 1997; Bagnères et al., 1991a; Vauchot et al., 1996). Presumably, HC are exchanged through physi-

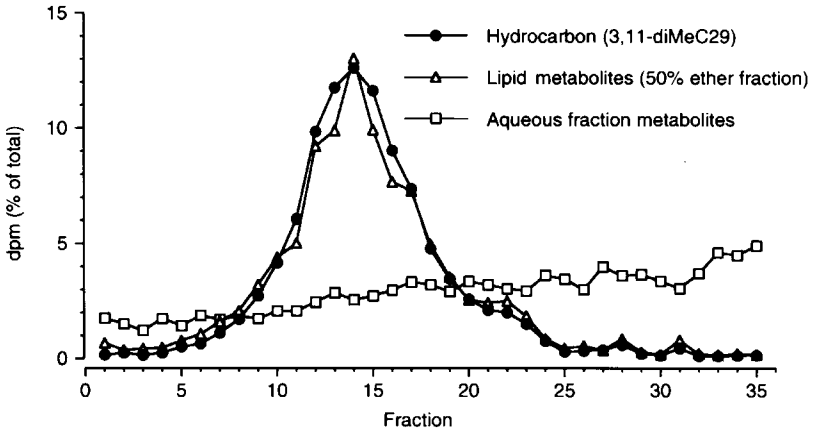

FIG. 12. In vivo uptake and metabolism of [11,12-^{3}H]dimethylnonacosane in worker *Z. nevadensis*. [^{3}H]HC (0.45 μCi in 1 μl hexane) was topically applied onto each of 70 workers and after 24 hr hemolymph was collected and the plasma subjected to KBr gradient ultracentrifugation. HC and polar lipid metabolites (hexane–ether, 50 : 50 fraction) were purified from each fraction and assayed for radioactivity by liquid scintillation spectrometry. Shown is the association of radiolabeled HC and its polar metabolites with hemolymph KBr fractions as a percentage of the respective total radiolabeled material recovered.

cal contact, possibly involving the postpharyngeal gland in ants (Bagnères and Morgan, 1991; Meskali et al., 1995; Soroker et al., 1994, 1995). However, cohabitation may alter both the quantity of the homospecific HC mixture on the cuticle as well as the relative amount of each component HC (Vauchot et al., 1996).

A critical unresolved question is by what mechanisms insects control the loading and unloading of lipophorin, resulting in unique HC profiles.

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