Journal of Chemical Ecology, Vol. 20, No. 5, 1994

COMPOSITION OF LARVAL SECRETION OF *Chrysomela lapponica* (COLEOPTERA, CHRYSOMELIDAE) AND ITS DEPENDENCE ON HOST PLANT

M. HILKER[1,*] and S. SCHULZ[2]

[1]*Lehrstuhl für Tierökologie II, Universität Bayreuth Postfach 101251, 95440 Bayreuth, Germany*

[2]*Institut für Organische Chemie, Universität Hamburg Martin-Luther-King-Platz 6, 20146 Hamburg, Germany*

(Received September 27, 1993; accepted January 3, 1994)

Abstract—The defensive secretion of *Chrysomela lapponica* larvae, which is produced by nine pairs of exocrine dorsal glands, has been chemically analyzed. The *C. lapponica* larvae were kept in the laboratory on leaves of either birch (*Betula pendula*), alder (*Alnus glutinosa*), or willow (*Salix fragilis*). Larvae developed normally on birch and willow, whereas those on alder died within a few days. GC-MS analyses of the secretion of larvae on birch and willow revealed that the composition of this secretion differs distinctly from the known ones of several other *Chrysomela* species feeding exclusively on Salicaceae. In the exocrine secretion of larvae on birch, 69 compounds were identified, which included the main components isobutyric acid, 2-methylbutyric acid, and esters of the two. Several of the esters have not been reported previously from nature. The alcoholic components of the esters may be hydrolysis products of *Betula* glycosides. Most components of the secretion of larvae feeding on birch were also found in the secretion of larvae feeding on willow. In addition, major amounts of benzoic acid and salicylalcohol were present in the secretion of the larvae feeding on willow. *C. lapponica* obviously acquires salicylalcohol by hydrolysis of salicin from willow leaves. However, in contrast to other *Chrysomela* species, *C. lapponica* larvae oxidize only traces of salicylalcohol to salicylaldehyde. The repellent activity of single authentic compounds of the secretion of larvae feeding on birch and willow, respectively, was tested in laboratory bioassays with ants (*Myrmica sabuleti*). Biosynthetic pathways to some identified compounds are suggested and discussed under evolutionary and functional aspects.

Key Words—Coleoptera, Chrysomelidae, *Chrysomela lapponica*, larval secretion, defense.

*To whom correspondence should be addressed.

INTRODUCTION

It is well known from several chrysomelid taxa that larvae defend themselves against enemies by oozing exocrine secretion from dorsal glands when attacked. The species of the subtribes Chrysomelina and Phyllodectina (Chrysomelinae, Chrysomelini) possess nine pairs of dorsal exocrine glands located in the meso- and metathorax and in the abdominal segments 1–7. Iridoid monoterpenes have been identified as main components of their larval glandular secretions for many of these species (review: Pasteels et al., 1988a,b). These compounds are produced de novo by the larvae via the acetate–mevalonate pathway (Lorenz et al., 1993). On the other hand, a few chrysomeline species use direct host plant precursors for production of main components of their larval secretions. *Gastrolina depressa* feeds on Juglandaceae and contains juglone in its larval secretion, which is obviously derived from the host plant (Matsuda and Sugawara, 1980). Several *Chrysomela* species and *Phratora vitellinae*, which feed on Salicaceae, use salicin of their host plants, hydrolyze this phenolglycoside, and oxidize the resulting aglycone by a specific enzyme to salicylaldehyde, which is discharged by the exocrine larval glands (Pasteels et al., 1982, 1984, 1990; Wain, 1943). In contrast to these *Chrysomela* species feeding exclusively on Salicaceae, *C. interrupta* larvae, which feed on alder (Betulaceae), contain 2-phenylethyl isobutyrate and 2-phenylethyl 2-methylbutyrate as the main components of their exocrine secretions (Blum et al., 1972). These compounds or possible precursors are unknown from alder leaves. Nevertheless, the glycosides of 2-phenylethylalcohol are believed to be common constituents of the green parts of plants (Stahl-Biskup et al. 1993).

According to Brown (1956), the nearctic *C. interrupta* and its sibling species, which feed on Betulaceae and/or Salicaceae, have often been confused with the European *C. lapponica*, for which birch and alder (both Betulaceae) as well as willow and poplar (Salicaceae) are cited as host plants (Brown, 1956; Mohr, 1966). The chemistry of the larval secretion of *C. lapponica* has not been studied before, whereas the morphology of the larval dorsal glands of this species has been known for a long time (Garb, 1915). Since several other *Chrysomela* species have specialized on willows and use mainly one particular allelochemical of their host plant (salicin), *C. lapponica* is an appropriate species to examine how a switch from one host plant family to another one with different characteristic plant allelochemicals may influence the composition of larval secretion. Summaries of known allelochemicals of Salicaceae and Betulaceae are given by Hegnauer (1964, 1973), Merkx and Baerheim Svendson (1990), Palo (1984), and Thieme (1971). Will *C. lapponica* larvae use the different plant allelochemicals for the production of their exocrine secretion compounds? Does the larval secretion of *C. lapponica* differ from the known one of its closely

related species *C. interrupta*? In order to answer these questions we conducted the study presented here.

METHODS AND MATERIALS

Larvae of *C. lapponica* (L1, L2) feeding on *Betula pendula* were collected in June 1992 near Selb, Bavaria. The larvae were kept in the laboratory in climate chambers (20°C, light–dark cycle: 16 hr/8 hr) on *B. pendula*, *Alnus glutinosa*, and *Salix fragilis*. Small twigs of the host plants placed in water-filled vials were offered to the larvae. After one to two weeks, the exocrine secretion of the dorsal glands was analyzed by GC-MS. Living larvae were placed under a stereomicroscope, disturbed with forceps, and the emerging secretion was directly collected with a microsyringe. This secretion was immediately analyzed by the following GC-MS systems:

System I. A VG 70/250 S mass spectrometer coupled to a Hewlett-Packard HP 5890 A gas chromatograph with splitless injection (injector temperature 250°C) or on-column injection, equipped with a 30-m × 0.32-mm Rt_x-5 (Restek) fused-silica column programmed from 50°C to 300°C at 5°C/min. EI (70 eV) and CI mass spectra (70 eV, isobutane) were recorded. The same column and conditions were used for GC analyses. Enantiomer separations were performed using this GC-MS system equipped with a 50-m fused silica capillary coated with a 1:1 mixture of heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrine and OV-1701 (König et al. 1992).

System II. EI mass spectra (70 eV) were obtained using a Carlo Erba Vega Series 2 gas chromatograph with splitless injection (injector temperature 220°C) coupled to a Finnigan MAT Ion Trap Detector ITD 800. A 12.5-m × 0.32-mm FS-OV-1701 (Chrompack, Frankfurt, Germany) column programmed from 60°C to 280°C at 10°C/min was used.

Helium was used as the carrier gas in both systems.

The structures of the identified compounds were confirmed by comparison with synthetic samples. Esters of isobutyric acid and 2-methylbutyric acid were synthesized by conventional methods (Tietze and Eicher, 1991). Mono- and diesters were separated by column chromatography, if necessary. (*S*)-2-Methylbutyric acid was prepared by hydrolysis of commercially available (*S*)-2-methylbutyric anhydride. (*S*)-1,2-Propanediol was obtained from ethyl (*S*)-lactate. (*R*)-1,3-Hexandiol was synthesized by reduction of ethyl 3-oxohexanoate with baker's yeast yielding ethyl (*R*)-3-hydroxyhexanoate, followed by reduction with lithium aluminum hydride (Dillon et al., 1991). (*R*,*E*)-8-Hydroxylinalool [(*R*,*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol] was obtained by catalytic selenium dioxide oxidation (Umbreit and Sharpless, 1977) of (*R*)-(−)-linalool (ICN, Meckenheim, Germany).

Microreductions were performed to obtain further information on the nature of the secretions. Collected larval secretions were injected into 200 μl of absolute diethyl ether and 5 mg prewashed $LiAlH_4$ added. After stirring for 3 hr, ice was added and the aqueous phase extracted three times with diethyl ether. After filtration of the combined organic phases over prewashed anhydrous Na_2SO_4, the solvent was reduced to a volume suitable for GC-MS analyses, or treated with 100 μl MSTFA (*N*-Methyl-trimethylsilyltrifluormethylacetamide) for 3 hr. Careful removal of solvent and excess reagent furnished a modified extract suitable for GC-MS analyses.

In order to examine the biological significance of the compounds identified, the repellent activity of single synthetic secretion components against ants was tested in laboratory bioassays with *Myrmica sabuleti*, which is known as generalized entomophagous predator (Hölldobler and Wilson, 1990). One microliter of a pure, fluid, synthetic component (purity: 99%) was placed on a small piece of glass (5 mm × 10 mm), which was deposited in one arm of a T-shaped tube of Plexiglas (T axis: 8 cm long; each T arm: 7.5 cm long; inner size of tube: 1 × 1 × 1 cm, openings of the T tube at the base of the T axis and at both ends of the T arms). When testing the repellent activity of the solids benzoic acid (Roth AG, Basel, Switzerland) and salicylalcohol (Sigma GmbH, Deisenhofer, Germany), 1 mg was placed onto the small piece of glass. The repellent activity of the natural larval secretion was examined by collecting the secretion of one larva with a small piece of filterpaper (5 × 10 mm) and by placing it into a T arm.

M. sabuleti workers of a laboratory colony were released at the base of the T tube. The ants that ran to the T arm with the synthetic component and natural secretion (test side) or to the opposite control side were counted. Untreated pieces of glass (synthetic components) or filter paper (natural secretions) were located in the control T arm. Reactions of 20 ants were tested for each component. After monitoring the reaction of one individual, the tube was cleaned before testing the next one. Only those ants were counted that immediately ran to the openings of one of the T arms. Ants that left the T tube at the base where they were released were discarded. The sign test for paired observations was used for statistical analysis (Lorenz, 1988).

RESULTS

C. lapponica larvae developed normally on birch or willow, whereas larvae that were fed with alder leaves died within a week. Thus, only the secretions of larvae feeding on birch or willow (referred to below as birch larvae and willow larvae) were investigated. The total ion current chromatogram of the secretion of both types of larvae is shown in Figure 1. A list of the identified compounds is given in Table 1.

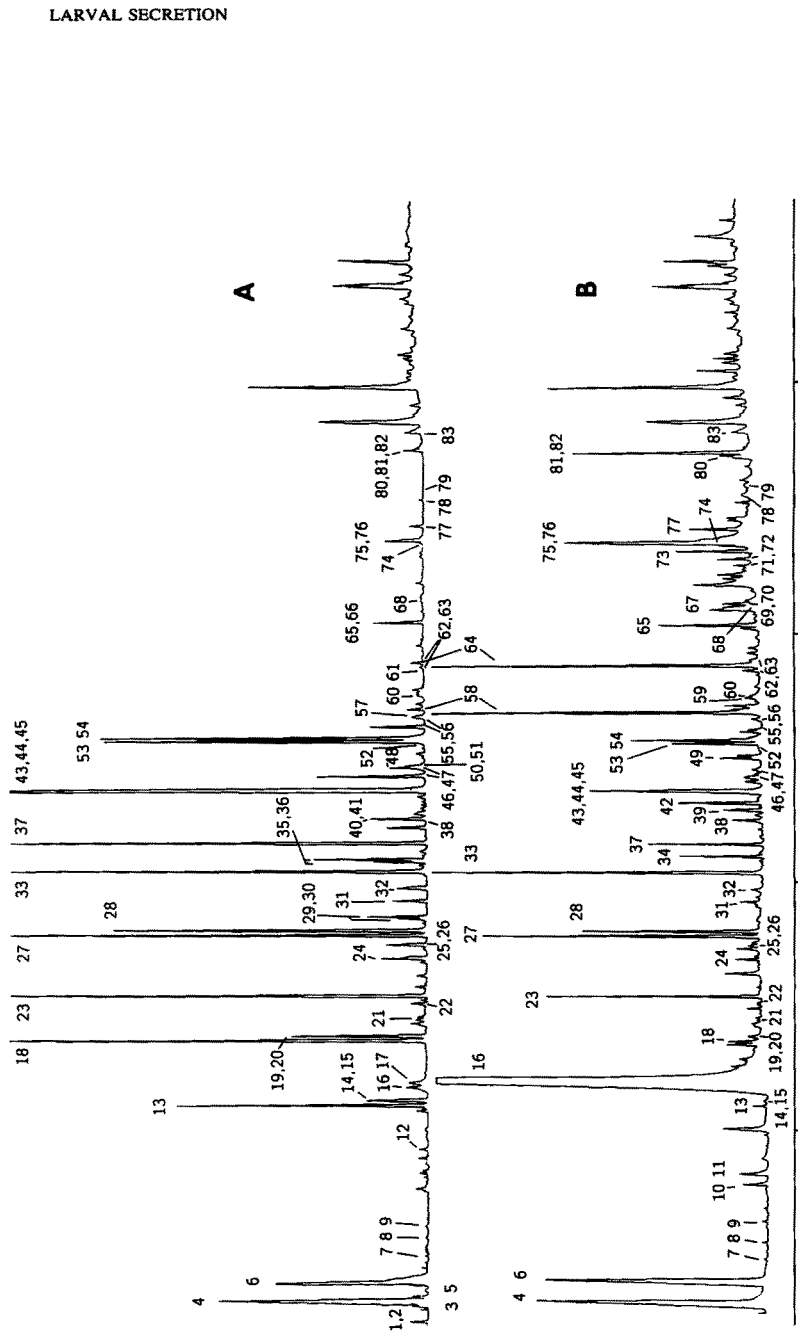

FIG. 1. Total ion current chromatograms (TIC) of exocrine larval secretion of *Chrysomela lapponica* larvae feeding on birch, *Betula pendula* (A) or willow, *Salix fragilis* (B). Both chromatograms were obtained with GC-MS system I (see text); numbering of compounds according to Table 1.

TABLE 1. COMPOUNDS IDENTIFIED FROM EXOCRINE SECRETION OF *Chrysomela lapponica* LARVAE FEEDING ON BIRCH (*Betula pendula*) OR WILLOW (*Salix fragilis*)[a]

	Compound	*Betula*	*Salix*
1	2-Methylbutanal	+	
2	3-Methylbutanal	+	
3	Ethyl isobutyrate	+	
4	Isobutyric acid	+++	+++
5	Ethyl 2-methylbutyrate	+	
6	2-Methylbutyric acid	+++	+++
7	2-Methylbutyl isobutyrate	+	+
8	Phenylacetaldehyde	+	+
9	2-Methylbutyl 2-methylbutyrate	+	+
10	Salicylaldehyde		+
11	Benzyl alcohol		+
12	Linalool oxide	+	+
13	(*Z*)-3-Hexenyl isobutyrate	++	+
14	Hexyl isobutyrate	++	+
15	5-Hexenyl isobutyrate	++	+
16	Benzoic acid		+++
17	6-Ethenyl-2,6,6-trimethyltetrahydropyran-3-ol	+	
18	(*Z*)-3-Hexenyl 2-methylbutyrate	++	+
19	Hexyl 2-methylbutyrate	++	+
20	5-Hexenyl 2-methylbutyrate	++	+
21	1,2-Ethandiyl diisobutyrate	++	+
22	1,2-Propandiyl diisobutyrate	+	+
23	Benzyl isobutyrate	+++	+++
24	1,2-Ethandiyl 1-isobutyrate 2-methylbutyrate	+	
25	1,2-Propandiyl 1-isobutyrate 2-(2-methylbutyrate)	+	+
26	1,2-Propandiyl 2-isobutyrate 1-(2-methylbutyrate)	+	+
27	Benzyl 2-methylbutyrate	+++	+++
28	2-Phenylethyl isobutyrate	+++	+++
29	*cis*-2-Ethenyl-2,6,6-trimethyltetrahydropyran-3-yl isobutyrate	++	
30	*trans*-2-Ethenyl-2,6,6-trimethyltetrahydropyran-3-yl isobutyrate	++	
31	1,2-Ethandiyl bis(2-methylbutyrate)	+	+
32	1,2-Propandiyl bis(2-methylbutyrate)	+	+
33	2-Phenylethyl 2-methylbutyrate	+++	+++
34	Geranyl isobutyrate		++
35	*cis*-2-Ethenyl-2,6,6-trimethyltetrahydropyran-3-yl 2-methylbutyrate	++	
36	*trans*-2-Ethenyl-2,6,6-trimethyltetrahydropyran-3-yl 2-methylbutyrate	++	
37	1,3-Hexandiyl diisobutyrate	+++	++
38	(*Z*)-3-Hexenyl benzoate	+	++
39	Hexyl benzoate		+
40	(*Z*)-8-Isobutyryloxylinalool	++	
41	1,4-Hexandiyl diisobutyrate	+	
42	Geranyl 2-methylbutyrate		++

TABLE 1. CONTINUED

	Compound	*Betula*	*Salix*
43	1,3-Hexandiyl 1-isobutyrate 3-(2-methylbutyrate)	+++	++
44	1,3-Hexandiyl 3-isobutyrate 1-(2-methylbutyrate)	+++	++
45	(*E*)-8-Isobutyryloxylinalool	+++	+++
46	*cis*-1,4-Cyclohexandiyl diisobutyrate	+	+
47	*trans*-1,4-Cyclohexandiyl diisobutyrate	+	+
48	(*Z*)-8-(2-Methylbutyryloxy)linalool	++	
49	1,2-Ethandiyl 1-benzoate 2-isobutyrate		+
50	1,4-Hexandiyl 1-isobutyrate 4-(2-methylbutyrate)	+	
51	1,4-Hexandiyl 4-isobutyrate 1-(2-methylbutyrate)	+	
52	2-(4-Hydroxyphenyl)ethyl isobutyrate	+	+
53	1,3-Hexandiyl bis(2-methylbutyrate)	+++	++
54	(*E*)-8-(2-Methylbutyryloxy)linalool	+++	+++
55	*cis*-1,4-Cyclohexandiyl isobutyrate 2-methylbutyrate	+	+
56	*trans*-1,4-Cyclohexandiyl isobutyrate 2-methylbutyrate	+	+
57	1,4-Hexandiyl bis(2-methylbutyrate)	+	
58	Benzyl benzoate	+	+++
59	1,2-Ethandiyl benzoate 2-methylbutyrate		+
60	2-(4-Hydroxyphenyl)ethyl 2-methylbutyrate	+	+
61	Rhododendryl isobutyrate	+	
62	*cis*-1,4-Cyclohexandiyl bis(2-methylbutyrate)	+	+
63	*trans*-1,4-Cyclohexandiyl bis(2-methylbutyrate)	+	+
64	2-Phenylethyl benzoate	+	+++
65	Methyl palmitate	++	++
66	Rhododendryl 2-methylbutyrate	+	
67	Geranyl benzoate		++
68	Ethyl palmitate	+	+
69	1,3-Hexandiyl 1-benzoate 3-isobutyrate		++
70	1,3-Hexandiyl 3-benzoate 1-isobutyrate		++
71	1,3-Hexandiyl 3-benzoate 1-(2-methylbutyrate)		++
72	1,3-Hexandiyl 1-benzoate 3-(2-methylbutyrate)		++
73	(*E*)-8-Benzoyloxylinalool		++
74	Methyl linoleate	++	++
75	Methyl linolenate	++	+
76	Methyl oleate	+	+
77	Methyl stearate	+	+
78	Ethyl linolenate	+	+
79	Ethyl stearate	+	+
80	Methyl eicosadienoate	++	+
81	Methyl eicosatrienoate	++	+
82	Methyl eicosenoate	++	+
83	Methyl eicosanoate	++	+
84	Salicylalcohol		+++

[a] + (trace components), ++ (minor components), +++ (major components).

Isobutyric and 2-methylbutyric acids could be easily identified by their mass spectra. Most of the other compounds exhibited mass spectra with prominent ions at m/z = 43, 71, and 89, or at m/z = 57, 85, and 103. In some spectra both ion groups occurred (Figure 2). These spectra indicated the presence of mono- and diesters of the mentioned acids in the secretion. Molecular weights were obtained by CI-MS. Reduction of the secretion, followed by silylation led to the identification of the following alcohols in the derivatized extracts: (*E*)- and (*Z*)-8-hydroxylinalool, 1,3-hexandiol, benzylalcohol, and 2-phenylethanol were major components, while ethandiol, 1,2-propandiol, 1,4-hexandiol, hexanol, (*Z*)-3-hexenol, 5-hexenol, *cis*- and *trans*-1,4-cyclohexandiol, pyranoid *cis*- and *trans*-linalool oxide (2,2,6-trimethyl-6-vinyltetrahydropyran-3-ol), 4-(4-hydroxyphenyl)-butan-2-ol (rhododendrol), and fatty alcohols occurred in smaller amounts. The naturally occurring compounds represent, therefore, mono- and diesters of these alcohols with isobutyric and 2-methylbutyric acids except for the fatty alcohols. These were formed during the derivatization procedure by reduction of methyl and ethyl esters of fatty acids present in the secretion. Unsymmetrical diols such as 1,3-hexandiol occurred as four different types of diesters (Table 1 and Figure 3).

Generally, only primary and secondary alcohols are esterified, while tertiary alcohol functions remain free. For example, 8-hydroxylinalool is esterified at the primary hydroxy group at C-8 only, and no diester could be detected in the natural secretion. While small amounts of the furanoid linalool oxide with a free alcohol group were present, no respective ester could be identified. The esters and the acids were the predominant constituents of the secretion, but small amounts of free alcohols and some aldehydes were also present. Thus, the secretion was made up by a complex mixture of more than 60 compounds. Unambiguous proof of the structures was obtained by comparison with synthetic samples (see Methods and Materials). Some compounds, which represent also esters of the mentioned acids, remained unidentified (see below).

Most of the compounds identified from the secretion of birch larvae were also present in willow larvae (Figure 1A and B, and Table 1). The major difference was the occurrence of large amounts of benzoic acid and salicylalcohol in the secretion of willow larvae. Benzoic acid esters could be readily identified in the secretion by their diagnostic ions at m/z = 77 and 105. This acid was esterified with the alcohols present in birch larvae esters, except for the linalool oxides, esters of which were absent in willow larvae. In addition, also esters of geraniol occurred in willow larvae. Benzyl benzoate and 2-phenylethyl benzoate became major components in their secretion.

Salicylalcohol could not be identified by GC-MS in extracts investigated on a 30-m fused silica column coated with an apolar phenylmethylsilicone phase using splitless injection or on-column injection. This may be due to the very polar character of the molecule, being unable to dissolve in the apolar silicone.

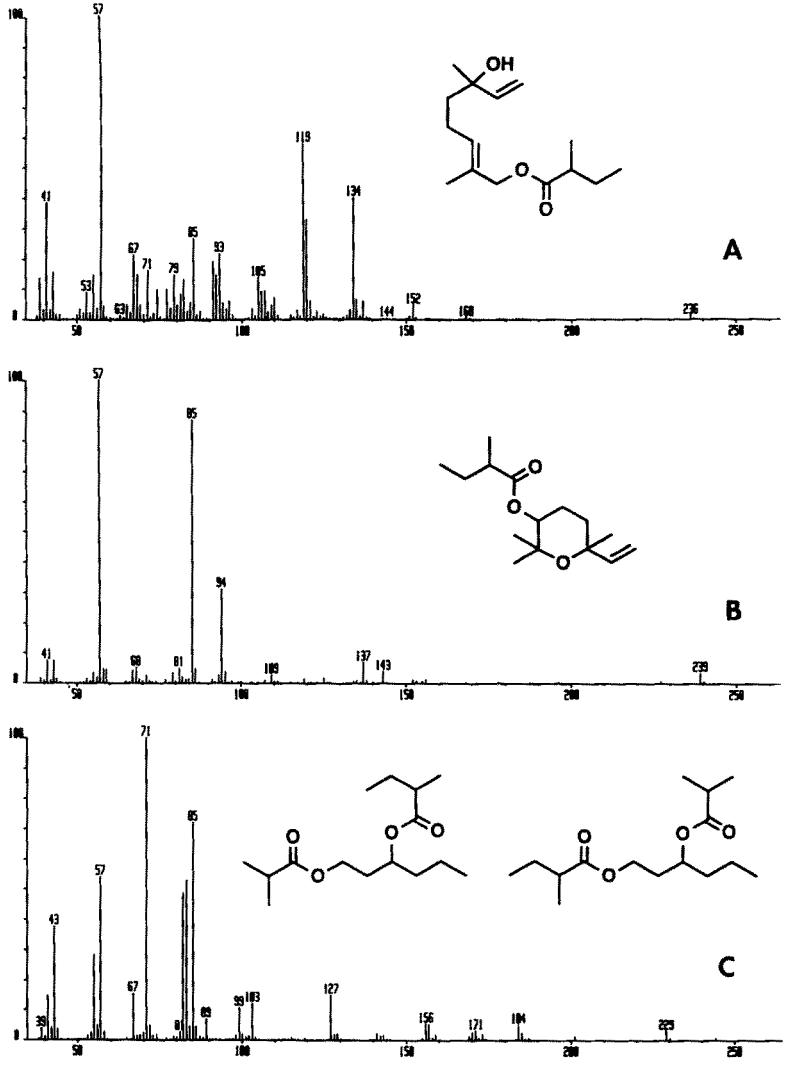

FIG. 2. Mass spectra of compounds 54 (A), 35 or 36 (B), and a mixture of 43 and 44 (C). Compounds 43 and 44 were not separated by GC-MS. Careful analyses of all scans of the respective peak nevertheless showed differences in ion intensities between early and late scans, which could be attributed to slightly different retention volumes of the two compounds. A synthetic mixture of 43 and 44 showed the same behavior.

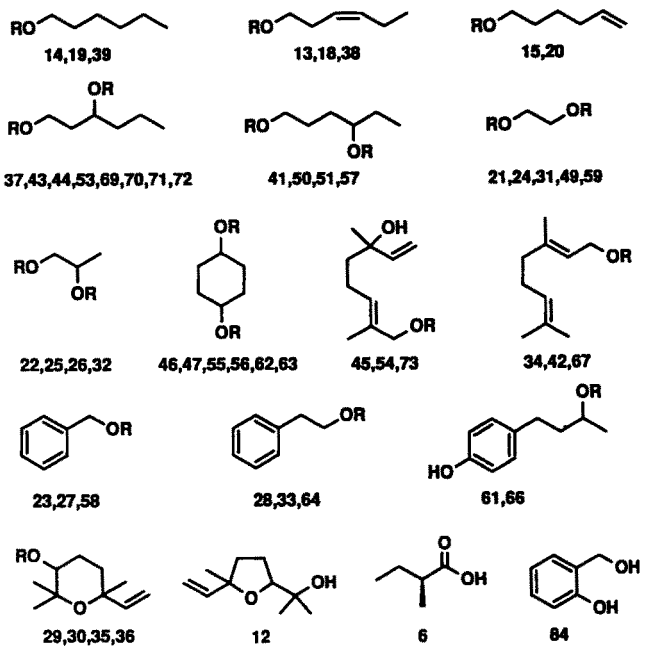

FIG. 3. Compounds identified from the exocrine secretion of *Chrysomela lapponica* larvae. R may be isobutyryl, 2-methylbutyryl, or benzoyl, depending on the actual composition of the compounds (compare Table 1).

Even relatively large amounts of pure compound eluted as a broad "hill" only. By using a 12.5-m fused silica column coated with medium polar OV-1701, the presence of this compound could be clearly demonstrated (Figure 4). Surprisingly, no ester of salicylalcohol but small amounts of salicylaldehyde, a major component of the larval secretion of other *Chrysomela* species feeding exclusively on Salicaceae, could be identified.

The absolute configuration of some representative compounds of the secretion of birch larvae was elucidated by using a GC-MS system equipped with a fused silica capillary coated with a modified cyclodextrine (see Methods and Materials) as chiral stationary phase. The proof of the identity of a given peak by its mass spectrum was necessary because of the high number of compounds present in the secretions. Comparison of racemates and synthetic enantiomers revealed the results given in Table 2. (*S*)-2-Methylbutyric acid was present as pure enantiomer, while the 8-hydroxylinalyl esters and the 1,3-hexandiyl diisobutyrate surprisingly were present as nonracemic mixtures (7:3) of enantiomers. The main enantiomer of 1,2-propandiyl diisobutyrate exhibited the (*S*) config-

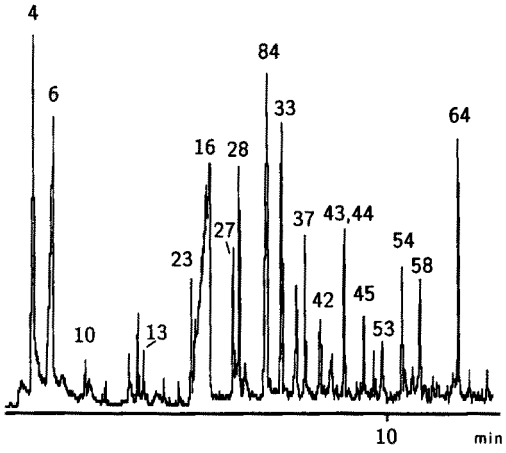

FIG. 4. Total ion current chromatogram of exocrine larval secretion of *Chrysomela lapponica* larvae feeding on *Salix fragilis*; this analysis was performed with GC-MS system II (see text); numbering of compounds according to Table 1.

TABLE 2. RELATIVE PROPORTIONS OF ENANTIOMERS (%) OF SELECTED COMPOUNDS PRESENT IN LARVAL SECRETION OF *Chrysomela lapponica*

Compound	*S*	*R*
2-Methylbutyric acid	>95	
1,2-Propandiyl diisobutyrate	+	–
1,3-Hexandiyl diisobutyrate	70	30
(*E*)-8-Isobutyryloxylinalool	30	70
(*E*)-8-(2-Methylbutyryloxy)linalool	30	70

uration, while the low abundance of this compound did not allow determination of whether the *R*-enantiomer was also present (Figure 5).

The total ion current chromatograms of both the secretion of birch larvae and willow larvae showed several late eluting peaks. The structures of these compounds are still unknown. However, mass spectra with fragments at m/z = 43, 57, 71, and 85 indicated these compounds to be polyesters, too.

Chiral compounds were tested as racemates only. Bioassays with ants were conducted with the compounds and secretions listed in Table 3. The exocrine secretion of larvae on both birch and willow significantly repelled ants. With the exception of benzoic acid and salicylalcohol, each compound tested revealed a repellent activity against the ants.

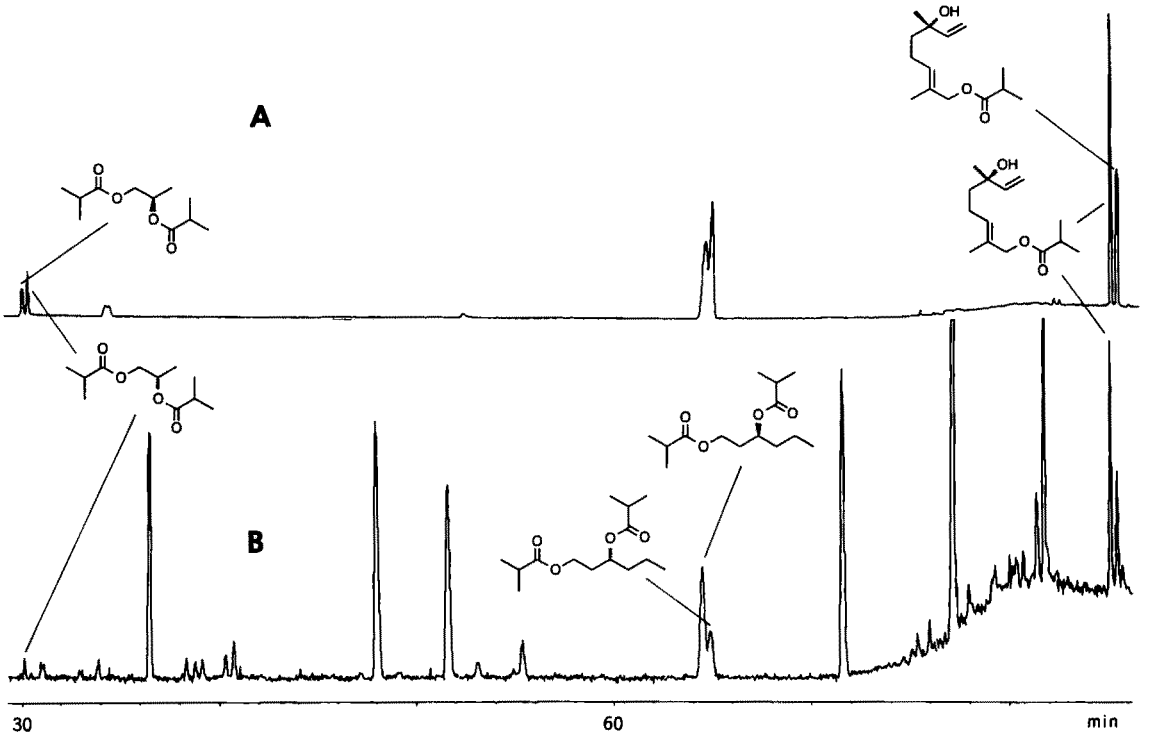

FIG. 5. Enantiomer separation of compounds 22, 37, and 45 on a 50-m heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrine chiral phase (TIC). (A) Mixture of synthetic references. (B) Secretion of larvae feeding on birch. Programmed from 60°C to 100°C at 30°C/min, 20 min isothermal, then at 30°C/min to 115°C, 60 min isothermal, then at 5°C/min to 170°C.

TABLE 3. REPELLENT ACTIVITY OF SECRETION OF *Chrysomela lapponica* LARVAE FEEDING ON BIRCH (*Betula pendula*) OR WILLOW (*Salix fragilis*) AND SYNTHETIC MAIN COMPONENTS OF SECRETIONS AGAINST *Myrmica sabuleti*[a]

Compound/secretion	% ants		Significance
	At test side	At control side	
Secretion of larvae on birch	15	85	**
Secretion of larvae on willow	10	90	***
Isobutyric acid	10	90	***
2-Methylbutyric acid	10	90	***
Hexyl isobutyrate	25	75	*
1-Hexenyl isobutyrate	10	90	***
(*Z*)-3-Hexenyl isobutyrate	5	95	***
1,3-Hexandiyl diisobutyrate	5	95	**
Benzyl isobutyrate	0	100	***
Benzyl 2-methylbutyrate	10	90	***
2-Phenylethyl isobutyrate	5	95	***
2-Phenylethyl 2-methylbutyrate	15	85	**
(*E*)-8-Isobutyryloxylinalool	15	85	***
Benzoic acid	60	40	NS
Salicylalcohol	40	60	NS
Salicylaldehyde[b]	5	95	***

[a] 100% ants: $N = 20$; for details see text. Statistical analysis: two-sided sign test for paired observations. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; NS, not significant.
[b] This compound is present only in traces in the secretion of *C. lapponica* larvae feeding on willow.

DISCUSSION

The larval secretions of *C. lapponica* feeding on willow or birch contain many compounds that have not been reported from nature before. The diesters of the aliphatic diols represent new natural products. To our knowledge, this class of compounds has not been reported from any other insect source before. The related, nonesterified 1,3-nonandiol is a component of the rectal gland secretions of fruit flies (Kitching et al., 1986, Nishida et al. 1990). The esters of (*E*)- and (*Z*)-8-hydroxylinalool and the pyranoid linalool oxides are also new natural products. Free 8-hydroxylinalool has been reported from scentless plant bugs (Aldrich et al., 1990), while the linalool oxides have been identified in hairpencils of danaine butterflies (Schulz et al., 1988, 1993).

The alcoholic components of several of the detected esters may be derived from plant glycosides by hydrolyzation or directly from plant alcohols. Glucosides of (*E*)- and (*Z*)-8-hydroxylinalool and rhododendrol are known as constituents of *Betula* leaves (Klischies and Zenk, 1978; Tschesche et al., 1977).

In *Salix fragilis*, glycosides of 3-hexenol, benzylalcohol, 2-phenylethanol, linalool, and other terpene alcohols have been identified from fresh leaves after enzymatic hydrolysis with β-glucosidase (Merkx and Baerheim Svendsen, 1990). Linalool and 2-phenylethanol are also major constituents of the larval secretion of *Gonioctena viminalis* feeding upon *Salix* spp. (Dettner and Schwinger, 1987). Glycosides of (Z)-3-hexenol, hexanol, benzyl alcohol, and 2-phenylethanol as well as linalool and geraniol are believed to be common plant constituents (Merkx and Baerheim Svendsen 1990, Stahl-Biskup et al. 1993). Glycosides of 1,4-cyclohexandiol, 1,2-propandiol, and 1,2-ethandiol have not been reported from any plant source, but 1,2-cyclohexandiol occurs glycosidically bound in Salicaceae. Nevertheless, we could not identify diesters of 1,2-cyclohexandiol with certainty in the secretion.

Free C_6 alcohols are common plant constituents (Visser et al., 1979; Visser, 1986). For production of the diesters, C_6 diols are necessary, which are not known as constituents of willow or birch leaves. These diols are most probably biosynthetically derived from 3-hexenol. The occurrence of 1,3- and 1,4-hexandiol should exclude a normal acetate pathway to these compounds, because such a mechanism leads exclusively to the 1,3-product. We hypothesize that oxidation of 3-hexenol with a monooxygenase gives 3,4-epoxyhexanol, which could be enzymatically isomerized to 3- and 4-oxohexanols. Reduction would yield the 1,3- and 1,4-hexandiols (Figure 6). The observed enantiomer composition can be explained by a not very enantioselective enzymatic reduction of the ketone intermediates. Whether this transformation is performed by the host plant or by larvae is unknown. We were not able to identify esters of 3,4-epoxyhexanol, the key intermediate of the hypothesized pathway, in the secre-

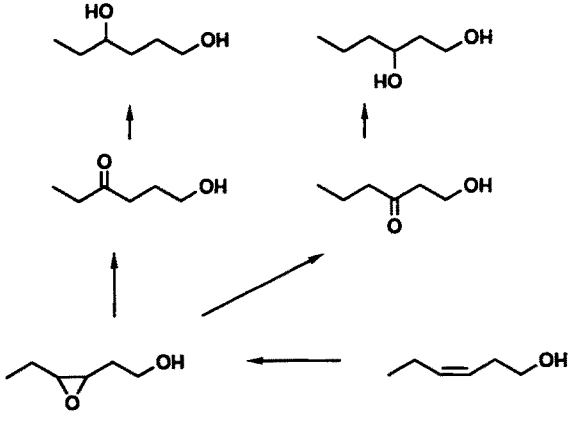

FIG. 6. Proposed biosynthetic pathway leading to 1,3- and 1,4-hexandiol.

tion of *C. lapponica* larvae. Nevertheless, esters of the postulated intermediates have been identified in different organisms. 3,4-Epoxyhexyl acetate is a floral scent component of *Jasminum sambac* (Kaiser, 1986). Esters of 3-oxohexanol have been identified in the scent organs of *Heliconius* butterflies (Schulz, unpublished results).

Another monoxygenase transformation leading to compounds present in the larval secretion seems to take place exclusively in the plants. The linalool oxides and 8-hydroxylinalool are biosynthesized from linalool. While allylic oxidation of linalool yields 8-hydroxylinalool, monooxygenase oxidation yields the relatively unstable 6,7-epoxylinalool, which is known to cyclize easily to the furanoid and pyranoid linalool oxides. The absence of the linalool oxide esters in the secretion of willow larvae points to the plant origin of the linalool oxides in birch larvae and, thus, to the occurrence of monooxygenase transformations in the host plants rather than in the larval secretion.

Although many precursors of the secretion constituents are obviously present in the host plant, we cannot exclude larval de novo synthesis of them. Alcoholic components of some esters are very likely derived from amino acids: phenylethanol from phenylalanine and hydroxyphenylethanol from tyrosine. 8-Hydroxylinalool may be plant-derived or biosynthesized de novo by *C. lapponica* via the acetate–mevalonate pathway, but the presence of its glycoside, betulalboside, in *Betula* leaves points to plant origin. Betulalbosides have been identified in several plants (Stahl-Biskup et al., 1993) and may thus also be present in willow, which would explain its presence in the secretion of willow larvae.

The aliphatic carboxylic acids detected in the secretion of *C. lapponica* larvae may be derived from amino acids (Blum, 1987). Isobutyric acid and 2-methylbutyric acid, which are also known as constituents of the exocrine glandular secretion of the lepidopteran larvae *Papilio aegeus*, have been proved to be biosynthesized in this species from valine and isoleucine, respectively (Seligman and Doy, 1973). Most probably these compounds are biosynthesized de novo by *C. lapponica* larvae.

A striking difference between the secretion of *Chrysomela lapponica* larvae feeding on willow and birch is the presence of benzoic acid and salicylalcohol as main components in the secretion of willow larvae. Salicylalcohol is obviously derived from salicin from the host plant (*Salix fragilis*) by hydrolysis. In contrast to other *Chrysomela* spp. feeding on Salicaceae, *C. lapponica* larvae only hydrolyze salicin, but hardly oxidize the resulting salicylalcohol to salicylaldehyde. The occurrence of large amounts of free and esterified benzoic acid is characteristic for Salicaceae (Hegnauer, 1973), and thus the benzoic acid may be obtained from the host plant. Our results strongly suggest that *C. lapponica* larvae liberate plant allelochemicals from the glandular secretions.

The results of the present study prompt the hypothesis that *C. lapponica*

larvae produce the main components of their exocrine glandular secretions on a metabolic pathway that has not yet been described for the biosynthesis of chrysomelid larval secretions. The known pathways are:

1. De novo biosynthesis of iridoid monoterpenes via the acetate–mevalonate pathway (Lorenz et al., 1993).

2. Hydrolysis of plant glycosides by a β-glucosidase and oxidation of the resulting aglycone by a specific oxidase (proved for the production of salicylaldehyde, suggested for juglone) (Pasteels et al. 1983, 1984; Matsuda and Sugawara, 1980).

For major compounds in the larval secretion of *C. lapponica*, we suggest the following pathway: De novo synthesis of isobutyric and 2-methylbutyric acid from amino acids, followed by esterification with alcohols derived from the host plant (obtained most probably by hydrolysis of plant glycosides) or with de novo synthesized alcohols. This hypothesis needs to be proved by precise biogenetic studies with labelled precursors.

The de novo synthesis of iridoid monoterpenes in chrysomelid larval secretions is considered a widespread ancestral character by Pasteels et al. (1990), whereas the use of plant allelochemicals as direct precursors for the production of larval secretions is regarded as a derived character occurring in a few chrysomelid taxa. If further studies will confirm the pathway of biogenesis suggested above for the larval secretion of *C. lapponica*, this species would connect de novo synthesis of glandular components (production of carboxylic acids from amino acids) with the use of direct plant precursors for the production of carboxylic acid esters (see, e.g., 8-hydroxylinalyl esters or rhododendryl esters). The presence of carboxylic acid esters as larval allomones is, up to now, only known in two *Chrysomela* species, *C. lapponica* and *C. interrupta*, whereas in seven other *Chrysomela* species salicylaldehyde is a major component. Larvae of *Linaeidea aenea*, a species feeding on alder, produce iridoid monoterpenes in their defensive glands (Sugawara et al., 1979). This species is closely related to the genus *Chrysomela*. Formerly, *Linaeidea* had been considered as a subgenus of the current genus *Chrysomela* (Hennig, 1938; Seeno and Wilcox, 1982). Thus, within this group of closely related species both ancestral (monoterpenes) and derived (salicylaldehyde) characters occur when following the trait of evolution outlined by Pasteels et al. (1990).

How does the chemical composition of the larval secretion of *C. lapponica* fit into this evolutionary sequence? Up to now, it has been impossible to judge whether a de novo synthesis of carboxylic acids from amino acids in the larval exocrine glands is an ancestral or a derived character. The use of amino acids for the production of larval secretion components could either have been reduced in other *Chrysomela* species or have evolved alternatively to the development of a specific oxidase of salicylalcohol. Pasteels et al. (1989) suggested the use of amino acids for the production of exocrine glandular secretions in chrysome-

line adults (glands at the pronotum and elytra) as an ancestral character because of its widespread occurrence. For a careful evolutionary consideration of larval secretion components of *C. lapponica*, further knowledge on their biosynthesis and the enzymes involved will be necessary.

The bioassays with ants revealed that the repellent activity of the larval secretion is independent of whether larvae feed on birch or willow. Since the larval secretion was not analyzed quantitatively, the synthetic components were not tested in natural concentrations. Therefore, these bioassays provide only preliminary information on the actual defensive efficiency of single compounds of the natural secretion. Nevertheless, the bioassays show that benzoic acid and salicylalcohol, major components of the secretion of willow larvae, display no repellent effect against ants. In contrast, larvae of other *Chrysomela* species are able to convert high amounts of salicylalcohol to salicylaldehyde and, thus, to a significant repellent against ants. In contrast, larvae of other *Chrysomela* species are able to convert high amounts of salicylalcohol to salicylaldehyde and, thus, to a significant repellent against ants (Table 3). While salicylalcohol is inactive as a repellent against ants, Pasteels et al. (1983) demonstrated that it acts as a significant feeding deterrent against *Myrmica rubra*. In *Plagiodera versicolora*, which is also a chrysomelid *Salix* feeder, neither salicylalcohol nor salicylaldehyde is present in the larval secretion, but iridoid monoterpenes, which also significantly repel ants (Meinwald et al., 1977), are present; this species excretes salicin and salicylalcohol with the feces (Pasteels et al., 1990). Further detailed investigations on the metabolism of plant components within the gut, fat body, and hemolymph of chrysomeline larvae could elucidate which physiological parameters "decide" about the discharge of plant allelochemicals by the alimentary tract or exocrine glands.

Acknowledgments—We thank Wittko Francke, University Hamburg, and Jacques Pasteels, University Brussels, for helpful discussions and critical review of the manuscript. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

REFERENCES

ALDRICH, J.R., CARROLL, S.P., OLIVER, J.E., LUSBY, W.R., RUDMAN, A.A., and WATERS, R.M. 1990. Exocrine secretions of scentless plant bugs: *Jadera*, *Boisea* and *Niesthrea* species (Hemiptera: Heteroptera: Rhopalidae). *Biochem. Syst. Ecol.* 18:369–376.

BLUM, M.S. 1987. Biosynthesis of arthropod exocrine compounds. *Annu. Rev. Entomol.* 32:381–413.

BLUM, M.S., BRAND, J.M., WALLACE, J.B., and FALES, H.F. 1972. Chemical characterization of the defensive secretion of a chrysomelid larva. *Life Sci.* 11:525–531.

BROWN, W.M. 1956. The new world species of *Chrysomela* L. (Coleoptera: Chrysomelidae). *Can. Entomol.* 88(Suppl. 3):3–54.

DETTNER, K., and SCHWINGER, G. 1987. Chemical defense in the larvae of the leaf beetle *Gonioctena viminalis* L. (Coleoptera: Chrysomelidae). *Experientia* 43:458–460.

DILLON, M.P., HAYES, M.A., SIMPSON, T.I., and SWEENEY, J.B. 1991. The synthesis of isotopically labelled *N*-acetylcysteamine thio esters utilizing a baker's yeast reduction in deuterium oxide. *Bioorg. Med. Chem. Lett.* 1:223–226.

GARB, G. 1915. The eversible glands of a chrysomelid larva, *Melasoma lapponica*. *J. Entomol. Zool.* 7:88–97.

HEGNAUER, R. 1964. Chemotaxonomie der Pflanzen, Vol. 3, Betulaceae. Birkhäuser Verlag, Basel. pp. 255–268.

HEGNAUER, R. 1973. Chemotaxonomie der Pflanzen, Vol. 6, Salicaceae. Birkhäuser Verlag, Basel. pp. 241–258.

HENNIG, W. 1938. Übersicht über die wichtigsten deutschen Chrysomelinen. *Arb. Physiol. Ang. Entomol. Berlin-Dahlem* 5:85–136.

HÖLLDOBLER, B., and WILSON, E.O. 1990. The Ants. Springer Verlag, Berlin. 732 pp.

KAISER, R. 1986. New volatile constituents of *Jasminum sambac* (L.) Aiton, pp. 669–684 in B.M. Lawrence, B.D. Mookherjee, and B.J. Willis (eds.). Flavors and Fragrances: A World Perspective. Proceedings 10th International Congress of Essential Oils, Fragrances and Flavors, Washington, D.C. Elsevier Science Publishers, Amsterdam.

KITCHING, W., LEWIS, J.A., FLETCHER, M.T., DREW, R.A.I., MOORE, C.J., and FRANCKE, W. 1986. Spiroacetals in rectal gland secretions of Australian fruit fly species. J. Chem. Soc. Commun. 1986:853–854.

KLISCHIES, M., and ZENK, M.H. 1978. Stereochemistry of C-methylation in the biosynthesis of rhododendrin in *Alnus* und *Betula*. *Phytochemistry* 17:1281–1284.

KÖNIG, W.A., GEHRCKE, B., ICHELN, D., EVERS, P., DÖNNECKE, J., and WANG, W. 1992. New, selectively substituted cyclodextrins as stationary phases for the analysis of chiral constituents of essential oils. *J. High Resolut. Chromatogr.* 14:367–372.

LORENZ, M., BOLAND, W., and DETTNER, K. 1993. Biosynthese von Iridodialen in Wehrdrüsen von Blattkäferlarven (Chrysomelinae). *Angew. Chem.* 32:912–914.

LORENZ, R.J. 1988. Grundbegriffe der Biometrie, 2nd ed. Fischer Verlag, Stuttgart. 241 pp.

MATSUDA, K., and SUGAWARA, F. 1980. Defensive secretion of chrysomelid larvae *Chrysomela vigintipunctata costella* (Marseul), *C. populi* L. and *Gastrolina depressa* Baly (Coleoptera: Chrysomelidae). *Appl. Entomol. Zool.* 15:316–320.

MEINWALD, J., JONES, T.H., EISNER, T., and HICKS, K. 1977. New methyl-cyclopentanoid terpenes from the larval defensive secretion of a chrysomelid beetle (*Plagiodera versicolora*). Proc. Natl. Acad. Sci. U.S.A. 74:2189–2193.

MERKX, Y.M., and BAERHEIM SVENDSEN, A. 1990. Glycosidically bound volatile aliphatic and aromatic alcohols—a common feature in the vegetable kingdom? *J. Essent. Oil Res.* 2:207–208.

MOHR, K.-H. 1966. Chrysomelidae, pp. 95–280, *in* H. Freude, K.W. Harde, and G.A. Lohse (eds.). Die Käfer Mitteleuropas. Bd. 9. Goecke and Evers, Krefeld.

NISHIDA, R., TAN, K.H., TAKAHASHI, S., and FUKAMI, H. 1990. Volatile components of male rectal glands of the melon fly, *Dacus cucurbitae* Coquillet (Diptera: Tephritidae). *Appl. Entomol. Zool.* 25:105–112.

PALO, R.T. 1984. Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *J. Chem. Ecol.* 10:499–520.

PASTEELS, J.M., BRAEKMAN, J.-C., and DALOZE, D. 1982. Chemical defense in chrysomelid larvae and adults. *Tetrahedron* 38:1891–1897.

PASTEELS, J.M., ROWELL-RAHIER, M., BRAEKMAN, J.-C., and DUPONT, A. 1983. Salicin from host

plant as precursor of salicylaldehyde in defensive secretion of chrysomeline larvae. *Physiol. Entomol.* 8:307–314.

PASTEELS, J.M., ROWELL-RAHIER, M., BRAEKMAN, J.-C., and DALOZE, D. 1984. Chemical defences in leaf beetles and their larvae: The ecological, evolutionary and taxonomic significance. *Biochem. Syst. Ecol.* 12:395–406.

PASTEELS, J.M., ROWELL-RAHIER, M., BRACKMAN, J.-C., DALOZE, D., and DUFFEY, S. 1989. Evolution of exocrine chemical defense in leaf beetles (Coleoptera, Chrysomelidae). *Experientia* 45:295–300.

PASTEELS, J.M., BRAEKMAN, J.-C., and DALOZE, D. 1988a. Chemical defense in the Chrysomelidae, pp. 233–252, *in* P. Jolivet, E. Petitpierre, and T.H. Hsiao (eds.), Biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht.

PASTEELS, J.M., ROWELL-RAHIER, M., and RAUPP, M.J. 1988b. Plant-derived defense in chrysomelid beetles, pp. 235–272, *in* P. Barbosa and D. Letourneau (eds.). Novel Aspects of Insect–Plant Interactions. Wiley, London.

PASTEELS, J.M., DUFFEY, S., and ROWELL-RAHIER, M. 1990. Toxins in chrysomelid beetles. Possible evolutionary sequence from de novo synthesis to derivation from food-plant chemicals. *J. Chem. Ecol.* 16:211–222.

SCHULZ, S., FRANCKE, W., and EDGAR, J. 1988. Volatile compounds from androconial organs of danaine and ithomiine butterflies. *Z. Naturforsch* 43c:99–104.

SCHULZ, S., BOPPRÉ, M., and VANE-WRIGHT, R.I. 1993. Specific mixtures of secretions from male scent organs of African milkweed butterflies (Danainae). *Phil. Trans. R. Soc. Lond. Ser. B* 342:161–181.

SEENO, T.N., and WILCOX, J.A. 1982. Leaf beetle genera (Coleoptera: Chrysomelidae). *Entomography* 1:1–221.

SELIGMAN, I.M., and DOY, F.A. 1973. Biosynthesis of defensive secretions in *Papilio aegeus*. *Insect Biochem.* 3:205–215.

STAHL-BISKUP, E., INTERT, F., HOLTHUIJZEN, J., STENGELE, M., and SCHULZ, G. 1993. Glycosidically bound volatiles—a review 1986–1991. *Flav. Frag. J.* 8:61–80.

SUGAWARA, F., MATSUDA, K., KOBAYASHI, A., and YAMASHITA, K. 1979. Defensive secretion of chrysomelid larvae *Linaeidea aenea* Linné and *Plagiodera versicolora distincta* Baly. *J. Chem. Ecol.* 5:929–934.

THIEME, H. 1971. Vorkommen und Verbreitung von Phenolglykosiden in der Familie der Salicaceen. *Herba Pol.* 17:248–257.

TIETZE, L.F., and EICHER, Th. 1991. Reaktionen und Synthese. Thieme, Stuttgart. 636 pp.

TSCHESCHE, R., CIPER, F., and BREITMAIER, E. 1977. Monoterpen-Glucoside aus den Blättern von *Betula alba* und den Früchten von *Chaenomeles japonica*. *Chem. Ber.* 110:3111–3117.

UMBREIT, M.A., and SHARPLESS, K.B. 1977. Allylic oxidation of olefins by catalytic and stochiometric selenium dioxide with *tert.*-butyl hydroperoxide. *J. Am. Chem. Soc.* 99:5526–5528.

VISSER, J.H. 1986. Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* 31:121–144.

VISSER, J.H., VAN STRATEN, S., and MAARSE, H. 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado potato beetle, *Leptinotarsa decemlineata*. *J. Chem. Ecol.* 5:13–25.

WAIN, R.L. 1943. The secretion of salicylaldehyde by the larvae of the brassy willow beetle (*Phyllodecta vitellinae*). Annu. Rep. Agric. Nat. Stn. pp. 108–110.