

Chrysomelidial in the Opisthontal Glands of the Oribatid Mite, *Oribotritia berlesei*

Günther Raspotnig · Rene Kaiser ·
Edith Stabentheiner · Hans-Jörg Leis

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Abstract Gas chromatographic–mass spectrometric analyses of whole body extracts of *Oribotritia berlesei*, a large-sized soil-dwelling oribatid mite, revealed a consistent chemical pattern of ten components, probably originating from the well-developed opisthontal glands. The three major components of the extract were the iridoid monoterpene, (3*S*,8*S*)-chrysomelidial (about 45% of the extract), the unsaturated hydrocarbon 6,9-heptadecadiene, and the diterpene β -springene (the latter two, each about 20–25% of the extract). The remaining minor components (together about 10% of the extract) included a series of hydrocarbons (tridecene, tridecane, pentadecene, pentadecane, 8-heptadecene, and heptadecane) and the tentatively identified 9,17-octadecadienal. In contrast, analysis of juveniles showed only two compounds, namely a 2:1 mixture of (3*S*,8*S*)-chrysomelidial and its epimer, epi-chrysomelidial (3*S*,8*R*-chrysomelidial). Unexpectedly, neither adult nor juvenile secretions contained the so-called astigmatid compounds, which are considered characteristic of secretions of oribatids above moderately derived Mixonomata. The chrysomelidials, as well as β -springene and octadecadienal, are newly

identified compounds in the opisthontal glands of oribatid mites and have chemotaxonomic potential for this group. This is the first instance of finding chrysomelidials outside the Coleoptera.

Keywords Iridoid monoterpene · Oil gland · Chemosystematics · β -Springene · Oribatida

Introduction

The largest and most conspicuous exocrine system in the acariform mite suborders Oribatida and Astigmata is the paired opisthontal glands (syn. oil glands). These glands, according to a current and widely accepted hypothesis, evolved only once within ancient Oribatida (after the near-basal oribatid groups, Palaeosomata and Enarthronota, had split-off), and are considered characteristic of the four more-derived oribatid groups, Parhyposomata, Mixonomata, Desmonomata, Brachypylina, and also of astigmatid mites. The presence of opisthontal glands not only constitutes a major argument for a proposed monophyletic unit of so-called glandulate Oribatida, including Astigmata (Norton 1998), but also provides evidence for the evolutionary origin of astigmatid mites in an ancient opisthontal gland-bearing oribatid group.

In addition to the presence or absence of opisthontal glands, the secretions of these glands provide phylogenetic information as they include a rich variety of hydrocarbons, terpenes, aromatics, and alkaloids in species-specific or group-specific patterns. These secretions, derived from a homologous gland system, allow the tracing of evolutionary lineages from near-basal to more-derived glandulate Oribatida. Apart from the well-studied opisthontal gland secretions of Astigmata (Kuwahara 2004), the secretions of a selection

G. Raspotnig (✉) · R. Kaiser
Institute of Zoology, Karl-Franzens-University,
Universitätsplatz 2,
8010 Graz, Austria
e-mail: guenther.raspotnig@uni-graz.at

G. Raspotnig · R. Kaiser · H.-J. Leis
Department of Biochemical Analysis and Mass Spectrometry,
Medical University, University Children's Hospital,
Auenbruggerplatz 30,
8036 Graz, Austria

E. Stabentheiner
Institute of Plant Sciences, Plant Physiology,
Schubertstrasse 51,
8010 Graz, Austria

of species of several major groups of glandulate oribatids have been investigated, including representatives of Parhyposomata (Sakata and Norton 2001), a few species of Mixonomata and Desmonomata (Sakata et al. 1995, 2003; Raspotnig et al. 2001, 2005a, b; Shimano et al. 2002; Sakata and Norton 2003), and also examples of Brachypylylina (Takada et al. 2005; Saporito et al. 2007). While Parhyposomata produce phenols and naphthols, the so-called astigmatid compounds (sensu Sakata and Norton 2001) evolved within ancient mixonomatans, and hence, are possibly found in all the above groups. These astigmatid compounds comprise a set of terpenes and aromatics, namely neral, geranial, neryl formate, 2-hydroxy-6-methylbenzaldehyde, and γ -acaridial. The distribution of astigmatid compounds in oribatids is of special interest since these compounds not only provide chemical support for the evolutionary origin of astigmatid mites within ancient Oribatida (Norton 1998), but also define a presumably monophyletic subunit within glandulate Oribatida, the “astigmatid compounds-bearing” oribatids (Raspotnig 2006).

Several chemotaxonomic studies that examined these presumptive astigmatid compound-bearing groups have been undertaken and have yielded a fragmented picture of the distribution of astigmatid compounds. These compounds are difficult to detect in the more derived groups of Desmonomata (apart from Trhypochthoniidae) and generally in Brachypylylina, suggesting that, in these groups, astigmatid compounds have been prone to evolutionary reduction and replacement. In some cases, desmonomatan and brachypylyline species are chemically “dimorphic”, with astigmatid compounds present only in juveniles and completely replaced in adults by novel compounds such as toxic alkaloids (Takada et al. 2005; Saporito et al. 2007). In contrast, the full (or nearly full) set of astigmatid compounds seems to characterize highly derived mixonomatans, low to moderately derived representatives of Desmonomata, and Astigmata. Specifically, these compounds have been detected in opisthontal gland secretions of *Collohmanna gigantea* (Raspotnig et al. 2001), in all Trhypochthoniidae so far investigated (e.g., Sakata et al. 1995, 2003), and in astigmatid mites (e.g., Kuwahara 2004). However, there are large gaps in our knowledge of the distribution of astigmatid compounds: the most conspicuous gap being in the Euphthiracarodea, a speciose group of box mites whose opisthontal gland chemistry has yet to be investigated. Euphthiracaroids are considered a group of highly derived mixonomatans that, together with phthiracaroids, constitute a possible sister group of *Collohmanna*. Thus, euphthiracaroids are likely candidates for producing astigmatid compounds. The study of the chemistry of opisthontal glands in euphthiracaroids is important not only to further our understanding of the evolution of astigmatid compounds, but also because this group is a crucial link for tracing a

possible evolutionary lineage from Oribatida to Astigmata. With respect to the latter, some (not all!) recent molecular studies (e.g., Murell et al. 2005; Domes et al. 2007) conflict with morphological data. Therefore, studying the opisthontal gland chemistry may resolve this problem.

In the present paper, we provide the first chemical analysis of the opisthontal gland secretion of *Oribotritia berlesei*, a large-sized oribatid mite from the near-basal euphthiracaroid family Oribotritiidae.

Methods and Materials

Mites and Mite Extracts Specimens of *O. berlesei* (Michael 1898) (mixonomatan Oribatida: Euphthiracarodea, Oribotritiidae) were collected from the litter and fermentation layer of a mixed forest near Ferlach (Carinthia, Austria). Individuals were collected by hand or extracted from soil samples by using Berlese-Tullgren funnels. In all, 35 adults (all in the course of one collection in September 2006) were used for chemical analyses, five adults were used for scanning electron microscopy, and 30 adults were transferred to Petri dishes (equipped with Plaster of Paris and dead wood) and kept in the dark for a period of about 12 months either at room temperature (one Petri dish) or at 10°C (second Petri dish), respectively. Individuals of both Petri dishes laid eggs, and the offspring were reared to deutonymphal and tritonymphal instars. Since immatures are burrowers in dead wood, pieces of dead wood (provided in the rearing dishes) were checked for immatures at weekly intervals. Ten deutonymphs and eight tritonymphs were used in the chemical studies.

Extracts (containing opisthontal gland secretions) of adults were prepared by immersing freshly collected, living, individuals in hexane (one individual per 100 μ l) for 30 min (Raspotnig et al. 2001, 2005a, b). For juveniles, individual and pooled extracts (containing two to six individuals) were prepared similarly. The extracts were used without further cleanup.

Chemical Analysis: Gas Chromatography—Mass Spectrometry A trace gas chromatograph (GC) coupled to a Voyager mass spectrometer (MS; both from Thermo, Vienna, Austria) and equipped with a ZB-5MS fused silica capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness, Phenomenex, Germany) was used for the analyses. Injection was splitless with helium (at a constant flow rate of 1.5 ml min⁻¹) as a carrier gas. The column temperature was programmed from 50°C (held for 1 min) to 200°C at 10°C min⁻¹, and then to 300°C at 15°C min⁻¹. The ion source of the mass spectrometer and the transfer line were kept at 150°C and 310°C, respectively. Electron impact (EI) spectra were recorded at 70 eV.

Derivatization, Syntheses, Reference Compounds, and Other Chemicals Tri-, penta-, and heptadecane, as well as citral (60% geranial, 40% neral) and selenium dioxide for the preparation of oxocitral (see Bellesia et al. 1986), were purchased from Aldrich (Vienna, Austria). For determination of double bonds in the heptadecadiene, dimethyl-disulfide (DMDS) adducts were generated according to the method described by Vincenti et al. (1987). The opisthonotal gland secretion of *Platynothrus peltifer* (Raspotnig et al. 2005b) was used as a natural source for 6,9-heptadecadiene and 8-heptadecene for chromatographic comparisons. In order to clarify further the structure of chrysolmelidial, the *O*-methyl-oxime derivatives were prepared with methoxyamine hydrochloride (MOX.HCl; 2% in pyridine): 200 μ l of MOX was added to 50 μ l of extract and left at 70°C for 1 h. Products were cleaned in H₂O, extracted with hexane, and analyzed. Stereoisomers of chrysolmelidial and dehydroiridodial were synthesized according to Bellesia et al. (1986). Secretions from eversible defense glands of larvae of the chrysolmelid beetles, *Gastrophya viridula* and *Plagioderma versicolora* (collected in the surroundings of Graz, Austria), known to contain natural (3*S*,8*S*)-chrysolmelidial (e.g., Pasteels et al. 1982), were collected on small filter paper pieces, extracted in hexane (one individual in 100 μ l), and used as sources for authentic reference compounds. Hexane extracts of fresh leaves of *Actinidia polygama* (obtained from the botanical garden in Tübingen, Germany) were taken as a natural source for (3*R*,8*S*)-dehydroiridodial (Yoshihara et al. 1978).

Scanning Electron Microscopy Air-dried mites were mounted on aluminum stubs, sputtercoated (AGAR sputtercoater, Gröpl, Tulln, Austria), and examined with a

Philips XL30 ESEM (Philips/FEI, Vienna, Austria) at high vacuum mode and 20 kV accelerating voltage.

Results

Extracts of Adult *O. berlesei* A total of ten components (peaks a–j) were detected in extracts of adult *O. berlesei* (Fig. 1a). The main component in extracts, component c (RT=13.36 min; ca. 45% of the total extract, based on peak areas) had an EI fragmentation pattern resembling a methyl-cyclopentene monoterpene (Fig. 2a), with a molecular ion at m/z 166 (7) and characteristic fragments at m/z 151 (6), 148 (46), 138 (25), 133 (9), 123 (15), 120 (12), 119 (10), 109 (49), 108 (40), 105 (30), 95 (19), 91 (27), 81 (100), 79 (78), and 77 (38). The spectrum was consistent with that of chrysolmelidial from the literature (e.g., Blum et al. 1978; Oldham et al. 1996) and with a compound from the NIST library (“2-formyl- α ,3-dimethyl-2-cyclopentene-1-acetaldehyde”; CAS no. 75332-42-2: “2-methyl-5(1-methyl-2-oxoethyl)-1-cyclopentene-1-carbaldehyde”). In order to prove the dialdehyde structure, we generated *O*-methyl-oxime derivatives, resulting in the elimination of peak c and the formation of two new peaks, c' and c'', with longer retention times (RT=14.92 and 15.11 min). The two compounds had identical mass spectra, with molecular ions at m/z 224 (4), and fragment ions at m/z 193 (13), 178 (22), 146 (25), 138 (100), 107 (38), 106 (95), 91 (18), 87 (21), 79 (47), and 77 (29). This pattern indicated *O*-methyl-oximation of compound c, forming two isomeric dimethylloximes, which confirmed the proposed dialdehydic structure (addition of two *O*-methyl-oxime groups to compound c = 166 plus 2 \times 29=new molecular ion at m/z 224).

Fig. 1 Typical mass chromatograms of extracts of *Oribotritia berlesei*: **a** adult individual. **b** Deutonymph. Peaks *a* (tridecene), *b* (tridecane), *c*₁ (“epi-chrysolmelidial” = 3*S*,8*R*-chrysolmelidial), *c* (“chrysolmelidial” = 3*S*,8*S*-chrysolmelidial), *d* (pentadecene), *e* (pentadecane), *f* (6,9-heptadecadiene), *g* (8-heptadecene), *h* (heptadecane), *i* (tentatively, β -springene), *j* (tentatively, 9,17-octadecadienal)

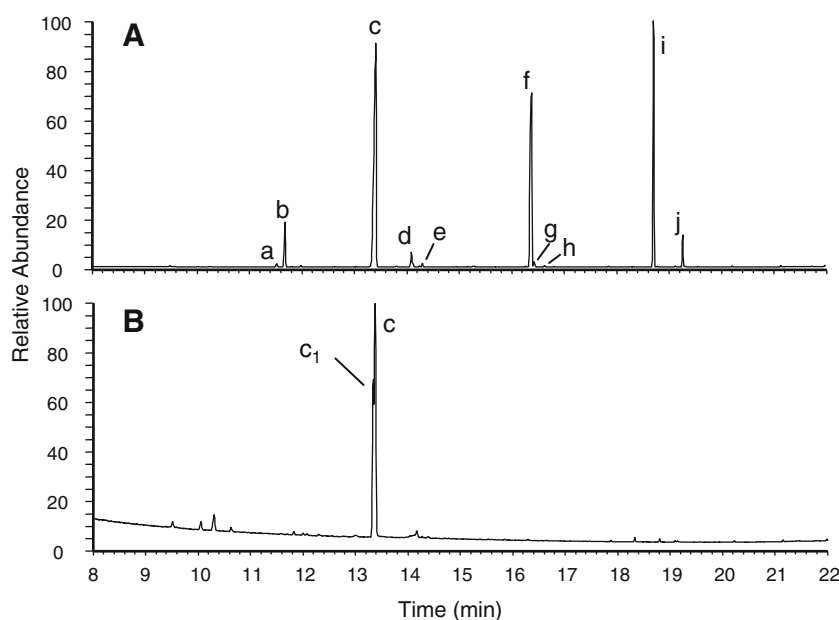
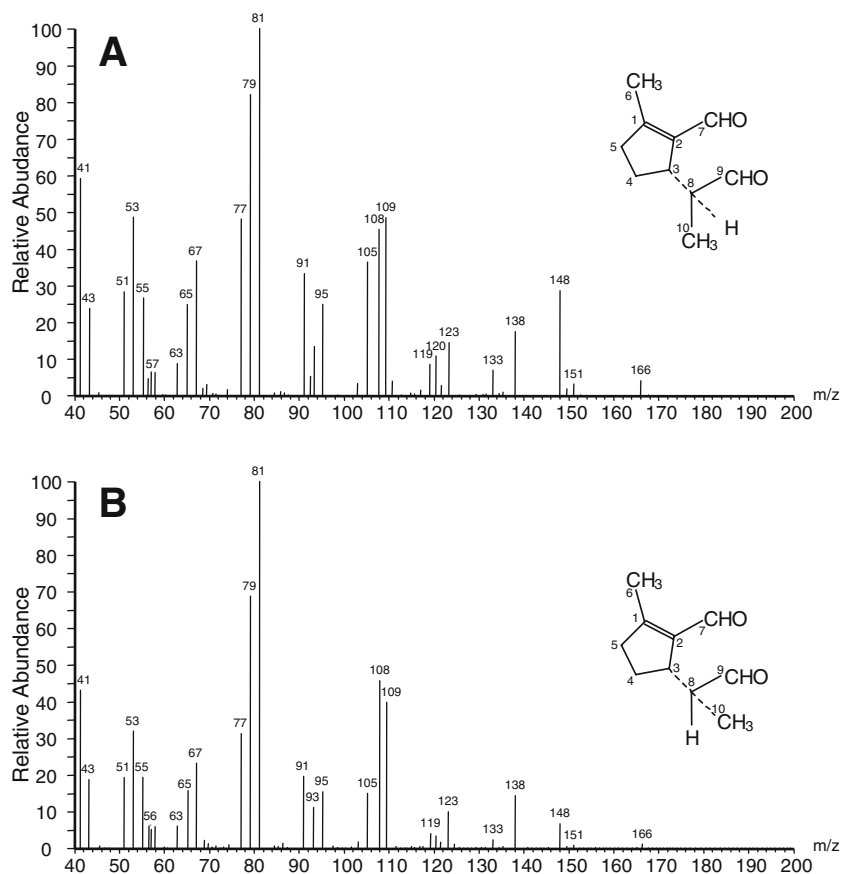


Fig. 2 Electron impact mass spectra of chrysolmelidials in extracts of *Oribotria berlessei*.

a Compound **c**, tentatively identified as (3*S*,8*S*)-chrysolmelidial. **b** Compound **c**₁, tentatively identified as epi-chrysolmelidial = (3*S*,8*R*)-chrysolmelidial. Note the differences in relative intensities of ions at *m/z* 148 ($M^+ - H_2O$) and *m/z* 138 ($M^+ - CO$). Further diagnostic fragments are assigned to: $M^+ - H_2O - CO$ (*m/z* 120); $C_7H_9O^+$ (*m/z* 109), $C_7H_7^+$ (*m/z* 91), and $C_6H_9^+$ (*m/z* 81: base peak = the methylcyclopentene moiety)



For a comparison of compound **c** to authentic chrysolmelidial and its characterization, we used (1) a synthetic mixture of chrysolmelidial-stereoisomers, and (2) naturally occurring (3*S*,8*S*)-chrysolmelidial. The synthetic mixture was assumed to contain four stereoisomers of chrysolmelidial and dehydroiridodial (according to Bellesia et al. 1986), namely two diastereomeric pairs of enantiomers, i.e., (3*S*,8*S*)-chrysolmelidial, (3*R*,8*R*)-dehydroiridodial, and (3*S*,8*R*)-chrysolmelidial (= epi-chrysolmelidial), (3*R*,8*S*)-dehydroiridodial. However, the mixture exhibited only two, poorly separable peaks at RT=13.32 and 13.36 min, each peak corresponding to one pair of enantiomers, with single enantiomers not separable under the chromatographic conditions. The later-eluting peak of the mixture corresponded to compound **c** from the *Oribotritia* extract, with respect to both retention time and EI fragmentation pattern. Larval secretions of the leaf beetle *Gastrophysa viridula* (see “Methods and Materials”), contain mainly (3*S*,8*S*)-chrysolmelidial (“original chrysolmelidial”). Analysis of this secretion showed only one abundant component that eluted at RT=13.36 min, corresponding to the later-eluting peak of the synthetic mixture and to compound **c** of the mite extracts. Thus, the later-eluting peak of the synthetic mixture was assigned as either (3*S*,8*S*)-chrysolmelidial or its enantiomer, (3*R*,8*R*)-dehydroiridodial. Since dehydroiridodials are considered of plant origin only

(see “Discussion”), compound **c** was tentatively identified as (3*S*,8*S*)-chrysolmelidial.

The mass spectrum of peak **f** (RT=16.36 min), the second most abundant compound in extracts (about 25% of total), suggested a doubly unsaturated C17-hydrocarbon, based on a molecular ion at *m/z* 236 and a series of unsaturated hydrocarbon fragments. The DMDS adduct of the heptadecadiene showed a molecular ion at *m/z* 362 and diagnostic ions at *m/z* 231, 203, 183, 159, 155 (base peak), and 131, consistent with 6,9-heptadecadiene (see Raspotnig et al. 2005b). Compound **i** (RT=18.70 min; ca. 20% of the total) showed a terpene fragmentation pattern with a molecular ion at *m/z* 272 (4) and further *m/z* of 257 (3), 229 (4), 203 (5), 187 (10), 161 (17), 133 (31), 120 (15), 119 (17), 107 (20), 93 (50), 81 (37), 69 (100), 55 (16), 53 (17), and 41 (59). The compound was tentatively identified as β -springene on the basis of mass spectral comparison to β -springene spectra from literature and from the NIST library. The remaining peaks **a**, **b**, **d**, **e**, **g**, and **h**, all of them minor components, appeared to be a series of hydrocarbons, with compounds **b** (RT=11.69), **e** (RT=14.32 min), and **h** (RT=16.65 min) exhibiting the spectra of saturated *n*-alkanes with molecular ions at *m/z* 184, 212, and 240, respectively. The compounds were identified as tri-, penta-, and heptadecane by comparison of retention times to authentic

standards. Compounds a (RT=11.54), d (RT=14.10 min), and g (RT=16.44 min) had mass spectra of mono-unsaturated analogs, with molecular ions at m/z 182, 210, and 238, corresponding to tri-, penta-, and heptadecene. The position of the double bonds was not determined because, due to their low amounts, the compounds were not recovered after DMDS derivatization. The retention time of compound g was the same as that of 8-heptadecene (from extracts of *P. peltifer*, see “Methods and Materials”). Compound j (RT=19.27 min), also a minor component, had the following mass spectrum: m/z 264 (11), 235 (3), 221 (4), 207 (4), 165 (4), 151 (7), 137 (10), 123 (10), 109 (22), 95 (61), 81 (16), 79 (52), 67 (100), 55 (38), 54 (32), 44 (32), and 41 (69). The compound was tentatively identified as 9,17-octadecadienal on the basis of these mass spectral data.

The pattern of the ten compounds was consistently detected in 30 of the 35 individuals of adult *O. berlesei* (Fig. 1a), with relatively low inter-individual variation with respect to the relative abundance of components (data not shown). The other five extracts did not contain any of the components.

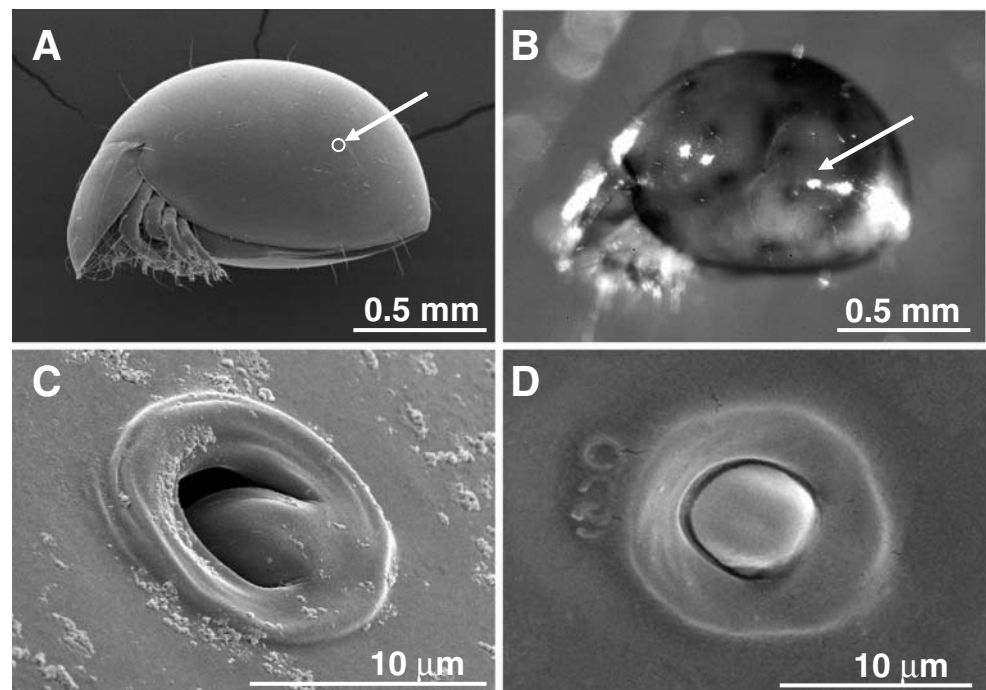
Extracts of Juvenile *O. berlesei* Extracts of juveniles (exclusively deut- and tritonymphs) contained only two compounds in a 1:2 ratio (peak c_1 and c in Fig. 1b): The major component c corresponded to tentatively identified (3*S*,8*S*)-chrysolmelidial from the adult extracts, based on identical retention time and mass spectrum. The minor component c_1 eluted earlier (RT=13.32 min) but was not fully separable from (3*S*,8*S*)-chrysolmelidial. The mass

spectrum of compound c_1 appeared to be similar to that of (3*S*,8*S*)-chrysolmelidial (Fig. 2); one difference, however, was the greater intensity of the ion at m/z 138 in relation to the ion at m/z 148 [c.f., in (3*S*,8*S*)-chrysolmelidial the ion at m/z 148 is more intense]. These data suggested the compound to be one of the other possible stereoisomers of chrysolmelidial, most likely an epimeric diastereomer of (3*S*,8*S*)-chrysolmelidial. Moreover, the retention time and mass spectrum of compound c_1 fully corresponded with those of the earlier-eluting peak of the synthetic chrysolmelidial mixture, limiting the possibilities to epi-chrysolmelidial (3*S*,8*R*-chrysolmelidial) and its enantiomer, (3*R*,8*S*)-dehydroiridodial. In addition, its retention time and mass spectrum matched those of plant-derived (3*R*,8*S*)-dehydroiridodial. Thus, again assuming that dehydroiridodials are exclusively plant-derived compounds, compound c_1 was tentatively identified as epi-chrysolmelidial (3*S*,8*R*-chrysolmelidial).

From a total of eight extracts of juvenile *O. berlesei* investigated (derived from 18 individuals), seven extracts consistently showed these two stereoisomers only; the other extract (an extract of a single individual) apparently did not contain any compounds at all.

Potential Secretory Glands The most conspicuous exocrine gland system of *O. berlesei* is the paired opisthonotal glands that, in light microscopic observations, are visible through the cuticle of the notogaster as kidney-shaped sacs of considerable size (maximal diameter about 0.5 mm: i.e., ca. 1/3 of body length; see Fig. 3b). The glands open to the notogastral surface via one single flap-provided pore orifice on either side of the body (Fig. 3a,c,d). The flap,

Fig. 3 Topography and external morphology of opisthonotal glands in *Oribotritia berlesei*. **a** Scanning electron micrograph (SEM) of an adult individual, lateral view. The arrow marks the orifice of the left opisthonotal gland. **b** Opisthonotal glands are visible through the integument under light microscopy. **c** SEM of an opisthonotal gland pore with flap open. **d** SEM of the pore of another individual, orifice closed



presumably an external closing mechanism, was found either in an opened (Fig. 3c) or a closed position (Fig. 3d) in different individuals. No other comparably large glands or pore orifices are present in this species. Opisthotal glands are present in adults and in all juvenile stages.

Discussion

Occurrence of Chrysomelidial Even though iridoid monoterpenes are widely distributed in exocrine secretions of a diverse range of arthropods, chrysomelidial, so far, has only been reported from leaf beetle larvae (Chrysomelidae) and from certain rove beetles (e.g., Pasteels et al. 1982; Weibel et al. 2001). Originally, chrysomelidial was found as a major constituent of the larval eversible glands of the chrysomelids, *Gastrophysa cyanea* (Blum et al. 1978) and *Plagioderma versicolora* (Meinwald et al. 1977), and its stereochemistry later elucidated by Meinwald and Jones (1978). According to these studies, and to current knowledge, chrysomelidial in leaf beetles is a mixture of two diastereomers, (3*S*,8*S*)-chrysomelidial (= the original chrysomelidial) and (3*S*,8*R*)-chrysomelidial (= epi-chrysomelidial), with the former being the predominant isomer in the majority of chrysomelidial-producing species (e.g., Sugawara et al. 1979; Pasteels et al. 1982). In contrast, the remaining two possible stereoisomers (there are four stereoisomers due to the two asymmetric carbon atoms at C₃ and C₈) are termed “dehydroiridodials” and are considered of plant origin only (Yoshihara et al. 1978; Sugawara et al. 1979). Under the conditions used in this study, we were able to separate the diastereomers but not the respective enantiomers of chrysomelidial and dehydroiridodial. Assignments of chrysomelidials and dehydroiridodials are often misinterpreted in the literature. For instance, Bellesia et al. (1986) described the chrysomelidials as a pair of enantiomers, whereas they are in fact diastereomers. In the present paper, we use the classification of Sugawara et al. (1979) for these compounds, but also use the more common nomenclature of Bellesia et al. (1986). Thus, assuming dehydroiridodials are found exclusively in plants, the compounds in *O. berlesei* extracts are (3*S*,8*S*)-chrysomelidial and (3*S*,8*R*)-chrysomelidial (epi-chrysomelidial).

Our analysis of *O. berlesei* is not only the first example of the chemistry of exocrine glands of Euphthiracarodea but also the only report of chrysomelidials found outside the Coleoptera. In *O. berlesei*, chrysomelidials most likely originate from the opisthotal glands: these glands are quite large in *O. berlesei*, and thus probably capable of producing compounds in the amount detected. Furthermore, some of the components, especially 6,9-heptadecadiene and some other hydrocarbons, found in the extract are characteristic constituents of opisthotal glands of many oribatid (and astigmatid) mite species (Kuwahara 2004; Rasputnig

2006). Functionally, at least in Chrysomelidae, iridoid monoterpenes such as chrysomelidials are considered defensive or for microbial protection; these functions are also generally attributed to opisthotal glands of Oribatida (Rasputnig et al. 2003).

Chemosystematic Impact With respect to chemosystematic studies that use opisthotal gland profiles of Oribatida, the finding of chrysomelidials in *O. berlesei* is significant. In preliminary investigations, chrysomelidials have been found also in other species of Euphthiracarodea (data unpublished), suggesting that this chemical class may be characteristic for species of Oribotritiidae, possibly representing an important chemical “synapomorphy” of this family. Also, two further compounds in extracts of *O. berlesei*, if our tentative identifications are correct, would be novel for oribatid and astigmatid mite opisthotal gland secretions. The diterpene, β -springene, which we tentatively identified as one of the three main components of the secretion, was originally found in secretions of springboks (Burger et al. 1978), and has also been detected in exocrine secretions of further vertebrates, especially mammals and reptiles (e.g., Waterhouse et al. 1996; Schulz et al. 2003), and invertebrates such as certain hymenopterans (e.g., Howard et al. 2003; Cruz-Lopez et al. 2005), but not in arachnids. The other novel compound (for oribatid and astigmatid mite opisthotal gland secretions) is the tentatively identified 9,17-octadecadienal, a minor constituent of the *O. berlesei* extract. Both tentatively identified compounds, β -springene and 9,17-octadecadienal, may be of chemosystematic value in Oribatida. Their unequivocal identification and possible distribution across other species of Euphthiracarodea remain to be studied.

Astigmatid Compounds Perhaps the most surprising result of our study is the lack of “astigmatid compounds” in the opisthotal gland extract of *O. berlesei*. These compounds are common secretion constituents in Mixonomata–Desmonomata and Astigmata, and are regarded as characteristic of all groups above middle-derived mixonomatans. In Collohmanniidae (proposed close relatives of euphthiracaroids), astigmatid compounds dominate opisthotal gland secretions. Astigmatid compounds, at least in some higher-derived desmonomatans and in brachypyelines, may be reduced and replaced by other (novel) compounds in adult secretions of certain groups as in, for example, *Hermannia convexa* (Rasputnig et al. 2005a) and *Schelorbates* ssp. (Takada et al. 2005). Thus, there is a chemical dimorphism between juveniles and adults, with juvenile secretions being distinguished by the plesiomorphic, astigmatid compounds-rich condition. This chemical dimorphism was tested in *O. berlesei*; however, neither adults nor juveniles contained any astigmatid compounds.

Even though the lack of astigmatid compounds in *O. berlesei* may be explained by complete evolutionary reduction, this situation may suggest a possible polyphyletic origin of astigmatid compounds in opisthontal glands in general. The lack of astigmatid compounds is even more surprising in light of the classification system proposed by Haumann (1991), in which oribotritiids are regarded as basal euphthiracaroids. Astigmatid compounds-free opisthontal gland secretions in adults and juveniles also occur in several groups of brachypylinines (Raspotnig, unpublished data), but these groups are considered highly derived. The lack of astigmatid compounds in Oribotritiidae could be reconciled by a systematic replacement of euphthiracaroids below the Collohmanniidae, corresponding to a possible split from the oribatid stem-line before astigmatid compounds evolved. However, this is not supported by morphological characters (Grandjean 1969). The lack of astigmatid compounds may not necessarily be true for all Oribotritiidae, and any extrapolation from data for *O. berlesei* remains speculative. Our major aim for future studies is the search for astigmatid compounds in other representatives of euphthiracaroid families.

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