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DIHYDROMATRICARIATE-BASED TRIGLYCERIDES, GLYCERIDE ETHERS, AND WAXES IN THE AUSTRALIAN SOLDIER BEETLE, *Chauliognathus lugubris* (COLEOPTERA: CANTHARIDAE)

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Abstract—The soldier beetle *Chauliognathus lugubris* is shown to contain triglycerides and glyceride ethers of 8-dihydromatricaria acid, and waxes of the C_{12} homolog of this acid, as well as the previously reported free acid. The triglycerides contain one, two, or three dihydromatricariate moieties, with any remaining positions esterified with normal fatty acids. The glyceride ethers were monostearyl ethers of glycerol esterified with dihydromatricaria acid and oleic or linoleic acid. The waxes, which also include a dihydromatricaria chromophore in the alcohol moiety, occur only in the females and are present in paired accessory glands in the abdomen. The ethers are restricted to females and appear to be associated with developing eggs. The triglycerides are much more abundant in females than males. Triglycerides, glyceride ethers, and waxes represent about 95% of the dihydromatricariate moiety (average, ca. 590 μg) in females with free acid the remainder; in males free acid is present to over 50% (ca. 22 μg) and the remainder is triglyceride (ca. 15 μg). Larvae contain mainly tridihydromatricariate-substituted triglyceride and a smaller quantity of the free acid.

Key Words—Dihydromatricaria acid, triglyceride, (Z)-dec-8-ene-4,6-diynoic acid, glyceride ethers, waxes, antifeedant, *Chauliognathus lugubris*, Coleoptera, Cantharidae, accessory glands, defense.

INTRODUCTION

The presence of (Z)-8-dihydromatricaria acid [(Z)-dec-8-ene-4,6-diynoic acid] in soldier beetles was first reported by Meinwald et al. (1968) for *Chauliog-*

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nathus lecontei. These authors showed that the acid was produced as a milky secretion in a series of defensive glands in the prothorax and the first eight abdominal segments. The same acid, along with precoccinelline and related alkaloids, was also found in the Australian species *C. lugubris* (F.) [=*C. pulchellus* (Macleay)] (Moore and Brown, 1978). Subsequently Eisner et al. (1981) reported dihydromatricaria acid in glands and hemolymph of *C. pennsylvanicus* and demonstrated its feeding deterrency to jumping spiders (*Phidippus* spp.). In the present paper we report the additional presence in *C. lugubris* of triglycerides and glyceride ethers of dihydromatricaria acid, and waxes of the C_{12} homolog of this acid with C_{12} and C_{14} alcohols also including the dihydromatricaria chromophore. The waxes and ethers occur in females only.

Nomenclature. The term "dihydromatricaria chromophore" is used to describe the chromophore associated with the conjugated ene-diyne system present in dihydromatricaria acid (Figure 1), which gives rise to the UV spectrum described by Eisner et al. (1981) [233 nm, (log ξ = 3.03), 240 (3.45), 254 (3.78) 267 (3.99) and 282 (3.90)]. The term "total dihydromatricariate" is used for all material bearing the above chromophore regardless of the chemical nature or chain length. It is normally expressed quantitatively as the corresponding amount of free dihydromatricaria acid.

METHODS AND MATERIALS

Gas Chromatography. Gas chromatography (GC) was conducted on a Varian 2100 instrument with a Varian CDS 111 data processor, and with flame ionization detectors and a nitrogen flow of 25 ml/min. The following glass columns were used: column 1, 2 m × 2 mm ID of 3% (w/w) OV-101 on Chromosorb W; column 2, 2 m × 3 mm ID of 5% (w/w) OV-101 on Gas-Chrom Q; column 3, 4 m × 3 mm ID of 5% (w/w) Carbowax 20 M on Gas-Chrom Z; column 4, 2 m × 3 mm ID of Porapak P; and column 5, 2 m × 3 mm ID of 1.5% (w/w) OV-17 on Gas-Chrom Q.

Samples for mass spectra or other purposes were collected from the gas chromatograph in capillary tubes cooled, when necessary, by carbon dioxide snow. Kovats' indices (I) were measured isothermally using saturated alkanes as standards. Percentage compositions, when quoted, are uncorrected for the response factors of the individual components.

Thin-Layer Chromatography. Thin-layer chromatography (TLC) was performed using two main systems: system 1, 200-mm glass plates coated with silica gel HR with zinc sulfide fluor and developed with dichloromethane; and system 2, 200-mm glass plates coated with aluminum oxide G with zinc sulfide fluor developed with hexane–ether 2:1. Visualization was by ultraviolet (UV) irradiation (254 nm) or iodine staining. When required, the components were

recovered from the plates by excision of the spot or band, extraction of the material with dichloromethane–methanol 9 : 1, filtration of the extract, and concentration to the desired volume in a stream of nitrogen.

High-Performance Liquid Chromatography. High-performance liquid chromatography (HPLC) was performed on a Waters Associates instrument with two M6000 pumps, a model 720 system controller, and a model 450 variable wavelength UV detector. The column was a Regis Workhorse octadecylsilyl 300 × 4.6 mm ID reversed phase. Gradient programmed elution (system A) was used with two solvents: water (A) and methanol–2-propanol 7 : 3 (B). The stages used were: initially 25% A, 75% B; at 8 min, 10% A, 90%B; at 23 min, 100% B, at 30 min, 100% B; at 31 min, 25% A, 75% B. All gradients were linear, the flow rate was 2 ml/min, and the wavelength was set to 267 nm. Alternatively, an isocratic system (system B), with methanol–dichloromethane 85 : 15, a flow rate of 2 ml/min, and simultaneous UV and refractive index (RI) detection, was used. Samples for mass spectroscopy and other purposes were obtained by collection and evaporation of the eluant.

The quantities quoted in Tables 1 and 2 (except for the free acid) are the HPLC peak height at 267 nm for a 50-μl injection at sensitivity ×1.0. The solvent gradient was such that, within experimental error and except for the free acid, the peak widths at half height were constant. For the free acid, the width was only one third that of the other components and its quantity was calculated accordingly.

Mass Spectrometry. Mass spectra were determined on a VG Micromass 70-70 mass spectrometer interfaced to a Hewlett Packard 5790A gas chromatograph and a VG 11-250 data system. Volatile samples were introduced via GC with a 25-m bonded-phase 5% phenyl-, methyl-silicone capillary column (SGE, BP5, 0.5 μm film thickness). Components of low volatility, after separation by TLC or HPLC, were analyzed by flash volatilization from an extended gold support (Brown et al., 1985), or a polyimide probe tip, with rapid data acquisition. This method markedly increased the relative abundance of molecular parent ions compared with conventional probe techniques, simplified the fragmentation patterns, and facilitated the analysis of unresolved mixtures. Electron ionization (EI) mass spectra were determined with an ionization energy of 70 eV and trap current of 100 μA. Chemical ionization (positive ion, PCI, and negative ion, NCI) mass spectra were obtained at 50 eV ionization energy and 200 μA emission current. Methane, isobutane, and ammonia were used as reagent gases at estimated source pressures of 100, 80, and 60 Pa, respectively. Source temperatures were 180–210°C.

Ultraviolet Spectroscopy. Ultraviolet spectroscopy was performed on a Pye Unicam SP 8000 recording spectrophotometer. Dichloromethane and methanol were the solvents in 1-mm or 10-mm quartz cells.

Insects. Batches of *C. lugubris* beetles were obtained from beneath flow-

ering *Eucalyptus* spp. trees according to season in the Canberra area. Larvae were obtained from soil and litter samples also collected in the Canberra area.

Treatment of C. lugubris. The secretion from the gland was obtained as described previously (Moore and Brown, 1978).

Total extraction, *C. lugubris* beetles, or parts thereof, were crushed in dichloromethane and left in this solvent at 5°C for 24 hr. The dichloromethane extract was separated by filtration, the residue reextracted with dichloromethane and filtered again, and the filtrates combined. The dichloromethane solution was diluted to 25 ml for UV spectroscopy or concentrated for TLC analysis. Estimates of total dihydromatricariate were made by measurements of the UV absorbance of the untreated dichloromethane extracts using the extinction coefficient given by Eisner et al. (1981). The relative abundances of the different components were determined by a TLC separation followed by UV absorbance measurements. Samples for HPLC were purified by chromatography on a silica Sep-Pak eluting with dichloromethane–methanol 9:1. The solution was then evaporated, the residue taken up in methanol–2-propanol 7:3 (500 μl), and the resulting solution filtered through a nylon membrane (pore size 0.45 μm).

Transesterification. Methanolysis reactions were carried out by heating in 1% sulfuric acid in methanol (50 μl) in a Reacti-vial at 100°C for 15 min. The product was used directly for GC or TLC analysis. Acetic acid transesterifications were carried out similarly using 1% sulfuric acid in acetic acid at 90°C for 2 hr.

Hydrolysis of Glyceride Ethers. A sample of the glyceride ether was treated with methanolic sodium hydroxide (1%, 50 μl) at 70°C for 30 min in a sealed Reacti-vial. The resulting solution was extracted with hexane and the hexane extract treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (25 μl) at 70°C for 30 min. The hexane solution was analyzed directly by GC.

Hydrogenation. Hydrogenations were carried out in septum-sealed Reacti-vials (with a vent) with a trace of Adams platinic oxide catalyst and methanol (20–30 μl) as solvent. The hydrogen was introduced by means of a syringe and bubbled through the mixture to provde agitation.

Ozonolysis. Microzonolyses were conducted in carbon disulfide solution in a Reacti-vial cooled by carbon dioxide snow. Ozonized oxygen was bubbled through using a 10-ml syringe, which previously had been charged from the ozonizer, till a starch iodide paper turned blue. The ozonide was then decomposed with a trace of triphenylphosphine and the resulting solution analyzed directly by GC (column 3 at 70°C and column 4 at 120°C).

Reference Compounds. (*Z*)-8-Dihydromatricaria acid and its methyl ester used as reference compounds were from samples previously characterized (Moore and Brown, 1978) from *C. lugubris* glands. Tripalmitin was obtained from Calbiochem (La Jolla, California) and batyl alcohol from Sigma Chemical Company (St. Louis, Missouri).

RESULTS

Chromatographic Separations. Previous work on various species of *Chauliognathus* involved either analysis of secretion obtained directly from the defensive glands or chemical extractions, specifically aimed at isolating only free acids. However, TLC analysis (on silica with dichloromethane–methanol 9:1) of a dichloromethane extract of whole *C. lugubris* beetles showed, in addition to the free acid, a great deal of UV-absorbing material of high R_f. Subsequently TLC (system 1) showed the free dihydromatricaria acid at the origin, a major UV absorbing spot (fraction A) at R_f 0.70, and a partially resolved series of components from R_f 0.60 to 0.30. Exposure to iodine vapor gave rise to strong staining at R_f 0.55 probably due to the normal fats (tripalmitin had R_f of 0.55). Methyl dihydromatricariate had R_f of 0.64 but was not present in the extract. The UV spectrum of fraction A (in methanol) and that of the partially resolved band showed the characteristic dihydromatricaria chromophore (cf. Eisner et al., 1981). Absorbance measurements showed that the free acid represented about 5% of total dihydromatricariate, fraction A about 45%, and the other band about 50%.

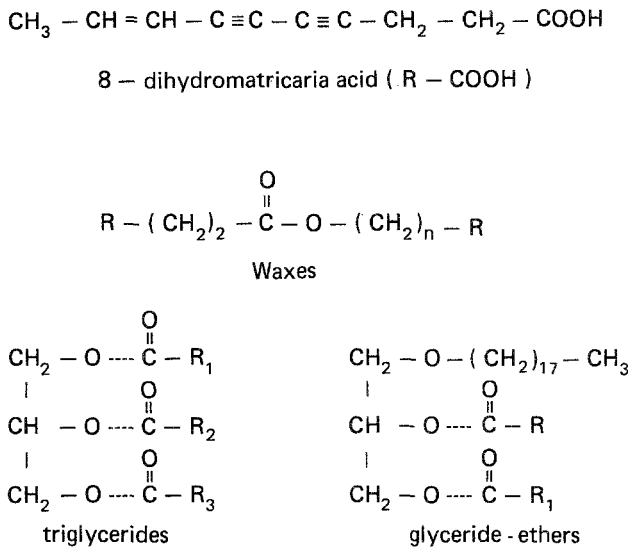

FIG. 1. Formulas for *C. lugubris* components. A dotted line indicates that the position of the substituent may be undetermined. Waxes: A1, $n = 3$; *A2*, $n = 5$. Glyceride ethers: B1, R_1 = linoleyl, B2, R_1 = oleyl. Triglycerides: C, $R_1 = R$, $R_2 = R_3$ = palmityl, stearyl, oleyl or linoleyl; D1, $R_1 = R_2 = R$, R_3 = linoleyl; D2, $R_1 = R_2 = R$, R_3 = palmityl or oleyl; D3, $R_1 = R_2 = R$, R_3 = stearyl; E, $R_1 = R_2 = R_3 = R$.

TLC with system 2 gave better resolution and produced four clearly resolved spots. These had R_f values of 0.74 (ca. 60% of the total absorbance), 0.6 (7%), 0.43 (15%), and 0.23 (15%), and there was a little free acid at the origin. All of these fractions showed the dihydromatricaria chromophore. The strong iodine-staining area coincided with the spot at R_f 0.74. Subsequent TLC of the components from these spots on system 1 showed that the R_f 0.74 spot comprised fraction A and another fraction (B) with R_f 0.53; the 0.6 material had R_f 0.47 (fraction C); the 0.43 material had R_f 0.43 (fraction D); and the 0.23 material had R_f 0.35 (fraction E).

Reversed-phase HPLC of the *C. lugubris* mixture achieved greater resolution of the components and the observed peaks have been correlated with HPLC analyses of the separate TLC fractions A–E (Table 1). Normal-phase HPLC added little to the information derived from silica TLC and was not continued.

Identification of Fraction A. GC analysis of TLC fraction A (column 1, 250°C) showed four major peaks with retention times 7.95 min (15%, I = 2903), 9.01 min (5%, I = 2951), 13.50 min (51%, I = 3106), and 15.26 min (28%, I = 3155). These indices suggested that the components were two pairs of homologs with a chain-length difference of two carbon atoms. Separate GC analyses of HPLC peaks A1 and A2 showed that the first two GC components

TABLE 1. HPLC OF *C. lugubris* DICHLOROMETHANE EXTRACT

R_t (min)	Peak height, cm (%)[a]	Fraction	Mol wt[b]
1.4	3.0 (3.8)	free acid	162
6.4	12.3 (15.7)	E	524
7.7	9.0 (11.5)	A1	348
9.4	22.5 (28.7)	A2	376
10.3	1.2 (1.5)	A3	
11.0	0.8 (1.0)	A4	
13.05	2.1 (2.7)	D1	642
13.95	14.6 (18.6)	D2	618 + 644
15.4	1.0 (1.3)	D3	646
17.2	0.5 (0.6)	C	
20.0	0.2 (0.3)	C	
21.4	0.4 (0.5)	C	
22.45	1.5 (1.9)	C	
23.25	0.3 (0.4)	C	
24.1	0.2 (0.3)	C	
24.6	1.7 (2.2)	B1	750
25.6	7.2 (9.2)	B2	752

[a] The peak height (cm) represents the total dihydromatricariate content of the component.
[b] From mass spectroscopic measurements.

were derived from A1 and the second pair from A2. The mass spectra of the material from peaks A1 and A2 (PCI, methane) showed the presence of components with molecular weights of 348 (MH^+ m/z 349) and 376 (MH^+ m/z 377), respectively.

Fraction A1 was methanolyzed and the product analyzed by TLC (system 1). Two UV-absorbing spots were resolved at R_f 0.59 [cf. methyl (Z)-8-dihydromatricariate at 0.60] and at R_f 0.23. Both of these components showed the characteristic dihydromatricaria UV spectrum. GC analysis (column 2, 150°C) of the R_f 0.59 band from methanolysis of A1 showed two peaks; the major one had R_t of 7.16 min (90%, I = 1698) and the minor had R_t 8.68 min (10%, I = 1747). After catalytic reduction, these two peaks collapsed to one, which was identified by GC R_ts (columns 2 and 3) as being due to methyl dodecanoate. GC-MS (EI) of the unreduced methyl ester fraction showed that both components had molecular weights of 204 and similar fragmentation patterns. The mass spectra were strongly reminiscent of that of methyl (Z)-8-dihydromatricariate and thus these components are almost certainly the Z and E isomers of the C_{12} homologue of methyl 8-dihydromatricariate. Ozonolysis of the major methyl ester component gave only acetaldehyde as a detectable product. The major component would be thus methyl (Z)-dodec-10-ene-6,8-diynoate and the minor component probably the E isomer. The stereochemistry was assigned by analogy with the C_{10} homologs, where Meinwald et al. (1968) have shown the Z isomer to predominate.

Material from the R_f 0.23 fraction from methanolysis of A1 was analyzed by GC on column 1 at 150°C and two components were detected; these had retention times of 5.87 min (76%, I = 1647), and 7.15 min (24%, I = 1698). After catalytic reduction only one component was detected and this was identified by GC R_ts (columns 2 and 3, 150°C) as dodecanol. GC-MS of the unreduced material showed molecular weight of 176 for the first eluting material. These components were therefore, presumably, the Z and E isomers of a C_{12} alcohol containing the dihydromatricaria chromophore. Ozonolysis led to the detection of only acetaldehyde showing the components of this methanolysis fraction to be (Z)- and (E)-dodec-10-ene-6,8-diynol. There was a greater proportion of the E isomer than is the case with the methyl esters. A1 therefore consisted of waxes derived from the above acids and alcohols.

The main component of fraction A1 was (Z)-dodec-10-ene-6,8-diynyl (Z)-dodec-10-ene-6,8-diynoate. E isomers (particularly in the alcohol moiety) were represented in less abundant waxes of A1. Similarly A2 was shown to contain waxes of the homologous (Z)- and (E)-tetradec-12-ene-8,10-diynol and (Z)- and (E)-dodec-10-ene-6,8-diynoic acid. The minor wax fractions, A3 and A4, appeared to be mixtures and were not studied further.

Identification of Fraction B. Fraction B was obtained by successive TLC on systems 1 and 2. However, the strong iodine staining, concurrent with the

UV absorption, indicated that the dihydromatricariate-containing components were cochromatographing with the ordinary fats. HPLC (system B) of fraction B showed two UV-absorbing peaks with retention times of 5.66 min (B1) and 6.52 min (B2). In addition to these components, the RI detector showed several other components, presumably fats, at longer retention times.

The mass spectrum of B2 (PCI, NH_3) showed an $(M + NH_4)^+$ ion at *m/z* 770, consistent with a molecular weight of 752, and major fragments at *m/z* 591 (M-161, loss of dihydromatricariate), and *m/z* 471 (M-281, loss of oleate). The negative ion spectrum (NCI, isobutane) showed a small ion at *m/z* 751 $[(M - H)^-]$ and major ions at *m/z* 281 (oleate) and *m/z* 161 (dihydromatricariate). These spectra were consistent with a monostearyl ether of glycerol esterified with dihydromatricaria acid and oleic acid.

The mass spectrum (PCI, NH_3) of B1 was rather similar and showed an ion at *m/z* 768, indicating a molecular weight of 750, and major ions at *m/z* 589 (M-161, loss of dihydromatricariate) and *m/z* 471 (M-279, loss of linoleate). Thus B1 was very similar to B2 except that the oleate moiety had been replaced by linoleate. This slightly more polar component would be expected to elute before B2 on reversed-phase HPLC.

Methanolysis of B2 followed by GC analysis (column 3 at 200°C) showed that the major methyl esters produced were in fact methyl oleate and methyl dihydromatricariate. The small quantities of other esters produced probably arose because the sample was not completely clear of the normal fats. There was some evidence of the latter in the form of small peaks at higher mass in the mass spectrum and in the fact that the ratio of dihydromatricariate to fatty ester was lower than expected. Methanolysis of B1 was not very helpful because of even greater interference from these fats. However, the abundance of methyl linoleate was much higher than in any of the other methanolysis reactions.

Transesterification of B2 with acetic acid–sulfuric acid followed by GC (column 1, 180°C programmed at 4°C/min) gave two major components with R_t 7.48 min and 18.64 min, respectively. The mass spectrum of the short R_t material (PCI, NH_3) showed a probable molecular weight of 368, consistent with replacement of one of the ester groups by acetate and elimination of the other. The mass spectrum of the long R_t material (PCI, NH_3) showed a probable molecular weight of 428 and was consistent with replacement of both the original ester moieties in the molecule by acetate.

Hydrolysis followed by silylation of B1 and B2 gave a product with an R_t (columns 1 and 5) identical with that of the trimethylsilyl ether of batyl alcohol (racemic 3-octadecyloxy-1,2-propanediol). This supported the presence of a stearyl ether moiety, probably in a primary position, in B1 and B2.

Identification of Fraction C. The mass spectrum of TLC fraction C (PCI, NH_3) showed prominent $(M + NH_4)^+$ ions at *m/z* 782 (mol wt 764) and 756 (mol wt 738) consistent with triglycerides with one dihydromatricariate and two

oleate moieties; and one dihydromatricariate, one oleate, and one palmitate, respectively. There were additional less intense ions representing glycerides with other possible combinations of fatty acids.

Methanolysis of fraction C followed by GC analysis (column 3 at 200°C) led to the detection of the following methyl esters: methyl palmitate (17%), methyl (*Z*)-8-dihydromatricariate (16%), methyl (*E*)-8-dihydromatricariate (5%), methyl stearate (7%), methyl oleate (51%), and methyl linoleate (3%). The ratio of dihydromatricariate to the normal fatty esters (allowing for chain length) was consistent with a 1 : 2 ratio of dihydromatricariate to fatty esters.

HPLC showed that fraction C was composed of a number of different components most of which were minor. Because of the small amounts of the components, individual mass spectra were not recorded. This greater nonhomogeneity was consistent with random distribution of fatty acid moieties in two positions of glycerol.

Identification of Fraction D. The mass spectrum (PCI, NH_3) of D2, the major HPLC peak of fraction D, indicated that at least two compounds were present. The major one had a molecular weight of 644 [$(M + NH_4)^+$ *m/z* 662] and fragments at *m/z* 483, 363, and 103; the minor component had a molecular weight of 618 [$(M + NH_4)^+$ *m/z* 636] and had fragments at *m/z* 457, 363, and 103. These mass spectra indicated that the major component was a triglyceride with two dihydromatricariate moieties and one oleate group (ions of *m/z* 483 represented M-dihydromatricariate and *m/z* 363 M-oleate). The minor component would be similar but with a palmitate group instead of the oleate.

The minor components of the fraction D (D1 and D3) showed molecular weights of 642 and 646, respectively, and were therefore considered to be triglycerides with linoleate and stearate in addition to the two dihydromatricariate groups.

Methanolysis of fraction D followed by GC analysis (columns 2 and 3) led to the detection of methyl (*Z*)-8-dihydromatricariate (45%), the *E* isomer (4%), methyl palmitate (10%), methyl oleate (34%), methyl stearate (5%), and methyl linoleate (1.5%). These data supported the mass spectral findings since the peak area ratio of the normal fatty esters to dihydromatricariate (ca. 1 : 1), after allowing for differences in molar responses to the detector because of chain length, was close to that expected for a triglyceride with two dihydromatricariate moieties and one fatty ester moiety.

Identification of Fraction E. The mass spectrum (PCI, NH_3) of fraction E showed a molecular weight of 524 [$(M + NH_4)^+$ *m/z* 542] and also major fragment ions at *m/z* 363 (M-161, loss of dihydromatricariate) and *m/z* 103. All these data indicated that fraction E was a triglyceride with dihydromatricariate in all three positions. This was confirmed by methanolysis of fraction E followed by GC analysis (column 2 at 150°C programmed at 4°C/min to 250°C and column 3 at 150°C) when only methyl (*Z*)-dihydromatricariate and about

10% of the *E* isomer were detected. Fraction E was therefore largely tri-(*Z*)-8-dihydromatricarin.

Biological Distribution of Components. To appreciate better the role of the various dihydromatricaria-containing components, an attempt was made to locate precisely their occurrence in the beetles. Earlier work had shown that the defensive glands contained little but the free acid. Males (3, average weight 40 mg) and females (3, average weight 60 mg) were assayed individually by UV spectroscopy. Males contained an average of 37 μg of total dihydromatricariate but females averaged much higher at 590 μg. TLC separation (system 1) followed by UV estimation of the resulting fractions showed that the males contained 22 μg of free acid and the remaining material was in the band representing fraction B–E. Females, however, contained only 24 μg of free acid; ca. 44% (260 μg) of the total dihydromatricariate was fraction A (waxes) and the remainder (306 μg) was in the band due to fractions B–E.

The above findings were subsequently supported and expanded by HPLC analyses of individual males and females. These results are summarized in Table 2. Males again were shown to contain much less dihydromatricariate material than females, and most of theirs was free acid; the remainder was triglyceride. Males did not appear to contain any waxes or ethers, and the small quantities of these components detected in some specimens could well have been acquired adventitiously through contact with females. It was again noticeable that the free acid content of both sexes was similar, but females had very much more

TABLE 2. HPLC ANALYSIS OF INDIVIDUAL BEETLES

			Fraction					
Beetle	Wt (mg)	Acid	A	B	C	D	E	Total
Females								
1	48.7	8.3	46.3	15.7	12.1	31.1	25.2	139
2	47.5	8.7	12.5		6.8	1.3	4.7	34
3	46.9	3.3	32.4	1.2	12.1	3.5	5.4	58
4	65.4	6.0	27.6	9.8	4.9	22.6	14.0	85
5	55.5	2.8	20.6	5.4	2.7	13.6	9.2	54
Males								
1	43.0	2.6			1.4	0.1	0.8	4.9
2	43.4	3.4	0.3	0.1	1.4		0.3	5.6
3	35.4	5.0	0.3		0.9	1.4		7.6
4	36.4	1.9		0.05	0.6	0.1	0.04	2.7
5	38.7	4.8	0.1		0.9		0.3	6.2

[a]The values quoted are the peak heights (cm) at 267 nm and $\times 1.0$ sensitivity for a 50-μl injection from 500 μl of solution.

of the bound dihydromatricariate. There was considerable variation in the proportions of the various components between individuals. This may have reflected the level of maturity as well as individual variation.

Since individual males were very low in dihydromatricariate, an extract of 40 males was analyzed by TLC (system 1). The bands corresponding to the free acid and triglyceride–glyceride ether were removed (no waxes were present) and, after extraction, the UV spectra of the resulting solutions were recorded. These showed that free acid represented ca. 55% of the total dihydromatricariate. Subsequently HPLC analysis of the triglyceride–glyceride ether band extract showed that it contained mainly triglycerides with a single dihydromatricariate moiety (C, 64%) with smaller quantities of disubstituted (D, 8.5%) and trisubstituted (E, 27%) triglycerides. No glyceride ethers were detected. These data were consistent with those for individual males (Table 2) and confirmed the absence of waxes and glyceride ethers in males.

The following parts of beetles were then assayed separately for dihydromatricariate: (1) Elytra, from males or females contained very little dihydromatricariate material. (2) The area from around the defensive gland of several combined males and females contained mainly free acid but with small quantities of the other components. (3) Eggs, removed by dissection, contained triglycerides (mainly with two or three dihydromatricariate moieties) and ethers (37%). No waxes or free acid were detected. (4) Viscera from males contained mainly triglycerides with a smaller quantity of the free acid.

Since the wax was present only in the females and was absent from the eggs, it seemed likely that it would occur in abdominal accessory glands and be used for coating the eggs immediately prior to oviposition. This was confirmed upon dissection when large paired colleteral glands were discovered. These glands, which were replete with a white wax like substance, filled much of the sixth and seventh abdominal segments and opened, via individual ducts, into the vagina. GC analysis of their contents revealed only the components of the wax fraction A, of the total extracts.

The very high level of glyceride ethers in eggs suggested that, in the extracts studied, these components were largely derived from eggs and hence would be absent from males. Also it might be expected that nongravid females would be low in ethers. To confirm this supposition, an extract of a number of females of low gravidity was examined. This sample showed a high level of wax (60%) but only about 6% of glyceride ethers (16%) after allowance for waxes and free acid; most of the remainder was triglyceride. This value for ethers was lower than for the bulk sample (21%), despite the fact that the latter contained males as well as females. These data indicated that the glyceride ethers were associated with eggs or possibly the ovarian system as a whole. Thus the level of glyceride ethers in individual females reflects the state of ovarian development.

Several batches of larvae of *Chauliognathus* spp., after extraction with dichloromethane, showed the characteristic dihydromatricariate UV spectrum. TLC and HPLC of this solution showed that the major UV-absorbing material was fraction E (trisubstituted triglyceride); there was a small quantity of free acid present, but none of the other components was detected.

Preliminary investigation of a female of *Chauliognathus nobilitatus* (Erichson) by HPLC showed a very similar distribution of dihydromatricaria-containing compounds. However, in this species, there was very little, if any, free acid, and the wax fraction (A) showed greater diversity in chain length.

DISCUSSION

The detection, in *C. lugubris*, of glycerides and waxes based on the dihydromatricariate moiety contributes to our knowledge of the defensive chemistry of these beetles. The results also demonstrate a significant difference between the sexes, which was not apparent in the case of the defensive glands. In males the free acid accounts for much of the total dihydromatricariate content, but in the females it constitutes only a very small proportion of the total.

The dihydromatricariate-containing triglycerides are probably associated with the fat body and would provide a further dose of antifeedant material to increase the discomfort of any predator naive enough to consume one of these beetles; they may also act as a reserve of the acetylenic moiety from which depleted defensive glands are replenished. The greater abundance of dihydromatricariate-substituted material in females than in males presumably reflects the larger bulk of fat body and the presence of accessory glands and developing eggs in the former sex.

An interesting discovery reported in this paper concerns the wax-containing accessory glands present only in females. The waxes have the dihydromatricaria chromophore incorporated into both their alcoholic and acidic components; they are present in very substantial quantities (ca. 300 μg/female or about 0.5% of body weight). The location of the glands and the nature of their secretion suggest that they dispense an egg protectant. The waxes contained in these glands would be deposited on the eggs as they are laid providing, possibly, both a bacteriostatic and antifeedant protective coating. It is noteworthy that the chain length of the acidic component of the wax is increased to C_{12} and that the alcoholic moiety to mainly C_{14} but with a smaller proportion of C_{12} material. This increase in chain length presumably enhances the properties of the wax in its role as a protective surface lipid.

We have no knowledge of the specific role of the glyceride ethers in eggs but they are presumably part of the protective mechanism. As far as we are aware, this is the first report of glyceride ethers from insects, although they

have been reported previously from marine sources, especially shark liver oil, and from mammals and microorganisms (see Downing, 1976).

REFERENCES

BROWN, W.V., JONES, A.J., LACEY, M.J., and MOORE, B.P. 1985. The chemistry of buprestins A and B. Bitter principles of jewel beetles (Coleoptera: Buprestidae). *Aust. J. Chem.* 38:197–206.

DOWNING, D.T. 1976. Mammalian waxes, pp. 18–48, *in* P.E. Kolattukudy (ed.). Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam.

EISNER, T., HILL, D., GOETZ, M., JAIN, S., ALSOP, D., CAMAZINE, S., and MEINWALD, J. 1981. Antifeedant action of (*Z*)-dihydromatricaria acid from soldier beetles (*Chauliognathus* spp.). *J. Chem. Ecol.* 7:1149–1158.

MEINWALD, J., MEINWALD, Y.C., CHALMERS, A.M., and EISNER, T. 1968. Dihydromatricaria acid: Acetylenic acid secreted by soldier beetle. *Science* 160:890–892.

MOORE, B.P., and BROWN, W.V. 1978. Precoccinelline and related alkaloids in the Australian soldier beetle, *Chauliognathus pulchellus* (Coleoptera: Cantharidae). *Insect Biochem.* 8:393–395.