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The Pheromone System of the Male Danaine Butterfly, *Idea leuconoe*

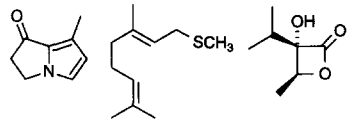
S. Schulz*[a] and R. Nishida[b]

[a]*Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany*
[b]*Pesticide Research Institute, Kyoto University, Kyoto, 606–01, Japan*

Abstract—Male *Idea leuconoe* butterflies release a complex mixture of volatiles from their pheromone glands (hairpencils) during courtship. The pheromone components geranyl methyl thioether (**2**), viridifloric β-lactone (**3**) and 6-hydroxy-4-dodecanolide (**10**) have been synthesized for the first time. Therefore, the structural assignment of these new natural products could be proved. Related 7-hydroxy-5-alkanolides are also present in the extract. The volatiles are embedded in a lipidic matrix with more than 150 components. This matrix consists of alkanes, alkenes, 2,5-dialkyltetrahydrofurans, secondary alkanols and alkenols as well as alkanones and alkenones. Several regioisomers of the oxidized hydrocarbons occur. The elucidation of double bond positions has been performed by MS using DMDS adducts. Copyright © 1996 Elsevier Science Ltd

Introduction

Male danaine butterflies possess striking eversible pheromone glands, so-called hairpencils, which are used during courtship.[1] About 30 years ago the first pheromone component of these butterflies, danaidone (**1**), could be identified[2] and its function as courtship pheromone proved.[3] Since then, many danaine species have been investigated and the chemical composition of their male pheromone glands elucidated.[1,4,5] Despite the fact that some of the scent bouquets consist of up to 60 components, no pheromonal function of any other component than **1** has been established.



1 **2** **3**

Figure 1. Pheromone components of *I. leuconoe*.

Recently we were able to show that male *I. leuconoe* butterflies emit a complex mixture of chemicals from their hairpencils. At least three of these components, danaidone (**1**), geranyl methyl thioether (**2**) and (*S,S*)-viridifloric-β-lactone (**3**), act as courtship pheromones (Fig. 1).[6] An artificial mixture of hairpencil compounds containing **1**, **2** and **3** as well as phenol, *p*-cresol, benzoic acid, a series of homologue 6-hydroxy-4-alkanolides ranging from C_{10} to C_{13}, (*E,E*)-farnesol, and (*Z*)-9-tricosene elicited the same courtship behavior as a crude hairpencil extract.[6] In addition, (−)-(*R*)-mellein (8-hydroxy-3-methyl-3,4-dihydroisocoumarin) and another β-lactone related to **3**, 2-ethyl-2-hydroxy-3-butanolide, could be identified in the hairpencil extracts. Besides their function as courtship pheromones, other types of interactions, such as a defensive warning odor or intermale recognition (for a discussion see reference 6), seem also to be associated with these chemicals. The volatile compounds identified are embedded into a complex lipidic matrix (see Fig. 2). In this paper we will report on the synthesis of some hairpencils constituents, the identification of additional components and the composition of the lipid matrix.

Key words: pheromones, Danainae, lipids, *Idea*, hairpencil.
Dedicated to Professor H. J. Bestmann on the occasion of his 70th birthday.

Results and Discussion

The structure of the previously unknown geranyl methyl thioether (**2**) was deduced from its mass spectrum (see Fig. 3). The presence of sulfur was detected by high-resolution mass spectroscopy, giving a molecular formula of $C_{11}H_{20}S$ ($M^+_{obs} = 184.1289$, $M^+_{calc} = 184.1286$). Ions at $m/z = 47$ (CH_3S^+) and 61 ($CH_3SCH_2^+$) and the loss of 48 amu (CH_3SH) from M^+ indicated a thiomethyl group in the molecule. Typical terpenic ions ($m/z = 41$, 69, 81, 93, 123 and 136) suggested that this molecule could be geranyl methyl thioether (**2**). For an unambiguous proof, **2** was synthesized by reaction of geranyl chloride with sodium thiomethanolate in boiling ethanol. The product showed identical mass spectrum and GC retention times on different stationary phases as the natural compound which is therefore (*E*)-3,7-dimethyl-2,6-octadienyl methyl thioether (**2**). The corresponding

(*Z*) isomer exhibited a similar mass spectrum, but a shorter retention time on an apolar phase.

Another pheromone component not reported from nature before is 2-hydroxy-2-(1-methylethyl)-3-butanolide (viridifloric β-lactone, **3**). Its identification based on MS, IR and NMR data has been described.[6] A racemic mixture of both diastereomers of **3** was synthesized for structural assignment according to Fig. 4. Starting from the dilithio salt of 3-methylbutyric acid, an alkylation with acetaldehyde yielded 3-hydroxy-2-(1-methylethyl)-butyric acid (**4**). The following dehydration with P_2O_5 gave a 3:2 (*E*/*Z*) mixture of 2-(1-methylethyl)-2-butenoic acids (**5**), which was converted into a 3:2 mixture of racemic trachelanthic [(2*R**,3*S**)-2,3-dihydroxy-2-(1-methylethyl)-butyric acid, **6a**] and viridifloric acids [(2*R**,3*R**)-2,3-dihydroxy-2-(1-methylethyl)-butyric acid, **6b**], using an osmium catalyzed dihydroxylation. Lactonization using mesyl chloride[7] furnished the desired β-lactones. The minor component, viridifloric β-lactone (**3**), exhibited identical mass spectra and retention times as the natural compound. The absolute configuration of this lactone could be established to be (2*S*,3*S*)-2-hydroxy-2-(1-methylethyl)-3-butanolide by comparison with an authentic sample.[8]

A series of homologue C_{10} to C_{13} γ-lactones were also identified in the hairpencils. Based on MS and NMR data, their structures were assigned to be 6-hydroxy-4-alkanolides,[6] a type of compound which to our knowledge has not been reported from nature before. The representative 6-hydroxy-4-dodecanolide (**10**) has been synthesized for unambiguous proof of the structure in the following manner (see Fig. 5): Methyl 4-oxobutyrate (**7**)[9] was allylated with allylzinc chloride according to reference 10. Ozonolysis of the resulting 6-hepten-4-olide (**8**)[10] led to the unstable 6-oxo-4-hexanolide (**9**), which was directly alkylated using hexylmagnesium bromide at 0 °C. Thus, a 2:1 mixture of the two diastereomers of **10** was obtained, which proved to be unseparable, even on an apolar gas chromatographic phase. The major compound showed identical NMR and MS data to the natural product. Further investigation by GC using chiral phases revealed its absolute configuration to be (4*S*,6*S*).[8]

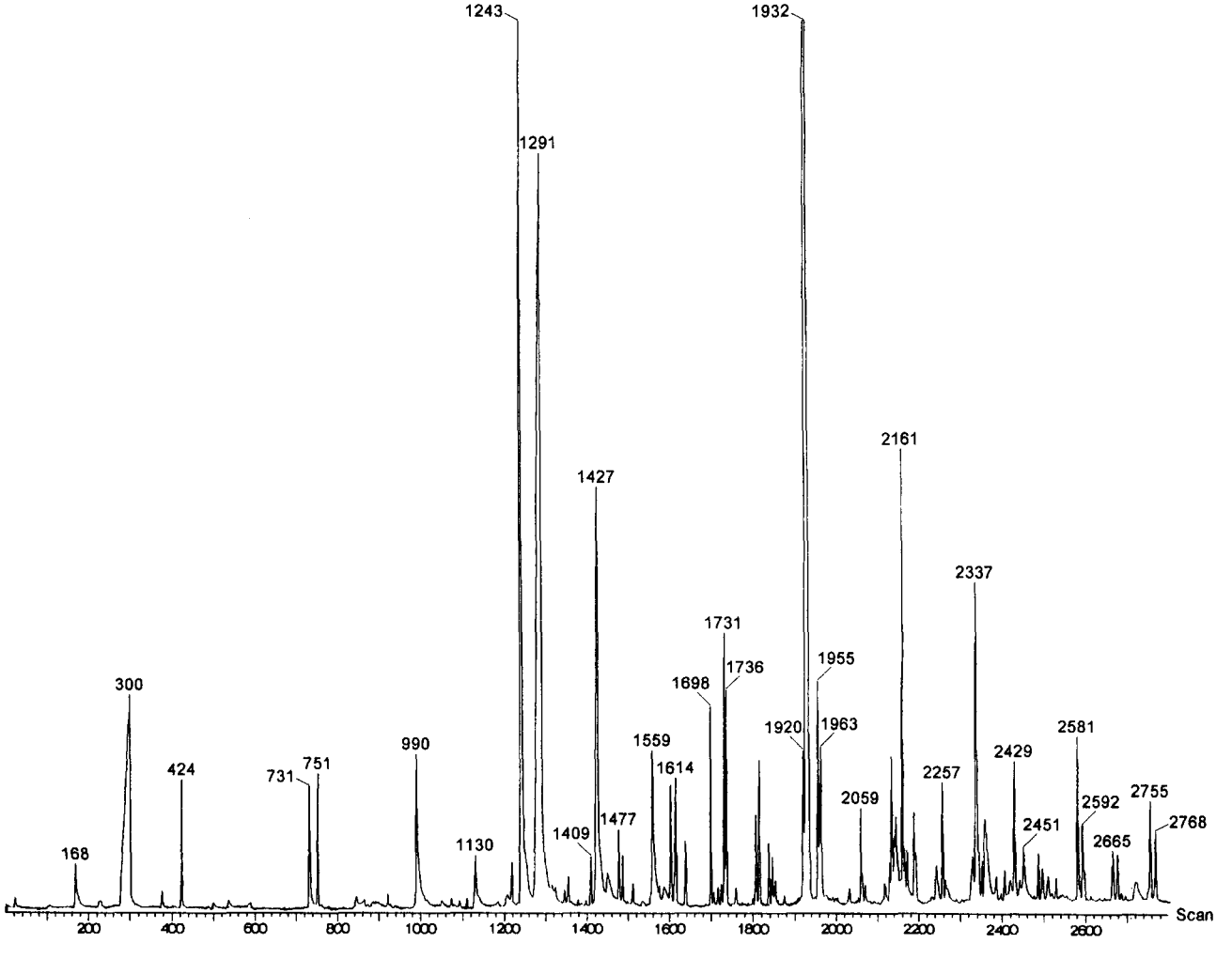


Figure 2. Total ion chromatogram of a hairpencil extract from *I. leuconoe*. The scan numbers are used for identification of compounds in Tables 1 and 2.

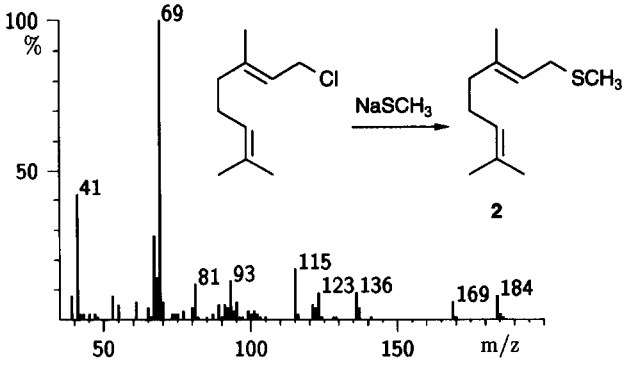


Figure 3. Mass spectrum and synthesis of (*E*)-3,7-dimethyl-2,6-octadienyl methyl thioether (geranyl methyl thioether, **2**).

The mass spectrum of **10** show prominent ions at $m/z = 85$, 100, 111 and 129 [see Fig. 6(A)], all of which contained the intact β-lactone ring. Shortly after these γ-lactones some small peaks occurred in the gas chromatogram of the natural extract exhibiting mass spectra with prominent ions at $m/z = 99$, 114, 125 and 143 [see Fig. 6(B)]. The shift of 14 amu could be explained by a δ-lactone structure instead of the γ-lactone found in **10**. Therefore, these compounds were identified as a series of previously unknown 7-hydroxy-5-alkanolides, with chain length ranging from C_{11} to C_{13}.

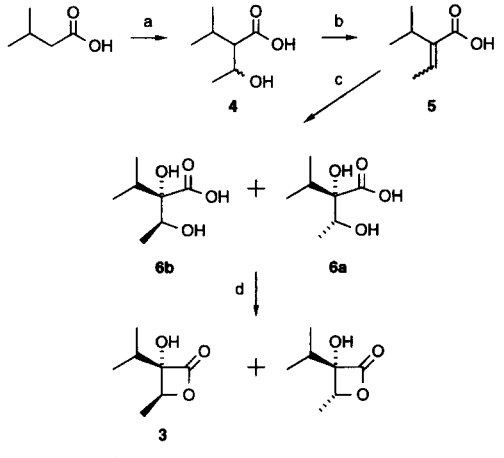


Figure 4. Synthesis of 2-hydroxy-2-(1-methylethyl)-3-butanolides (relative configurations). (a) 2 equiv *n*-BuLi, CH_3CHO; (b) P_2O_5; (c) $K_3Fe(CN)_6$, OsO_4; CH_3SO_2Cl, Na_2CO_3.

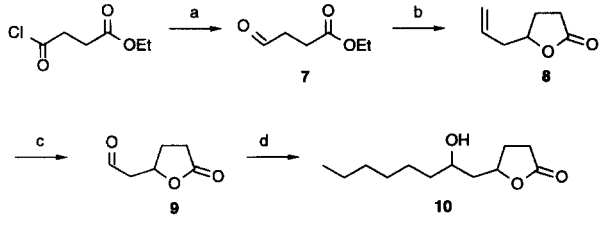


Figure 5. Synthesis of 6-hydroxy-4-dodecanolide. (a) H_2, Pd/C, lutidine; (b) $CH_2{=}CHCH_2ZnCl$, DMSO, then H^+; (c) O_3, than H_2, Pd/C; (d) $C_6H_{13}MgBr$.

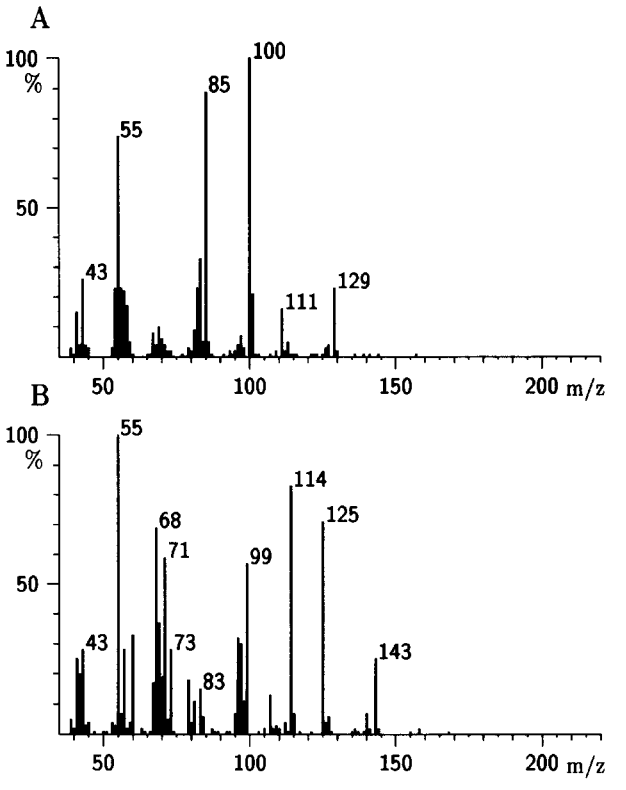


Figure 6. Mass spectrum of (A) 6-hydroxy-4-dodecanolide (**10**) and (B) 7-hydroxy-5-dodecanolide.

Several other volatiles were present in the hairpencils. Some of the phenolic compounds, such as *p*-nitrophenol, may be xenobiotics taken up by the butterflies. For a discussion, see reference 6. A list of identified compounds is presented in Table 1.

The volatile components of the hairpencil extracts were embedded in a lipidic matrix. This complex mixture was made up by more than 150 components consisting of straight chain alkanes, alkenes, saturated as well as unsaturated ketones and alcohols, and 2,5-dialkyltetrahydrofurans. The complete composition of this mixture is shown in Table 2.

Alkanes and 2,5-dialkyltetrahydrofurans (THFs) were readily identified by their mass spectra and gas chromatographic retention times.[4,11] The THFs occurred as mixtures of *cis* and *trans* isomers. The position of double bonds in alkenes was determined by MS investigation of their dimethyl disulfide (DMDS) adducts[12] and comparison of GC retention times with synthetic samples. The main compound proved to be (*Z*)-9-tricosene (**11**).

In addition to these lipids a series of ketones and secondary alcohols were identified by their MS. Their GC retention times indicated an unbranched carbon skeleton with the exception of hexahydrofarnesylacetone (6,10,14-trimethylpentadecan-2-one). Surprisingly, the oxygen-function was not located at one certain position. Instead, a mixture of regioisomeric ketones and alcohols were present bearing the functional group between C-2 and C-7 (see Table 2).

The location of the oxygen functionality could be deduced by analysis of the MS, despite the fact that no complete GC resolution was obtained. For example, the MS of the mixture of 4-, 5-, 6- and 7-pentacosanones [see Fig. 7(a)] exhibited the typical α-cleavage products on both sides of the keto group ($m/z = 323$, 309, 295, 281 and 71, 85, 99, 113), as well as the respective McLafferty-type rearrangement products in the low mass region ($m/z = 86/87$, 100/101, 114/115 and 128/129) and to a minor extent in the high mass region.

Similarly, the presence of the 4-, 5-, 6- and 7-pentacosanols could be deduced from the MS in Figure 7(b). Diagnostic ions characteristic for secondary alcohols were found at $m/z = 73$, 87, 101, and 115 as well as at $m/z = 283$, 297, 311, and 325, which were formed by loss of the smaller alkyl chain adjacent to the hydroxy group. The major alcohols and ketones were the pentacosanones and pentacosanols, followed by the respective C_{23}-compounds.

In addition, some unsaturated C_{23} ketones and alcohols were identified, eluting in the same region as the saturated ones (see Fig. 2). Respective C_{25}-compounds were present in trace amounts only. The location of the functional group was deduced by MS, as described for the saturated analogues. After separation of a hairpencil extract by column chromatography using solvents of different polarity,[6] fractions containing THFs as well as ketones (fraction 4 in reference 6, 3% methyl acetate in hexane) and alcohols (fraction 5, 4% methyl acetate in hexane) were obtained. These fractions were subjected to derivatization with DMDS.

Table 1. Compounds identified in the hairpencil extract of *I. leuconoe*. Lipids are tabulated in Table 2. Scan numbers refer to Figure 2. Concn: +++: major component; ++: minor component: +: trace component; (a): artifact formed during work up

Scan	Compound	Concn
22	Phenol	+
125	*o*-Cresol	+
168	*p*-Cresol	+
172	2-Ethyl-2-hydroxy-3-butanolide	+
300	2-Hydroxy-2-(1-methylethyl)-3-butanolide (**3**)	++
376	Benzoic acid	+
424	Ethyl viridiflorate (a)	++
731	Danaidone (**1**)	++
751	Geranyl thiomethyl ether (**2**)	++
842	*p*-Hydroxyacetophenone	+
990	Mellein	++
993	*p*-Nitrophenol	+
1130	6-Hydroxy-4-decanolide	+
1243	(*E*,*E*)-Farnesol	+++
1291	6-Hydroxy-4-undecanolide	+++
1320	7-Hydroxy-5-undecanolide	+
1409	Hexahydrofarnesylacetone	+
1427	6-Hydroxy-4-dodecanolide (**10**)	++
1448	7-Hydroxy-5-dodecanolide	+
1510	Octadecanal	+
1559	6-Hydroxy-4-tridecanolide	++
1575	Ethyl hexadecenoate (a)	+
1586	7-Hydroxy-5-tridecanolide	+
1602	Ethyl palmitate (a)	+
1758	Eicosanal	+
1798	Ethyl linoleate (a)	+
1806	Ethyl oleate (a)	+
1837	Ethyl stearate (a)	+
2051	Ethyl eicosanoate (a)	+
2720	Cholesterol	++
2730	α-Tocopherol	+

The resulting DMDS adducts of the different tricosenols in fraction 5 were inseparable by GC. Their MS showed intense ions at $m/z = 173$ (C_8H_{17}-$CHSCH_3^+$) and to a minor extent at $m/z = 243$ ($C_{13}H_{27}$-$CHSCH_3^+$), indicating two different saturated alkyl chains with 9 and 14 carbon atoms, respectively. Other ions arising from cleavage of the chain between the sulfur atoms and containing the hydroxyl group were found at $m/z = 259$ ($C_{13}H_{26}OHCHSCH_3^+$) and 189 ($C_8H_{16}OHCHSCH_3^+$). Loss of water from these fragments led to the intense ions at $m/z = 241$ and, to a minor extent, at $m/z = 171$. Therefore, the alkenols were a mixture of tricos-9- and 14-enols, in which the latter dominated.

The DMDS adducts of the alkenones found in fraction 4 showed similar results. In the spectrum of the tricosenones, obtained by adding all scans containing these ions (see Fig. 8), intense fragments at $m/z = 173$ (C_8H_{17}-$CHSCH_3^+$ or $C_7H_{15}O$—$CHSCH_3^+$) and 257 ($C_{14}H_{28}$-$CHSCH_3^+$ or $C_{13}H_{24}O$-$CHSCH_3^+$) were observed. In contrast to the spectra reported for the DMDS adducts of shorter chain unsaturated ketones,[13] no prominent ion arising from the loss of 48 amu from the fragment containing the keto group was observed.

Instead, small but diagnostic ions were found at $m/z = 199$ (257–58 [CH_3COCH_3]) and 208 (257–49 [CH_5S]), suggesting the oxygen-containing ion to be found at $m/z = 257$. This ion is also more intense than its counterpart at $m/z = 173$. Therefore, the alkenones were tentatively identified to be the tricos-14-enones, which can be easily formed by oxidation of **11**, the major alkene of the hairpencils. The other possible isomer consistent with the major, but not the minor, peaks observed, tricos-8-enone, is biogenetically not easily formed from the alkenes and alkenols present in the extract. Additional ions were found at $m/z = 187$ and 243, the former being more intense than the latter, suggesting small amounts of tricos-9-enones were also present in the mixture. The characteristic ion at $m/z = 138$ (187–49) could be observed, but the fragment at $m/z = 129$ (187–58) was missing. Generally, the identification of the fragment containing the oxygen may be difficult, especially when spectra with low intensity are obtained. An unambiguous proof is only possible by high-resolution MS of the DMDS adducts of the alkenes, which in our case could not be performed due to lack of material. The alkenones were accompanied by minor amounts of tetracos-15-enones and pentacos-16-enones (see Table 2).

Several other body parts of the butterflies were investigated for the presence of identified compounds. Hairs cut from hairpencils were analyzed to test whether

Table 2. Lipids identified in the hairpencil extract of *I. leuconoe*. NC: Number of carbon atoms in the alkyl chain; scan: scan numbers of Figure 1; FG: Position of the functional group. Details are given in the footnotes. Main compounds within a group of isomers are denoted by bold characters; concn: +++ main component of whole extract, ++ minor component, + trace

NC	Alkanes		Alenes			Ketones			Enones			Alcohols			Alkenols			Tetrahydrofurans		
	Scan	Concn	Scan	FG[a]	Concn	Scan	FG[b]	Concn	Scan	FG[b]	Concn	Scan	FG[c]	Concn	Scan	FG[d]	Concn	Scan	FG[e]	Concn
17	1218	+				1485	2	+												
18	1354	+	1320		+	1614	2	+												
19	1485	+	1477		+	1736	2	++												
20	1611	+				1852	2	+												
21	1731	++	1698	7,9	++	1963	2	++												
						1958	3	+												
22	1845	+	1814	7,8,**9**	++	2069	2	+				2081	2,3	+						
						2062	3	+												
23	1955	++	1932	7,***Z*9**	+++	2173	2	+	2138	2,3[f]	+	2183	2,3	+	2168	4,5,6,7	+			
			1920	diene	++	2162	3	+	2116	4,5,6,7[f]	+	2170	4,5,6	+	2172	2,3	+			
						2146	**4**,5,6,7	+												
24	2059	+	2032	7,9	+	2268	2	+	2232	[g]	+	2272	2,3	+						
						2264	3	+				2268	4,5,6,7	+						
						2245	4,**5**,**6**,**7**	+												
25	2161	++	2134	7,**9**	++	2337	4,5,**6**,**7**	++	2325	4,5,6[h]	+	2360	3,**4**,**5**,6	++						
	2193	+[i]																		
26	2257	+				2429	5,6,7	++				2451	5,6,7	+						
27	2352	+				2518	5,6,7	+				2550	5,6,7	+				2425	5–18,6–17,7–16,8–15,9–14	+
28																		2495	5–19,6–18,7–17,**8**–**16**,**9**–**15**	+
29																		2581	7–18,8–17,**9**–**16**,11–14	++
30																		2665	8–18,**9**–**17**	+
31																		2755	**9**–**18**,11–16,13–14	++
32																		2870	9–19,10–18,11–17,12–16,13–15	+
33																		2972	11–18,13–16	+
34																		3120	7–23,12–18,13–17,14–16	+
35																		3290	8–23,9–22,13–18,15–16	+

[a]Position and geometry of the double bond.
[b]Position of the keto group.
[c]Position of the hydroxyl group.
[d]Position of the hydroxyl group. The double bond is located at C-14; minor amounts of 9-tricosenols occur also.
[e]Location of the tetrahydrofuran ring along the chain. The entry 8–11 with NC = 27 thus indicates 2-heptyl-5-hexadecyltetrahydrofuran. Scan numbers refer to the earlier eluting *cis* component.
[f]The double bond is located at C-14; minor amounts of 9-enones occur also.
[g]Tetracos-15-enones with unknown position of the functional group.
[h]The double bond is located at C-16.
[i]13-Methylpentacosane.

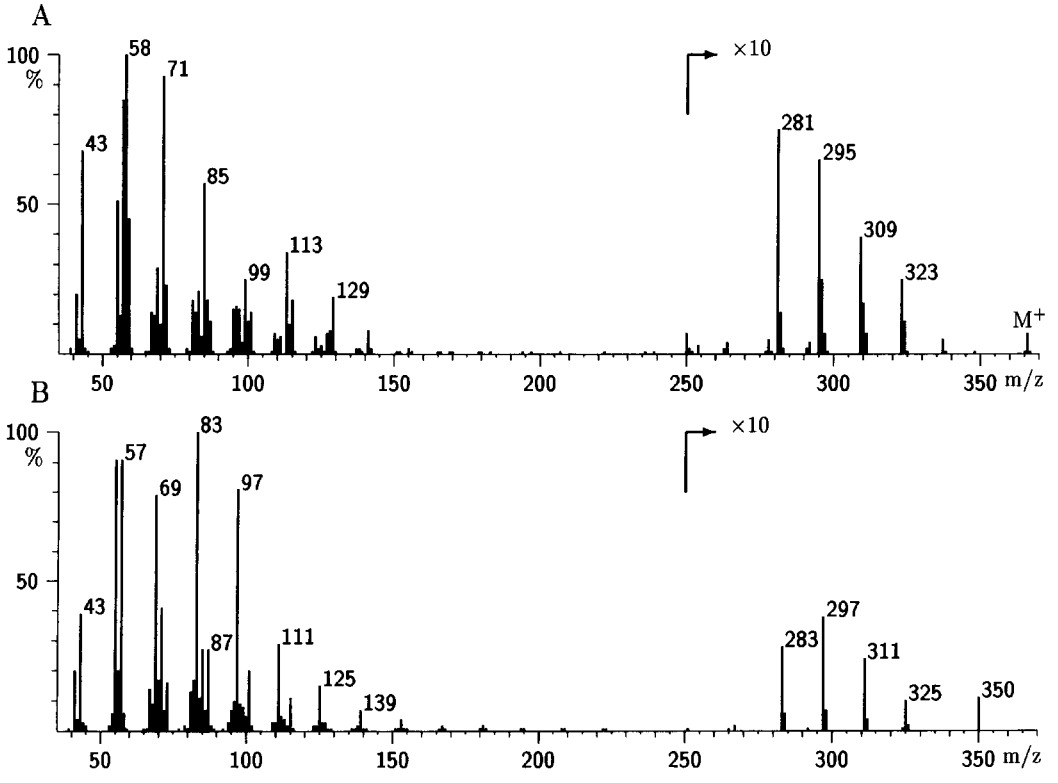


Figure 7. Mass spectrum of (A) a mixture of 4-, 5-, 6- and 7-pentacosanones and (B) a mixture of 4-, 5-, 6- and 7-pentacosanols.

some compounds may have originated from contamination by the hemolymph during preparation of the hairpencils. This seemed not to be the case because hairs only and whole hairpencils contained the same components. In addition, the hemolymph did not show any of these compounds in measurable amounts, with the exception of cholesterol and α-tocopherol as well as traces of the alkanes.

Wing extracts contained the alkanes and THFs as main components of their wax layer. Besides these compounds, straight-chain unbranched aldehydes and primary alcohols were present, but no ketones or secondary alcohols were detected.

The results presented above show that the hairpencil bouquet consists of several volatile compounds imbedded in a lipid layer of complex composition. The primary function of the hairpencils seems to be to mediate close range male–female courtship interactions. Nevertheless, additional roles may be warning signals for potential predators or male–male interactions.[6]

The courtship success of a male does not depend upon one single compound. At least the three components **1**, **2** and **3** seem to be essential. By use of the artificial mixture cited in the Introduction, responses of the females similar to those obtained from crude hairpencil extracts can be observed in greenhouse experiments.[6]

The surface lipids found on the *I. leuconoe* cuticle are composed mainly of alkanes and THFs, accompanied by primary alcohols and aldehydes. The alkanes and THFs are also present on the hairpencils. Hairpencil specific compounds are the alkenes, ketones and

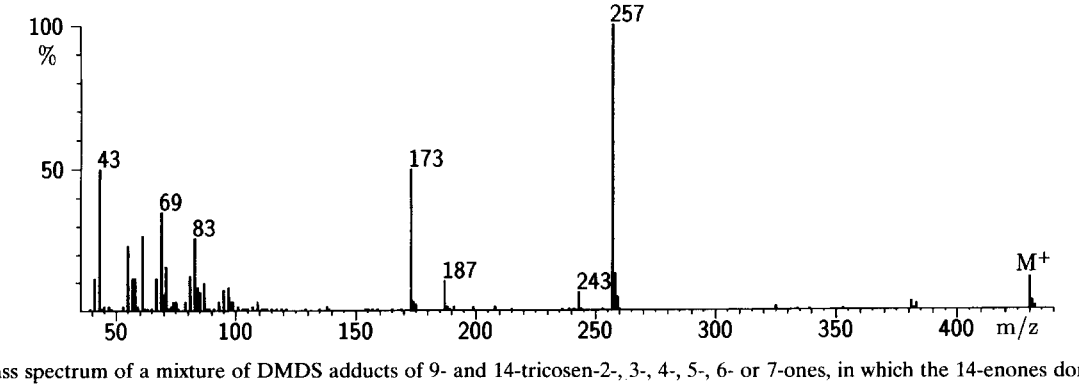


Figure 8. Mass spectrum of a mixture of DMDS adducts of 9- and 14-tricosen-2-, 3-, 4-, 5-, 6- or 7-ones, in which the 14-enones dominated.

secondary alcohols. The alcohols may be formed from the respective hydrocarbons by a not very site-specific oxidation near the end of the molecule. Further oxidation of the alcohols furnishes the ketones. Related acetates of regioisomeric secondary alcohols have been identified in the extracts of female screwworm flies, *Cochliomyia hominivorax*.[14] They play a role in stimulating copulation of the flies, acting in close range as it is the case in *Idea*. Other regioisomeric mixtures of long-chain secondary alcohols are rarely found in insects.[15]

The alkenols and alkenones possess the double bond at the same position as the main unsaturated alkene, (*Z*)-9-tricosene (**11**). Depending on which side the oxygen introduction occurs, tricos-9- or 14-enols and tricosenones are formed. The double bond may be introduced early in the biosynthetic pathway, so that **11** is the precursor for the unsaturated secondary alcohols and ketones. A similar sequence has been proved for the formation of **11** and (*Z*)-14-tricosen-10-one in *Musca domestica*.[16] While the saturated compounds are found with different chain length, unsaturated compounds possess predominantly a C_{23} skeleton, pointing to a specific process of formation.

The surface lipids may fulfil several roles in the pheromone system of *Idea*. Some of them, especially **11** as well as the unsaturated ketones and alcohols, may function as true pheromone constituents. The main component of these, (*Z*)-9-tricosene, is a pheromone of the housefly.[17] Unsaturated ketones of similar chain length are known as sex pheromones of female geometrid, carposinid and lymantrid moth.[18] The lipids are also useful as solvents for the volatiles. A more polar character, introduced by the oxidation of the hydrocarbons, may give better solubility for small polar compounds as the phenols or **3**. In addition, the lipids slow down the evaporation rate of very volatile compounds. They may also prevent degradation processes of hairpencil constituents together with the well-known antioxidant α-tocopherol, present in the hairpencils.

The volatile constituents are primarily transferred through air during courtship.[6] Nevertheless, the whole secretion is also painted onto the antennae of the female. It seems likely that the lipids act as a fixative for the volatiles on the antennae, thus providing a lasting stimulus. In another, more developed, danaine species, *Danaus gilippus*, (*E*,*E*)-3,7-dimethyldeca-3,7-diene-1,10-diol acts as a glue for pheromone transfer particles which have been transferred during courtship onto the females antennae.[3] In both cases the persistence of the volatiles on the antennae is increased by the mentioned compounds.

Experimental

Equipment

^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker WM 400 instrument. ^{1}H NMR standards: tetramethylsilane ($CDCl_3$, C_6D_6) or acetonitrile (D_2O, 1.98 ppm); ^{13}C NMR standards: $CDCl_3$ (77.00 ppm) or acetonitrile (D_2O, 118.20 ppm). MS (70 eV) were obtained with a VG 70/250 S mass spectrometer coupled to a Hewlett Packard HP 5890 A gas chromatograph and a Fisons MD-800 mass spectrometer coupled to a Fisons GC 8000. Analytical GLC analyses were carried out with a Carlo–Erba Fractovap 2101 gas chromatograph with a flame ionization detector and on-column injection. Separations were performed using a 30 m Rt_x-5 (i.d. = 0.32 mm, d_f = 0.25 μm) fused silica column with hydrogen as the carrier gas. Column chromatography was performed on silica gel (70–230 mesh, Merck).

Material

Males of *I. leuconoe* were collected on Ishigaki and Iriomote islands (Japan). Hairpencils were carefully cut, stored in ethanol until work up and partioned between water and hexane. The hexane layer was used for analysis after careful removal of most of the solvent at ambient temperature. Ethyl esters were formed during extraction and annotated with an (a) in Table 2. Separation of hairpencil components by column chromatography was performed as described in reference 8.

(*E*)-3,7-Dimethyl-2,6-octadienyl methyl thioether (2). A mixture of geranyl chloride (500 mg, 2.89 mmol) and sodium thiomethanolate (320 mg, 5.81 mmol) were heated under reflux in boiling ethanol (3 mL). During that time a white precipitate of sodium chloride occurred. After cooling, water was added and the mixture extracted three times with diethyl ether. Drying ($MgSO_4$) and removal of the solvent furnished a yellow oil which was subjected to column chromatography (silica, hexane/diethyl ether). Thus, 320 mg (1.75 mmol, 61 % yield) of **2** were obtained as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.60 (s, 3H, CH_3), 1.65 (d, 3H, CH_3, J = 1.2 Hz), 1.68 (d, 3H, CH_3, J = 1.2 Hz), 2.03 (s, 3H, S-CH_3), 2.00–2.15 (m, 4H, H-4,5), 3.12 (d, 2H, H-1, J = 7.8 Hz), 5.07–5.13 (m, 1H, H-6, J = 6.8 Hz), 5.23 (tq, 1H, H-2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.29 (CH_3), 16.02 (CH_3), 17.65 (CH_3), 25.65 (S—CH_3), 26.52 (CH_2), 31.11 (CH_2), 39.61 (CH_2), 120.28 (CH), 123.98 (CH), 131.60 (*C*=C), 138.80 (*C*=C). HR-MS: M^+_{obs} = 184.1281, M^+_{calc} = 184.1286 ($C_{11}H_{20}S$).

3-Hydroxy-2-(1-methylethyl)-butyric acid (4). According to the procedure of Moersch and Burkett for the alkylation of aliphatic acids,[19] the dilithium derivative of 3-methylbutyric acid was converted into **4** by reaction with acetaldehyde in 60% yield. Both diastereomers were formed in equal amounts. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.99 (s, 3H, CH_3, J = 6.6 Hz), 1.01 (d, 3H, CH_3, J = 6.6 Hz), 1.02 (d, 3H, CH_3, J = 6.6 Hz), 1.06 (d, 3H, CH_3, J = 6.4 Hz), 1.27 (d, 3H, CH_3, J = 6.2 Hz), 1.28 (d, 3H, CH_3, J = 6.6 Hz), 2.05–2.17 (m, 3H), 2.37 (t, 1H, H-2, J = 6.8 Hz), 4.07 (dq, 1H, H-3, J = 4.0, 6.2 Hz), 4.13 (quin, 1H, H-3, J = 6.6 Hz). ^{13}C NMR

($CDCl_3$, 100 MHz): δ 19.83 (CH_3), 20.03 (CH_3), 20.20 (CH_3), 20.73 (CH_3), 21.02 (CH_3), 22.01 (CH_3), 26.85 (CH), 27.69 (CH), 58.68 (C-2), 58.91 (C-2), 66.03 (C-3), 66.81 (C-3), 178.97 (C-1), 179.07 (C-1).

2-(1-Methylethyl)-2-butenoic acid (5). A 3:2 (*E*/*Z*) mixture of **5** was prepared in 55% yield by dehydration of **4** with P_2O_5 as described for the corresponding ethyl esters in reference 20. (*E*)-**5**: ^1H NMR ($CDCl_3$, 400 MHz): δ 1.19 [d, 6H, CH—($C\underline{H}_3$)$_2$, J=7.0 Hz], 1.84 (d, 3H, H4, J=7.0 Hz), 2.94 [sept, 1H, $C\underline{H}$—(CH_3)$_2$], 6.88 (q, 1H, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.02 (C-4), 20.53 [CH—(CH_3)$_2$], 26.72 [$\underline{C}$H—(CH_3)$_2$] 137.91 (C-2), 138.63 (C-3), 173.44 (C-1).

Compound (*Z*)-**5**: ^1H NMR ($CDCl_3$, 400 MHz): δ 1.08 [d, 6H, CH—($C\underline{H}_3$)$_2$, J=7.0 Hz], 1.99 (dd, 3H, H-4, J=7.0, 1.0 Hz), 2.74 (m, 1H, $C\underline{H}$—CH_3), 6.06 (dq, 1H, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.81 (C-4), 21.99 [CH—($\underline{C}H_3$)$_2$], 31.04 [$\underline{C}$H-(CH_3)$_2$], 134.63 (C-3), 138.57 (C-2), 174.50 (C-1).

(±)-Trachelanthic (6a) and (±)-viridifloric acids (6b). By oxidation of the acids **5** with catalytic amounts of osmium tetroxide and potassium hexacyanoferrate as cooxidant,[21] a 3:2 mixture of **6a** [(2*R**,3*S**)-2,3-dihydroxy-2-(1-methylethyl)-butanoic acid] and **6b** [(2*R**,3*R**)-2,3-dihydroxy-2-(1-methylethyl)-butanoic acid] was obtained.

Compound **6a**: ^1H NMR (400 MHz, D_2O): δ 0.84 (d, 3H, CH_3, J=6.6 Hz), 0.86 (d, 3H, CH_3, J=6.6 Hz), 1.11 (d, 3H, CH_3, J=6.6 Hz), 2.00 (sept, 1H, H-1′), 4.04 (q, 2H, H-3). ^{13}C NMR (100 MHz, D2O): δ 14.66 (CH_3), 15.73 (CH_3), 16.05 (CH_3), 31.18 [$\underline{C}$H—(CH_3)$_2$], 69.08 (C-3), 82.91 (C-2).

Compound **6b**: ^1H NMR (400 MHz, D_2O): δ 0.83 (d, 3H, CH_3, J=6.6 Hz), 0.86 (d, 3H, CH_3, J=6.6 Hz), 1.14 (d, 3H, CH_3, J=6.6 Hz), 2.03 (sept, 1H, H-1′), 4.02 (q, 2H, H-3). ^{13}C NMR (100 MHz, D2O): δ 14.89 (2 CH_3), 15.45 (CH_3), 31.19 [$\underline{C}$H—(CH_3)$_2$], 67.91 (C-3), 82.37 (C-2), 176.53 (C-1).

2-Hydroxy-2-(1-methylethyl)-3-butanolide (3). To a solution of **6a** and **6b** (50 mg, 0.31 mmol) in abs CH_2Cl_2 (3 mL), Na_2CO_3 (300 mg) was added, followed by mesyl chloride (150 mg, 1.69 mmol). After stirring overnight, H_2O (5 mL) was added, the phases separated and the aqueous phase extracted three times with CH_2Cl_2.[7] The combined organic phases were dried with $MgSO_4$, the solvent removed and the residue distilled at low temperature under reduced pressure yielding a clean mixture of the two β-lactones in 55% yield. Attempts to subject the crude product to column chromatography resulted in degradation of the product.

(2*R**,3*S**)-2-Hydroxy-2-(1-methylethyl)-3-butanolide: ^1H NMR (400 MHz, C_6D_6): δ 0.62 (d, 3H, CH_3, J=6.6 Hz), 0.75 (d, 3H, CH_3, J=6.6 Hz), 0.96 (d, 3H, CH_3, J=6.0 Hz), 1.52 (sept, 1H, H-1′), 3.88 (q, 1H, H-3).

(2*R**,3*R**)-2-Hydroxy-2-(1-methylethyl)-3-butanolide (**3**): ^1H NMR (400 MHz, C_6D_6): δ 0.58 (d, 3H, CH_3, J=6.6 Hz), 0.88 (d, 3H, CH_3, J=6.6 Hz), 1.02 (d, 3H, CH_3, J=6.0 Hz), 1.71 (sept, 1H, H-1′), 4.01 (q, 1H, H-3). ^{13}C NMR (100 MHz, C_6D_6): δ 13.70 (CH_3), 13.86 (CH_3), 14.91 (CH_3), 27.10 (C-1′), 80.64 (C-3), 87.55 (C-2), 170.11 (C-1). $C_7H_{12}O_3$ (144.2); calcd: C, 58.32; H, 8.39. Found: C, 58.01; H, 8.27.

Methyl 4-oxobutanoate (7). This compound was prepared according to reference 9. Bp.: 79–81 °C (15 torr). Yield: 51%. ^1H NMR (400 MHz, $CDCl_3$): δ 2.63 (t, 2H, CH_2), 2.81 (t, 2H, CH_2), 3.68 (s, 3H, CH_3), 9.79 (s, 1H, CHO).

6-Hepten-4-olide (8). This compound was prepared according to reference 10. Yield: 58% (lit. 66%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.90 (m, 1H, H-3), 2.23–2.53 (m, 5H), 4.54 (m, 1H, H-4), 5.14 (m, 2H, H-7), 5.76 (m, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.95 (CH_2), 28.55 (CH_2), 39.36 (C-5), 79.66 (C-4), 118.83 (C-7), 131.94 (C-6), 176.98 (C-1). MS (70 eV): *m/z* (%) 39 (11), 41 (9), 57 (18), 85 (100).

6-Oxo-4-hexanolide (9). This compound was prepared according to reference 10. Due to its instability, the aldehyde was immediately used in the next step without purification.

6-Hydroxy-4-dodecanolide (10). To a solution of **9** (0.5 g, 3.9 mmol) in abs diethyl ether (10 mL) was added an etheral 1 M hexylmagnesium bromide solution (3.9 mL) at 0 °C. The mixture was stirred overnight and hydrolyzed with satd NH_4Cl solution. The phases were separated, the aqueous phase extracted three times with diethyl ether and the combined organic phases dried ($MgSO_4$). After filtration and removal of the solvent, the product was purified by column chromatography (silica, CH_2Cl_2). The diastereomers were formed in ratios between 1:1 and 1.5:1, depending on the experiment. No separation by column chromatography could be obtained. Yield: 0.29 g (34%).

(*R**,*R**)-**10**: ^1H NMR (400 MHz, $CDCl_3$):δ 0.88 (t, 3H, H-12), 1.25–2.00 (m, 14H), 2.38 (m, 1H, H-2), 2.54 (m, 1H, H-2), 3.88 (m, 2H, H-6), 4.79 (dddd, 1H, H-4, J=3.2, 6.0, 8.4, 9.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02 (C-12), 22.55 (C-11), 25.40 (C-8), 28.50 (C-3), 28.86 (C-2), 29.18 (C-9), 31.76 (C-10), 38.05 (C-7), 43.15 (C-5), 68.54 (C-6), 78.12 (C-4), 176.86 (C-1).

(*R**,*S**)-**10**: ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, H-12), 1.25–2.00 (m, 14H), 2.38 (m, 1H, H-2), 2.54 (m, 1H, H-2), 3.81 (m, 2H, H-6), 4.69 (tt, 1H, H-4, J=6.0, 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.02 (C-12), 22.55 (C-11), 25.39 (C-8), 28.34 (C-3), 28.53 (C-2), 29.20 (C-9), 31.76 (C-10), 37.54 (C-7), 42.66 (C-5), 69.46 (C-6), 79.45 (C-4), 177.15 (C-1). $C_{12}H_{22}O_3$ (214.3); calcd: C, 67.26; H 10.35. Found: C, 67.11; H, 10.15.

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