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Aphrodisiac Pheromones from the Wings of the Small Cabbage White and Large Cabbage White Butterflies, *Pieris rapae* and *Pieris brassicae*

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The small and large cabbage butterflies, *Pieris rapae* and *P. brassicae*, are found worldwide and are of considerable economic importance. The composition of the male scent-producing organs present on the wings was investigated. More than 120 components were identified, but only a small portion proved to be male specific. Major components were the known beetle pheromone ferrulactone (**1**) in *P. rapae* and its previously unknown larger analogue, brassicalactone (**2**), in *P. brassicae*. The latter carries an additional isoprene unit and is closely related to **1**. Other components present in larger amounts on male relative to female wings were hexahydrofarnesylacetone (**18**) and phytol (**23**). Brassicalactone (**2**) was fully characterized by synthesis of its various diastereomers by using ring-closing metathesis. A similar approach to ferrulactone (**1**) failed, presumably because of its smaller ring size. Instead, this compound was synthesized by using a modified literature procedure. The biological activity of the compounds in the extract was tested by coupled gas chromatographic-electroantennographic (GC-EAD) analysis, which showed that both macrolides and the other major components of the wings can be detected by the antennae of the conspecific female butterflies. Other detectable compounds included several alkanes, which are typical constituents of the butterfly cuticula, derivatives of phytol (**23**) and long-chain secondary alcohols. Finally, bioassays with males showed that the mixture of **1** (*P. rapae*) or **2** (*P. brassicae*) together with **18** and **23** applied to freshly eclosed males increased mating success compared to untreated males. Therefore, the two macrolides **1** and **2** are aphrodisiac pheromone components of male small and large cabbage white butterflies, respectively.

Introduction

The small cabbage white butterfly, *Pieris rapae*, and the large cabbage white butterfly, *Pieris brassicae* (Lepidoptera: Pieridae), are common in many parts of the northern hemisphere. Their larval stages feed on plants of the cabbage family (Brassicaceae) and can cause considerable economic damage to *Brassica* crops. Despite their wide geographic distribution and agricultural importance, their intraspecific chemical communication system has received little attention. One reason might be the assumption that these brightly colored butterflies rely on visual cues for communication and defense. Nevertheless, the ground-breaking work of Brower and others on *Danaus gilippus* has demonstrated that male butterflies use pheromones during courtship behavior[1–4] or to attract females.[5]

Recently we became interested in the cuticular chemistry of Pierids, because polymorphism between the hydrocarbon profiles in the epicuticular wax layer of the antennae and other body parts of *P. rapae* can be observed; this seems to be of functional relevance.[6] Furthermore, it is well known that male Pierids possess androconial organs—modified scales containing odor-producing glandular structures—on their hind- and forewings.[7–11] It has been shown repeatedly that the chemical cues emanating from the wings are used as pheromones in the Pieridae. Rutowski showed that epicuticular components of *Eurema lisa* and *Colias philodice* males elicited the acceptance behavior of females.[12,13]

Male specific wing compounds involved in maintaining reproductive isolation between *Colias eurytheme* and *C. philodice* were identified.[14] Hexyl myristate, palmitate, and stearate were present exclusively in *C. philodice*, while 13-methylheptacosane was specific for *C. eurytheme*, and all four compounds were electrophysiologically active in the respective species. In addition, several unbranched alkanes, which are typical cuticular components, were present on the wings of both species. Behavioral experiments indicate that the esters act as species-recognition signals. Recently it was shown that UV reflectance of males of *C. eurytheme* seem more important for mating success than the pheromones.[15] Sappington showed individual variability of 13-methylheptacosane, heptacosane and nonacosane in *C. eurytheme*.[16,17]

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Males of the green-veined butterfly, *Pieris napi*, contain geranial and neral in their wing androconia.[8,11] These compounds act as the courtship sex pheromones of the males. Lack of this cue prevents successful courtship by the males.[11] In *P. melete* it has been shown that the male wing scent has sex pheromonal activity and is needed for successful mating.[18] It induces the transition from the initial refusal posture of females to acceptance of courting males. This behavior can also be observed in *P. rapae*, which refuses courting *P. melete*, probably because of the lacking or wrong male pheromone.[19]

During the analysis of the cuticular hydrocarbons of *P. rapae* and *P. brassicae* several heterosubstituted alkanes were found to be present on the exocuticle. Therefore, we became interested in their structure. Male-specific compounds on the wings of *P. rapae* and *P. brassicae* had been reported earlier by Bergström and Lundgren without information on their structure.[8] Indole (**26**) and three unknown compounds were reported as male specific wing components of *Pieris rapae crucivora*.[20]

A different type of chemical communication was recently discovered in *Pieris*. Males transfer volatile compounds—so-called antiaphrodisiacs—onto the females during copulation; these render them unattractive for other males. Methyl salicylate was identified as an antiaphrodisiac of *P. napi*,[21,22] while the related *P. rapae* uses both methyl salicylate and indole (**26**). In *P. brassicae* benzyl cyanide (**29**) serves this function.[23] Another antiaphrodisiac, (*E*)-ocimene, was found in the heliconiine butterfly, *Heliconius melpomene*.[24]

The identification, synthesis, and biological activity of the major male-specific compounds from wings of *P. rapae* and *P. brassicae* are reported in this article. In addition, an overview of the wing chemistry and compounds eliciting electroantennographic responses in the two species is reported.

Results

Wings of both sexes of *P. rapae* and *P. brassicae* were extracted with pentane for 10 min. These extracts were then analyzed by GC-MS, which revealed the presence of a male specific trace component (Figure 1 A, inset) in *P. rapae*. The mass spectrum proved to be identical to that of ferrulactone I (cucujolide I, **1**), a pheromone of the Rusty Grain beetle, *Cryptolestes ferrugineus* (Figure 2 A).[25] In the extract of *P. brassicae* an unknown compound (**A**) was found, which exhibited a mass spectrum with a molecular ion at *m/z* 262 (Figure 2 B).

These compounds were anticipated to originate from the scent scales located over the entire wing area of the males.[10] Therefore, the wings were extracted again with the more polar dichloromethane, which is able to penetrate deeper into the tissue than pentane. Thus, extracts containing higher concentrations of both **1** and **A** were obtained. Additionally, several other oxygenated compounds were enriched. Extracts prepared similarly from female wings showed that both **1** and **A**, as well as some other components, were male specific (Figure 3). The concentration of these compounds reached a maximum about ten days after emergence of the adults.

The mass spectral analysis of **1** and **A** showed similarities in their fragmentation patterns (Figure 2). The base peak at *m/z* 68, as well as the characteristic ions at *m/z* 99 and 127, occurred in both spectra. Although the structures of these ions are not known, a general similarity of the compounds was observed. The difference of 68 amu between the molecular ions

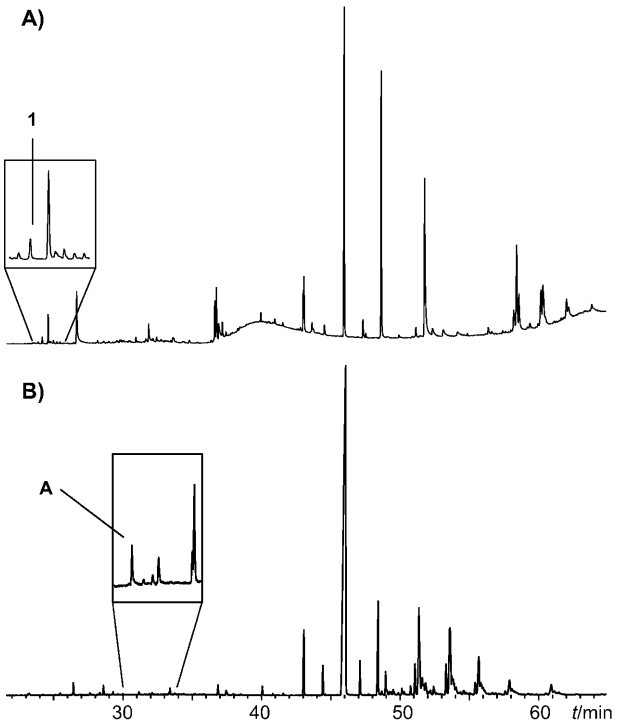


Figure 1. Gas chromatograms of short time pentane extracts of wings of freshly emerged: A) *P. rapae*, and B) *P. brassicae* males. The inserts show the low concentration of ferrulactone I (**1**) and brassicalactone (**A**).

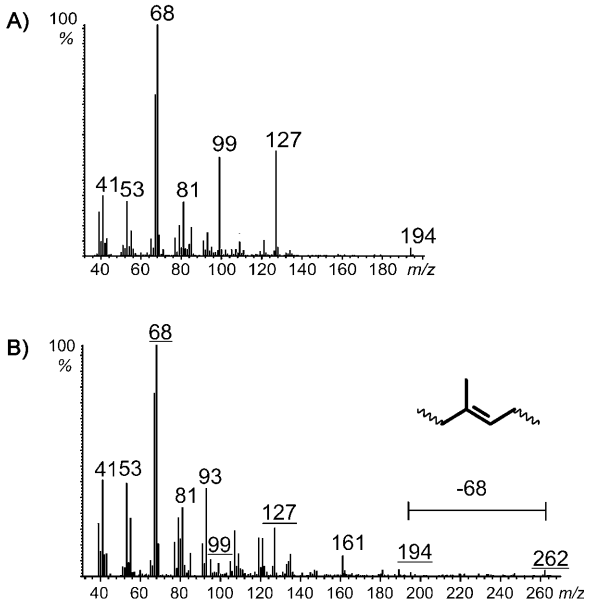


Figure 2. Mass spectra of: A) ferrulactone (**1**), and B) unknown compound **A**, which was later identified to be brassicalactone (**2**). Characteristic ions pointing to a structural similarity to **1** are underlined.

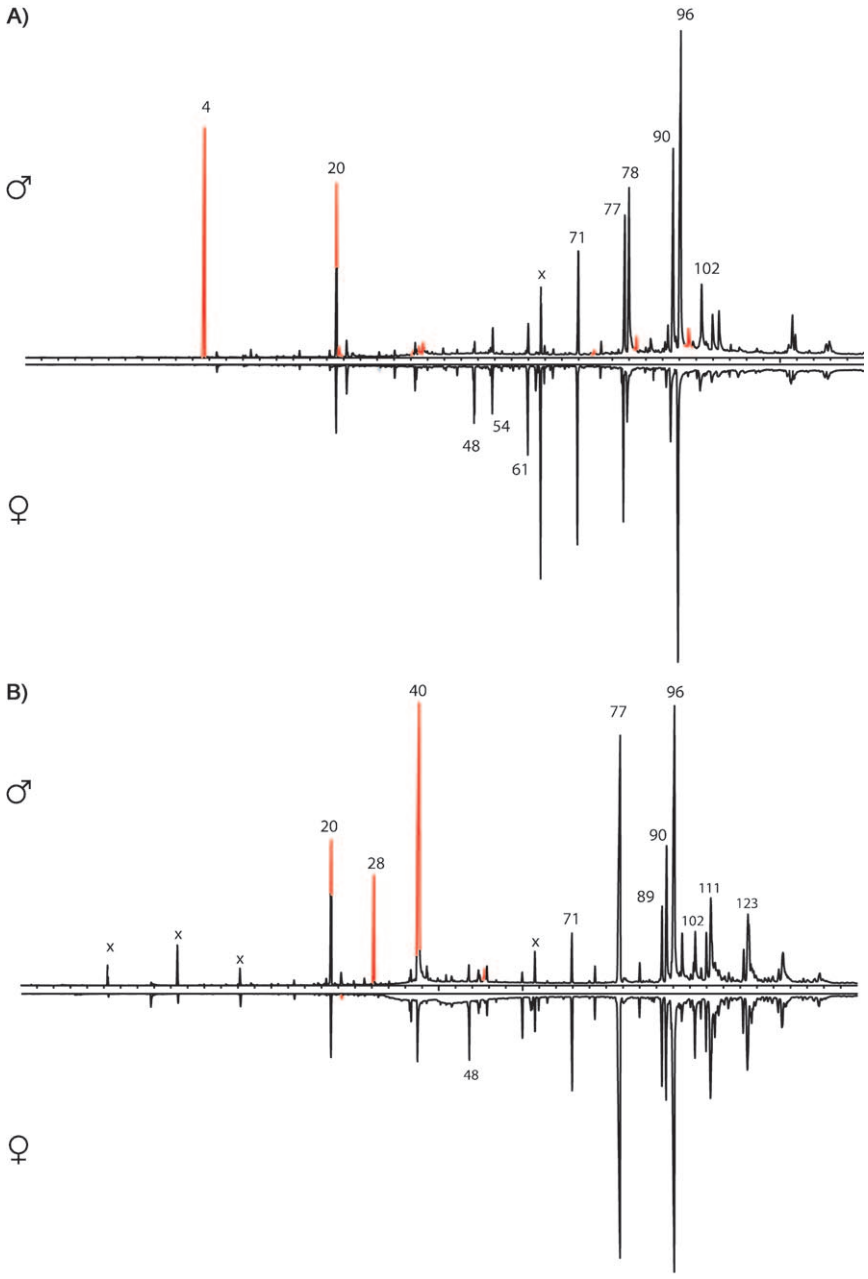


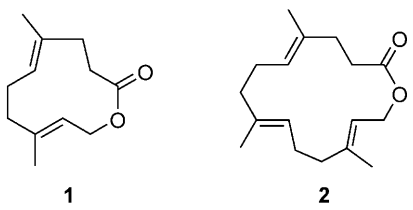
Figure 3. Gas chromatograms of one week old male (upper) and female (lower): A) *P. rapae* and B) *P. brassicae* CH_2Cl_2 extracts. The red regions indicate male-specific components or compounds occurring in higher concentration in males; numbers refer to entries in Table 1. An x marks any artefacts.

at *m/z* 194 and 262 can be explained by an additional methylbutenediyl unit, formally a terpene building block. Compound **A** could not be silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA); upon saponification with trimethylsulfonium hydroxide a hydroxylated methyl ester was formed, which points to a lactone structure of the parent compound. Therefore, it was postulated that **A** is an analogue of **1** with an additional terpene unit within the ring as in **2** (Figure 4). Hence, it was necessary to synthesize this compound to verify its structure and to determine its configuration.

The strategy relied on a ring-closing metathesis (RCM) as the key step in the synthesis of **2** (Scheme 1). (*E*,*E*)-Farnesyl acetate (**3**) was selectively dihydroxylated at the terminal double bond, followed by oxidative cleavage of the vicinal diol with $NaIO_4$ to afford the aldehyde **4**. Methenylation by a Wittig reaction furnished the terminal alkene **5**, which was reduced with $LiAlH_4$ to the alcohol **6**. The keto group of ethyl levulinate (**7**) was transformed into a terminal alkene through Wittig methodology, and transesterification with **6** and Bu_2SnO as catalyst yielded the RCM precursor **9**.[26] Finally, cyclization of the ester was successfully achieved in 50% yield by using a Grubbs catalyst of the second generation.[27] The 15-membered macrolide **2** was formed in a 3:1 mixture of the (4*E*,8*E*,12*E*)/(4*Z*,8*E*,12*E*) diastereomers.

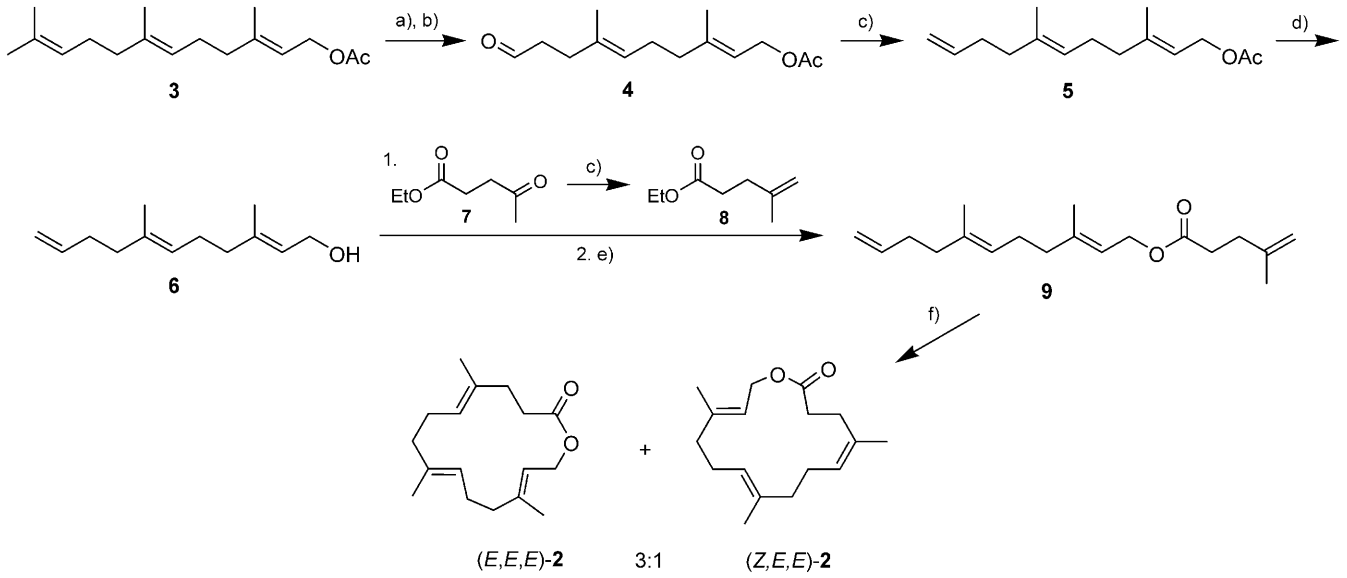
In a second synthesis *E*/*Z* isomers of the C-8=C-9 double bond were synthesized (Scheme 2). Geranyl acetate (**10**) was dihydroxylated with OsO_4,[28,29] followed by cleavage with $NaIO_4$ to yield aldehyde **11**. Again, a Wittig reaction introduced the terminal double bond to furnish the starting alcohol **12**.

(*E*)-Geranyl acetone (**13**) was then transformed into (*E*)-4,8-dimethyl-4,8-nonadienal (**14**) by the method described by Zoretic et al.[30] This aldehyde was directly converted into the methyl ester **15** with PDC and methanol in DMF.[31] The following transesterification with Bu_2SnO gave the precursor **16** for the final RCM by using the second generation Grubbs catalyst. In this synthesis a 42% yield of a 5:1 mixture of the (4*E*,8*E*,12*E*)- and (4*E*,8*Z*,12*E*)-diastereomers of **2** were obtained.

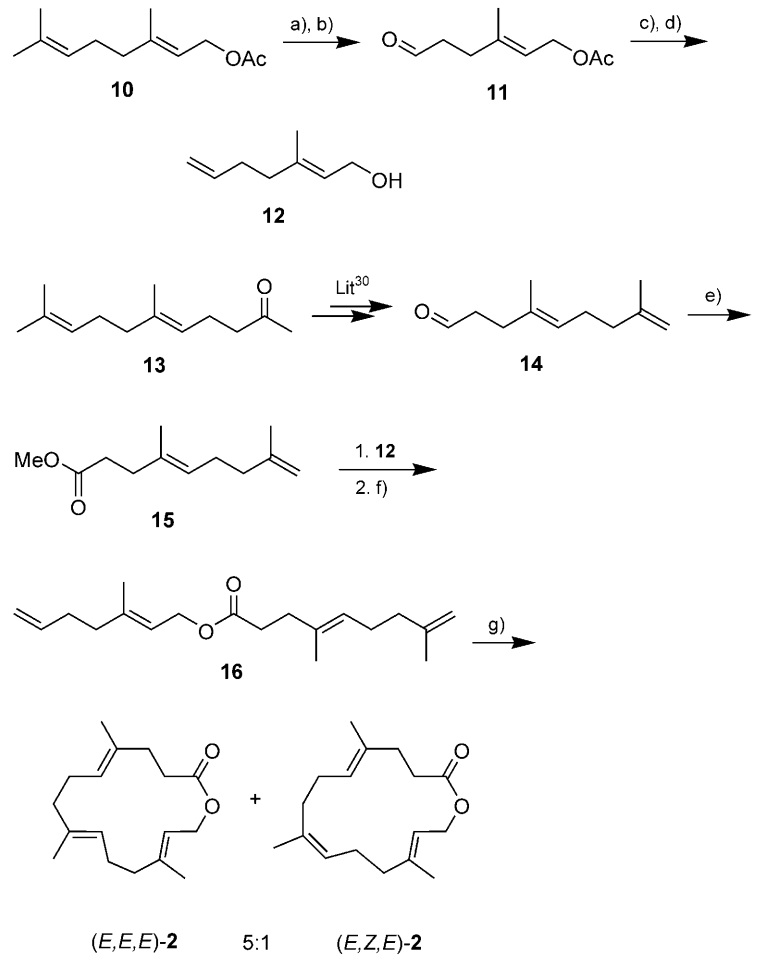


1 2

Figure 4. Structures of ferrulactone (**1**) and brassicalactone (**2**).



Scheme 1. a) OsO_4/NMO, acetone/H_2O, 50 °C; b) $NaIO_4$, 1,4-dioxane/H_2O, 0 °C to 5 °C; c) $MePPh_3Br$, BuLi, dimethoxyethane, −78 °C to room temperature; d) $LiAlH_4$, diethyl ether, reflux; e) Bu_2SnO, 80 °C; f) Grubbs 2, CH_2Cl_2, reflux.



Scheme 2. a) OsO_4/NMO, acetone/H_2O, 50 °C; b) $NaIO_4$, 1,4-dioxane/H_2O, 0 °C to 5 °C; c) $MePPh_3Br$, *n*BuLi, dimethoxyethane, −78 °C to room temperature; d) $LiAlH_4$, diethyl ether, reflux; e) PDC, MeOH, DMF, room temperature; f) Bu_2SNO, 80 °C; g) Grubbs 2, CH_2Cl_2, reflux; h) PDC, DMF/CH_3OH, room temperature; i) Bu_2SnO, **A**, 80 °C; j) Grubbs 2, CH_2Cl_2, reflux.

The major compound formed in both RCM reactions was the all-*E* macrolide, which showed identical mass spectra and gas chromatographic retention times to that of the natural product, **A**. Therefore, this compound is (4*E*,8*E*,12*E*)-4,8,12-trimethyl-4,8,12-tetradecatrien-14-olide (*E,E,E*-**2**), a previously not described natural product for which we propose the name brassicalactone.

Ferrulactone (**1**) was also synthesized to confirm the configuration of the double bonds in the natural product. Several attempts with RCM in a similar manner to that just described, failed, presumably because of the considerable strain of the 11-membered ring. Therefore, the original synthesis by Oehlschlager et al.,[32] starting from geranyl acetate (**10**), was used; this led to **17** in 21.4% overall yield as reported. In contrast, the synthesis of Cheskis et al., which we also carried out, gave **17** in 9.2% overall yield, only, albeit 81% were reported.[33] In the final cyclization step the method of Shiina et al.[34] proved to be superior to the Corey–Nicolaou process, and **1** was obtained in 25% yield (Scheme 3). The synthesis material was identical to the natural compound, and this confirmed the presence of ferrulactone with *E,E* configuration in male *P. rapae*.

Besides the macrolides, several alkanes, alkenes, primary and secondary alcohols, ketones, aldehydes as well as terpenoids related to (*E*)-phytol (**23**) occurred on the wings of both species. These compounds were identified by comparison of mass spectra and retention indices with those of reference compounds. A list of the identified components is given in Table 1. Long-chain secondary alcohols (**30**) and the respective ketones were identified as reported earlier.[35] In addition, internal alkane-1,2-diols (**31**)

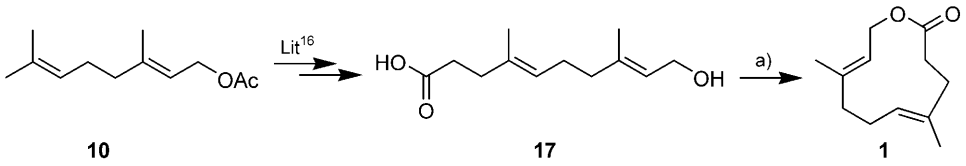
Table 1. Compounds identified on the forewings of *P. rapae* and *P. brassicae*, and results of GC-EAD experiments. E: number of positive electroantennographic responses of male (Em) or female (Ef) antennae to male extracts in different GC-EAD experiments (15–20 per group); f: female; m: male; RI: gas chromatographic retention index; M: molecular ion of unknown compounds; B: base peak of unknown compounds. Relative peak area to largest peak in extract: xxx: > 20%; xx: 5–20%; x: 0.5–5%; tr: < 0.5%.

	Compound	RI	*P. rapae*				*P. brassicae*			
			f	m	Ef	Em	f	m	Ef	Em
1	benzylcyanide (**29**)	1182						tr	5	3
2	decanal	1217					tr	tr		
3	1H-indole (**26**)	1343		tr						
4	ferrulactone (**1**)	1492		xxx	4	3		tr		
5	β-ionone	1492					tr	tr		
6	pentadecane	1500	tr	tr	3	6				
7	suspensolide (**22**)	1504						tr		
8	dihydroactinidiolide (**24**)	1560					tr	tr		
9	hexadecane	1600	x	x						
10	B81, M194	1625						tr		
11	tetradecanal	1629		x	4	4				
12	isopropyl dodecanoate	1631	tr	tr				tr		
13	heptadecane	1700	tr	tr			tr	tr		
14	B56	1726	tr	tr			tr	tr		
15	pentadecanal	1729	tr	tr			tr	tr		
16	octadecane	1800	tr	x			tr	x		
17	B43, M192	1812					tr	x		
18	isopropyl tetradecanoate	1831	tr	x						
19	neophytadiene isomer	1837					tr	x		
20	hexahydrofarnesylacetone (**18**)	1851	xx	xxx	7	6	xx	xxx	7	7
21	6,10,14-trimethylpentadecan-2-ol (**21**)	1857		x	6	6				
22	neophytadiene isomer	1862					tr	x		
23	hexadecanol	1898		tr	6	5				
24	nonadecane	1900	tr	x			tr	tr		
25	heptadecane-2-one	1920	tr	tr						
26	3-methyl-2-(3,7,11-trimethyldodecyl)furan	1920					tr	x		
27	isophytol	1952					x	x		
28	brassicalactone (**2**)	1981					tr	xx	3	
29	B81, M262	1986						tr		
30	icosane	2000	tr	tr						
31	B84	2003		tr				tr		
32	isopropyl hexadecanoate	2030	tr	x			x	tr		
33	octadecanal	2036	tr	tr						
34	B43	2069		x						
35	3-(4,8,12-trimethyltridecyl)-4-butanolide (**25**)[a]	2089	tr	x			tr	x		
36	octadecanol	2095	tr	tr	3	5				
37	henicosene	2095	x	x			x	x		
38	henicosane	2100	x	x	3	3	xx	x		
39	2-(4,8,12-trimethyltridecyl)-4-butanolide (**27**)[a]	2108	tr	tr			x	x		
40	phytol (**23**)	2120		x	3		xx	xxx	5	3
41	nonadecanal	2134	tr	x						
42	phytal	2150		x			xx	xx	3	
43	docosene	2195	tr				x	x		
44	phytyl acetate	2214		tr			x	x		
45	icosanal	2235	x	x			x	x		
46	octadecanol	2296		tr	4	2				
47	tricosene	2296	x	tr			x	tr		
48	tricosane	2300	xx	x	5	5	xx	x	5	6
49	9- and 11-methyltricosane	2336					x	x		
50	7-methyltricosane	2340					x	x	4	2
51	henicosanal	2342	tr	tr						
52	isopropyl octadecanoate	2352	tr							
53	B111, M308	2358	x	x			x	x		6
54	4,8,12,16-tetramethylheptadecan-4-olide (**20**)	2366	xx	x	2		xx	x		
55	octadecane-5-olide (**28**)	2380		tr						
56	hexyl hexadecanoate	2387	tr							
57	tetracosane	2400	tr	tr	2		x	x		
58	docosanal	2445	x	tr			x	tr		
59	B43	2468	tr	tr						
60	pentacosene	2497	x	x						
61	pentacosane	2500	xx	x	5	6	xx	x	3	6
62	octyl hexadecanoate	2505		tr						

Table 1. (Continued)

	Compound	RI	*P. rapae*				*P. brassicae*			
			f	m	Ef	Em	f	m	Ef	Em
63	9- and 11-methylpentacosane	2532	x	tr			x	tr		
64	7-methylpentacosane	2539	x	tr			x	tr		
65	7,11-dimethylpentacosane	2564	x	x			x	tr		
66	5,11-dimethylpentacosane	2576		tr						
67	hexyl octadecanoate	2589	x							
68	hexacosane	2600	x	x			x	tr		
69	tetracosanal	2647	tr	tr						
70	B69	2664	tr	tr			x	tr		
71	heptacosane	2700	xxx	xxx	6	6	xx	xx	3	
72	geranylgeraniol	2774					x	x		
73	octacosane	2800	x	x			x	x		2
74	squalene	2818	x	tr			x	tr		
75	B110	2820	tr	x	3		x	tr		
76	B110	2846	tr	x			x	tr		
77	nonacosane	2900	xxx	xxx	4	4	xxx	xxx		
78	5-, 6-, 7-, 8-, 9-, 10-, and 11-heptacosanol (**30**)	2918	xx	xxx[c]	4	4	tr	x	2	
79	7,8-, 8,9-, 9,10-, and 10,11-heptacosanediol (**31**)[b]	2933	tr	x[d]			x	tr		
80	6,8-, 7,9-, 8,10-, and 9,11-heptacosanediol[b]	2938	tr	x				tr		
81	5,8-, 6,9-, and 7,10-heptacosanediol[b]	2944	tr	tr				tr		
82	triacontane	3000	x	x	3					
83	B352	3017		x				tr		
84	6-, 7-, 8-, 9-, 10-, and 11-octacosanol (**30**)	3034	tr	tr	3					
85	octacosanal	3048	x	x			x	x		
86	B94, M394	3075		x						
87	15-nonacosanone	3088	x	x						
88	6-, 7-, 8-, 9-, and 10-nonacosanone	3095	x	x						
89	hentriacontane	3100	xx	x			xx	xx		
90	5-, 6-, 7-, 8-, 9-, 10-, and 11-nonacosanol (**30**)	3119	xx	xxx			x	xx[c]		
91	2-nonacosanone	3131	tr	tr						
92	7,8-, 8,9-, 9,10-, 10,11-, and 11-12-nonacosanediol (**31**)[b]	3136	tr	x			x	x		
93	7,9-, 8,10-, and 9,11-nonacosanediol[b]	3142	tr	x						
94	13,17-dimethylhentriacontane	3158					x	x		
95	α-tocopherol	3175	x	xxx			x	x		
96	cholesterol	3175	xxx	xxx			xxx	xxx		
97	β-cholesta-5,24-dien-3-ol	3201	x	x			xx	xx		
98	8-, 9-, 10-, and 11-triacontanol (**30**)	3228		x				tr		
99	hydrocarbon	3251					x	x		
100	B95	3258		x						
101	tricosanal	3261	x	x			x	x		
102	β-ergost-5-en-3-ol	3269	x	xx			xx	xx		
103	B95	3295		tr						
104	10- and 11-hentriacontanone	3297	x	tr						
105	cholest-4-en-3-one	3300	x	tr						
106	tritriacontane	3300	x	x			x	x		
107	9-, 10-, 11-, and 12-hentriacontanol (**30**)	3328	x	xx			tr	tr[e]		
108	11-, 13-, and 15-methyltritriacontane	3330					xx	xx		
109	2-hentriacontanone	3333	tr	tr			tr	tr		
110	9,10-, 10,11-, and 11,12-hentriacosanediol (**31**)[b]	3345	tr	tr						
111	15,19-dimethyltritriacontane	3357					xx	xx		
112	13,19- and 15,21-dimethyltritriacontane	3360					x	x		
113	β-stigmast-5-en-3-ol	3363	x	xx			x	x		
114	11,21-dimethyltritriacontane	3368					x	x		
115	tetratriacontane	3400					x	x		
116	10-, 11-, and 12-dotriacontanol (**30**)	3428	tr	tr						
117	hydrocarbon	3430					x	x		
118	B218, M424	3455	x	x						
119	dotriacontanal	3465	tr	tr				tr		
120	B217, M426	3511		tr						
121	10-, 11-, 12-, and 13-tritriacontanol (**30**)	3530	tr	x			tr	tr[f]		
122	11-, 13-, and 15-methylpentatriacontane	3530					xx	xx		
123	15,19-dimethylpentatriacontane	3557					xx	xx		
124	11,21-dimethylpentatriacontane	3568					x	x		

[a] Tentatively identified based on published mass spectra.[47] [b] Present as cyclic dimethylsilyl derivative in the extract. [c] Additionally, a 12-alkanol occurred. [d] Additionally, 6,7-heptacosanediol occurred. [e] Additionally, 8-hentriacontanol occurred. [f] Additionally, 9-tritriacontanol occurred.



Scheme 3. a) 2-Methyl-6-nitrobenzoic anhydride, DMAP, CH_2Cl_2.

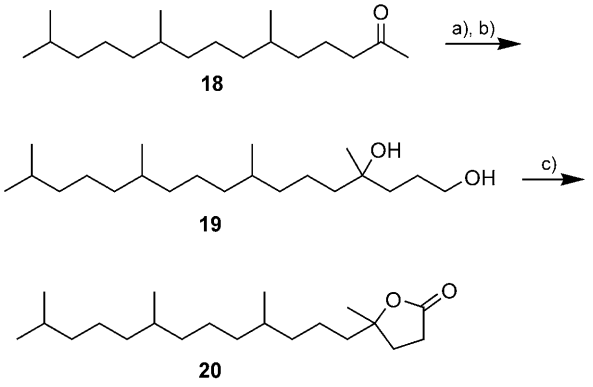
were identified, accompanied by small amounts of the respective 1,3- and 1,4-diols. These compounds were detected as their cyclic dimethylsilyl derivatives, which were formed by treatment of the diols with the methylsilicone GC phase. We have observed this surprising behavior of diols earlier,[36] which shows that the presumably inert apolar silicones used in GC can react with the analyte under certain conditions (for the mass spectra see the Supporting Information). Due to their poor gas chromatographic behavior, the free diols were not observed, but trimethylsilylation with MSTFA prior to analysis proved their presence. In such derivatized extracts the respective bis(trimethylsiloxy) compounds were detected.

Several of these compounds proved to be male specific; this suggested their occurrence in the scent glands. Male *P. rapae* contained large amounts of **1** as well as hexahydrofarnesylacetone (**18**), which occurred in females too, but in a markedly lower amount. Several trace components were present in males only as indole (**26**), 6,10,14-trimethylpentadecan-2-ol (**21**), 5-octadecanolide (**28**), tetradecanal, hexadecanol, octadecanol, (*E*)-phytol (**23**), its acetate, and the respective aldehyde. Furthermore, the secondary alcohols occurred in higher amounts on male wings compared to those of the female.

Major components on the wings of male *P. brassicae* besides **2**, were **18** and (*E*)-phytol (**23**). The latter two components were present on female wings too, but in markedly lower amounts. In addition, minor male-specific components were benzyl cyanide (**29**), **1**, and its isomer suspensolide (**22**), and some trace compounds of unknown identity.

We then tested the biological activity of the whole bouquet of both species using electroantennography coupled to gas chromatography (GC-EAD). Antennae of both male and female *P. rapae* and *P. brassicae* were used as detectors of a gas chromatograph, and the current elicited by the eluting compounds in the antennae was recorded. Special care was taken to record responses even from compounds with very low volatility, such as nonacosane.[37]

The experiments were repeated fifteen times and the results are shown in Table 1. Obviously, most of the major male-specific compounds can be detected by both sexes in each species. While ferrulactone (**1**) elicits a signal in both male and female antennae of *P. rapae*, brassicalactone (**2**) is only detected by female *P. brassicae*. On the contrary, other major components, such as **18** and **23**, and major hydrocarbons were detected by both species and both sexes. Minor compounds containing a terpenoid trimethylalkyl chain were also sometimes detected; these were **21** or the extended terpenoid 4,8,12,16-tetramethylheptadecan-4-olide (**20**). The latter compound was synthesized from **18** by the Grignard reaction of 3-(benzyloxy)propyl magnesium bromide; removal of the benzyl protecting group furnished the diol **19** (Scheme 4). Final oxidation under Ley conditions[38] yielded the desired lactone **20**. Several primary alcohols from *P. rapae* also elicited an electroantennographic response. Secondary alcohols with C_{27} chains were active in both species. Because several positional isomers eluted together, it remained unclear whether all isomers or only a specific compound elicited the response. The most abundant component of these alcohols was 9-heptacosanol.



Scheme 4. a) $BnO(CH_2)_3MgBr$; b) H_2, Pd/C; c) TPAP.

A mating-competition bioassay was then performed to prove the activity of the male-specific compounds. The three major male-specific components were used in mixtures in the proportions they were found on the wings. In the case of *P. rapae* the three components **1**, **18**, and **23** (Figure 5) were used in a 21:12:1 mixture while in the case of *P. brassicae* a 16:10:31 mixture of **2**, **18**, and **23** was used. The behavioral assay demonstrated that application of the mixture to the forewings of freshly emerged males in an amount that was estimated to be double the total amount naturally present on wings of ten day old males significantly enhanced their mating success. Thus, freshly emerged males with relatively low amounts of the tested compounds on their wings competed with chemically augmented "supermales". Out of the 44 *P. rapae* males that mated, 30 had received the ferrulactone mixture (binomial test $z = 3.32$; $P < 0.001$). In the case of *P. brassicae*, out of the 26 males that mated, 20 had received the brassicalactone mixture ($z = 2.55$; $P < 0.005$). Observation of mating over time revealed that macrolide-treated males of both species mated faster and their competitiveness was maintained over the 150 min test period (Figure 6).

Discussion

The major difference in the compound bouquet of the wings of *P. rapae* and *P. brassicae* are the two male-specific macro-

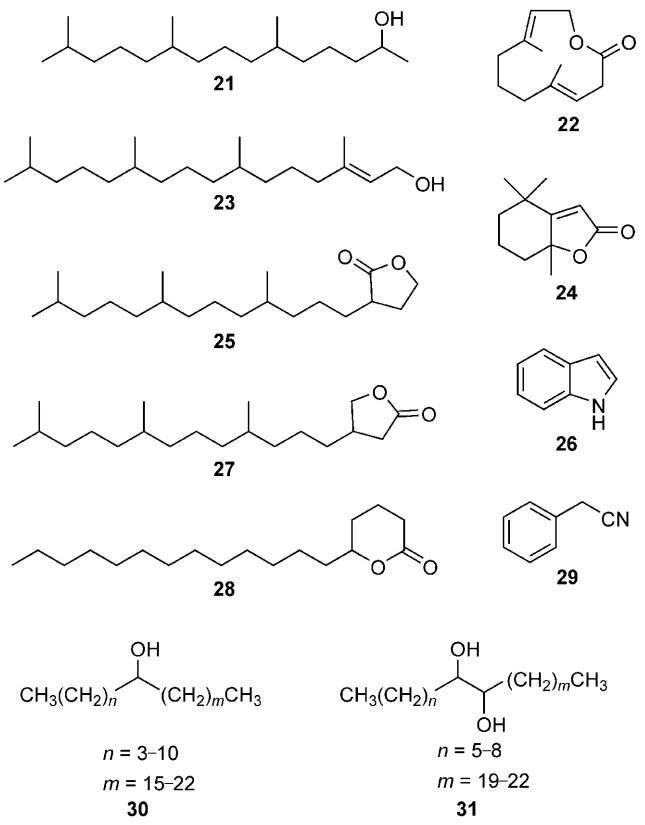


Figure 5. Structures of various metabolites from the wings of *Pieris* butterflies.

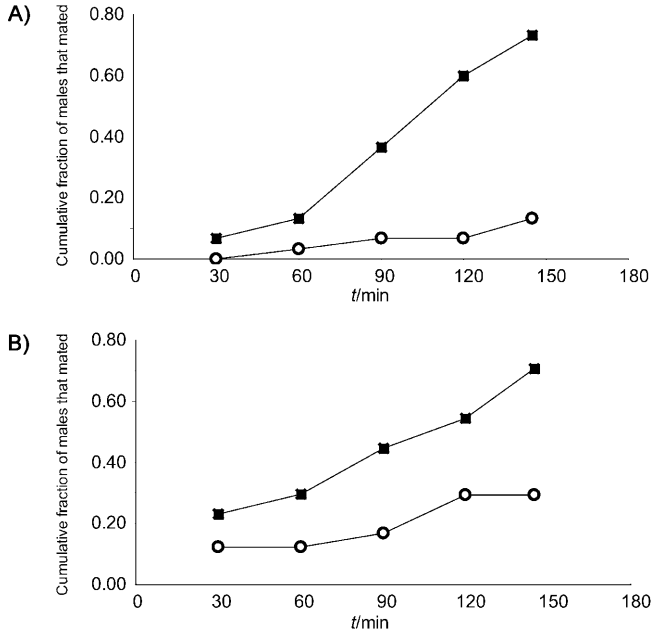


Figure 6. Cumulative mating success of freshly eclosed males without being supplemented with additional compounds (○) or with additional pheromone components (■). A) *Pieris brassicae*, B) *Pieris rapae*.

lides. While the known ferrulactone (**1**) occurs in the Small White, *P. rapae*, brassicalactone (**2**), which carries an additional isoprene unit, is used by the Large White. One of the unknown compounds reported earlier from *P. rapae crucivora*[20] is very likely **1**. The other components occur in both species and sexes, albeit in largely different concentrations. Hexahydrofarnesylacetone (**18**) has been reported several times from male scent organs of Lepidoptera,[35,39–41] but pheromonal activity has not been shown so far. In addition, it serves as a phagostimulant of Bermuda grass to larvae of the fall armyworm, *Spodoptera litoralis*.[42] The respective alcohol **21** is a male pheromone of the butterfly *Bicyclus anynana*,[43] while (*E*)-phytol (**23**) serves this function in males of the moth *Ephestia elutella*. It evokes female-courtship response in mixtures with 4-decanolide or 4-undecanolide.[44] Suspensolide (**22**) is a pheromone of fruit flies.[45] Long-chain secondary alcohols have been reported occasionally from the insect cuticle[46] or butterfly scent glands.[35] Electroantennographic activity has not been found for these compounds before. Tricosane was identified in wing extracts of male and female *P. rapae* and *P. brassicae*.[8] This compound and the other alkanes showing electroantennographic activity occur on all parts of the butterflies and are part of the epicuticular wax, contrary to **1** and **2** which are wing specific.[6]

The three-component blends consisting of **18**, **23**, and **1** or **2**, act similarly to those of other male Pierids (see the Introduction). While the compounds active in *C. philodice* are common hydrocarbons or long-chain esters,[14] the compounds reported here are of terpenoid origin. They seem to be degradation products of chlorophyll (**18**, **23**) or modified mono- or sesquiterpenes (**1**, **2**). The monoterpenes active in *P. napi* were not present.[8,11] The tested compounds function as typical butterfly aphrodisiac pheromones used by male butterflies in close vicinity to the females.[9] Females seem to reject or accept males during courtship depending on these cues, as described for *P. rapae*,[19] probably in a concentration-dependent manner. This behavior could be instrumental in avoiding heterospecific courtship and selecting the fittest partner. Whether the described activity in courtship is the sole function of the pheromone components remains to be elucidated.

Experimental Section

General remarks: ^{1}H and ^{13}C NMR spectra were recorded by using a Bruker DPX-400 (^{1}H 400 MHz, ^{13}C 100 MHz) instrument with $CDCl_3$ as solvent if not mentioned otherwise; the internal standard was tetramethylsilane (TMS). GC-MS analysis was performed with a Hewlett–Packard model 6890 gas chromatograph connected to a Hewlett–Packard model 5973 mass-selective detector. All reactions were carried out under nitrogen with oven-dried glassware and dry solvents were used. The chemicals were obtained from Sigma–Aldrich or Fluka and used without further treatment. All reactions were monitored by thin-layer chromatography (TLC) with Polygram SIL G/UV_{254} silica plates and viewed by use of heat-gun treatment with molybdatophosphoric acid (10%) in ethanol. For purification of the raw products column chromatography with Merck silica gel 60 (70–200 mesh) was used.

Biological material: *Pieris brassicae* and *P. rapae* butterflies were obtained from laboratory cultures reared on greenhouse-grown Brussels sprouts, *Brassica oleracea* L. var. *gemmifera* cv. Cyrus (Sluijs & Groot, Enkhuizen, The Netherlands), in climate controlled rooms

at 22±2, 50% relative humidity, with a 16:8 photo/scotoperiod. Male and female pupae were kept separately. After eclosion they were fed 10% sugar water for two weeks. Then the butterflies were frozen at −70 °C, the wings were separated from the body by using a razor blade and extracted first with pentane (500 μL; Merck, Suprasolv), then with CH_2Cl_2 (500 μL; Merck, Suprasolv) for 10 min each. The solvent was evaporated at ambient temperature to 20 μL final volume. These solutions were stored at −70 °C until analysis by GC-MS.

GC-EAD: Gas chromatography with electroantennographic detection was used to determine which of the volatile chemicals of the forewing extracts of *P. rapae* and *P. brassicae* were perceived by the male and female antennae. For each EAD experiment the tip of an excised antenna was cut off and the antenna was mounted between two glass-electrodes filled with insect ringer solution. The electrode at the antenna base was grounded via an Ag-AgCl wire and the electrode at the tip of the antenna was connected via an amplifier to a signal interface board (Syntech, Hilversum, The Netherlands) of a PC. One of the extracts was injected in splitless mode into a gas chromatograph HP6890 (Hewlett–Packard, Palo Alto, CA, USA) at 50 °C. After 1 min the split valve was opened and the temperature was raised by 10 °C min^{-1} to 310 °C. The GC was equipped with a DB5 capillary column (30 m×0.25 mm i.d., J&W Scientific) and a FID, and helium served as the carrier gas. The effluent was split with variable outlet splitter (SGE, Darmstadt, Germany). The split ratio FID/EAD was 1:3. The outlet for the EAD was placed in a cleaned and humidified airflow that was directed over the male or female antenna. The outlet was heated (310 °C) to avoid condensation of the effluent in the cooler airflow. EAD and FID signals were recorded simultaneously by using a PC that operated a GC-EAD program (Syntech, Hilversum, The Netherlands). A total of 70 GC-EAD runs were performed. GC-EAD active compounds were identified by GC-MS analysis.

Behavioral bioassay: A mating-competition assay was developed to assess the putative role of the macrolides as courtship sex pheromones. Freshly eclosed butterflies were collected from rearing cages within 4 h of eclosion, which ensured that mating had not yet occurred. Male and female butterflies were transferred to separate cages in a greenhouse, in which they had access to artificial flowers containing a solution of glucose (10%) in water. The next day male *P. rapae* butterflies were treated with hexane (2 μL) containing 0.2 μg of a 21:12:1 mixture of ferrulactone (**1**) hexahydrofarnesylacetone (**18**), and phytol (**23**), respectively. In bioassays with *P. brassicae*, males were treated with a 6:10:31 mixture (0.4 μg) of brassicalactone (**2**), **18**, and **23**, dissolved in hexane (2 μL). Control males received hexane (2 μL) only. Solutions or solvent were applied to the dorsal side of each of the two forewings. In one experiment the treated males, and in the following experiment the control males, were marked with a small red dot on the ventral side of their hindwing for recognition, by using a felt writer. After treatment, ten females were transferred to a new cage (dimensions 100×67×50 cm) in the greenhouse and ten treated males and ten control males were introduced into this cage simultaneously. The occurrence of copulas between females and either treated or control males was monitored every 15 min over a 2.5 h period in order to assess the time course of mating success. This assay was repeated seven times for *P. rapae* and three times for *P. brassicae*, and each experiment involved ten freshly eclosed females and 20 males.

(2*E*,6*E*)-10,11-Dihydroxy-3,7,11-trimethyl-2,6-dodecadienyl acetate: Farnesyl acetate (**3**, 4.57 g, 17.31 mmol) and *N*-methylmorpholin-*N*-oxide (3.3 g, 24.21 mmol) were dissolved in acetone (4 mL) and a mixture of H_2O (8 mL), OsO_4 (53 mg, 0.21 mmol) and *tert*-butanol (1 mL) was added. After being stirred for 24 h at 5 °C the reaction mixture was allowed to warm to room temperature and quenched with $NaHSO_3$ (1.36 g) and stirred for 1 h.[48] Water was added and the mixture was extracted several times with diethyl ether. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude diol was purified by flash chromatography (pentane/diethyl ether, 1:2); yield: 1.07 g (3.59 mmol, 20%); NMR spectroscopy data were identical to those published previously.[49] EI-MS: *m/z* (%): 220 [$M^+ - 2H_2O - CH_3CO$] (0.4), 179 (3), 161 (5), 143 (8), 135 (10), 121 (10), 107 (11), 93 (32), 81 (50), 68 (38), 59 (85), 55 (21), 43 (100).

(2*E*,6*E*)-3,7-Dimethyl-10-oxo-2,6-decadienyl acetate (4): A solution of diol **3** (1.07 g, 3.59 mmol) in 1,4-dioxane (9 mL) and H_2O (6 mL) was cooled in an ice bath. Sodium periodate (0.86 g, 4.06 mmol) was added and the mixture was stirred for 1.5 h at 0–5 °C.[50] The solid was filtered and the residue was washed three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash chromatography on silica (pentane/diethyl ether, 5:1); yield: 0.64 g (2.69 mmol, 75%). The ^{1}H NMR and MS data were identical to those already described.[51] ^{13}C NMR: δ = 16.0 (q), 16.3 (q), 21.0 (q), 26.0 (t), 31.7 (t), 39.2 (t), 42.0 (t), 61.3 (t), 118.5 (d), 124.7 (d), 133.4 (s), 141.7 (s), 171.0 (s), 202.5 ppm (d).

(2*E*,6*E*)-3,7-Dimethyl-2,6,10-undecatrienyl acetate (5): Methyltriphenylphosphonium bromide (1.14 g, 3.2 mmol) was dissolved in abs. 1,2-dimethoxyethane (DME, 1.12 mL) and stirred at −78 °C. A solution of *n*BuLi (1.6 M in hexane, 2.4 mmol, 1.58 mL) was added dropwise under a nitrogen atmosphere, and stirred for 1 h at 0 °C. Then the mixture was cooled again to −78 °C and a solution of **4** (0.6 g, 2.52 mmol) in abs. DME (2.5 mL) was added. The reaction mixture was allowed to warm to room temperature, stirred overnight, and quenched with water. Extraction with diethyl ether (three times) followed. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (pentane/diethyl ether, 40:1); yield: 0.54 g (2.26 mmol, 70.5%). ^{1}H NMR: δ = 1.60 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 2.05 (s, 3H, CH_3CO), 2.04–2.17 (m, 8H, CH_2), 4.58 (d, J = 7.1 Hz, 2H, CH_2OAc), 4.92–5.03 (m, 2H, CH_2=CH), 5.10 (dt, J = 1.2, 6.9 Hz, 1H, CH), 5.34 (dt, J = 1.2, 7.1 Hz, 1H, CH), 5.8 ppm (m, 1H, CH); ^{13}C NMR: δ = 16.0 (q), 16.4 (q), 21.0 (q), 26.1 (t), 32.3 (t), 39.0 (t), 39.5 (t), 61.4 (t), 114.3 (t), 118.3 (d), 123.9 (d), 138.7 (d), 134.9 (s), 142.1 (s), 171.1 ppm (s); EI-MS: *m/z* (%): 177 [M^+−OAc] (0.1), 161 (6), 135 (7), 119 (7), 93 (40), 81 (30), 67 (100), 55 (43), 43 (87).

(2*E*,6*E*)-3,7-Dimethyl-2,6,10-undecatrien-1-ol (6): A solution of **5** (0.43 g, 1.82 mmol) in abs. diethyl ether (2 mL) was added dropwise to a mixture of $LiAlH_4$ (137 mg, 3.60 mmol) and abs. diethyl ether (25 mL) under a nitrogen atmosphere. The mixture was heated to reflux for 1 h and then cooled to room temperature. Water and sulfuric acid (10% in water) were added until the $Al(OH)_3$ residue disappeared. The aqueous layer was separated and extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and the solvent was evaporated under reduced pressure. Then the crude product was purified by flash chromatography on silica with pentane/diethyl ether (2:1); yield: 310 mg (1.60 mmol, 88%). ^{1}H NMR: δ = 1.60 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.00–2.22 (m, 8H, CH_2), 4.15 (d, J = 6.9 Hz, 2H, CH_2OH), 4.92–5.05 (m, 2H, CH_2=CH), 5.12 (dt, J = 1.2, 6.9 Hz, 1H, CH), 5.42 (dt, J = 1.2, 6.9 Hz, 1H, CH), 5.79 ppm (m, 1H, CH); ^{13}C NMR: δ = 16.3 (q), 16.6 (q), 26.5 (t), 32.6 (t), 39.3 (t), 39.8 (t), 59.7 (t), 114.6 (t), 123.7 (d), 124.4 (d), 139.0 (d), 135.1 (s), 140.0 ppm (s); EI-MS: *m/z*

(%): 194 (0.1), 179 (2), 163 (6), 121 (8), 109 (23), 93 (27), 81 (34), 67 (100), 55 (52), 41 (85).

(2*E*,6*E*)-3,7-Dimethyl-2,6,10-undecatrienyl 4-methyl-4-pentenoate (9): According to the procedure of Baumhof et al.,[26] a solution of **6** (0.2 g, 1.22 mmol), ethyl 4-methyl-4-pentenoate (**8**; 0.12 g, 0.84 mmol)[52] and dibutyl tinoxide (20.8 mg, 0.084 mmol) was heated to 80 °C for 24 h. The mixture was allowed to cool to room temperature and hydrolyzed with saturated $NaHCO_3$ solution. The aqueous phase was extracted several times with diethyl ether and the combined organic layers were dried with $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography on silica (pentane/diethyl ether, 20:1); yield: 90 mg (0.31 mmol, 37%). ^{1}H NMR ($CDCl_3$ 400 MHz): δ = 1.60 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 2.03–2.48 (m, 12H, CH_2), 4.58 (d, J = 7.1 Hz, 2H, CH_2), 4.69 (s, 1H, CH_2), 4.74 (s, 1H, CH_2), 4.92–5.02 (m, 2H, CH_2), 5.11 (dt, J = 6.9, 1.2 Hz, 1H, CH), 5.34 (dt, J = 7.1, 1.2 Hz, 1H, CH), 5.69 ppm (m, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 16.0 (q), 16.4 (q), 22.5 (q), 26.1 (t), 32.3 (t), 32.7 (t), 32.74 (t), 39.0 (t), 39.5 (t), 61.3 (t), 110.3 (t), 114.2 (t), 118.5 (d), 124.0 (d), 134.9 (s), 138.7 (d), 142.1 (s), 144.1 (s), 173.3 ppm (s); EI-MS: *m/z* (%): 249 [M^+−41] (0.8), 176 (2), 161 (6), 147 (5), 121 (10), 109 (27), 93 (46), 81 (36), 67 (100), 55 (54), 41 (77).

(4*E*,8*E*,12*E*)- and (4*Z*,8*E*,12*E*)-4,8,12-Trimethyl-4,8,12-tetradecatrien-14-olide (brassicalactone, (*E*,*E*,*E*)- and (*Z*,*E*,*E*)-2): A solution of ester **9** (70 mg, 0.24 mmol) in abs. CH_2Cl_2 (10 mL) was added dropwise to a solution of Grubbs catalyst of the second generation, (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro-(*o*-isopropoxyphenylmethylene)ruthenium[27] (12 mg, 0.015 mmol, 6 mol%) in abs. CH_2Cl_2 (240 mL, 1 mM) under a nitrogen atmosphere. The reaction mixture was stirred at 40 °C for 7 h and the mixture was filtered through silica to remove the catalyst. The filtrate was evaporated under reduced pressure. The *E*,*E*/*Z*,*E*,*E* ratio of the crude product was 3:1. The product was purified by flash chromatography on silica (pentane/diethyl ether, 60:1); yield: 32 mg (0.12 mmol, 50%); (*E*,*E*,*E*)-**2**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.56 (s, 3H, CH), 1.61 (s, 3H, CH), 1.71 (s, 3H, CH), 2.04–2.07 (m, 2H, CH), 2.10–2.15 (m, 4H, CH), 2.17–2.22 (m, 2H, CH), 2.28–2.30 (m, 2H, CH), 2.40–2.43 (m, 2H, CH), 4.59 (d, J = 7.3 Hz, 2H, CH), 4.92–4.99 (m, 2H, CH), 5.23–5.30 ppm (m, J = 7.3, 1.3 Hz, 1H, CH); ^{13}C NMR (CDCl, 100 MHz): δ = 16.2 (q), 16.3 (q), 16.7 (q), 24.3 (t), 24.4 (t), 32.3 (t), 33.8 (t), 60.8 (t), 119.5 (d), 123.8 (d), 124.2 (d), 132.9 (s), 134.2 (s), 140.4 (s), 172.7 ppm (s); EI-MS: *m/z* (%): 262 (2), 247 (1), 194 (4), 161 (5), 135 (6), 127 (14), 107 (13), 93 (29), 79 (29), 68 (100), 67 (88), 53 (51), 41 (57).

(*E*)-3-Methyl-6-oxo-2-hexenyl acetate (11): The preparation of compound **11** was performed analogously to that of **4** starting from geranyl acetate; yield: 2.1 g (14.13 mmol, 47% over two steps). The NMR spectroscopy data were identical to those already described.[53] EI-MS: *m/z* (%): 127 [M^+−CH_3CO] (2), 126 (18), 110 (24), 95 (7), 84 (56), 81 (32), 67 (29), 55 (21), 43 (100), 41 (29), 39 (23).

(*E*)-3-Methyl-2,6-heptadienyl acetate: This compound was prepared analogously to compound **5** by a Wittig reaction from **11**: yield: 60%. ^{1}H NMR: δ = 1.71 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.01–2.23 (m, 4H, CH_2), 4.59 (d, J = 7 Hz, 2H, CH_2), 5.00 (m, 2H, CH_2), 5.35 (t, J = 7 Hz, 1H, CH), 5.80 ppm (m, 1H, CH); ^{13}C NMR: δ = 16.4 (q), 21.0 (q), 31.9 (t), 38.8 (t), 61.3 (t), 114.7 (t), 118.7 (d), 138.0 (s), 138.1 (d), 171.0 ppm (s); EI-MS: *m/z* (%): 127 [M^+−41] (1), 108 (13), 93 (49), 91 (11), 79 (21), 67 (19), 55 (14), 53 (12), 43 (100), 41 (29), 39 (25).

(*E*)-3-Methyl-2,6-heptadien-1-ol (12): This compound was prepared analogously to compound **6** by reduction of the ester described in the previous section; yield: 92%. ^{1}H NMR: δ = 1.68 (s, 3H, CH_3), 2.09–2.22 (m, 4H, CH_2), 4.15 (d, J = 7 Hz, 2H, CH_2), 4.99 (m, 2H, CH_2), 5.42 (t, J = 7 Hz, 1H, CH), 5.80 ppm (m, 1H, CH); ^{13}C NMR: δ = 16.2 (q), 32.0 (t), 38.8 (t), 59.3 (t), 114.6 (t), 123.8 (d), 138.3 (d), 138.6 ppm (s); EI-MS: *m/z* (%): 108 [M^+−H_2O] (10), 95 (32), 93 (37), 84 (12), 79 (16), 71 (39), 67 (29), 57 (31), 43 (23), 41 (100), 39 (62).

Methyl (*E*)-4,8-dimethyl-4,8-nonadienoate (15): According to the procedure of O'Conner and Just,[31] PDC (9.34 g, 24.90 mmol) was added to a solution of aldehyde **14**[30] (690 mg, 4.15 mmol) in methanol (1 mL) and abs. DMF (21 mL) and stirred for 20 h under a nitrogen atmosphere. The reaction mixture was poured into pentane (100 mL) and dist. H_2O (50 mL). The aqueous phase was separated, extracted three times with pentane and dried with $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (pentane/diethyl ether, 20:1); yield: 490 mg (2.04 mmol, 49%). ^{1}H NMR ($CDCl_3$ 400 MHz): δ = 1.62 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 2.02–2.10 (m, 2H, CH_2), 2.11–2.15 (m, 2H, CH_2), 2.28–2.32 (m, 2H, CH_2), 2.39–2.43 (m, 2H, CH_2), 3.66 (s, 3H, CH_3), 4.67 (s, 1H, CH_2), 4.70 (s, 1H, CH_2), 5.15 ppm (dt, J = 8.0, 1.3 Hz, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 15.9 (q), 22.4 (q), 26.1 (t), 33.0 (t), 34.6 (t), 37.6 (t), 51.4 (q), 109.9 (t), 124.9 (d), 133.4 (s), 145.6 (s), 173.8 ppm (s); EI-MS: *m/z* (%): 196 (0.3), 181 (0.4), 165 (3), 141 (7), 123 (4), 109 (32), 99 (32), 81 (100), 67 (23), 55 (16), 41 (19).

(*E*)-3-Methyl-2,6-heptadienyl (*E*)-4,8-dimethyl-4,8-nonadienoate (16): This ester was prepared by transesterification as described for **9**; yield: 32%. ^{1}H NMR ($CDCl_3$ 400 MHz): δ = 1.62 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.99–2.42 (m, 12H, CH_2), 4.58 (d, J = 7.0 Hz, 2H, CH_2), 4.67 (s, 1H, CH_2), 4.70 (s, 1H, CH_2), 4.94–5.04 (m, 2H, CH_2), 5.15 (dt, J = 6.8, 1.2 Hz, 1H, CH), 5.35 (t, J = 7.0 Hz, 1H, CH), 5.74–5.84 ppm (m, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 15.9 (q), 16.4 (q), 22.3 (q), 26.1 (t), 31.9 (t), 33.3 (t), 34.6 (t), 37.6 (t), 38.8 (t), 61.1 (t), 109.9 (t), 114.7 (t), 118.8 (d), 124.8 (d), 133.5 (s), 138.0 (d), 141.4 (s), 145.6 (s), 173.4 ppm (s); EI-MS: *m/z* (%): 249 [M^+−allyl] (0.2), 181 (24), 163 (8), 135 (3), 127 (22), 121 (33), 109 (50), 93 (32), 81 (95), 67 (100), 55 (73), 41 (55), 39 (25).

(4*E*,8*E*,12*E*)- and (4*E*,8*Z*,12*E*)-4,8,12-Trimethyl-4,8,12-tetradecatrien-14-olide (brassicalactone, (*E*,*E*,*E*)- and (*E*,*Z*,*E*)-2): The RCM was performed as described above, but by starting from **16**. A 5:1 mixture of (*E*,*E*,*E*)- and (*E*,*Z*,*E*)-**2** was formed. Pure (*E*,*E*,*E*)-**2** was isolated by column chromatography; yield: 42%.

6,10,14-Trimethyl-2-pentadecanone (18): Farnesylacetone (3 g, 11.43 mmol) was hydrogenated on Pd/C (0.2 g) in methanol (6 mL) under a pressure of 6 MPa in an autoclave for 3 h at 100 °C. The crude product was purified by flash chromatography on silica gel (pentane/diethyl ether, 40:1); yield: 49% (1.5 g, 5.6 mmol). NMR spectroscopy and MS data were identical to those already described.[54,55]

Benzyl 4-hydroxy-4,8,12,16-tetramethylheptadecyl ether: Benzyl 3-bromopropyl ether (1 g, 4.37 mmol) was treated with Mg (117 mg, 4.82 mmol) in abs. diethyl ether (5 mL). The resulting Grignard reagent was added dropwise to a solution of **18** (458 mg, 1.71 mmol) in abs. diethyl ether (5 mL) at 0 °C and stirred, overnight. Then crushed ice and sat. NH_4Cl were added to the mixture. The aqueous layer was extracted three times with diethyl ether. The combined organic phases were dried with $MgSO_4$. The crude product was purified by flash chromatography on silica (pentane/diethyl ether, 2:1); yield: 98% (700 mg, 1.67 mmol). ^{1}H NMR ($CDCl_3$,

400 MHz): $\delta = 0.83–0.88$ (m, 12H, CH), 1.16 (s, 3H, CH), 1.05–1.76 (m, 25H, CH, CH), 3.50 (t, 2H, CH, $J = 6.2$ Hz), 4.51 (s, 2H, CH), 4.69 (s, 1H, OH), 7.32 ppm (m, 5H, CH); ^{13}C NMR (CDCl, 100 MHz): $\delta =$ 19.64 (q), 19.67 (q), 19.7 (q), 19.74 (q), 21.4 (t), 22.6 (q), 22.7 (q), 24.3 (t), 24.5 (t), 24.9 (t), 26.9 (q), 26.93 (q), 28.0 (d), 32.8 (d), 37.3 (t), 37.46 (t), 37.5 (t), 37.8 (t), 38.71 (t), 38.74 (t), 39.43 (t), 42.5 (t), 71.0 (t), 72.3 (s), 73.0 (t), 127.0 (d), 127.6 (d), 127.7 (d), 128.4 (d), 138.4 ppm (s); EI-MS (70 eV): *m/z* (%): 403 (0.3) [$M^+ - 15$], 309 (3), 295 (2), 194 (3), 193 (20), 175 (2), 151 (19), 150 (2), 111 (6), 104 (7), 92 (11), 91 (100), 85 (39), 71 (12), 69 (10), 57 (15), 55 (10), 43 (17).

4,8,12,16-Tetramethylheptadecane-1,4-diol (19): The benzyl ether was cleaved according to the procedure of Kang et al.;[56] yield: 93%. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.84–0.87$ (m, 12H, CH), 1.01–1.70 (m, 25H, CH, CH), 1.19 (s, 3H, CH), 1.90 (s, 2H, OH), 3.67 ppm (t, $J = 6.0$ Hz, 2H, CH); ^{13}C NMR (CDCl, 100 MHz): $\delta = 19.63$ (q), 19.67 (q), 21.5 (t), 22.6 (q), 22.7 (q), 24.5 (t), 24.8 (t), 24.81 (t), 26.9 (q), 27.1 (t), 28.0 (d), 32.8 (d), 37.3 (t), 37.4 (t), 37.44 (t), 37.6 (t), 37.7 (t), 38.4 (t), 38.5 (t), 39.4 (t), 42.6 (t), 63.4 (t), 72.6 ppm (s); EI-MS (70 eV): *m/z* (%): 313 (1) [$M^+ - 15$], 295 (2), 269 (7), 139 (2), 125 (5), 113 (2), 111 (8), 109 (3), 103 (51), 97 (11), 85 (100), 83 (12), 81 (5), 71 (16), 67 (4), 58 (6), 57 (21), 55 (17), 43 (46), 41 (12).

4,8,12,16-Tetramethylheptadecan-4-olide (20): A suspension of 1,4-diol (420 mg, 1.28 mmol), NMO (260 mg, 1.92 mmol) and powdered molecular sieve (4 Å, 670 mg) was prepared. Tetrapropylammonium perruthenate (22.5 mg, 0.064 mmol) and abs. dichloromethane (4 mL) were added under a nitrogen atmosphere and the solution was stirred for 1 h.[38] The solid was filtered off and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica (pentane/diethyl ether, 2:1) as a mixture of diastereomers; yield: 84% (350 mg, 1.08 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.84–0.87$ (m, 12H, CH), 1.01–1.71 (m, 21H, CH, CH), 1.39 (s, 3H, CH), 1.93–2.01 (m, 1H, CH), 2.07–2.13 (m, 1H, CH), 2.53–2.67 ppm (m, 2H, CH); ^{13}C NMR (CDCl, 100 MHz): $\delta = 19.5$ (q), 19.57 (q), 19.6 (q), 19.63 (q), 19.66 (q), 19.7 (q), 21.4 (t), 22.6 (q), 22.7 (q), 24.4 (t), 24.79 (t), 24.8 (t), 25.62 (q), 25.64 (q), 28.0 (d), 29.2 (t), 32.67 (d), 32.7 (d), 32.76 (d), 32.78 (d), 32.97 (t), 33.0 (t), 37.21 (t), 37.27 (t), 37.32 (t), 37.35 (t), 37.37 (t), 37.39 (t), 39.4 (t), 41.3 (t), 86.9 (s), 176.8 ppm (s); EI-MS (70 eV): *m/z* (%): 324 (1), 306 (1), 254 (1), 196 (3), 184 (2), 166 (2), 151 (4), 141 (2), 140 (3), 127 (6), 126 (13), 125 (6), 114 (15), 111 (12), 100 (6), 99 (100), 97 (11), 95 (5), 85 (7), 83 (13), 81 (6), 71 (17), 70 (13), 69 (22), 68 (4), 67 (4), 57 (22), 56 (13), 55 (23), 43 (33), 41 (16), 39 (2).

(4*E*,8*E*)-4,8-Dimethyl-4,8-decadien-10-olide (ferrulactone, 1): According to the procedure of Shiina et al.,[34] a solution of the seco acid **17** (200 mg, 0.94 mmol) in abs. dichloromethane (40 mL) was added by a syringe pump over a period of 16 h at room temperature to a mixture of 2-methyl-6-nitrobenzoic anhydride (MNBA; 391 mg, 1.14 mmol) and 4-(dimethylamino)pyridine (DMAP; 277 mg, 2.26 mmol) in abs. dichloromethane (500 mL). Then the mixture was stirred for another hour. The reaction mixture was concentrated to 20 mL and subsequently washed with a saturated solution of $NaHCO_3$, H_2O, and brine. The organic layer was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica (pentane/diethyl ether, 40:1); yield: 24% (43 mg, 0.22 mmol). NMR and MS data were identical to those already described.[32]

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