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Composition of lipids from sunflower pollen (*Helianthus annuus*)

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Abstract

The contents of the pollen lipids of the sunflower *Helianthus annuus* are described. The major component is the *seco*-triterpene helianyl octanoate, followed by new β-diketones as second major group of compounds. They exhibit a shorter chain length and often other positions of the functional group compared to already known β-diketones. Of particular note are the 1-phenyl-β-diketones, not previously reported from nature. Further lipid classes present are related hydroxyketones and diols. Interestingly, new β-dioxoalkanoic acids are present in the extracts, which most likely are biogenetic precursors of the diketones. Additionally, we investigated the composition of the pollen coat which resembles the total extract, but lacks the dioxoalkanoic acids and certain estolides.

Keywords: *Helianthus annuus*; Compositae; Sunflower; Pollen; Composition; Lipids; Triterpenes; Alkanediones; Alkanediols; Hydroxyalkanones; Helianyl octanoate; Dioxoalkanoic acids

1. Introduction

The sunflower moth, *Homeosoma electellum*, lays its eggs onto the pollen of the sunflower, *Helianthus annuus*. Oviposition, as well as different aspects of female reproductive biology are stimulated by chemical cues originating from the pollen (Delisle et al., 1989; McNeil and Delisle, 1989). For investigation of the chemical basis of this phenomenon, we have been analyzing hexane extracts of sunflower pollen in an effort to identify the compounds that induce egg laying behavior in this moth species. Recently, a *seco*-triterpene alcohol, helianol (Akihisa et al., 1996; revised structure in Akihisa et al., 1998b), and C_{21}, C_{23}, C_{25}, and C_{27}–C_{35} *syn*-6,8-alkanediols (Akihisa et al., 1998a) were isolated from methanolic extracts of tubular and ligulate *H. annuus* flowers. In the present paper, we report on the identity of new types of lipids in sunflower pollen, as well as on the composition of the pollen grain coat.

2. Results and discussion

Helianthus annuus pollen was extracted with hexane by ultrasound or with an soxhlet apparatus. The resulting, very similar, extracts were subjected to GC–MS analysis and separated by column chromatography on silica. The lipids of the extract comprised of several compound classes: triterpenes, β-diketones, hydroxyketones, 1,3-alkanediols, alkanes, carboxylic acids, esters, and β-dioxoalkanoic acids. These classes will be discussed in the following sections. In addition, the lipid composition of the pollen coat was determined. Also, methylation, silylation, and transesterification procedures were performed with the extracts to help in the GC–MS identification of the constituents. More than 200 compounds could be identified by these methods. Several representative compounds were synthesized to proof assignments.

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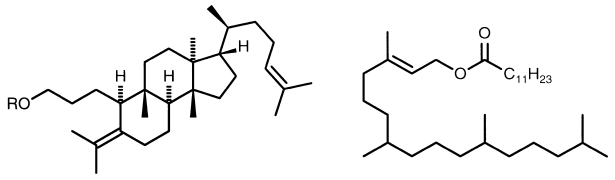


1: R = H
2: R = COC_7H_{15}

3

Scheme 1.

2.1. Terpenes

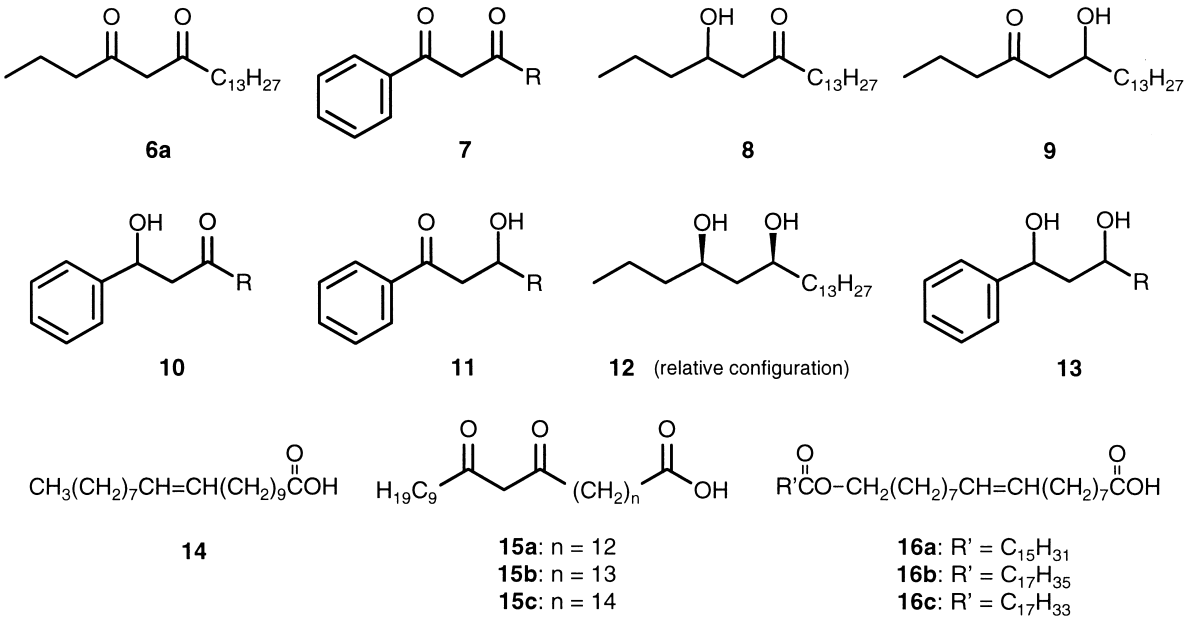
The major component of the whole extract exhibited a molecular ion at m/z = 554 in its mass spectrum. Characteristic ions at m/z = 127 and 145 pointed to an octanoate ester. This ester was cleaved with sodium methanoate, as it could not be completely separated from accompanying β-diketones. The alcohol part was isolated by column chromatography and subjected to extensive NMR analysis. It proved to be identical with helianol (**1**), recently identified in tubular flowers of *H. annuus* (Akihisa et al., 1996). Esterification of **1** with octanoyl chloride re-established the original compound and proved that helianyl octanoate (**2**) is the major naturally occurring compound. It is accompanied by small amounts of the corresponding hexanoate and of the free alcohol **1**, as well as trace amounts of the decanoate and dodecanoate. Akihisa et al. saponified their extract prior to analysis; it seems likely that even in whole tubular flowers most of **1** occurs in an esterified form. Finally, an isomer of **2** is present in trace amounts, exhibiting a very similar mass spectrum, but eluting earlier in GC. Other terpenes identified were minor amounts of phytyl dodecanoate (**3**), ac-

Table 1
β-Diketones identified in lipid extracts of *Helianthus annuus* pollen[a]

Chain length	4,6	5,7	6,8	7,9	8,10	10,12	11,13	12,14	Phenyl[b]
C_{18}									x
C_{19}	xx								
	6a								
C_{20}	x	x	xx						
C_{21}	xx		xxx						x
	6b		**6c**						
C_{22}	x	x	xx	x					xxx
									7a
C_{23}	xxx		xx						xx
	6d		**6e**						
C_{24}	xxx	x	xx	x		x			xx
									7b
C_{25}	xxx		xxx	x		xx			x
	6f		**6g**						
C_{26}	xx	x	xx	x		x	x		xx
C_{27}	xx		xx		x	xx		x	x
C_{28}		x	x	x	x	x	x	x	x
C_{29}			x		x	xx		xx	
						6h			
C_{30}				x		x	x	x	
C_{31}					x	xx		x	
						6i			
C_{32}						x	x	x	
C_{33}						x		x	

[a] xxx: Major component; xx: minor component; x: trace component.

[b] Chain length corresponds to all carbon atoms of the molecule, e.g. C_{18} denotes 1-phenyl-1,3-dodecanedione.



Scheme 2.

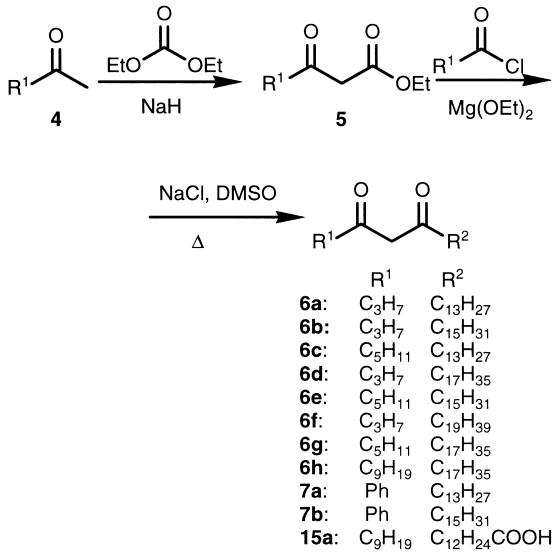


Fig. 1. Synthesis of β-diketones identified in sunflower pollen.

companied by trace amounts of the respective tetradecanoate, hexadecanoate, and free phytol. Additionally, trace amounts of β-sitosterol, stigmasterol, (iso)fucosterol, neophytadiene, kaur-16-ene, and kaur-16-ene acid were identified by GC–MS.

2.2. β-Diketones

After **2**, β-diketones are the most prominent class of lipid compounds of the pollen, showing characteristic ions at m/z = 43, 85, and 100 in their mass spectra. The position of the diketo group in the chain can be unequivocally deduced from ions arising by α- and γ-cleavage next to the carbonyl groups. They are accompanied by McLafferty rearrangement type ions, which subsequently lose water and acetone (Trka and Streibl, 1973; Hamilton, 1995). Nine different positional types of β-diketones occur in the pollen lipids

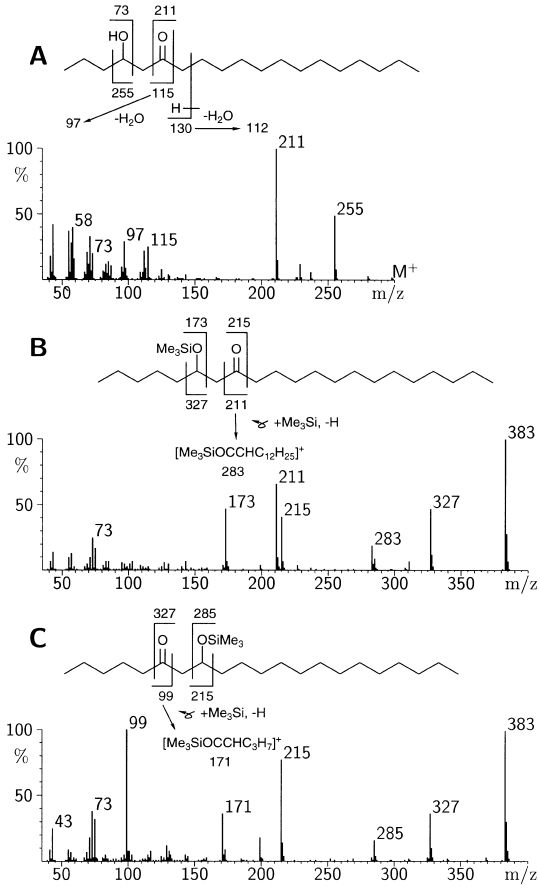


Fig. 2. Mass spectra and fragmentation scheme of A: 4-hydroxy-6-nonadecanone (**8**), B: TMS-ether of 6-hydroxy-8-henicosanone, C: TMS-ether of 8-hydroxy-6-henicosanone.

(see Table 1). Major constituents are the 4,6-diones and 6,8-diones and, to a lesser degree, the 10,12-diones. In addition, previously unknown 1-phenylalkane-1,3-diones were identified. Their mass spectra are characterized by intense ions at m/z = 105 $[PhCO]^+$, 120 $[PhCOCH_3]^{+\cdot}$, 147 $[PhCOCH_2CO]^+$, and 162 $[PhCOCH_2COCH_3]^{+\cdot}$. The chain length can be deduced

Table 2
Hydroxyketones identified in lipid extracts of *Helianthus annuus* pollen[a]

Chain length	4HO,6O	6HO,4O	5HO,7O	6HO,8O	8HO,6O	Ph3HO1O[b]	Ph1HO3O[b]
C_{19}	xx	x					
	8	**9**					
C_{20}	x		x				
C_{21}	xx	x		x	x		
C_{22}	x		x	x	x	x	x
C_{23}	xx	x		x	x	x	
C_{24}	x		x			x	x
C_{25}	x	x		x			
C_{26}				x		x	
C_{27}	x			x		x	

[a] xx: Minor component; x: trace component.
[b] Chain length corresponds to all carbon atoms of the molecule, e.g. C_{22} Ph3HO1O denotes 3-hydroxy-1-phenyl-1-hexadecanone.

by the M^+ and M^+–18 ions. The major aromatic diketone is 1-phenylhexadecane-1,3-dione (**7a**).

The position of the diketo group and the chain length can be readily established from the mass spectra obtained by capillary GC–MS, even when present in low amounts and complex mixtures. There is no need to perform ^{13}C-NMR experiments (Tulloch, 1985) or silylation (Tulloch and Hogge, 1978), as this actually leads to more complex chromatograms, due to the formation of four different isomers by monosilylation. In addition, disilylation can occur, which further complicates analysis.

The major diketones were synthesized (Fig. 1) to confirm our assignments and for future biological testing. An appropriate methyl ketone **4** was converted into the β-ketoester **5** by alkylation with diethyl carbonate (Cadman et al., 1995). Subsequent acylation with an acyl chloride (Reynolds and Hauser, 1963), followed by Krapcho decarbethoxylation (Krapcho, 1982) furnished the β-diketones **6** or **7**. The natural compounds proved to be identical to the synthetic products.

To our knowledge, most of the diketones reported here are of considerably shorter chain length than those from other natural sources (Bianchi, 1995). Also, the position of the oxygen atoms, very near to one end of the chain in some cases, is unique.

2.3. β-Hydroxyketones

The β-diketones are accompanied by small amounts of β-ketols. The most abundant is 4-hydroxy-6-nonadecanone (**8**), readily identified by its mass spectrum (Fig. 2). The identification of this compound was verified by comparison with a synthetic sample (Schulz and Arsene, 2000). The better elution properties of the silylated compounds allow us to identify 31 ketols (see Table 2). The hydroxy group is preferentially located near the short chain end of the molecule, but the regioisomers like **9** also occur in trace amounts. A migration of the silyl-group to the keto-group with hydrogen abstraction explains the diagnostic $[Me_3SiOC_nH_{2n-2}]^+$ ions in the spectra. This type of rearrangement has been observed earlier in spectra of other silylated compounds containing a carbonyl group (e.g. Draffan et al., 1968).

The respective 1-hydroxy-1-phenyl-3-alkanones **10** could also be identified based on the mass spectra of their silyl ethers. An α-cleavage next to the carbonyl and silyloxy groups leads to diagnostic fragments for the 1-hydroxy-1-phenyl-3-alkanones at m/z = 221 and 179, still containing the phenyl group. The length of the alkyl chain can be determined by the M^+–15 ion and the above stated silyl-rearrangement ion (acyl + 72). The mass spectra of the positional isomers, 3-hydroxy-1-phenyl-1-alkanones **11**, are dominated by the ion m/z = 105 (rearrangement ion at m/z = 177) and the phenyl containing ion m/z = 221 [$PhCOCH_2$-CHOTMS]$^+$. The alkyl chain length can be deduced from the M^+–15 ion and the α-cleavage next to the silyloxy group.

The ketols are accompanied by trace amounts of enones. These may be artifacts, formed in the injection port of the gas chromatograph, because silylation lowers their concentration, as does the use of a PTV-injector or on-column-injector instead of a splitless injector. To the best of our knowledge, 1,3-ketols have so far only been identified as wax constituents of *Brassica* (Holloway and Brown, 1977a; Holloway et al., 1977b) and *Eucalyptus* (Osawa and Namiki, 1985). The ketols described here have not been reported from nature before.

2.4. 1,3-Diols

Complete reduction of the β-diketo group is found

Table 3
Diols identified in lipid extracts of *Helianthus annuus* pollen[a]

Chain length	4,6	5,7	6,8	10,12	Phenyl-1,3[b]
C_{19}	xxx				
	12				
C_{20}	x	x			
C_{21}	xx		x		
C_{22}	x	x	x		x
C_{23}	xx		x		
C_{24}	x	x	x		x
C_{25}	x		x	x	

[a] xxx: Major component; xx: minor component; x: trace component.

[b] Chain length corresponds to all carbon atoms of the molecule, e.g. C_{22} denotes 1-phenyl-1,3-hexadecanediol.

Table 4
β-Dioxoalkanoic acids identified in lipid extracts of *Helianthus annuus* pollen[a]

Chain length	ω-6,8	ω-10,12	ω-12,14
C_{24}		x	
C_{25}	x	xx	
		15a	
C_{26}	x	xxx	
		15b	
C_{27}		xxx	x
		15c	
C_{28}		x	x
		15d	
C_{29}		x	x

[a] xxx: major components; xx: minor component; x: trace component. Position of keto groups relative to the alkyl end are described as e.g. ω-10,12.

in the 1,3-diols, which are important constituents of the pollen lipids. The structure can be deduced from the mass spectra, preferably from those of the disilyl ethers, which have superior elution characteristics in gas chromatography. The spectra correlated well to those described in the literature for other internal 1,3-diols (Akihisa et al., 1994; Hamilton, 1995). To confirm identifications, 4,6-nonadecanediol (**12**) was synthesized starting from 4,6-nonadecanedione by reduction with $NaBH_4$. The synthetic mixture of *syn*- and *anti*-diols are separable by GC as silyl ethers, showing the presence of only one diastereomer in the extracts. Natural 4,6-nonadecanediol was isolated. Its ^{13}C-NMR spectrum showed a sum of chemical shifts of C-4 and C-6 of 146.2 ppm, which, compared to the value of the unnatural isomer (138.7 ppm), allowed the assignment of a *syn*-diol structure (Hoffmann and Weidmann, 1985). This assignment was verified by stereoselective synthesis (Schulz and Arsene, 2000). The major diol class present in the lipids is the 4,6-diol group (see Table 3), accompanied by small amounts of 5,7-, 6,8-, 10,12-, and 1-phenyl-1,3-diols **13**. As the latter compounds occurred in trace amounts, they could only be identified by the mass spectra of the respective disilyl ethers. The spectra were characterized by a base peak at m/z = 179 $[PhCHOSiMe_3]^+$, accompanied by ions at 205 $[PhCHOSiMe_3CH_2CHOSiMe_3–90]^+$, M^+–90, and $[alkyl\text{-}CHOSiMe_3]^+$. Contrary to the alkanediols, both diastereomers of the aromatic diols occur in the extracts.

2.5. Fatty acids

A full range of saturated acids from hexanoic to nonacosanoic acids with the exception of the C_7 and C_{27} acids were detected in the extracts. Unsaturated acids present were 9-hexadecenoic acid (9–16:1), 9–18:1, 11–18:1, 9,12–18:2, 18:3, 11–20:1, 11,14–20:2, and 13–22:1. Position of double bonds in mono- and diunsaturated acids were determined by analysis of dimethyldisulfide adducts of the methyl esters (Leonhardt and DeVilbiss, 1985; Vincenti et al., 1987). The major acid and a major component present in the extracts is 11-eicosenoic acid (**14**), as all other acids occur in minor or trace amounts.

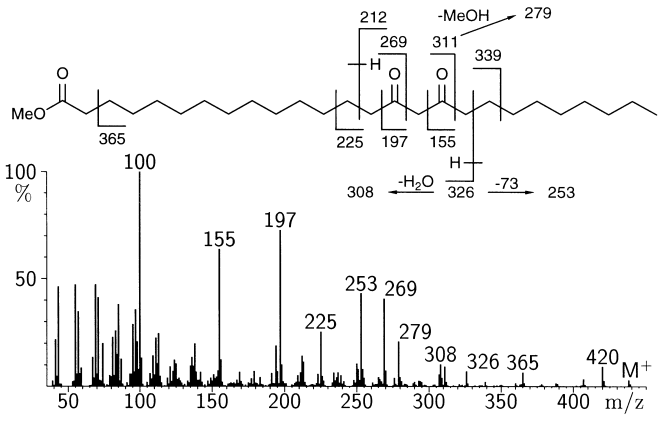


Fig. 3. Mass spectrum and fragmentation scheme of methyl 15,17-dioxohexacosanoate.

2.6. 1,3-Dioxoalkanoic acids

Surprisingly, a number of dioxoalkanoic acids were also identified in the extracts (see Table 4). The major groups are acids with the dioxo group at the 10,12-position relative to the alkyl end, as in the major members 15,17-dioxohexacosanoic acid (**15b**) and 16,18-dioxoheptacosanoic acid (**15c**). They were identified in methylated extracts by GC–MS as, in the underivatized form, they show bad elution properties. The methyl esters of **15** exhibit mass spectra with combined features of diketones and methyl esters (see Fig. 3). Besides the typical ions m/z = 100 (diketone) and m/z = 74 and 87 (methyl ester) prominent ions arise from cleavage around the diketo group and loss of methyl ester fragments from some of these ions. The 14,16-dioxopentacosanoic acid (**15a**) was synthesized according to the general scheme shown in Fig. 1 starting from methyl 3-oxododecanoate and methyl 13-(chloroformyl)tridecanoate, verifying our assignment.

There has been a controversy on the biogenetic origin of β-diketones in plant waxes. While it has been suggested that diketones are formed by a Claisen reaction between a β-ketoacyl compound and a normal fatty acyl compound followed by decarboxylation (Bianchi, 1995), the more likely route involves chain elongation of a β-ketoester (von Wettstein-Knowles, 1995). Like in polyketide biosynthesis, the β-dioxo group is retained in chain elongation, which is performed by special enzymes that differ from the enzymes for the elongation of normal fatty acids (von Wettstein-Knowles, 1995). Finally, the respective diketoacids yield the diketones after decarboxylation or decarbonylation. The diketoacids identified here are crucial intermediates for the β-diketones and provide strong support for the latter hypothesis. Furthermore, to the best of our knowledge, they have not been previously identified from any natural source.

Another interesting feature is the apparent lack of chain length specificity. Free diketones with an odd number of carbons in the chain (odd-numbered chain) occur in larger amounts compared to the diketones with an even number of carbons (even-numbered chain), which is consistent with the proposed pathway. Nevertheless, the corresponding acids did not show such a preference. The ratio of the C_{25}–C_{28} compounds is 26:45:26:3, exhibiting a total length peak rather than an odd/even preference. This may reflect

the status as a biosynthetic intermediate for the acids and raises the question on the biosynthetic origin of the acids with an odd-numbered chain.

Chain initiation by a propionyl unit instead of an acetyl unit is unlikely, because the diketo-group would be at the ω-11,13 position rather than at the ω-10,12 position in that case. Therefore, the odd-numbered diketoacids seem to be formed by α-oxidation (Shine and Stumpf, 1974; Khan and Kolattukudy, 1974) of the even-numbered acids. This is in contrast to recent findings that odd-numbered fatty acids up to C_{17} of epi-cuticular waxes from tobacco and *Brassica* spp. are formed by a propionate starter (Kroumova and Wagner, 1999). Alternatively to α-oxidation, elongation by one carbon atom of an even-numbered acids by an α-ketoacid elongase system has been postulated in the biosynthesis of short chain acids (Kroumova et al., 1994), but this report has recently been questioned (van der Hoeven and Steffens, 2000). Nevertheless, propionate seems to work as a starter in the biosynthesis of 5,7-, 7,9-, and 11,13-diketones, which occur only with even-numbered chains, and thus require odd-numbered diketoacids as precursors (see Table 4). It is possible, due to their low abundance, that these precursor acids could not be detected in the extracts. The phenyl diketones require benzoic acid as starter. The respective diketoacids could also not be detected.

2.7. Esters

Minor amounts of dodecyl palmitate, octyl eicosenoate, and octyl eicosadienoate could be identified in total extracts, as well as methyl esters of the fatty acids mentioned above. More surprising was the detection of esters of hydroxy fatty acids of the estolide type. The mass spectrum of the major component of these acids showed a molecular ion at m/z = 550 after methylation, accompanied by a base peak at M–32. Characteristic ions at m/z = 239 $[C_{15}H_{31}CO]^+$ and 257 $[C_{15}H_{31}CO_2H_2]^+$, as well as 322 $[M–C_{14}H_{28}–CH_3OH]^+$, 294 $[C_{17}H_{32}CO_2CH_3]^+$, 280 $[M–C_{14}H_{28}–CH_3OCOH{=}CH_2]^+$, and 262 $[M–C_{14}H_{28}–CH_3OCOH{=}CH_2–H_2O]^+$ confirm the assignment of hexadecanoyloxyoctadecenoic acid for this compound. Transesterification of a total extract with sodium methanoate and silylation furnished 18-hydroxyoctadecenoic acid as a major product, accompanied by a series of other hydroxyacids. Therefore, the major estolide in the extract was tentatively identified as 18-(hexadecanoyloxy)octadecenoic acid (16:0–18:1, **16a**). Further, ω-estolides could be identified as 12:0–18:1,

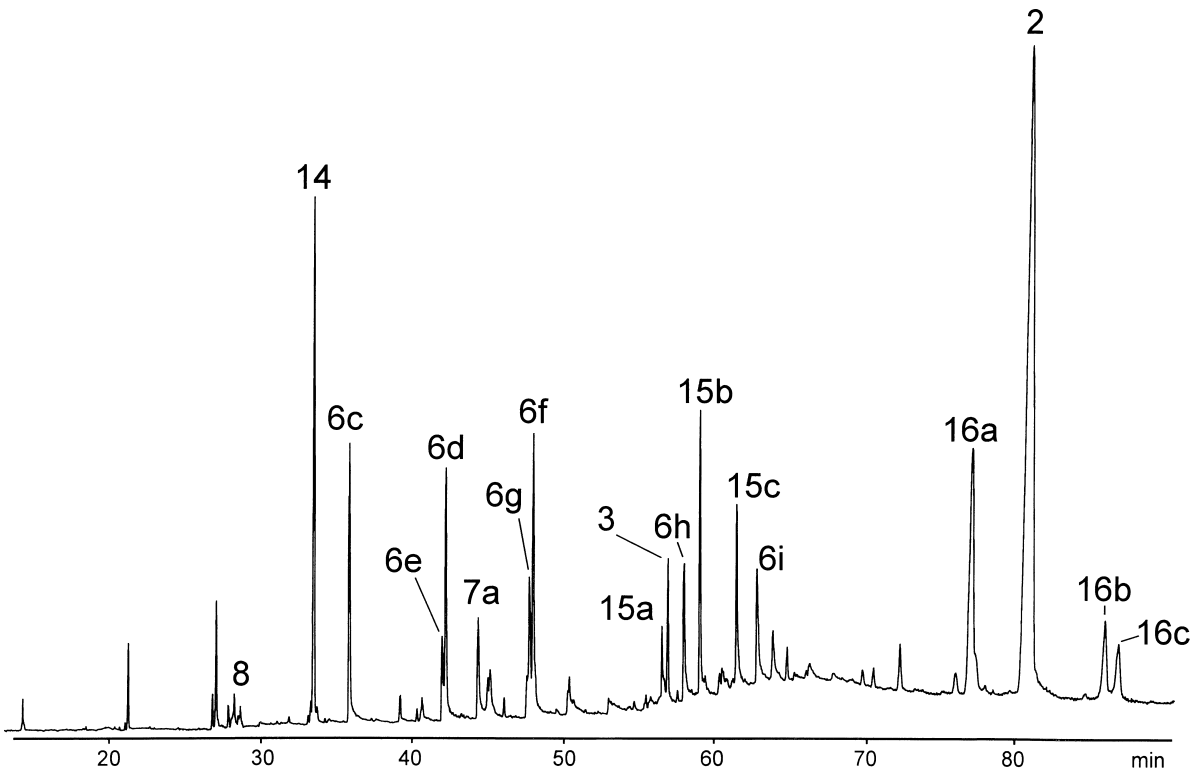


Fig. 4. Gas chromatogram of a methylated hexane extract of *Helianthus annuus* pollen. 25 m BPX-5 capillary column, on-column injection, programmed from 60–320°C with 4°C/min. Compounds mentioned in the text are indicated.

14:0–18:1, 18:0–18:1 (**16b**), and 18:1–18:1 (**16c**). These cutin-type compounds show the presence of cutin oligomers in the extract. After transmethylation and silylation, several cutin monomers could be identified in the total extract by GC–MS and derivatization: a series of C_{14} to C_{18}-α,ω-diacids, 3-hydroxyacids (3HO14:0, 3HO16:0, and 3HO18:0), ω-hydroxyacids (16HO16:0, 18HO18:0, 18HO18:1, 18HO18:2, and 18HO18:3) as well as 12- and 13-hydroxytetradecanoic acids. Of these, 18HO18:1 is the major component followed by 18HO18:3 and 18HO18:2, while all others occur in traces only. Finally, traces of the macrolides octadecen-18-olide and octadecatrien-18-olide are present in underivatized pollen extracts.

2.8. Alkanes

Low amounts of linear C_{25}, C_{27}, C_{29}, C_{31}, and C_{33} are present in the extracts.

2.9. Composition of the pollen grain coat

A gas chromatogram of a whole pollen extract is shown in Fig. 4. In contrast to the extensive extraction of whole pollen grains, the lipids of the pollen coat were obtained by a short (15 s) extraction of the pollen with pentane. Such a methodology has been shown to extract mainly the pollen coat lipids (Piffanelli et al., 1998). Qualitatively, these extracts are very similar to the ones obtained by longer extraction. They lack the dioxoalkanoic acids and the estolide-type compounds. Diketones, diols, and hydroxyketones remain major components in the proportions reported above, accompanied by dodecanoic acid, eicosenoic acid and phytyl dodecanoate. However, the dioxoalkanoic acids and the estolide-type compounds are absent and the concentrations of the triterpenes and the long chain diketones ($C \geq 32$) are markedly reduced. Increased amounts of α- and γ-tocopherol, which are also found in total extracts, are also present.

The lipids of the pollen and especially the pollen coat have so far only rarely been investigated in detail, despite the fact that they seem to trigger important biological processes (Meuter-Gerhards et al., 1999; Piffanelli et al., 1998). In most studies, only the qualitative composition of these lipids have been investigated (see, for example, Piffanelli et al., 1997; Dobson, 1988; for a general discussion, see Piffanelli et al., 1998), rather than the characterization of individual chemical species, as was the case for maize pollen (Bianchi et al., 1990). Predominant classes are free fatty acids, triterpenes and their esters, alkanes, alkenes, and wax type esters, as well as triglycerides. None of the diketones or related compounds have been reported from pollen before. Interestingly, long chain diketones have been shown to posses strong antioxidative properties (Osawa and Namiki, 1985), and may thus serve to protect pollen constituents, together with the tocopherols also present in the pollen coat. Also, helianol and its derivatives have so far only been detected in *H. annuus* and *Camellia* seed oil (Akihisa et al., 1998b). Sunflower pollen is less preferred by bees compared to pollen from other plants. This effect has been related to its lipids (Singh et al., 1999). The effect of the compounds discussed on the egg-laying behavior of *H. electellum* will be reported elsewhere.

3. Experimental

3.1. General methods

3.1.1. Instruments

MS: VG 70/250 S mass spectrometer coupled to a Hewlett-Packard HP 5890 A gas chromatograph and HP-MSD 5973, splitless injection, EI mode. GC: Carlo-Erba Fractovap 2101 gas chromatograph, flame ionization detector, on-column injection. CE Instruments GC 8000, split/splitless-injection or PTV-injection (Gerstel KAS 3). Separations were performed on 25 m BPX-5 (SGE, internal diameter 0.22 mm, film thickness 0.25 μm) capillary columns, programmed from 60–320°C with 4°C/min (H_2 carrier gas). NMR: ^{1}H- and ^{13}C-NMR spectra were obtained with Bruker WM 400 or AC 250P instruments. TMS was used as internal standard. For the interpretation of ^{13}C-NMR spectra, see Tulloch (1985). Chromatography: separations were performed on silica (Merck).

3.1.2. Derivatizations

Silylations were performed by adding 50 μl MSTFA (*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide) to 50 μl of the hexane extract. After 30 min at 60°C, the silylated samples were concentrated under a stream of nitrogen and then analyzed. Performing the reaction at room temperature and overnight resulted in complete disilylation of diketo groups.

Dimethyldisulfide adducts were formed by addition of 10 μl 5% I_2 in diethyl ether to a mixture of 100 μl extract and 100 μl dimethyl disulfide. This mixture was held overnight at 60°C. A saturated solution of $Na_2S_2O_3$ was added, the mixture thoroughly shaken, the phases separated, the water phase extracted twice with 100 μl pentane, and the combined organic phases dried with NaCl. Finally, excess reagent was removed by evaporation under a stream of N_2 (Vincenti et al., 1987).

Transesterification was carried out by dissolving 0.1 ml of a CH_2Cl_2 pollen extract in a 0.5 ml 1% NaOMe in absolute methanol, and heating of this mixture for 1 h to 60°C in a closed vial. The mixture was acidified with acetic acid and extracted with hexane.

Methylations were performed by addition of a solution of distilled diazomethane to hexane extracts.

3.1.3. Pollen

Pollen was collected from *H. annuus* var. Saturn, grown between 1989 and 1999 in Quebec City, Canada.

3.1.4. Extraction for analysis

About 5 g of pollen were extracted overnight with 50 ml hexane using a soxhlet apparatus, yielding about 100 mg of a red oil after concentration with a rotary evaporator. A similar extract was obtained by sonication of 5 g pollen for 15 min, which was repeated three times. After filtration, combination of the organic phases and removal of the solvent, about 120 mg of red oil were obtained. For determination of the pollen coat lipids, 350 mg of pollen were shaken for 15 s with 1 ml of pentane and rapidly filtered, using a cotton plug placed in a pipette.

*3.2. Isolation of compounds **2** and **12***

3.2.1. Extraction

Sonication of 90 g sunflower pollen in 300 ml CH_2Cl_2 was performed three times for 30 min. The combined extracts were filtered and the solvent removed (8.3 g red oil). This extract was separated by column chromatography (300 g silica) with hexane, followed by hexane/diethyl ether mixtures. The fractions containing both diketones **6** and terpene esters **2** and **3** were combined (1.9 g yellow oil). The diketones can be removed by transformation into potassium salts by shaking of 100 mg of this fraction with a mixture of 10 ml pentane and 15 ml methanol containing 250 mg solid KOH. The solvent is removed and the solid residue taken up in pentane, leaving the potassium diketonates as a solid. The esters in the pentane fraction can be purified by chromatography on silica with pentane/CH_2Cl_2. By this procedure a fraction containing 10 mg of **2**, accompanied by small amounts of **3** and some residual diketones is obtained. Transesterification with NaOMe furnished **1** and methyl octanoate as major products.

*3.2.2. Isolation of helianol (**1**)*

To 12 ml of a 5:1 methanol/diethyl ether mixture, 400 mg of the diketone/ester fraction and 300 mg KOH were added. After stirring for 2 h, the solution was neutralized with 1 N HCl, the solvent removed, and the residue taken up in diethyl ether. The crude saponified mixture was separated by chromatography on silica (40 g) with hexane/ethyl acetate 85:15, yielding 80 mg pure **1**, containing minor amounts of phytol. The ester **2** could be reconstituted by reaction of octanoyl chloride with **1** at 0°C in pyridine.

The NMR values of **1** and **2** were identical to those reported for **1** and its acetate (Akihisa et al., 1996). ^{13}C-NMR signals of the octanoyl side chain in **2** (100.6 MHz, $CDCl_3$): $\delta = 14.1$ (C-8′, *q*), 22.6 (C-7′, *t*), 25.1 (C-3′, *t*), 29.7 (C-4′ and C-5′, 2*t*), 31.7 (C-6′, *t*), 34.4 (C-2′, *t*), 174.0 (C-1′, *s*).

3.2.3. Isolation of (R, S*)-4,6-nonadecanediol (**12**)*

A fraction containing pure **12** (10 mg) could be isolated under the chromatographic conditions reported for the separation of **2**. ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, $J = 7$ Hz, H-19), 0.93 (3H, *t*, $J = 7$ Hz, H-1), 1.20–1.32 (24H, *m*, CH_2), 1.36–1.48 (4H, *m*, H-3 and H-7), 1.60 (1H, *bt*, H-5), 1.63 (1H, *bt*, H-5), 1.95 (1H, *br s*, 4-OH or 6-OH), 2.85 (H, *br s*, 4-OH or 6-OH), 3.86 (2H, *m*, H-4, H-6). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 14.0$ (*q*), 14.1 (*q*), 18.5 (*t*, C-2), 22.7 (*t*, C-18), 25.3 (*t*, C-8), 29.3–29.7 (*t*, C-9–C-16), 31.9 (*t*, C-17), 38.2 (*t*, C-7), 40.3 (*t*, C-3), 42.8 (*t*, C-5), 72.9 (*d*), 73.3 (*d*). EIMS 70 eV (rel. int.): 282 $[M-H_2O]^+$ (0.3), 257 (6), 239 (16), 213 (20), 117 (100), 99 (66), 81 (50), 73 (50), 70 (56), 45 (6), 43 (28). Disilyl ether EIMS 70 eV (rel. int.): 429 $[M-15]^+$ (0.3), 374 (1), 359 (4), 311 (10), 285 (62), 219 (5), 186 (5), 171 (16), 147 (23), 145 (100), 131 (5), 103 (6), 75 (8), 73 (32).

3.2.4. Relative proportions of the different compound classes in the extracts

Concrete numbers were difficult to obtain because of overlapping peaks and different chromatographic behavior in GC as well as column chromatography. Therefore, the following numbers for the whole pollen extract are only estimates, derived from GC with on-column injection (numbers for the pollen coat in parentheses): terpenes and terpene esters 35% (13%), diketones 17% (32%), fatty acids 8% (14%), ketols 8% (14%), diols 12% (20%), dioxoalkanoic acids 4% (1%), esters 12% (–), alkanes 2% (4%), others 2% (2%).

3.3. Synthesis of reference compounds

3.3.1. Synthesis of β-diketones

The required β-ketoesters were obtained commercially or by condensation of an appropriate methyl ketone **4** with diethyl carbonate (Cadman et al., 1995). The products were then transformed into the β-diketones by the following procedure (Krapcho, 1982; Reynolds and Hauser, 1963).

3.3.2. General procedure for synthesis of β-diketones

Magnesium turnings (165 mg, 6.8 mmol) were added to 0.35 ml ethanol followed by a drop of tetrachloromethane to prepare $Mg(OEt)_2$. After the reaction has started, a solution of the respective ethyl β-oxo-car-

boxylate (6.8 mmol) in 0.7 ml ethanol and 2.7 ml diethyl ether was added, keeping the mixture under reflux. Then a solution of the acid chloride (6.8 mmol) in anhydrous diethyl ether (0.7 ml) was added slowly. The mixture was stirred overnight and hydrolyzed with ice water (3 ml) and conc. H_2SO_4 (0.15 ml). The phases were separated, the aqueous phase extracted twice with diethyl ether, the organic phases combined, washed with $NaHCO_3$, and dried ($MgSO_4$) (Cadman et al., 1995). The crude product was filtered, the solvent removed, and finally added to a solution of NaCl (0.34 g, 5.8 mmol) in water (0.3 ml) and DMSO (4.4 ml). This mixture was then heated for 8 h under reflux. Water (20 ml) was added to the cooled mixture, which was then extracted thrice with diethyl ether, and the combined organic phases dried ($MgSO_4$) (Krapcho, 1982). After filtration and removal of the solvent, the product was purified by column chromatography (silica, light petroleum/diethyl ether). The yields varied between 40 and 60%.

*3.3.3. 4,6-Nonadecanedione (**6a**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, H-19), 0.95 (3H, *t*, J = 7.1 Hz, H-1), 1.21–1.34 (20H, *m*, H-9–H-18), 1.52–1.71 (4H, *m*, H-2 and H-8), 2.25 (2H, *t*, J = 7.6 Hz, H-7), 2.26 (2H, *t*, J = 7.6 Hz, H-3), 2.48 (2H, *t*, J = 7.6 Hz, H-7, enol), 2.49 (2H, *t*, J = 7.6 Hz, H-3 enol), 3.55 (2H, *s*, H-5), 5.47 (1H, *s*, H-5 enol), 15.5 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.7$ (*q*, C-1), 14.1 (*q*, C-19), 16.8 (*t*, C-2), 19.1 (*t*, C-2 enol), 22.7 (*t*, C-18), 23.4 (*t*, C-8), 25.7 (*t*, C-8 enol), 29.0–29.7 (*t*, C-9–C-16), 31.9 (*t*, C-17), 38.4 (*t*, C-7 enol), 40.2 (*t*, C-3 enol), 43.8 (*t*, C-7), 45.6 (*t*, C-3), 57.1 (*t*, C-5), 99.1 (*d*, C-5 enol), 194.1 (*s*, C-4 enol), 194.7 (*s*, C-6 enol), 204.2 (*s*, C-4), 204.4 (*s*, C-6). EIMS 70 eV (rel. int.): 296 $[M]^+$ (0.8), 278 $[M–H_2O]^+$ (1), 253 (5), 211 (1), 141 (5), 128 (31), 113 (23), 100 (10), 85 (18), 71 (100), 43 (26).

*3.3.4. 4,6-Henicosanedione (**6b**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, H-19), 0.95 (3H, *t*, J = 7.1 Hz, H-1), 1.22–1.36 (24H, *m*, H-9–H-20), 1.55–1.70 (4H, *m*, H-2 and H-8), 2.25 (2H, *t*, J = 7.6 Hz, H-7), 2.26 (2H, *t*, J = 7.6 Hz, H-3), 2.48 (2H, *t*, J = 7.6 Hz, H-7 enol), 2.49 (2H, *t*, J = 7.6 Hz, H-3 enol), 3.55 (2H, *s*, H-5), 5.46 (1H, *s*, H-5 enol), 15.5 (1H, *br s*, OH, enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.8$ (*q*, C-1), 14.1 (*q*, C-21), 16.9 (*t*, C-2), 19.2 (*t*, C-2 enol), 22.7 (*t*, C-20), 23.4 (*t*, C-8), 25.7 (*t*, C-8 enol), 29.0–29.7 (*t*, C-9–C-22), 31.9 (*t*, C-19), 38.5 (*t*, C-7 enol), 40.3 (*t*, C-3 enol), 43.8 (*t*, C-7), 45.6 (*t*, C-3), 57.2 (*t*, C-5), 99.1 (*d*, C-5 enol), 194.1 (*s*, C-4 enol), 194.8 (*s*, C-6 enol), 204.2 (*s*, C-4), 204.4 (*s*, C-6). EIMS 70 eV (rel. int.): 324 $[M]^+$ (1), 306 $[M–H_2O]^+$ (4), 281 (17), 239 (6), 141 (28), 128 (74), 113 (77), 100 (53), 85 (52), 71 (100), 43 (77).

*3.3.5. 6,8-Henicosanedione (**6c**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7 Hz, CH_3), 0.89 (3H, *t*, J = 7 Hz, CH_3), 1.21–1.30 (24H, *m*, H-2 and H-3, H-11–H-20), 1.52-1.67 (4H, *m*, H-4 and H-10), 2.26 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9), 2.50 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9 enol), 3.54 (2H, *s*, H-7), 5.49 (1H, *s*, H-7 enol), 15.5 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.9$ (*q*, C-1), 14.1 (*q*, C-21), 22.4 (*t*, C-2), 22.7 (*t*, C-20), 23.1 (*t*, C-4), 23.4 (*t*, C-10), 25.4 (*t*, C-4 enol), 25.7 (*t*, C-10 enol), 29.0–29.7 (*t*, C-11–C18), 31.3 (*t*, C-3), 31.4 (*t*, C-3 enol), 31.9 (*t*, C-19), 38.4 (2*t*, C-5 and C-9 enol), 43.8 (2*t*, C-5 and C-9), 57.2 (*t*, C-7), 99.0 (*d*, C-7 enol), 194.5 (2*s*, C-6 and C-8 enol), 204.3 (2*s*, C-6 and C-8). EIMS 70 eV (rel. int.): 324 $[M]^+$ (0.7), 306 $[M–H_2O]^+$ (1), 253 (3), 211 (5), 169 (4), 156 (12), 141 (21), 113 (10), 100 (39), 99 (50), 71 (23), 43 (100).

*3.3.6. 4,6-Tricosanedione (**6d**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, CH3), 0.95 (3H, *t*, J = 7.1 Hz, CH_3), 1.20-1.31 (28H, *m*, H-9–H-22), 1.54-1.67 (4H, *m*, H-2 and H-8), 2.25 (2H, *t*, J = 7.6 Hz, H-7), 2.27 (2H, *t*, J = 7.6 Hz, H-3), 2.48 (2H, *t*, J = 7.6 Hz, H-7 enol), 2.49 (2H, *t*, J = 7.6 Hz, H-3 enol), 3.55 (2H, *s*, H-5), 5.47 (1H, *s*, H-5 enol), 15.52 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.7$ (*q*, C-1), 14.1 (*q*, C-23), 16.8 (*t*, C-2), 19.1 (*t*, C-2 enol), 22.7 (*t*, C-22), 23.4 (*t*, C-8), 25.7 (*t*, C-8 enol), 28.6 (*t*), 29.0-29.7 (*t*, C-9-C-20), 31.9 (*t*, C-21), 38.5 (*t*, C-7 enol), 40.2 (*t*, C-3 enol), 43.8 (*t*, C-7), 45.6 (*t*, C-3), 57.2 (*t*, C-5), 99.1 (*d*, C-5 enol), 194.1 (*s*, C-4 enol), 194.8 (*s*, C-6 enol), 204.1 (*s*, C-4), 204.4 (*s*, C-6). EIMS 70 eV (rel. int.): 352 $[M]^+$ (1), 306 $[M–H_2O]^+$ (1), 309 (5), 267 (2), 141 (32), 128 (91), 113 (88), 100 (65), 85 (49), 71 (100), 43 (78).

*3.3.7. 6,8-Tricosanedione (**6e**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, CH_3), 0.90 (3H, *t*, J = 7.1 Hz, CH_3), 1.21-1.30 (28H, *m*, H-2 and H-3, H-11–H-22), 1.52–1.67 (4H, *m*, H-4 and H-10), 2.26 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9), 2.49 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9 enol), 3.54 (2H, *s*, H-7), 5.49 (1H, *s*, H-7 enol), 15.5 (1H, *br s*, OH, enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.9$ (*q*, C-1), 14.1 (*q*, C-23), 22.4 (*t*, C-2), 22.7 (*t*, C-22), 23.1 (*t*, C-4), 23.4 (*t*, C-10), 25.4 (*t*, C-4 enol), 25.7 (*t*, C-10 enol), 29.0–29.7 (*t*, C-11–C20), 31.3 (*t*, C-3), 31.4 (*t*, C-3 enol), 31.9 (*t*, C-21), 38.4 (2*t*), C-5 and C-9 enol), 43.8 (2*t*, C-5 and C-9), 57.2 (*t*, C-7), 99.0 (*d*, C-7 enol), 194.5 (2*s*, C-6 and C-8 enol), 204.3 (2*s*, C-6 and C-8). EIMS 70 eV (rel. int.): 352 $[M]^+$ (0.7), 334 $[M–H_2O]^+$ (2), 281 (6), 239 (4), 169 (9), 156 (21), 141 (36), 113 (11), 100 (50), 99 (62), 71 (18), 43 (100).

*3.3.8. 4,6-Pentacosanedione (**6f**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, CH_3), 0.95 (3H, *t*, J = 7.1 Hz, CH_3), 1.21–1.32 (30H, *m*, H-9–H-24), 1.53–1.69 (4H, *m*, H-2 and H-8), 2.25 (2H, *t*, J = 7.6 Hz, H-7), 2.27 (2H, *t*, J = 7.6 Hz, H-3), 2.49 (2H, *t*, J = 7.6 Hz, H-7 enol), 2.50 (2H, *t*, J = 7.6 Hz, H-3 enol), 3.55 (2H, *s*, H-5), 5.48 (1H, *s*, H-5 enol), 15.5 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.7$ (*q*, C-1), 14.1 (*q*, C-25), 16.8 (*t*, C-2), 19.1 (*t*, C-2 enol), 22.7 (*t*, C-24), 23.4 (*t*, C-8), 25.7 (*t*, C-8 enol), 28.6 (*t*), 29.0–29.7 (*t*, C-9–C-22), 31.9 (*t*, C-23), 38.5 (*t*, C-7 enol), 40.2 (*t*, C-3 enol), 43.8 (*t*, C-7), 45.6 (*t*, C-3), 57.2 (*t*, C-5), 99.1 (*d*, C-5 enol), 194.1 (*s*, C-4 enol), 194.8 (*s*, C-6 enol), 204.1 (*s*, C-4), 204.4 (*s*, C-6). EIMS 70 eV (rel. int.): 380 $[M]^+$ (0.8), 362 $[M–H_2O]^+$ (3), 337 (8), 295 (2), 141 (19), 128 (75), 113 (70), 100 (41), 85 (42), 71 (100), 43 (67).

*3.3.9. 6,8-Pentacosanedione (**6g**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7.1 Hz, CH_3), 0.90 (3H, *t*, J = 7.1 Hz, CH_3), 1.21–1.30 (32H, *m*, H-2 and H-3, H-11–H-24), 1.52–1.67 (4H, *m*, H-4 and H-10), 2.26 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9), 2.49 (4H, 2*t*, J = 7.6 Hz, H-5 and H-9 enol), 3.54 (2H, *s*, H-7), 5.49 (1H, *s*, H-7 enol), 15.53 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 13.9$ (*q*, C-1), 14.1 (*q*, C-25), 22.4 (*t*, C-2), 22.7 (*t*, C-24), 23.1 (*t*, C-4), 23.4 (*t*, C-10), 25.4 (*t*, C-4 enol), 25.7 (*t*, C-10 enol), 29.0–29.7 (*t*, C-11-C22), 31.3 (*t*, C-3), 31.4 (*t*, C-3 enol), 31.9 (*t*, C-23), 38.4 (2*t*), C-5 and C-9 enol), 43.8 (2*t*, C-5 and C-9), 57.2 (*t*, C-7), 99.0 (*d*, C-7 enol), 194.5 (2*s*, C-6 and C-8 enol), 204.3 (2*s*, C-6 and C-8). EIMS 70 eV (rel. int.): 380 $[M]^+$ (0.5), 362 $[M–H_2O]^+$ (0.7), 309 (2), 267 (1), 169 (5), 156 (16), 141 (27), 113 (13), 100 (78), 99 (100), 71 (46), 43 (49).

*3.3.10. 10,12-Nonacosanedione (**6h**)*

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.88–0.93 (6H, 2*t*, J = 7 Hz, H-1 and H-29), 1.2–1.31 (40H, *m*, H-2–H-7, H-15–H-28), 1.52–1.64 (4H, *m*, H-8 and H-14), 2.24–2.29 (4H, 2*t*, J = 7.6 Hz, H-9 and H-13 enol), 2.49 (4H, 2*t*, J = 7.6 Hz, H-9 and H-13), 3.55 (2H, *s*, H-11), 5.47 (1H, *s*, H-11 enol), 15.54 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 14.1$ (2*q*, C-1 and C-29), 22.7 (2*t*, C-2 and C-28), 23.5 (2*t*, C-8 and C-14), 25.8 (2*t*, C-8 and C-14 enol), 28.9–29.8 (*t*, C-4–C-7 and C-15—C-26), 31.9 (2*t*, C-3 and C-27), 38.4 (2*t*), C-9 and C-13 enol), 43.8 (2*t*, C-9 and C-13), 57.3 (*t*, C-11), 99.0 (*d*, C-11 enol), 194.5 (2*s*, C-10 and C-12 enol), 204.3 (2*s*, C-10 and C-12). EIMS 70 eV (rel. int.): 436 $[M]^+$ (3), 418 $[M–H_2O]^+$ (9), 337 (4), 309 (12), 306 (10), 281 (8), 278 (7), 267 (10), 252 (4), 248 (9), 239 (9), 225 (17), 222 (6), 212 (8), 197 (22), 194 (10), 183 (16), 164 (7), 155 (42), 142 (9), 138 (19), 124 (10), 113 (19), 100 (100), 97 (21), 85 (33), 71 (46), 43 (42).

*3.3.11. 1-Phenyl-1,3-hexadecanedione (**7a**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 6.9 Hz, H-16), 1.20–1.42 (20H, *m*, H-6–H-15), 1.64–1.72 (2H, *m*, H-5), 2.42 (2H, *t*, J = 7.6 Hz, H-4), 2.57 (2H, *t*, J = 7.6 Hz, H-4 enol), 4.08 (2H, *s*, H-2), 6.18 (1H, *s*, H-2 enol), 7.4–7.9 (5H, *m*, ar), 15.54 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$), only the enol form could be observed: $\delta = 14.1$ (*q*, C-16), 22.7 (*t*, C-15), 25.9 (*t*, C-5), 29.3–29.7 (*t*, C-6–C-13), 31.9 (*t*, C-14), 39.3 (*t*, C-4), 96.1 (*d*, C-2), 127.0 (2*d*, ar), 128.6 (2*d*, ar), 132.2 (*d*, ar), 135.1 (*s*, ar), 183.5 (*s*, C-1), 197.0 (*s*, C-3). EIMS 70 eV (rel. int.): 330 $[M]^+$ (0.4), 312 $[M–H_2O]^+$ (0.8), 175 (3), 162 (29), 147 (22), 120 (11), 105 (100), 77 (24).

*3.3.12. 1-Phenyl-1,3-octadecanedione (**7b**)*

^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 6.9 Hz, H-18), 1.20–1.34 (24H, *m*, H-6–H-17), 1.64–1.72 (2H, *m*, H-5), 2.42 (2H, *t*, J = 7.6 Hz, H-4), 2.57 (2H, *t*, J = 7.6 Hz, H-4), 4.10 (2H, *s*, H-2), 6.16 (2H, *s*, H-2 enol), 7.4–7.9 (5H, *m*, ar), 15.54 (1H, br *s*, OH enol). 13 C-NMR (100.6 MHz, $CDCl_3$), only the enol form could be observed: $\delta = 14.1$ (*q*, C-18), 22.7 (*t*, C-17), 25.9 (*t*, C-5 enol), 29.3–29.7 (*t*, C-6–C-15), 31.9 (*t*, C-16), 39.3 (*t*, C-4 enol), 96.1 (*d*, C-2 enol), 127.0 (2*d*, ar), 128.6 (2*d*, ar), 132.2 (*d*, ar), 135.1 (*s*, ar), 183.5 (*s*, C-1 enol), 197.0 (*s*, C-3). EIMS 70 eV (rel. int.): 358 $[M]^+$ (1), 340 $[M–H_2O]^+$ (3), 175 (4), 162 (34), 147 (27), 120 (10), 105 (100), 77 (21).

*3.3.13. 14,16-dioxopentacosanoic acid (**15a**)*

This acid was synthesized according to the general procedure by condensation of methyl 3-oxododecanoate with the acid chloride of monomethyl tetradecandioate. The methyl ester group was partially saponified during decarbmethoxylation, leading to a mixture of the free acid and the methyl ester, which was separated by column chromatography on silica. ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (3H, *t*, J = 7 Hz, H-25), 1.2–1.31 (28H, *m*, H-4–H-11, H-19–H-24), 1.52–1.68 (6H, *m*, H-3, H-12, and H-18), 2.27 (4H, *t*, J = 7.6 Hz, 12.H-13 and H-17 enol), 2.34 (2H, *t*, J = 7.6 Hz, H-2), 2.49 (4H, *t*, J = 7.6 Hz, H-13 and H-17), 3.55 (2H, *s*, H-15), 5.47 (1H, *s*, H-15 enol), 10.0 (1H, *br s*, COOH), 15.54 (1H, *br s*, OH enol). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 14.1$ (*q*, C-25), 22.6 (*t*, C-24), 23.4 (2*t*, C-12 and C-18), 24.7 (*t*, C-3), 25.7 (*t*, C-12 and C-18 enol), 29.0–29.5 (*t*, C-4–C-11 and C-19–C-22), 31.8 (*t*, C-23), 33.9 (*t*, C-2), 38.4 (2*t*, C-13 and C-17 enol), 43.8 (2*t*, C-13 and C-17), 57.2 (*t*, C-15), 99.0 (*d*, C-15 enol), 179.4 (*s*, C-1), 194.6 (2*s*, C-14 and C-16 enol), 204.5 (2*s*, C-14 and C-16). EIMS 70 eV (rel. int.) of methyl ester: 424 $[M]^+$ (2), 406 (8), 351 (6), 312 (6), 297 (9), 294 (10), 265 (20), 255 (42), 239 (41), 225 (22), 212 (11), 197 (66), 155 (60), 100 (100), 87 (11), 85 (39), 84 (15), 83 (27), 74 (18), 71 (45), 69 (51), 59 (10).

*3.3.14. 4,6-Nonadecanediol (**12**)*

A solution of $NaBH_4$ (52 mg, 1.4 mmol) and NaOH (11 mg, 0.3 mmol) in water (2.2. ml) was added dropwise to a solution of 4,6-nonadecanedione (0.1 g, 0.3 mmol) in 2.2 ml ethanol. The reaction mixture was stirred at room temperature for 12 h, during which a white precipitate was formed. The solvent was removed under reduced pressure and the residue taken up with water. The aqueous solution was extracted twice with diethyl ether and the combined organic phases dried with $MgSO_4$. After filtration and removal of the solvent, the product was purified by column chromatography (silica, light petroleum/diethyl ether). Yield: 70 mg (69%), d.r. ($4R^*$, $6S^*$)/($4R^*$, $6R^*$) = 78:22. ^{1}H-NMR: $\delta = 0.88$ (3H, *t*, J = 7 Hz, CH_3), 0.93 (3H, *t*, J = 7 Hz, CH_3), 1.21–1.33 (24H, *m*, H-2, H-9–H-18), 1.37–1.5 (5H, *m*, H-3, H-5, H-7), 1.58–1.63 (1H, *m*, H-5), 2.27 (2H, *br s*, 4-OH or 6-OH), 2.87 (2H, *br s*, 4-OH or 6-OH), 3.86 (2H, *m*, H-4 and H-6, *syn*), 3.95 (2H, *m*, H-4 and H-6, *anti*). ^{13}C-NMR (100.6 MHz, $CDCl_3$): $\delta = 14.0$ (*q*), 14.1 (*q*), 18.1 (*t*, C-2 anti), 18.5 (*t*, C-2 *syn*), 22.6 (*t*), 25.3 (*t*), 25.7 (*t*), 29.3–29.7 (*t*), 31.9 (*t*, C-17), 37.5 (*t*, C-7 *anti*), 38.2 (*t*, C-7 *syn*), 39.6 (*t*, C-3 *anti*), 40.3 (*t*, C-3 *syn*), 42.1 (*t*, C-5 *anti*), 42.8 (*t*, C-5 *syn*), 69.1 (*d*, *anti*), 69.5 (*d*, *anti*), 72.9 (*d*, *syn*), 73.3 (*d*, *syn*).

3.4. Mass spectral data of selected representative compounds

*3.4.1. Phytyl dodecanoate (**3**)*

EIMS 70 eV (rel. int.): 478 $[M]^+$ (3), 296 (10), 278 (29), 263 (4), 201 (2), 183 (30), 137 (15), 123 (100), 109 (30), 95 (74), 82 (61), 68 (81), 60 (1), 57 (62).

*3.4.2. 4-Hydroxy-6-nonadecanone (**8**)*

EIMS 70 eV (rel. int.): 298 $[M]^+$ (2), 280 $[M–H_2O]^+$ (4), 255 $[M–43]^+$ (49), 211 $[RCO]^+$ (100), 115 $[C_3H_7CHOHCH_2CO]^+$ (25), 112 $[C_3H_7CHOHCH_2$-$CHOHCH_2–H_2O]^+$ (22), 97 $[115–H_2O]^+$ (29), 73 $[C_3H_7CHOH]^+$ (20), 58 $[C_3H_6O]^+$ (40). EIMS 70 eV (rel. int.) of silyl ether: 355 $[M–15]^+$ (100), 327 (38), 311 (4), 283 (24), 211 (45), 187 (37), 145 (39), 75 (18), 73 (24).

*3.4.3. 6-Hydroxy-4-nonadecanone (**9**)*

EIMS 70 eV (rel. int.): 298 $[M]^+$ (0.5), 280 (3), 255 (16), 115 (59), 97 (26), 71 (100), 58 (7). EIMS 70 eV (rel. int.) of silyl ether: 355 $[M–15]^+$ (100), 327 (30), 311 (1), 285 (9), 237 (2), 211 (5), 187 (64), 171 (11), 145 (9), 143 (29), 75 (20), 73 (22), 71 (54).

3.4.4. 1-Hydroxy-1-phenyl-3-hexadecanone

EIMS 70 eV (rel. int.) of silyl ether: 404 $[M]^+$ (3), 389 (14), 283 (13), 221 (48), 205 (7), 179 (100), 127 (3), 104 (3).

3.4.5. 3-Hydroxy-1-phenyl-1-hexadecanone

EIMS 70 eV (rel. int.) of silyl ether: 404 $[M]^+$ (0.4), 389 (71), 325 (6), 314 (3), 285 (7), 269 (5), 221 (77), 205 (23), 177 (39), 143 (6), 135 (7), 105 (100), 77 (13), 75 (16), 71 (13).

3.4.6. 1-Phenyl-1,3-hexadecanediol

EIMS 70 eV (rel. int.) of disilyl ether: 463 $[M–15]^+$ (0.2), 388 (29), 285 (5), 253 (2), 205 (19), 179 (100), 147 (13), 91 (2), 73 (16).

*3.4.7. 15,17-Dioxohexacosanoic acid (**15b**)*

EIMS 70 eV (rel. int.) of methyl ester: 438 $[M]^+$ (3), 420 (9), 365 (7), 326 (7), 311 (9), 308 (10), 279 (21), 269 (41), 253 (43), 225 (25), 212 (14), 197 (73), 155 (64), 100 (100), 87 (13), 85 (38), 84 (15), 83 (26), 74 (20), 71 (41), 69 (48), 59 (9). EIMS 70 eV (rel. int.) of enol-TMS ether TMS ester: 568 $[M]^+$ (2), 553 (26), 441 (37), 297 (16), 269 (100), 172 (19), 73 (88).

*3.4.8. 16,18-Dioxoheptacosanoic acid (**15c**)*

EIMS 70 eV (rel. int.) of methyl ester: 452 $[M]^+$ (3), 434 (9), 379 (7), 340 (6), 325 (7), 322 (8), 293 (16), 283 (30), 267 (33), 225 (25), 212 (12), 197 (57), 155 (52), 100 (100), 87 (13), 85 (41), 84 (16), 83 (30), 74 (21), 71 (47), 69 (49), 59 (19).

*3.4.9. 18-(Hexadecanoyloxy)octadecenoic acid (**16a**)*

EIMS 70 eV (rel. int.) of methyl ester: 550 $[M]^+$ (9), 518 (78), 500 (13), 322 (25), 294 (14), 280 (62), 262 (57), 257 (24), 239 (69), 123 (39), 109 (58), 95 (95), 87 (20), 81 (97), 74 (21), 73 (18), 69 (87), 55 (100). EIMS 70 eV (rel. int.) of silyl ester: 608 $[M]^+$ (5), 593 (3), 518 (32), 500 (7), 337 (32), 329 (17), 322 (9), 313 (57), 280 (31), 262 (20), 257 (17), 239 (32), 129 (50), 117 (37), 95 (66), 81 (69), 73 (100), 61 (6), 60 (7).

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