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Homologous long-chain δ-lactones in leaf cuticular waxes of *Cerinthe minor*

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Abstract

A homologous series of eleven δ-lactones (1,5-alkanolides) was identified in cuticular waxes from leaves of *Cerinthe minor* L., six of them representing novel compounds. They accounted for 79% of the total coverage of 41 μg wax per cm^2 leaf area. Various chemical transformations with product identification by GC-mass spectrometry and GC-FTIR were employed to assign the structures. The chain-lengths of the δ-lactones ranged from C_{22} to C_{32} and even-numbered homologues were prevalent. Additionally, aldehydes (C_{26}–C_{30}), alkanes (C_{23}–C_{29}), primary alcohols (C_{26}–C_{32}), alkanoic acids (C_{20}–C_{32}), wax esters (C_{40}–C_{56}) and lupeol were detected.

Keywords: *Cerinthe minor*; Boraginaceae; Cuticular wax; Leaves; Lactones; 1,5-alkanolides; Hydroxy fatty acids

1. Introduction

The genus *Cerinthe* comprises 10 species occurring in Europe, predominantly in the Mediterranean area. Some of them, e.g. *C. major* (honeywort), are cultivated as ornamental plants. Their attractiveness is in part due to the glaucous appearance of their leaves, caused by an array of tubular wax crystals on the cuticle surface, which scatters visible light (Clark & Lister, 1975). On various plant species, comparable crystals have been described (Neinhuis & Barthlott, 1997). Their presence enlarges the exposed hydrophobic surface thereby rendering the leaf highly unwettable (Holloway, 1970). This has two important ecological functions: (1) by forcing water droplets to pearl off, dirt particles are washed away (Barthlott & Neinhuis, 1997) and (2) by preventing the formation of macroscopic water films, germination of pathogenic microorganisms is inhibited (Deising, Nicholson, Haug, Howard & Mendgen, 1992).

In the course of ongoing studies on the chemical composition and molecular structure of epicuticular wax crystals (Jetter & Riederer, 1994a, 1994b), the cuticular wax mixture of *C. minor* leaves has been analyzed. Wax analyses of members of the Boraginaceae have so far been scarce. *Cordia rothii* and *Ehretia buxifolia* waxes have been reported to consist exclusively of usual wax compound classes (Rao & Reddy, 1980; Behari & Gupta, 1980). However, the cuticular wax of *C. minor* was found to consist predominantly of a series of homologues with unknown spectral properties. We therefore attempted to identify these constituents by various chemical transformations and product structure assignment by GC-mass spectrometry and GC-FTIR.

2. Results and discussion

The leaf surface extract of *C. minor* was dominated by a series of homologous compounds characterized as carboxylic esters by the pattern of low mass spectral peaks and an IR band at 1773 cm^{-1}. The most prominent high mass signals could be interpreted as the

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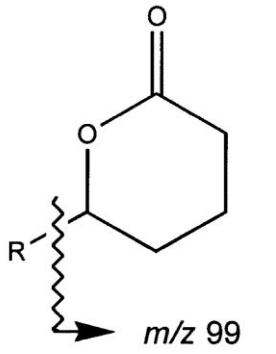
$[M]^+$ ($C_nH_{2n-2}O_2$) and a fragment ($C_nH_{2n-4}O$) generated by the loss of H_2O. Finally, a base peak of m/z 99 in their mass spectra indicated the presence of a cyclic ester group ($C_5H_7O_2$), i.e. a δ-lactone (*alias* 1,5-alkanolide *alias* 3,4,5,6-tetrahydro-alkyl-2H-pyran-2-one) structure (Budzikiewicz, Djerassi & Williams, 1967). This assignment could not be auth-enticated directly, because spectral data of very long-chain δ-lactones have not been published previously. In order to verify the structures, the wax mixture was subjected to various chemical transformations and the respective products were separated and identified by GC-mass spectrometry and GC-FTIR.



$R = C_nH_{2n+1}$

1 - 11: n = 17 -27

Reduction of the esters with $LiAlH_4$ and transformation into TMSi ether derivatives yielded a series of homologous compounds. In their mass spectra, the combination of ions with m/z 73, 75 and 147 indicated the presence of at least two hydroxyl functions. Their exact positions were deduced from characteristic α-fragments with m/z 103 ($[CH_2(OTMSi)]^+$ indicative for primary OH), m/z 247 ($[C_5H_9(OTMSi)_2]^+$ indicative for 1,5-diol) and m/z 157 ($[C_5H_9(OTMSi)_2]^+$ - [HOTMSi], also indicative for 1,5-diol). The base peak, m/z 85, could be rationalized as a cyclic fragment $[C_5H_9O]^+$ originating from the bis-TMSi-1,5-diol moiety but a corresponding ion has not been reported for comparable compounds, e.g. the bis-TMSi ether of nonacosane-6,10-diol (Franich, Gowar & Volkman, 1979). While all the homologous reduction products shared the fragmentation pattern discussed so far, they differed in another α-fragment m/z 369, 383, 397 etc. corresponding to $[C_nH_{2n}OTMSi]^+$ with n ranging from 20 to 27. From a combination of the latter fragment with the 1,5-diol unit the overall chain lengths of the homologues could be inferred. They were confirmed by the presence of respective $[M-15]^+$ ions. Besides, traces of docosane-1,5-diol, tricosane-1,5-diol, hentriacontane-1,5-diol and dotriacontane-1,5-diol were detected by SIM of their α-fragments. Thus, the reduction products were identified as (unbranched) C_{22}–C_{32} 1,5-diols. The same carbon skeletons, number and position of the functional groups could accordingly be assigned to the original ester homologues. To our knowledge, all the alkanediols synthesized and identified here (except hexacosane-1,5-diol, cf. Subramanian, Mohanraj, Kulanthaivel & Srinivasan, 1977), represent novel compounds.

In a second experiment, the *Cerinthe* wax mixture was transesterified with BF_3–MeOH over 48 h. The mass spectra showed the characteristic fragmentation pattern of carboxylic esters and a fragment $[M-15]^+$, as expected for methyl esters. Besides, two characteristic ions at m/z 145 and at m/z 297, 311, 325, etc., respectively, could be interpreted as α-fragments on both sides of a methoxyl group. Thus, the transesterification products represent a series of (novel) methyl 5-methoxyalkanoates.

A series of 5-hydroxyalkanoates was formed after treatment of the *Cerinthe* wax mixture with BF_3–MeOH for only 1 h. Corresponding TMSi ether derivatives were identified by mass spectral peaks at m/z 73 ($[TMSi]^+$, indicative for OH), m/z 203 (α-fragment containing the methyl ester group), m/z 355, 369, 383, etc. (α-fragment containing the alkyl terminus) and m/z 441, 456, 470, etc. ($[M-15]^+$), respectively. It is noteworthy that transesterification with BF_3–MeOH yielded free hydroxyl groups after short treatment, while methoxyl groups were formed after longer incubation. Similar results have been reported especially for esters of secondary and cyclic alcohols, due to a AAL1 cleavage mechanism (Moore & Schwab, 1991).

Combining the results of the transesterification and reduction experiments with the spectral information of the original compounds, the latter are unambiguously identified as δ-lactones **1**–**11** (5-alkanolides). In the leaf wax of *C. minor*, lactone homologues with even carbon numbers, especially hexacosanolide and octacosanolide, prevailed (Tables 1 and 2. With a coverage of 32 $\mu g\,cm^{-2}$, they accounted for 79% of the total wax mixture. In view of this high percentage it seems plausible that the tubular wax crystals on *C. minor* leaves consist of δ-lactones (Gülz, Müller, Schmitz, Marner & Güth, 1992).

Intramolecular esters of C_6–C_{18} hydroxy fatty acids have repeatedly been described as important food flavor compounds (Vajdi, Nawar & Merritt, 1979), flower oil constituents (van Dort, Jagers, ter Heide & van der Weerdt, 1993) and insect pheromones (Bestmann,

Table 1
Compound classes in *Cerinthe minor* leaf cuticular waxes and their homologous composition (% of fraction) and total coverages ($\mu g\,cm^{-2}$). Individual components of the wax mixture were quantitated by GC-FID after formation of TMSi derivatives.

Carbon number	Alkanes	Aldehydes	Primary alkanols	Alkanoic acids	δ-lactones
20	–	–	–	1	–
21	–	–	–	tr	–
22	–	–	–	2	tr
23	9	–	–	1	tr
24	–	–	–	8	1
25	15	–	–	2	6
26	6	tr	tr	1	41
27	7	–	tr	3	14
28	15	tr	10	12	35
29	48	tr	3	11	1
30	–	tr	74	53	1
31	–	–	5	3	tr
32	–	–	8	3	tr
Total coverage	0.1	tr	0.4	0.8	32

tr, less than 0.5% detectable.

Haak, Kern, Hölldobler, 1995). Homologous C_{22}–C_{28} γ-lactones have been detected in the flower wax of *Rosa damascena* (Hadjieva *et al.*, 1974). In contrast, information on the occurrence, properties and ecological roles of the δ-lactone homologues identified here is very scarce. The C_{26} and C_{28} compounds have been reported from the leaf wax of *Heliotropium curassavicum* (Boraginaceae) (Subramanian *et al.*, 1977). δ-Lactones are highly interesting plant wax constituents, because the C_9–C_{24} homologues from other sources (*Trichoderma harzianum* and *de novo* synthesis) showed fungicidal and plant growth stimulating activities (Besnard, Gilbert & Davet, 1994).

Table 2
Isomer composition of wax esters in *Cerinthe minor* leaf waxes: percentage of different *n*-alkanoic acids in esters of various chain lengths. The intensities of the acid fragments $RCOOH_2^+$ in the mass spectrum of the respective homologue were used to calculate its isomer composition

Carbon number	Wax esters
40	2
41	2
42	8
43	6
44	15
45	10
46	22
47	7
48	11
49	3
50	4
51	2
52	3
53	2
54	3
55	tr
56	tr
Total coverage	7

tr, less than 0.5% detectable.

Total wax coverage of *C. minor* leaves was 41 $\mu g\,cm^{-2}$ or 11 $mg\,g^{-1}$ dry wt. Aldehydes (C_{26}–C_{30}), alkanes (C_{23}–C_{29}), primary alcohols (C_{26}–C_{32}), alkanoic acids (C_{20}–C_{32}) and traces of lupeol together accounted for only 3% (1.3 $\mu g\,cm^{-2}$) of the wax mixture (Table 1). Intermolecular alkyl esters (C_{40}–C_{56}, even carbon numbers prevailing) constituted 17% (7 $\mu g\,cm^{-2}$) of the surface extracts (Table 2). The isomer composition of individual ester homologues (Table 3) could be calculated from the intensities of the acid fragments, $RCOOH_2^+$ (Gülz, Markstädter & Riederer, 1994). Ester chain length diversity was mainly caused by variations in acid chain length; C_{10}–C_{22} alkanoic acids were esterified predominantly to 1-triacontanol. Besides, short-chain esters also contained high portions of 1-octacosanol and longer ester homologues also comprised 1-dotriacontanol and higher alcohols. Odd-numbered esters consisted of odd-numbered carbon acids or alcohols.

Table 3
Isomer composition of wax esters in *Cerinthe minor* leaf waxes: percentage of different *n*-alkanoic acids in esters of various chain lengths. The intensities of the acid fragments $RCOOH_2^+$ in the mass spectrum of the respective homologue were used to calculate its isomer composition

Ester carbon number	Alkanoic acid carbon number											
	10	12	13	14	15	16	17	18	19	20	21	22
40	1	81	6	7	1	3	–	–	–	–	–	–
41	–	54	28	9	6	3	1	–	–	–	–	–
42	tr	61	7	24	2	5	1	1	–	–	–	–
43	–	12	22	21	25	10	3	3	1	3	1	1
44	–	6	3	35	7	42	1	3	–	tr	–	2
45	–	tr	4	6	34	36	13	6	tr	tr	tr	1
46	–	1	–	4	2	69	4	19	–	tr	–	1
47	–	3	tr	3	7	22	32	27	4	1	tr	tr
48	–	1	–	1	2	19	3	63	2	4	4	1
49	–	–	–	–	–	10	17	23	18	9	9	14
50	–	–	–	–	–	–	–	29	–	71	–	–

tr, less than 0.5% detectable.

3. Experimental

3.1. Plant material

Plants were grown in the Botanical Garden of the University of Würzburg in 1997. At fruit maturity, 10 leaves were cut and immediately used for wax extraction. The surface area extracted was subsequently measured digitally by scanning photocopies of the leaves. The corresponding dry wt was finally determined after drying the same leaves for 12 d at 55°C.

3.2. Wax extraction and analysis

Leaf blades were immersed twice for 20 s in $CHCl_3$ at room temp. The resulting solutions of cuticular waxes were dried, filtered and the solvent removed under red. pres. Compounds containing hydroxyl groups were transformed (with bis-*N*,*O*-trimethylsilyltrifluoroacetamide in pyridine, 30 min at 70°C) to TMSi derivatives. Qualitative analyses were carried out by GC-mass spectrometry and GC-FTIR (30 m OV-1 WCOT i.d. 320 μm, on-column-injection at 50°C, oven 2 min at 50°C, 40°C min^{-1} to 200°C, 2 min at 200°C, 3°C min^{-1} to 300°C, 30 min at 300°C and He carrier gas inlet pressures 8 min at 40 kPa, 2 kPa min^{-1} to 150 kPa, 8 min at 150 kPa), quantitation of individual compounds by GC-FID (as above, but carrier gas H_2).

3.3. δ-Lactones

GC-FTIR ν_{max} cm^{-1}: 2934, 2866, 1773, 1458, 1235, 1055, 847. GC-EIMS (70 eV) *m*/*z* (rel. int.): *1,5-Tetracosanolide* $[M]^+$ 366 (2), $[M–H_2O]^+$ 348 (10), 330 (1), $[M–H_2O–CO_2]^+$ 304 (6), $[C_6H_{10}O_2]^+$ 114 (31), 111 (23), $[C_5H_7O_2]^+$ 99 (100), 97 (49), 83 (47), 71 (47), 69 (43), 57 (44), 55 (57). *1,5-Pentacosanolide* $[M]^+$ 380 (2), $[M–H_2O]^+$ 362 (11), 344 (1), $[M–H_2O–CO_2]^+$ 318 (5), $[C_6H_{10}O_2]^+$ 114 (31), 111 (26), $[C_5H_7O_2]^+$ 99 (100), 97 (53), 83 (52), 71 (50), 69 (45), 57 (55), 55 (65). *1,5-Hexacosanolide* $[M]^+$ 394 (2), $[M–H_2O]^+$ 376 (14), 358 (2), $[M–H_2O–CO_2]^+$ 332 (6), $[C_6H_{10}O_2]^+$ 114 (35), 111 (28), $[C_5H_7O_2]^+$ 99 (100), 97 (59), 83 (56), 71 (56), 69 (53), 57 (64), 55 (73). *1,5-Heptacosanolide* $[M]^+$ 409 (2), $[M–H_2O]^+$ 390 (14), 372 (2), $[M–H_2O–CO_2]^+$ 346 (5), $[C_6H_{10}O_2]^+$ 114 (37), 111 (31), $[C_5H_7O_2]^+$ 99 (100), 97 (64), 83 (60), 71 (59), 69 (57), 57 (72), 55 (77). *1,5-Octacosanolide* $[M]^+$ 423 (3), $[M–H_2O]^+$ 405 (16), 386 (2), $[M–H_2O–CO_2]^+$ 360 (5), $[C_6H_{10}O_2]^+$ 114 (38), 111 (33), $[C_5H_7O_2]^+$ 99 (100), 97 (70), 83 (66), 71 (65), 69 (61), 57 (79), 55 (81). *1,5-Nonacosanolide* $[M]^+$ 447 (1), $[M–H_2O]^+$ 419 (8), 400 (1), $[M–H_2O–CO_2]^+$ 374 (2), $[C_6H_{10}O_2]^+$ 114 (35), 111 (28), $[C_5H_7O_2]^+$ 99 (100), 97 (54), 83 (47), 71 (49), 69 (55), 57 (69), 55 (64). *1,5-Triacontanolide* $[M]^+$ 461 (1), $[M–H_2O]^+$ 433 (13), 414 (1), $[M–H_2O–CO_2]^+$ 388 (5), $[C_6H_{10}O_2]^+$ 114 (39), 111 (41), $[C_5H_7O_2]^+$ 99 (100), 97 (73), 83 (74), 71 (75), 69 (71), 57 (85), 55 (79).

3.4. 1,5-Alkanediols

The wax mixture (*ca.* 1 mg, 2.5 μmol) was reduced with $LiAlH_4$ (7.6 mg, 200 μmol) in refluxing THF (200 μl) over 48 h. The mixture of LiAl-alcoholate complexes was hydrolyzed with 10% H_2SO_4, the alco-

hols obtained by extraction of the solution with Et_2O and transformed into the corresponding TMSi ethers. GC-EIMS of bis-TMSi ethers *m/z* (rel. int.): *Tetracosane-1,5-diol* $[M-15]^+$ 500 (0.1), 369 (28), 247 (2), 157 (6), 149 (6), 147 (11), 129 (14), 103 (8), 85 (100), 73 (36). *Pentacosane-1,5-diol* $[M-15]^+$ 514 (0.1), $[C_nH_{2n}OTMSi]^+$ 383 (26), 247 (2), 157 (6), 149 (6), 147 (10), 129 (14), 103 (6), 85 (100), 73 (34). *Hexacosane-1,5-diol* $[M-15]^+$ 528 (0.1), $[C_nH_{2n}OTMSi]^+$ 397 (33), 247 (2), 157 (6), 149 (6), 147 (10), 129 (14), 103 (6), 85 (100), 73 (34). *Heptacosane-1,5-diol* $[M-15]^+$ 542 (0.1), 411 (25), 247 (2), 157 (6), 149 (7), 147 (10), 129 (14), 103 (6), 85 (100), 73 (32). *Octacosane-1,5-diol* $[M-15]^+$ 556 (0.1), 426 (26), 247 (2), 157 (6), 149 (6), 147 (10), 129 (13), 103 (6), 85 (100), 73 (31). *Nonacosane-1,5-diol* $[M-15]^+$ 570 (0.1), 440 (16), 247 (2), 157 (5), 149 (7), 147 (10), 129 (14), 103 (5), 85 (100), 73 (31). *Triacontane-1,5-diol* $[M-15]^+$ 584 (0.1), 454 (12), 247 (2), 157 (5), 149 (17), 147 (11), 129 (17), 103 (11), 85 (100), 73 (43).

3.5. 5-Methoxy fatty acid Me esters

The wax mixture (*ca.* 1 mg, 2.5 μmol) was transesterified with BF_3–MeOH (200 μl) for 48 h at 70°C. The resulting mixture was diluted with 1 ml of Et_2O and washed with 3×1 ml H_2O. GC-EIMS *m/z* (rel. int.): *Me 5-methoxytetracosanoate* $[M-15]^+$ 397 (0.5), 381 (0.3), 348 (0.5), 311 (6), 145 (100), 113 (19), 97 (7), 83 (8), 71 (2), 69 (8), 57 (9), 55 (9). *Me 5-methoxypentacosanoate* $[M-15]^+$ 411 (0.4), 395 (0.3), 362 (0.5), 325 (5), 145 (100), 113 (18), 97 (7), 83 (8), 71 (25), 69 (7), 57 (9), 55 (8). *Me 5-methoxyhexacosanoate* $[M-15]^+$ 425 (0.5), 409 (0.4), 376 (0.6), 339 (6), 145 (100), 113 (19), 97 (8), 83 (8), 71 (26), 69 (8), 57 (11), 55 (10). *Me 5-methoxyheptacosanoate* $[M-15]^+$ 439 (0.4), 423 (0.3), 390 (0.5), 353 (5), 145 (100), 113 (17), 97 (7), 83 (7), 71 (24), 69 (7), 57 (10), 55 (8). *Me 5-methoxyoctacosanoate* $[M-15]^+$ 453 (0.4), 437 (0.3), 404 (0.6), 367 (5), 145 (100), 113 (17), 97 (8), 83 (8), 71 (24), 69 (7), 57 (11), 55 (9). *Me 5-methoxynonacosanoate* $[M-15]^+$ 467 (0.4), 451 (0.3), 418 (0.4), 381 (4), 145 (100), 113 (15), 97 (7), 83 (8), 71 (24), 69 (8), 57 (16), 55 (9). *Me 5-methoxytriacontanoate* $[M-15]^+$ 481 (0.2), 465 (0.2), 432 (0.3), 395 (3), 145 (100), 113 (14), 97 (8), 83 (7), 71 (20), 69 (7), 57 (11), 55 (9).

3.6. 5-Hydroxy fatty acid Me esters (TMSi ethers)

The wax mixture (*ca.* 1 mg, 2.5 μmol) was transesterified with BF_3–MeOH (200 μl) for 1 h at 70°C. The reaction product was diluted with 1 ml of Et_2O, washed with 3×1 ml H_2O and concentrated in a stream of N_2. The resulting solid mixture was derivatized with bis-*N*,*O*-trimethylsilyltrifluoroacetamide in pyridine, 30 min at 70°C. GC-FTIR ν_{max} cm^{-1}: 2934, 2866, 1758, 1452, 1362, 1257, 1167, 1084, 847. GC-EIMS of TMSi ethers *m/z* (rel. int.): *Me 5-hydroxytetracosanoate* $[M-15]^+$ 456 (0.6), 439 (0.7), 423 (0.9), 397 (0.1), 369 (15), 348 (0.5), 203 (100), 174 (5), 171 (9), 146 (6), 129 (14), 113 (7), 103 (3), 99 (10), 75 (10), 73 (23), 57 (10), 55 (11). *Me 5-hydroxypentacosanoate* $[M-15]^+$ 470 (0.5), 454 (0.6), 447 (0.1), 411 (0.1), 383 (13), 362 (0.3), 203 (100), 174 (5), 171 (9), 146 (6), 129 (13), 113 (7), 103 (5), 99 (8), 75 (9), 73 (28), 57 (12), 55 (10). *Me 5-hydroxyhexacosanoate* $[M-15]^+$ 484 (2), 468 (0.7), 452 (1), 425 (0.1), 398 (17), 376 (1), 203 (100), 174 (6), 171 (9), 146 (6), 129 (13), 113 (7), 103 (3), 99 (8), 75 (9), 73 (23), 57 (10), 55 (10). *Me 5-hydroxyheptacosanoate* $[M-15]^+$ 498 (1), 482 (0.5), 466 (0.6), 412 (12), 390 (0.4), 203 (100), 174 (6), 171 (9), 146 (6), 129 (13), 113 (6), 103 (3), 99 (7), 75 (9), 73 (21), 57 (10), 55 (9). *Me 5-hydroxyoctacosanoate* $[M-15]^+$ 512 (1), 496 (0.4), 480 (0.6), 426 (13), 405 (0.5), 203 (100), 174 (6), 171 (8), 146 (6), 129 (12), 113 (6), 103 (3), 99 (7), 75 (9), 73 (21), 57 (11), 55 (10). *Me 5-hydroxynonacosanoate* $[M-15]^+$ 526 (1), 510 (0.3), 494 (0.2), 440 (8), 419 (0.3), 203 (100), 174 (5), 171 (8), 146 (7), 129 (13), 113 (6), 103 (3), 99 (7), 75 (8), 73 (22), 57 (11), 55 (8). *Me 5-hydroxytriacontanoate* $[M-15]^+$ 540 (1), 524 (0.2), 508 (0.2), 454 (7), 432 (0.3), 203 (100), 174 (6), 171 (7), 146 (6), 129 (11), 113 (6), 103 (2), 99 (5), 75 (6), 73 (18), 57 (10), 55 (7).

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References

Barthlott, W., & Neinhuis, C. (1997). *Planta, 202,* 1.

Behari, M., & Gupta, C. (1980). *Acta Ciencia Indica Chemica, 6,* 226.

Besnard, O., Gilbert, R., & Davet, P. (1994). *Chemical Abstracts, 121,* 868 Abstract.

Bestmann, H. J., Haak, U., Kern, F., & Hölldobler, B. (1995). *Naturwissenschaften, 82,* 142.

Budzikiewicz, B., Djerassi, C. and Williams, D. H. (1967). In *Mass spectrometry of organic compounds.* (p. 205). San Francisco, Holden–Dey, 1967, p. 205.

Clark, J. B., & Lister, G. R. (1975). *Plant Physiology, 55,* 407.

Deising, H., Nicholson, R. L., Haug, M., Howard, R. J., & Mendgen, K. (1992). *Plant Cell, 4,* 1101.
Franich, R. A., Gowar, A. P., & Volkman, J. K. (1979). *Phytochemistry, 18,* 1563.
Gülz, P.-G., Müller, E., Schmitz, K., Marner, F.-J., & Güth, S. (1992). *Zeitschrift für Naturforschung C, 47,* 516.
Gülz, P.-G., Markstädter, C., & Riederer, M. (1994). *Phytochemistry, 35,* 79.
Hadjieva, P., Stoianova-Ivanova, B., & Danieli, B. (1974). *Chemistry and Physics of Lipids, 12,* 60.
Holloway, P. J. (1970). *Pesticide Science, 1,* 156.
Jetter, R., & Riederer, M. (1994a). *Botanica Acta, 108,* 111.
Jetter, R., & Riederer, M. (1994). *Planta, 195,* 257.
Moore, J. A., & Schwab, J. M. (1991). *Tetrahedron Letters, 32,* 2331.
Neinhuis, C., & Barthlott, W. (1997). *Annals of Botany, 79,* 667.
Rao, J. V. S., & Reddy, K. R. (1980). *Indian Journal of Experimental Biology, 18,* 495.
Subramanian, P. S., Mohanraj, S., Kulanthaivel, P., & Srinivasan, J. (1977). *Experientia, 33,* 707.
Vajdi, M., Nawar, W. W., & Merritt, C. J. (1979). *Journal of the American Oil Chemists Society, 56,* 906.
van Dort, H. M., Jägers, P. P., ter Heide, R., & van der Weerdt, A. J. A. (1993). *Journal of Agricultural and Food Chemistry, 41,* 2063.