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Phytochemistry 67 (2006) 80–90
PHYTOCHEMISTRY
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Cuticular waxes from potato (*Solanum tuberosum*) leaves

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Received 1 June 2005; received in revised form 6 October 2005
Available online 28 November 2005

Abstract

The qualitative and quantitative compositions of leaf cuticular waxes from potato (*Solanum tuberosum*) varieties were studied. The principal components of the waxes were very long chain *n*-alkanes, 2-methylalkanes and 3-methylalkanes (3.1–4.6 μg cm^{-2}), primary alcohols (0.3–0.7 μg cm^{-2}), fatty acids (0.3–0.6 μg cm^{-2}), and wax esters (0.1–0.4 μg cm^{-2}). Methyl ketones, sterols, β-amyrin, benzoic acid esters and fatty acid methyl, ethyl, isopropyl and phenylethyl esters were found for the first time in potato waxes. The qualitative composition of the waxes was quite similar but there were quantitative differences between the varieties studied. A new group of cuticular wax constituents consisting of free 2-alkanols with odd and even numbers of carbon atoms ranging from C_{25} to C_{30} was identified.

Keywords: *Solanum tuberosum*; Solanaceae; Potato; Cuticular waxes; HPLC; GC and GC–MS analyses; 2-Alkanols; Methyl ketones

1. Introduction

The aerial surfaces of all higher plants are covered by a layer of cuticular waxes (Bianchi, 1995). These are composed mainly of very long chain aliphatic components, the chemical composition of which is species-, organ- and plant-ontogeny-specific. It can be also affected by environmental factors.

Although the primary role of cuticular lipids is to prevent uncontrolled water loss, a more interesting function is their contribution to plant–insect interactions (Juniper, 1995). The chemical composition of plant cuticular waxes can affect the resistance of plants to herbivores and may influence herbivore behaviour.

The potato (*Solanum tuberosum*) is widely cultivated in temperate climates. Studies have been done of variations in the chemistry and morphology of cuticular waxes of some solanaceous species, including *Solanum tuberosum* (Sen, 1987). SEM study of the potato leaf surface has demonstrated the ribbon-like distribution of crystalline wax. Unfortunately, there is very little information available on the chemical composition of potato leaf cuticular waxes. Only the main classes of waxes have been identified so far. Long chain *n*-alkanes and 2- and 3-methylalkanes in the C_{25}–C_{33} range have been identified in the leaf cuticular waxes of six potato varieties (Dubis et al., 1987).

The aim of the present work was to study the qualitative and quantitative compositions of leaf cuticular waxes in the potato. It yielded data on the chemical variability of cuticular waxes in four potato cultivars.

2. Results and discussion

Potato leaf waxes were obtained by 10-s extraction in CH_2Cl_2, and separated into 7 fractions by HPLC on a silica gel column (Fig. 1). The potato waxes and HPLC-separated fractions were then GC and GC–MS analysed. HPLC separation of cuticular waxes has a better resolution and is more efficient than column chromatography.

The wax yield of the cuticular wax layers on the leaves of the potato varieties studied were as follows: 5 μg cm^{-2} – Perkoz, 6 μg cm^{-2} – Aster and Maryna, and 7 μg cm^{-2}

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doi:10.1016/j.phytochem.2005.10.012

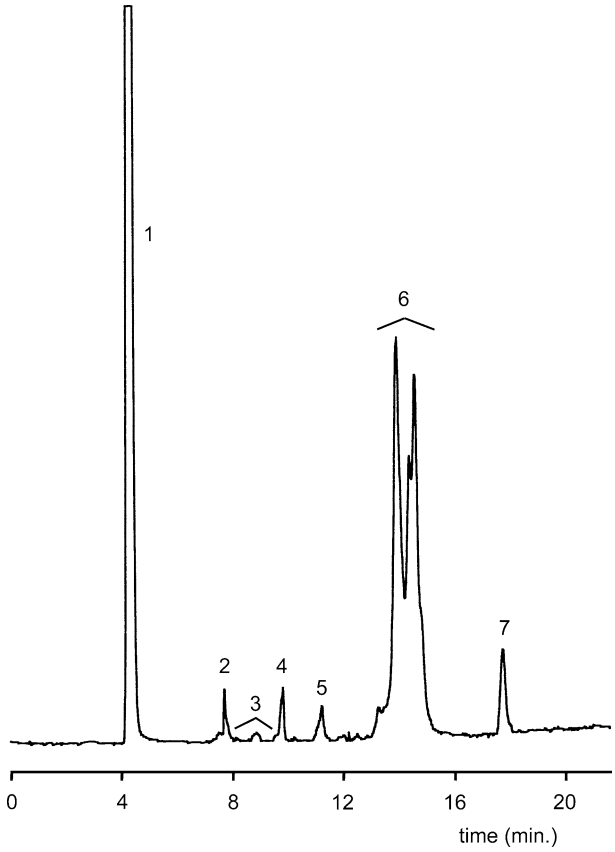

Fig. 1. HPLC-LSD chromatogram of potato leaf cuticular waxes of the Maryna variety. (1) Hydrocarbons; (2) wax esters; (3) benzoic acid esters, fatty acid methyl, ethyl, isopropyl and phenylethyl esters, aldehydes, and ketones; (4) methyl ketones and unknown; (5) unknown; (6) fatty acids, primary and secondary alcohols; (7) sterols.

– Ibis. The gravimetrically estimated yields of the wax are only approximate. The total quantities of GC-identified compounds were 4.1 μg cm^{-2} (Perkoz), 5.3 μg cm^{-2} (Aster), 5.3 μg cm^{-2} (Maryna), and 6.4 μg cm^{-2} (Ibis). The quantities of the unidentified compounds were approximately 0.9 μg cm^{-2} (Perkoz), 0.7 μg cm^{-2} (Aster), 0.7 μg cm^{-2} (Maryna), and 0.6 μg cm^{-2} (Ibis).

The potato surface extract contained significant amounts of sesquiterpene hydrocarbons and alcohols, the composition of which is described in Szafranek et al. (2005). *S. tuberosum* possesses type A glandular trichomes that release volatile substances, among them sesquiterpenes (Ave et al., 1987). Solvent extraction removes non-polar compounds from the surface of potato leaves. In consequence, the extract is a mixture of cuticular waxes and other compounds, in particular, trichome sesquiterpenes. The latter did not contribute to the quantities of wax components identified in the present work.

2.1. Alkanes

The principal components of the potato waxes were very long chain alkanes (Table 1), mainly *n*-alkanes (65–73% of the total alkanes), but also 2-methylalkanes (21–25%) and 3-methylalkanes (6–10%). Total hydrocarbons lay between 3.1 and 4.6 μg cm^{-2}. The individual alkanes ranged in length from C_{23} to C_{34}, with odd-numbered compounds predominating. In all four potato varieties, the most abundant hydrocarbons were *n*-hentriacontane, 2-methyltriacontane, *n*-nonacosane and *n*-heptacosane. The relative composition of the alkanes was very similar in the four

Table 1
Hydrocarbons in leaf cuticular waxes from potato varieties[a]

Hydrocarbon carbon no.[b]	Amount (ng cm^{-2})[c]			
	Aster	Ibis	Maryna	Perkoz
n – 23	6.5 ± 0.1	14.7 ± 0.7	6.9 ± 0.2	2.7 ± 0.1
n – 24	2.1 ± 0.1	6.7 ± 0.3	5.4 ± 0.2	1.1 ± 0.1
n – 25	55.0 ± 0.3	206.1 ± 8.2	209.4 ± 1.2	54.9 ± 2.2
n – 26	18.9 ± 0.9	43.9 ± 2.2	44.7 ± 0.7	19.6 ± 1.0
2-Me-26	2.5 ± 0.1	14.9 ± 0.7	5.7 ± 0.2	3.0 ± 0.2
n – 27	302.5 ± 1.0	560.0 ± 0.2	455.9 ± 3.3	212.4 ± 4.2
3-Me-27	1.4 ± 0.1	5.3 ± 0.3	2.9 ± 0.2	1.7 ± 0.1
n – 28	27.8 ± 1.4	43.6 ± 2.2	35.1 ± 0.7	26.4 ± 1.0
2-Me-28	65.4 ± 2.4	87.1 ± 4.4	86.7 ± 1.9	65.5 ± 2.7
n – 29	425.2 ± 14.8	463.0 ± 4.6	465.0 ± 1.3	379.2 ± 12.4
3-Me-29	45.8 ± 2.6	32.6 ± 1.2	48.2 ± 1.7	40.9 ± 1.0
n – 30	54.4 ± 2.4	76.8 ± 4.0	49.8 ± 0.4	50.7 ± 1.2
2-Me-30	542.2 ± 11.6	579.5 ± 15.7	516.8 ± 3.6	516.0 ± 5.8
n-31	1325.3 ± 0.1	1531.9 ± 0.1	1107.7 ± 0.1	1056.2 ± 0.1
3-Me-31	222.2 ± 1.8	174.4 ± 3.3	200.9 ± 3.2	210.5 ± 0.5
n – 32	61.3 ± 0.4	91.7 ± 5.1	45.9 ± 0.2	47.2 ± 0.3
2-Me-32	220.2 ± 0.7	275.1 ± 18.5	160.1 ± 1.5	176.1 ± 3.2
n – 33	256.4 ± 1.3	308.1 ± 15.4	175.5 ± 1.4	157.5 ± 5.9
3-Me-33	50.7 ± 0.3	51.5 ± 2.6	46.7 ± 1.3	44.5 ± 2.0
Total	3685.9	4566.7	3669.3	3066.1

[a] The results are the mean values from four GC–FID analyses.
[b] The number of carbon atoms in the main chain.
[c] Mean ± SD.

varieties, and resembled that obtained in earlier work (Dubis et al., 1987). The similar patterns found in these four varieties, cultivated under different conditions, suggest that they are characteristic of potato plants in general.

The cuticular components in tobacco leaves are also mostly very long chain *n*-alkanes, 2-methylalkanes and 3-methylalkanes (Severson et al., 1984). Similar alkanes were found in tomato leaf (Smith et al., 1996) and fruit (Bauer et al., 2004) waxes and in the surface waxes from the skins of bell peppers and aubergines (Bauer et al., 2005). The most common hydrocarbons in plant cuticular waxes are *n*-alkanes, ranging in carbon number from 21 to 33 (Bianchi, 1995). However, the cuticular waxes in the leaves of solanaceous plants are unusual in that they contain significant quantities of branched-chain hydrocarbons in addition to normal hydrocarbons.

2.2. Alcohols and acids

The second major class of potato wax compounds consists of primary alcohols. The HPLC-separated fraction No. 6 was analysed in the form of TMSi derivatives (results – see Table 2). Primary alcohols made up between 0.3 and 0.7 μg cm^{-2}. The fraction comprised a homologous series of primary alcohols with odd and even numbers of carbon atoms from C_{18} to C_{32}. The even-numbered homologues were dominant, the major components being 1-tetracosanol (12–18% of the total 1-alkanols), 1-hexacosanol (39–42%) and 1-octacosanol (24–29%). The distribution pattern was very similar in all four potato varieties. Free primary alcohols are widespread components of plant waxes (Bianchi, 1995), and their distribution pattern with three principal homologues is typical of all plants.

Fatty acids were present in potato leaf waxes in quantities from 0.3 to 0.6 μg cm^{-2}. The fraction was composed of a homologous series of saturated, straight-chain fatty acids with odd and even numbers of carbon atoms from C_{16} to C_{30} (Table 3). The most abundant acids were tetracosanoic (32–46% of the total fatty acids) and hexacosanoic acid (19–25%). Three of the potato varieties displayed similar distributions of fatty acid homologues but the Perkoz contained a relatively higher percentage of triacontanoic acid (20%). Free aliphatic fatty acids are common components of leaf waxes, but are usually present in low concentrations (Bianchi, 1995). The amount and composition of the acid fraction can be affected by environmental conditions (Bianchi, 1995). The distribution pattern of fatty acids from potato waxes is quite typical of plants in general.

A homologous series of very long chain secondary alcohols was identified in the potato waxes. The fragmentation patterns of the mass spectra of native 2-alkanols derived from potato waxes (Fig. 2) are similar to those of the 2-hexadecanol and 2-tricosanol standards (Ubik et al., 1975). The expected ions with *m/z* 45, 57, 71, 83, 97, 111, as well as $[M - (H_2O + 28)]^+$ and $[M - H_2O]^+$ are all present. The ion with *m/z* 45 forms the base peak in secondary alcohols substituted with a methyl group on the α-carbon atom (Beynon et al., 1968).

The potato 2-alkanols were also analysed in the form of acetate derivatives, and their mass spectra (Fig. 2) were compared with the literature data (Naccarato et al., 1972). The mass spectra of acetates possess characteristic ions with *m/z* 43, 61 equivalent to $[CH_3COOH_2]^+$, and $[M - 60]^+$ (Beynon et al., 1968; Christie, 1994). Straight-chained 1- and 2-alkoxyacetates have similar mass spectra but the acetate derivatives of 2-alkanols can be distinguished by their contents of *m/z* 87 and 102 ions (Naccarato et al., 1972).

The TMSi ethers of secondary alcohols produce significant ions as a result of α-cleavages, which allows the position of the hydroxyl groups in the alkyl chain to be assigned (Ubik et al., 1975; Evershed, 1992). The mass spectra of the TMSi ethers of the potato 2-alkanols (Fig. 2), the mass spectrum of the TMSi ether of the 2-hexadecanol standard, and that of the TMSi ether of 2-tricosanol published by Ubik et al. (1975) all showed the expected ions with *m/z* 73, 75, 117

Table 2
Primary alcohols in leaf cuticular waxes from potato varieties[a]

Alcohol carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
18	3.3 ± 0.1	3.3 ± 0.2	8.9 ± 0.4	1.9 ± 0.1
20	3.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	1.4 ± 0.1
22	22.7 ± 0.7	14.0 ± 0.2	12.8 ± 0.2	6.1 ± 0.1
23	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	1.2 ± 0.1
24	126.8 ± 4.6	95.3 ± 2.2	90.6 ± 0.3	41.6 ± 2.2
25	19.1 ± 0.8	23.7 ± 0.8	27.6 ± 0.1	12.8 ± 0.6
26	285.3 ± 8.0	291.7 ± 9.0	261.2 ± 1.8	131.0 ± 3.0
27	40.0 ± 1.2	38.3 ± 1.0	43.1 ± 0.2	21.8 ± 0.8
28	173.0 ± 6.0	201.6 ± 8.0	197.3 ± 3.4	78.7 ± 3.0
29	8.9 ± 0.3	9.2 ± 0.1	9.3 ± 0.1	7.4 ± 0.4
30	4.9 ± 0.2	4.4 ± 0.2	5.0 ± 0.2	14.1 ± 0.3
31	2.0 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
32	12.6 ± 0.6	6.3 ± 0.3	11.7 ± 0.6	12.3 ± 0.7
Total	704.8	694.5	674.8	332.6

[a] The results are the mean values from four GC–FID analyses.
[b] Mean ± SD.

Table 3
Fatty acids in leaf cuticular waxes from potato varieties[a]

Fatty acid carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
16	19.9 ± 0.4	15.0 ± 0.8	18.3 ± 0.4	17.2 ± 0.8
18	7.8 ± 0.2	6.1 ± 0.3	8.1 ± 0.3	7.3 ± 0.4
20	3.6 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	3.6 ± 0.1
22	34.6 ± 0.5	39.0 ± 0.2	51.2 ± 1.0	17.4 ± 0.9
23	5.6 ± 0.2	6.9 ± 0.1	6.7 ± 0.3	3.7 ± 0.1
24	225.4 ± 3.7	271.5 ± 5.0	285.7 ± 2.0	108.4 ± 4.0
25	8.6 ± 0.1	13.0 ± 0.4	12.5 ± 0.6	9.1 ± 0.5
26	120.7 ± 1.1	156.9 ± 4.7	148.1 ± 4.6	64.2 ± 2.0
28	43.9 ± 0.9	55.8 ± 1.4	48.9 ± 2.0	38.8 ± 2.0
30	44.1 ± 2.0	52.5 ± 2.8	44.8 ± 2.0	69.2 ± 3.0
Total	514.2	620.7	628.5	339.0

[a] The results are the mean values from four GC–FID analyses.
[b] Mean ± SD.

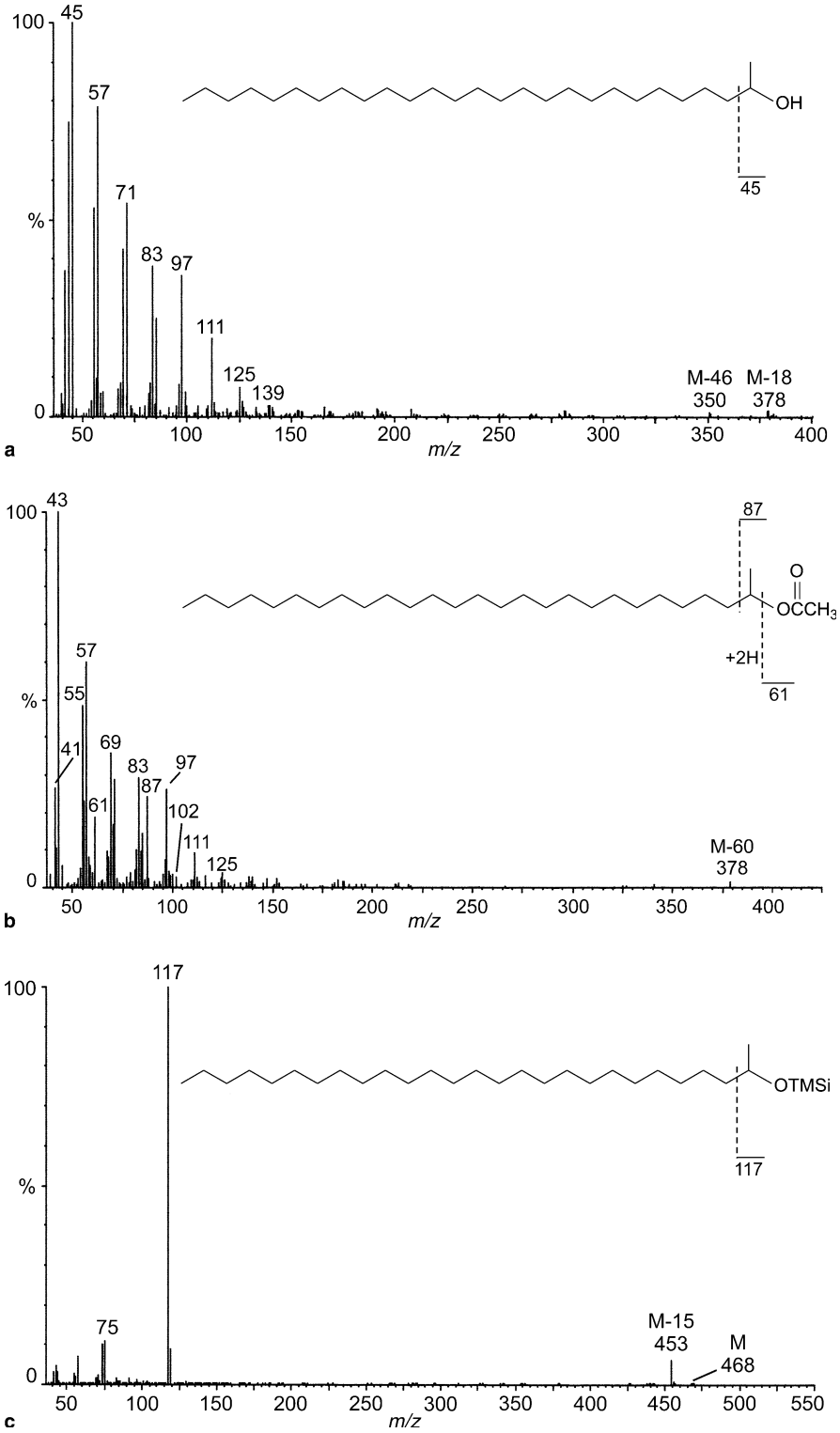

Fig. 2. Mass spectra (70 eV) of 2-heptacosanol identified in potato waxes: (a) native compound, (b) acetate derivative, and (c) TMSi ether.

and $[M - 15]^+$. The base peak of *m/z* 117 corresponding to $[CH_3CHOSi(CH_3)_3]^+$ is due to α-cleavage to the TMSi group.

Free secondary alcohols are not as abundant as primary alcohols in potato waxes. The total amount of 2-alkanols was fairly similar in three varieties (41–68 ng cm^{-2}), but the Ibis contained relatively small quantities of these compounds (3 ng cm^{-2}) (Table 4). Only one type of hydroxylation occurred in the four varieties; this was asymmetrical at position 2 – no other isomers could be detected. The secondary alcohols consisted of homologous series of straight-chained alkan-2-ols with odd and

Table 4
Secondary alcohols in leaf cuticular waxes from potato varieties[a]

Alcohol carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
25	3.0 ± 0.1	1.1 ± 0.1	11.2 ± 0.2	3.6 ± 0.1
26	2.8 ± 0.1	tr[c]	4.8 ± 0.3	3.1 ± 0.2
27	28.5 ± 1.1	1.7 ± 0.1	44.2 ± 0.7	29.0 ± 1.3
28	1.6 ± 0.1	tr	2.1 ± 0.1	2.1 ± 0.1
29	5.2 ± 0.1	tr	5.4 ± 0.3	6.1 ± 0.4
30	0.3 ± 0.1	nd[d]	nd	0.4 ± 0.1
Total	41.4	3.0	67.8	44.3

[a] The results are the mean values from four GC–FID analyses.
[b] Mean ± SD.
[c] tr, trace (< 0.1 ng cm^{-2}).
[d] nd, not detected.

Table 5
Terpenoids in leaf cuticular waxes from potato varieties[a]

Compound	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
Cholesterol[c]	60 ± 8	44 ± 4	15 ± 2	1 ± 0.1
β-Sitosterol[d]	16 ± 1	14 ± 1	15 ± 1	2 ± 0.1
β-Amyrin	34 ± 2	23 ± 1	35 ± 2	25 ± 1

[a] The results are the mean values from four GC–FID analyses.
[b] Mean ± SD.
[c] Calculated from the relative composition of the sterol fraction 7.
[d] Calculated from the analysis of the wax extract without prior HPLC separation. Co-elutes with C_{30} primary alcohol; yields calculated by subtraction of the C_{30} alcohol content.

even numbers of carbon atoms from C_{25} to C_{30}, the main component being 2-heptacosanol (57–69%). Their percentage distribution was similar in all the potato varieties.

The detection of the secondary alcohols is interesting because these compounds are major constituents of waxes from other plant families, for example Papaveraceae (Jetter and Riederer, 1996). In these other plants, however, the secondary alcohol is usually a single dominant component, such as nonacosan-10-ol. Moreover, the substitution is generally asymmetrical, with the hydroxyl in position 8, 9, 10, 12 or 14 but not 2.

To our knowledge, there are no previous reports on plant cuticular waxes with a free alkan-2-ol content. However, alkan-2-ol esters are common constituents of plant waxes (Bianchi, 1995). Alkan-2-ols (C_{17}, C_{19}) occur as aliphatic monomers covalently attached to cutin and suberin polymers, for example, in the leaves of corn (maize), peas, lettuce and barley (von Wettstein-Knowles, 1995). Significant amounts of C_{17}–C_{25} alkan-2-ols also occur free in the suberin of parsnips.

The proposed pathway for the biosynthesis of alkan-2-ols and their esters consists of decarboxylation, reduction and esterification (von Wettstein-Knowles, 1995; Bianchi, 1995). Interestingly, we found both free alkan-2-ols and methyl ketones in the potato waxes, but their distribution patterns were different. These findings contrast with the proposed biosynthetic pathway from methyl ketone precursors to alkan-2-ols.

The HPLC-separated fraction No. 6 also contained between 23 and 35 ng cm^{-2} of the triterpene alcohol β-amyrin (Table 5). This terpenoid is often found in plant waxes (Bianchi, 1995).

2.3. Sterols

The sterol fraction consisted of two constituents only – see Table 5. We found cholesterol (1–60 ng cm^{-2}) in our potato varieties, which is unexpected in plant waxes. Phytosterols have been found in a number of plant waxes, with sitosterol, campesterol and stigmasterol usually being the major ones (Bianchi, 1995). Cholesterol, the major sterol in mammals, is also found in plants (Hartmann, 1998), and generally accounts for a few percent of the sterol mixture of most plants, though some families (e.g., Solanaceae) contain higher amounts. In the cuticular waxes of rape leaves, cholesterol makes up ca. 70% of the total sterol content.

2.4. Ketones and aldehydes

A homologous series of methyl ketones (2-ketones, alkan-2-ones) with chain lengths from C_{25} to C_{33} was present in the potato waxes (Table 6). They were GC–MS identified using their characteristic *m/z* 43 $[R_1CO]^+$, 58 (McLafferty rearrangement), 59 (2H rearrangement) fragments, and weak M^+ and $[M-15]^+$ ions (McLafferty and Tureček, 1993) (Fig. 3). The peak intensities of the *m/z* 58 and *m/z* 59 varied with the chain length of the homologues. Because alkanes and methyl ketones overlap

Table 6
Ketones in leaf cuticular waxes from potato varieties[a]

Ketone carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
Methyl ketones				
25	0.4 ± 0.1	0.8 ± 0.1	1.6 ± 0.2	0.7 ± 0.1
26	nd[c]	nd	nd	0.3 ± 0.1
27	4.4 ± 0.4	4.1 ± 0.4	12.5 ± 0.9	10.8 ± 0.8
28	nd	nd	nd	0.4 ± 0.1
29	2.7 ± 0.3	4.6 ± 0.4	8.4 ± 0.7	7.1 ± 0.6
30	nd	nd	nd	0.7 ± 0.1
31	8.6 ± 0.6	11.4 ± 0.9	23.4 ± 1.6	34.6 ± 1.7
32	nd	0.9 ± 0.1	nd	2.0 ± 0.3
33	10.7 ± 0.9	17.2 ± 1.9	23.7 ± 2.4	34.0 ± 3.4
Total	26.8	38.9	69.5	90.6
Ketones with the carbonyl group located at position 8, 10, 12, 14 and 16				
29	1.9 ± 0.3	2.2 ± 0.4	4.0 ± 0.6	4.5 ± 0.6
31	4.5 ± 0.5	10.3 ± 0.9	11.1 ± 1.1	15.0 ± 1.9
33	3.0 ± 0.6	6.4 ± 1.0	8.5 ± 1.3	9.7 ± 1.6
Total	9.4	18.9	23.6	29.2

[a] The results are the mean values from two GC–MS analyses.
[b] Mean ± SD.
[c] nd, not detected.

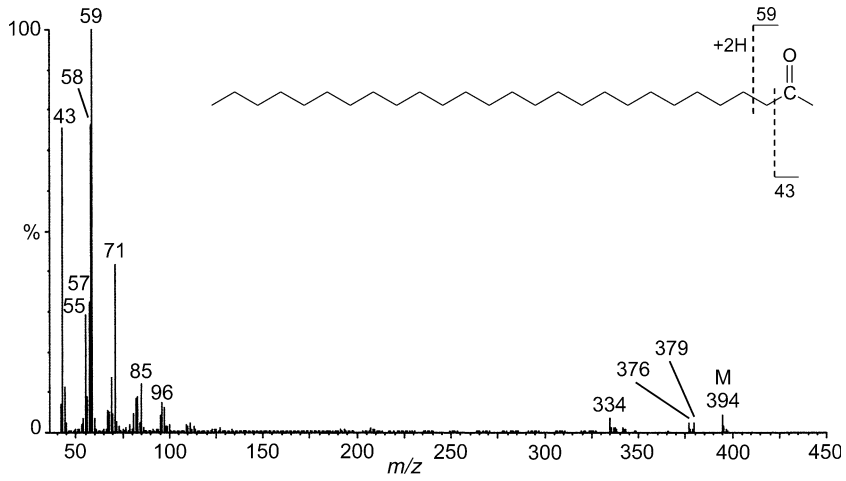

Fig. 3. Mass spectrum (70 eV) of 2-heptacosanone identified in potato waxes.

on the non-polar capillary column, their identification would be quite impossible without prior HPLC separation.

Methyl ketones were identified unequivocally by GC–MS analysis following their conversion to the corresponding 2-alkanol TMSi ethers. Their mass spectra and retention times correspond to those of the secondary alcohols found in the potato waxes.

The methyl ketones were mostly odd-numbered ones, the principal component being C_{33}. Minor amounts of even-numbered homologues were found in the Perkoz and Ibis varieties. The distribution patterns were similar for all potato varieties but the total amount of methyl ketones varied from 27 to 91 ng cm^{-2}.

As far as we know, methyl ketones have previously been reported as components of plant cuticular waxes from only two species: *Avicennia marina* (Mohan et al., 1998) and *Eucalyptus globulus* (Rapley et al., 2004). 2-Tridecanone, a short-chain methyl ketone occurring in the wild tomato, exhibited toxicity towards some insect pests (Dimock et al., 1982; Antonious et al., 2003). Very long chain methyl ketones were also identified in the soils, peat, sediments and clay mineral sealing of a waste disposal site (Püttmann and Bracke, 1995, and the references cited therein). They may well be formed from plant wax esters under aerobic conditions.

Potato methyl ketones were accompanied by ketones with the carbonyl group in positions 8, 10, 12, 14 and 16 (Tables 6 and 7). GC–MS on capillary columns with a non-polar stationary phase separates ketones according to the number of carbon atoms. The positions of the carbonyl groups in the different isomeric ketones were identified by GC–MS from $[RCO]^+$ and $[RC(OH)CH_3]^+$ ions. The relative compositions of the individual co-eluting ketones within each GC peak were estimated from the intensities of $[R_1CO]^+$ and $[R_2CO]^+$ ions according to the formula described by Netting and Macey (1971). Ketones with odd numbers of carbon atoms – 29, 31, and 33 – were found in the potato waxes, their yields varying from 9 ng cm^{-2} (Aster) to 29 ng cm^{-2} (Perkoz).

Table 7
Distribution of positional isomers (%) in ketones in leaf cuticular waxes from potato varieties[a]

Ketone	Aster	Ibis	Maryna	Perkoz
Nonacosanone				
Nonacosan-8-one	15	13	nd[b]	9
Nonacosan-10-one	16	12	13	13
Nonacosan-12-one	45	53	56	55
Nonacosan-14-one	23	22	31	23
Hentriacontanone				
Hentriacontan-8-one	21	21	nd	8
Hentriacontan-10-one	20	15	17	25
Hentriacontan-12-one	20	24	32	30
Hentriacontan-14-one	29	30	38	26
Hentriacontan-16-one	10	10	13	11
Tritriacontanone				
Tritriacontan-8-one	18	13	15	9
Tritriacontan-10-one	27	27	21	30
Tritriacontan-12-one	17	23	19	33
Tritriacontan-14-one	9	7	9	4
Tritriacontan-16-one	29	30	36	24

[a] The quantities of the individual isomers were estimated from the intensities of $[R_1CO]^+$ and $[R_2CO]^+$ ions.
[b] nd, not detected.

Potato waxes also contained detectable levels of very long chain aldehydes (13–17 ng cm^{-2}) with carbon atom numbers from C_{22} to C_{32} (Table 8). The most abundant of these were tetracosanal, hexacosanal, octacosanal and triacontanal. The distribution patterns of individual aldehydes were all quite similar in three of the four potato varieties studied; the exception was the Perkoz variety, which contained larger amounts of triacontanal and less hexacosanal.

2.5. Esters

Homologous esters of very long chain fatty acids with long primary alcohols (wax esters) were present in the cuticular waxes of the four potato varieties, but at different levels

Table 8
Aldehydes in leaf cuticular waxes from potato varieties[a]

Aldehyde carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
22	0.3 ± 0.1	0.1 ± 0.1	nd[c]	nd
23	0.3 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.3 ± 0.1
24	2.0 ± 0.2	1.8 ± 0.1	1.9 ± 0.3	1.5 ± 0.1
25	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	0.5 ± 0.1
26	4.9 ± 0.4	5.1 ± 0.5	4.9 ± 0.4	2.6 ± 0.3
27	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.1
28	4.5 ± 0.4	3.3 ± 0.3	5.1 ± 0.5	2.9 ± 0.4
29	0.8 ± 0.1	0.6 ± 0.1	nd	0.7 ± 0.1
30	2.2 ± 0.2	1.5 ± 0.2	2.4 ± 0.3	3.0 ± 0.2
31	0.1 ± 0.1	0.2 ± 0.1	nd	0.4 ± 0.1
32	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
Total	16.6	15.4	17.2	13.2

[a] The results are the mean values from two GC–MS analyses.
[b] Mean ± SD.
[c] nd, not detected.

between 76 and 365 ng cm^{-2} (Table 9). They were GC-separated according to the number of carbon atoms. Potato cuticular waxes contained a wide range of different wax ester isomers with chain lengths from C_{36} to C_{52}. The principal wax esters were those with carbon numbers C_{44} and C_{46}. The HPLC-separated wax esters were hydrolysed and analysed as TMSi derivatives. The wax ester constituents were saturated straight-chain fatty acids and primary alcohols from C_{14} to C_{28} and from C_{20} to C_{28}, respectively. The main esters were those of hexadecanoic (ca. 20%), octadecanoic (10%), eicosanoic (30%), docosanoic (15%) and tetracosanoic (8%) acids. The distribution patterns of the fatty acids in the wax esters differed from those of the free fatty acids in potato waxes. The alcohols liberated from the wax esters consisted predominantly of docosanol (ca. 17%), tetracosanol (20%), hexacosanol (30%) and octacosanol (15%). Their distribution pattern, showing a high content of docosanol, was unlike that of the free primary alcohols in the potato waxes. The composition of wax esters found in potato cuticular waxes is typical of plants (Bianchi, 1995).

Table 9
Wax esters in leaf cuticular waxes from potato varieties[a]

Wax ester carbon no.	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
36	2.6 ± 0.1	2.3 ± 0.1	1.2 ± 0.1	1.6 ± 0.1
37	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
38	7.5 ± 0.1	7.1 ± 0.3	3.4 ± 0.1	3.3 ± 0.1
39	0.8 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
40	16.0 ± 0.3	20.1 ± 1.0	6.7 ± 0.3	5.2 ± 0.3
41	2.1 ± 0.1	3.2 ± 0.2	1.3 ± 0.1	0.8 ± 0.1
42	31.4 ± 0.6	46.1 ± 2.8	14.4 ± 1.6	8.7 ± 0.5
43	3.7 ± 0.2	6.7 ± 0.3	2.0 ± 0.1	1.0 ± 0.1
44	42.8 ± 1.3	75.8 ± 5.3	22.2 ± 2.0	13.2 ± 0.9
45	5.1 ± 0.2	12.7 ± 1.1	3.5 ± 0.3	1.8 ± 0.2
46	36.2 ± 0.7	83.8 ± 6.7	20.7 ± 1.0	14.7 ± 1.2
47	4.6 ± 0.1	14.2 ± 1.4	3.3 ± 0.3	2.1 ± 0.2
48	20.1 ± 0.6	56.6 ± 5.1	13.3 ± 0.3	11.4 ± 1.0
49	0.7 ± 0.1	6.6 ± 0.5	1.3 ± 0.1	1.3 ± 0.1
50	8.9 ± 0.1	20.2 ± 0.6	5.2 ± 0.1	6.0 ± 0.2
51	1.7 ± 0.1	2.3 ± 0.2	1.0 ± 0.1	0.8 ± 0.1
52	4.6 ± 0.2	5.9 ± 0.5	1.9 ± 0.2	3.2 ± 0.2
Total	189.3	365.2	102.1	75.7

[a] The results are the mean values from two GC–FID analyses.
[b] Mean ± SD.

Careful examination of HPLC-separated fraction No. 3 revealed benzoic acid esters and methyl, ethyl, isopropyl and 2-phenyl-ethyl esters of fatty acids (Table 10), all of which are unusual components of cuticular wax (Bianchi, 1995).

Benzoic acid esters of very long chain alcohols were present in potato waxes (4–18 ng cm^{-2}). Their mass spectra showed significant ions with m/z 123, 105 and 77, characteristic of these compounds (Gülz et al., 1987). Benzoic

Table 10
Esters in leaf cuticular waxes from potato varieties[a]

Compounds	Amount (ng cm^{-2})[b]			
	Aster	Ibis	Maryna	Perkoz
Benzoic acid esters				
22[c]	tr[d]	0.4 ± 0.1	nd[e]	0.1 ± 0.1
24	1.0 ± 0.2	1.6 ± 0.2	1.5 ± 0.3	3.6 ± 0.4
25	1.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	4.5 ± 0.7
26	4.0 ± 0.5	2.2 ± 0.2	1.6 ± 0.3	8.2 ± 1.1
27	0.3 ± 0.1	0.3 ± 0.1	nd	1.1 ± 0.2
28	0.3 ± 0.1	0.2 ± 0.1	nd	0.4 ± 0.1
Total	6.6	5.4	4.0	17.6
Methyl esters				
16[f]	1.1 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	1.1 ± 0.2
18:2	2.0 ± 0.3	0.5 ± 0.1	0.8 ± 0.2	1.9 ± 0.3
18:3	1.8 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	1.6 ± 0.2
18	0.6 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.8 ± 0.1
20	0.4 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
22	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.1
24	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
26	nd	tr	0.1 ± 0.1	0.1 ± 0.1
Total	6.6	1.9	3.2	7.6
Ethyl esters				
Total	0.05	0.04	0.1	0.2
Isopropyl esters				
14[f]	0.7 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
16	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Total	1.2	1.0	1.0	0.8
Phenylethyl esters				
16[f]	nd	0.1 ± 0.1	nd	nd
20	nd	0.4 ± 0.1	tr	0.1 ± 0.1
22	0.1 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
24	0.2 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
26	0.1 ± 0.1	tr	nd	0.2 ± 0.1
Total	0.4	1.7	0.4	1.1

[a] The results are the mean values from two GC–MS analyses.
[b] Mean ± SD.
[c] Fatty alcohol carbon number.
[d] tr, trace (< 0.05 ng cm^{-2}).
[e] nd, not detected.
[f] Fatty acid carbon number.

acid hexacosanyl, tetracosanyl and pentacosanyl esters predominated in the potato waxes (Table 10). Benzoic acid esters of fatty alcohols had been found earlier in *Citrus halimii* and *Euphorbia dendroides* leaf waxes (Gülz et al., 1987; references cited therein).

Potato waxes from all four varieties contained methyl esters of the even-carbon-numbered acids from C_{16} to C_{26} with yields from 2 to 8 ng cm^{-2} (Table 10). Their distribution pattern differed from that of the free fatty acids. Besides the methyl esters of saturated fatty acids, methyl linoleate and methyl linolenoate were also present. The most prominent methyl esters were those of low molecular weight fatty acids ($C_{16:0}$, $C_{18:2}$, $C_{18:3}$, $C_{18:0}$). In addition, ethyl esters of saturated fatty acids (C_{16}–C_{26}) – mainly the ethyl esters of hexadecanoic, octadecanoic, eicosanoic and tetracosanoic acids – were found as minor components. Methyl esters had previously been found in the cuticular waxes of *Abies balsamea* and *Picea glauca* (Tulloch, 1987), *Picea abies* (Sümmchen et al., 1995) and in the flower waxes of the red raspberry (Griffiths et al., 2000). Methyl and ethyl eicosanoate were found in *Eucalyptus* waxes (Steinbauer et al., 2004). There are many possible interpretations of the ecological significance of the esters. Methyl esters of saturated and unsaturated fatty acids (C_{16}, C_{18}) have been found to act as pheromones for some insect species, for example, worker bees (Nelson and Blomquist, 1995).

We found the isopropyl esters of only two fatty acids – tetradecanoic and hexadecanoic – in the potato waxes (Table 10). The identification was confirmed by co-injections with synthesised standard compounds and by comparing their MS data. The mass spectrum of tetradecanoic acid isopropyl ester from potato waxes is shown in Fig. 4. A characteristic feature of the esters of higher alcohols are the abundant ions corresponding to the protonated carboxylic acid residue $[RCOOH_2]^+$ originating from a two-proton transfer fragmentation (Murphy, 1993; Kitson et al., 1996). The McLafferty rearrangement for isopropyl esters is shifted to *m/z* 102.

To our knowledge, the presence of isopropyl esters in plant cuticular waxes has not been reported so far in other species. The egg surface coating of honeybee queen-laid eggs contains isopropyl tetradecanoate and hexadecanoate (Katzav-Gozansky et al., 2003). The methyl, ethyl and isopropyl esters of fatty acids have also been reported from the roots of boraginaceous species with medicinal applications (Papageorgiou and Assimopoulou, 2003).

Phenylethyl esters were found to be minor components of potato waxes (0.4–1.7 ng cm^{-2}). There were esters of even-numbered fatty acids with chain lengths from C_{16} to C_{26} (Table 10). Their mass spectra showed characteristic dominant ions with *m/z* 104 (Gülz and Marner, 1986). Phenylethyl esters had previously been found in the cuticular waxes of *Jojoba* (Gülz and Marner, 1986), Papaveraceae species (Jetter and Riederer, 1996) and *Eucalyptus* (Steinbauer et al., 2004; Rapley et al., 2004).

These analyses of the four potato varieties show that potato leaf cuticular waxes are more complex than previously thought (Sen, 1987; Dubis et al., 1987). Methyl ketones, alkan-2-ols, sterols, β-amyrin, benzoic acid esters and fatty acid methyl, ethyl, isopropyl and phenylethyl esters were found for the first time in potato leaf waxes. The qualitative composition of the potato waxes was quite similar in these four varieties. There were quantitative differences in the content of the wax constituents between the potato varieties, which were grown under identical conditions. Further studies are needed to find out whether there is any correlation between the potato wax components, insect infestation and feeding.

3. Experimental

3.1. Plant material and wax extraction

Solanum tuberosum plants (cv. Aster, Ibis, Maryna, and Perkoz) were field-grown from certified seed tubers. The three-week-old plants were harvested, transferred immediately

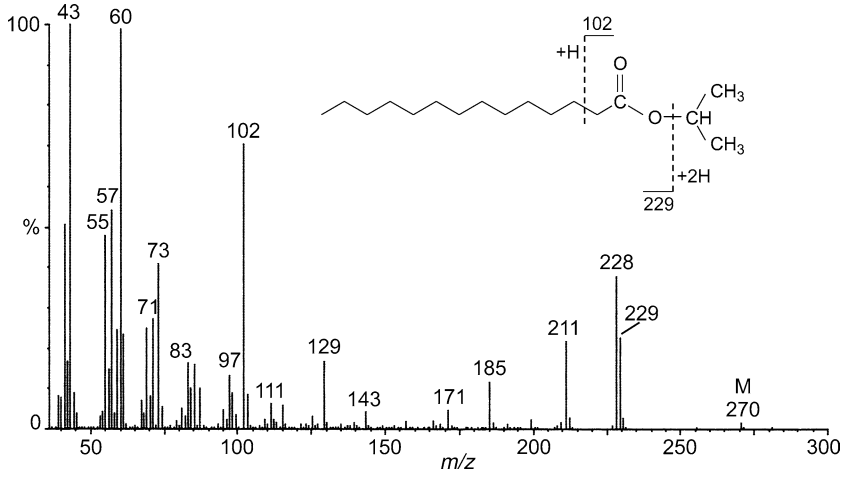

Fig. 4. Mass spectrum (70 eV) of tetradecanoic acid isopropyl ester from potato waxes.

to the laboratory, weighed and extracted. The leaf cuticular waxes were extracted by dipping and shaking the leaves (100 g) in CH_2Cl_2 for 10 s. For quantitative studies, the solvent was spiked with internal standards (*n*-docosane, *n*-hexatriacontane, *n*-propyl hexadecanoate, 19-methyleicosanoic acid). During the extraction, care was taken to avoid immersing any damaged or cut leaves in solvent. The wax extracts were then filtered, dried with Na_2SO_4 and concentrated. The total surface areas of the leaves was estimated by photocopying the leaves, cutting out the paper copies and weighing them.

3.2. Wax separations and analyses

HPLC separations of waxes were performed on a silica gel column (250 × 10 mm i.d., Alltech) attached to a Shimadzu chromatograph equipped with a light-scattering detector. A binary gradient elution system with solvent A (*n*-hexane) and solvent B (CH_2Cl_2–Me_2CO, 85:15) was applied with linear gradient formation from 2 to 100% of solvent B during 20 min. The flow-rate was 3.5 ml min^{-1}. Fractions were identified by co-injections with standards and collected. Fractions 3 and 4 were collected together for quantitative analyses. Additionally, the HPLC-separated fraction 6 (fatty acids and alcohols) was HPLC re-separated on an analytical silica gel column (4.6 × 10 mm i.d., Alltech) with isocratic elution (*n*-hexane–CH_2Cl_2–Me_2CO, 85:13:2) at a flow-rate of 0.8 ml min^{-1} to yield secondary alcohols.

The GC–FID analyses were carried out on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with an FID detector. Argon was used as carrier gas. The split ratio was 1:30 and the injection volume for the samples was ca. 1 μl.

Mass spectra (70 eV) were recorded on a TRIO-2000 quadrupole mass spectrometer. The samples were introduced through a Hewlett–Packard 5890 gas chromatograph. The carrier gas was helium.

The alkanes, esters, aldehydes and ketones were analysed directly, whereas the polar compounds were analysed as TMSi derivatives.

The GC–MS and GC–FID analyses of fraction 1 (alkanes), as well as TMSi derivatives of potato wax extracts and fraction 6 (fatty acids, β-amyrin, secondary and primary alcohols), fraction 7 (sterols), and fatty acids and alcohols liberated from the wax esters were performed using 30 m × 0.25 mm i.d., film thickness 0.25 μm RTX-1 WCOT capillary column (Restek). The oven temperature was programmed to rise from 200 to 320 °C at a rate of 4 °C min^{-1} and then held for 15 min. The carrier gas pressure was 80 kPa. The temperatures of the injector and the detector were 330 °C.

Esters, aldehydes and ketones (fractions 3 and 4) and secondary alcohols (as native compounds and acetate derivatives) were GC–MS analysed only (oven 6 min at 40 °C, 4 °C min^{-1} to 320 °C, 30 min at 320 °C; injector 330 °C; capillary column as above).

Wax esters (fraction 2 and native potato wax extract) were GC–FID analysed only (oven at 200 °C, 4 °C min^{-1} to 380 °C, 20 min at 380 °C; injector 380 °C; detector FID with a ceramic flame jet, temp. 400 °C; carrier gas at 95 kPa; 30 m × 0.25 mm i.d., film thickness 0.1 μm DB-1HT WCOT capillary column, J&W Scientific).

Compounds were identified by comparing their mass spectra and retention times with published data (Ubik et al., 1975; MSDC, 1991; Evershed, 1992; Murphy, 1993; Christie, 1994; Hamilton, 1995; Kitson et al., 1996) and commercial standards. The following standards were used: 16:0, 18:0, 20:0, 22:0, 24:0, 26:0 fatty acids, and 18:0, 20:0, 24:0, 26:0 fatty alcohols, β-sitosterol, cholesterol, behenic acid behenyl ester, behenic acid arachidyl ester, methyl linoleate (Sigma, Poland), 2-hexadecanol (Aldrich, Poland), 2-pentadecanone (Lancaster, Poland), β-amyrin (Fluka). MS data and retention times of the potato benzoic acid esters, fatty acid isopropyl and phenylethyl esters were compared with those from synthesised esters.

Internal standards representing three compound classes (*n*-docosane, *n*-hexatriacontane, *n*-propyl hexadecanoate, 19-methyleicosanoic acid) were added early during the wax extractions. They were retrieved in the HPLC fractions 1, 3, and 6. Moreover, fractions 3 and 4 were HPLC-collected together to facilitate quantitative analyses using the same internal standard (*n*-propyl hexadecanoate). Only wax esters and β-sitosterol were quantified during the analysis of the cuticular wax extract without HPLC separation. The amount of cholesterol was calculated from the percentage composition of the HPLC-separated sterol fraction 7.

Fraction 1 (alkanes), fraction 6 (fatty acids, alcohols, and β-amyrin), and fraction 7 (sterols) were identified by GC–MS and also analysed by GC–FID. GC–FID quantitative analysis was performed in four replicates. The relative standard deviation (RSD) of the quantitative results was $\leqslant$5%. The jointly collected fractions 3 and 4 (aldehydes, ketones and esters) were GC–MS quantified in two replicates (RSD ca. 10–15%). The wax esters were GC–FID quantified in two replicates (RSD $\leqslant$10%).

3.3. Synthesis of standards and derivatives for analyses

Isopropyl esters. The general procedures for the syntheses of esters involved a slightly modified version of the H_2SO_4 acid-catalysed esterification described by Christie (1994). The samples of tetradecanoic or hexadecanoic acids (ca. 10 mg, Sigma) were dissolved in *iso*-PrOH (4 ml) containing 0.05 ml of conc. H_2SO_4. The mixture was left overnight in a glass vial at 50 °C, after which water was added (10 ml) and the esters extracted with hexane (3 × 10 ml). The hexane layer was shaken with water (10 ml) containing $KHCO_3$ (2%), dried over Na_2SO_4, concentrated under N_2 and purified by LC (SiO_2, CH_2Cl_2–hexane 1:9).

Benzoic acid esters and phenylethyl esters. Benzoic acid esters were synthesised from 1-eicosanol, 1-tetracosanol, and 1-hexacosanol and phenylethyl esters from 16:0, 20:0,

22:0, 24:0 and 26:0 fatty acids according to the procedures described by Gülz et al. (1987) and by Gülz and Marner (1986), respectively.

The resulting esters were GC–MS analysed under the conditions used for the analyses of potato compounds.

TMSi derivatives. A silylation procedure was employed for sterols, alcohols and fatty acids using a mixture of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and trimethylchlorosilane (TMCS) (85:15, v/v) at 70 °C for 30 min.

Secondary alcohol acetylation. The potato 2-alkanols and 2-hexadecanol (Aldrich) were acetylated according to the procedure described by Blau (1995). The sample (ca. 0.1 mg) was dissolved in $CHCl_3$ (0.5 ml), and Ac_2O (0.1 ml) in HOAc (0.2 ml) was added. The sample was left overnight in a glass vial at 50 °C, and excess reagents were removed in a stream of N_2.

Wax ester hydrolysis. The hydrolysis–silylation procedure for potato wax esters (fraction 2) involved saponification of esters in ethanolic KOH solution, evaporation in a stream of nitrogen to dryness and then silylation of the hydrolysis products (Brüschweiler and Hautfeune, 1990).

Methyl ketone reduction. The potato methyl ketones and 2-pentadecanone (Lancaster) were reduced with $NaBH_4$ according to the modified procedure described by Kitson et al. (1996) to yield 2-alkanols. This modified procedure was tested by the reduction and analysis of 2-pentadecanone. The sample of methyl ketones (ca. 0.2 mg) was dissolved in EtOH (0.06 ml) and water (0.01 ml). $NaBH_4$ (2 mg) was added and the solution was left at room temperature for 1 h. $NaBH_4$ was destroyed by adding HOAc until no more gas evolved. The solution was evaporated to dryness under N_2. MeOH (0.5 ml) was added to the sample and the solution evaporated to dryness again (4×). The resulting 2-alkanols were GC–MS analysed after their conversion to TMSi derivatives.

3.4. GC retention times and mass spectral data of selected compounds

GC–EIMS (70 eV), retention time, *m*/*z* (rel. int.): *Isopropyl ester of tetradecanoic acid*: 43.4 min, 270 $[M]^+$ (2), 229 $[RCOOH_2]^+$ (24), 228 $[RCOOH]^+$ (38), 211 $[M - OCH(CH_3)_2]^+$ (22), 185 (11), 129 (17), 102 (70), 73 (41), 60 (100), 43 (100). *Isopropyl ester of hexadecanoic acid*: 48.3 min, 298 $[M]^+$ (1), 257 $[RCOOH_2]^+$ (30), 256 $[RCOOH]^+$ (40), 239 $[M - OCH(CH_3)_2]^+$ (21), 185 (8), 129 (21), 102 (63), 73 (50), 60 (100), 43 (100).

TMSi ethers of alkan-2-ols: 2-pentacosanol: 25.0 min, $[M]^+$ not detectable, 425 $[M - 15]^+$ (7), 117 $[CH_3CHOSi(CH_3)_3]^+$ (100), 73 (14), 75 (13), 57 (7), 43 (5). *2-Hexacosanol*: 26.7 min, $[M]^+$ not detectable, 439 $[M - 15]^+$ (5), 117 $[CH_3CHOSi(CH_3)_3]^+$ (100), 73 (13), 75 (14), 57 (6), 43 (5). *2-Heptacosanol*: 28.5 min, 468 $[M]^+$ (0.5), 453 $[M - 15]^+$ (8), 117 $[CH_3CHOSi(CH_3)_3]^+$ (100), 73 (12), 75 (15), 57 (7), 43 (5). *2-Octacosanol*: 30.0 min, $[M]^+$ not detectable, 467 $[M - 15]^+$ (6), 117 $[CH_3CHOSi(CH_3)_3]^+$ (100), 73 (11), 75 (15), 57 (8), 43 (4). *2-Nonacosanol*: 31.8 min, $[M]^+$ not detectable, 481 $[M - 15]^+$ (6), 117 $[CH_3CHOSi(CH_3)_3]^+$ (100), 73 (10), 75 (15), 57 (8), 43 (5).

Ketones (Perkoz variety): Nonacosanone (mixture of isomers): 69 min, 422 $[M]^+$ (2), 323 $[R_1CO]^+$ (2), 127 $[R_2CO]^+$ (3), 295 $[R_1CO]^+$ (3), 155 $[R_2CO]^+$ (4), 267 $[R_1CO]^+$ (15), 183 $[R_2CO]^+$ (16), 239 $[R_1CO]^+$ (5), 211 $[R_2CO]^+$ (8), 95 (17), 85 (23), 71 (70), 57 (90), 43 (100). *Hentriacontanone*: 72 min, 450 $[M]^+$ (2), 351 $[R_1CO]^+$ (2), 127 $[R_2CO]^+$ (5), 323 $[R_1CO]^+$ (10), 155 $[R_2CO]^+$ (13), 295 $[R_1CO]^+$ (10), 183 $[R_2CO]^+$ (17), 267 $[R_1CO]^+$ (11), 211 $[R_2CO]^+$ (13), 239 $[R_1CO]^+$ (10), 95 (20), 85 (30), 71 (80), 57 (80), 43 (100). *Tritriacontanone*: 75 min, 478 $[M]^+$ (2), 379 $[R_1CO]^+$ (1), 127 $[R_2CO]^+$ (2), 351 $[R_1CO]^+$ (6), 155 $[R_2CO]^+$ (4), 323 $[R_1CO]^+$ (5), 183 $[R_2CO]^+$ (6), 295 $[R_1CO]^+$ (0,5), 211 $[R_2CO]^+$ (1), 267 $[R_1CO]^+$ (4), 239 $[R_2CO]^+$ (4), 95 (15), 85 (27), 71 (70), 57 (88), 43 (100).

Methyl ketones: *2-heptacosanone*: 66.2 min, 394 $[M]^+$ (5), 379 $[M - 15]^+$ (3), 376 $[M - 18]^+$ (3), 85 (13), 71 (45), 59 (100), 58 (80), 43 (77); *2-Nonacosanone*: 69.6 min, 422 $[M]^+$ (4), 407 $[M - 15]^+$ (2), 404 $[M - 18]^+$ (3), 85 (20), 71 (62), 59 (100), 58 (82), 43 (64); *2-Hentriacontanone*: 72.7 min, 450 $[M]^+$ (5), 435 $[M - 15]^+$ (2), 432 $[M - 18]^+$ (3), 85 (23), 71 (62), 59 (100), 58 (60), 43 (57); *2-Tritriacontanone*: 76.1 min, 478 $[M]^+$ (5), 463 $[M - 15]^+$ (2), 460 $[M - 18]^+$ (3), 85 (24), 71 (63), 59 (100), 58 (59), 43 (57).

Acknowledgements

The work was partly financed by Grants PBZ-KBN-060/T09/2001/11 and KBN-125/P06/95/08 from the Polish State Committee for Scientific Research, and by Grant BW 8000-5-0290-0 from the University of Gdańsk. We are grateful to the two anonymous referees for their comments on the manuscript.

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