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EPICUTICULAR AND INTRACELLULAR LIPIDS OF *Hippophae rhamnoides* LEAVES

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It has been established that the bulk of the epicuticular lipids of common sea buckthorn leaves is represented by the class of higher aliphatic esters, while higher fatty acids, other acids of the aliphatic series, and cyclic acids of triterpene nature have also been detected. The intracellular lipids consist mainly of esters of various alcohols with higher fatty acids, while the same alcohols are found in the form of acetates and in the free state. In addition, the composition of the intracellular lipids includes hydrocarbons, triacylglycerols, free fatty acids, carotenoids, aldehydes, and acids of triterpene nature.

With the aim of developing an analog of sea buckthorn oil from cheap raw material, we have investigated the unsaponifiable part of an ethereal extract of common sea buckthorn leaves [1]. It was found that it contains the same group of compounds as the unsaponifiable part of the pericarp lipids [2] and, in addition to these, phytol, campesterol, cycloartenol, nortriterpene alcohols, and polyprenols. The polyprenols, which possess a known biological activity [3, 4] amounted to 12% of the weight of the unsaponifiable substances of the leaves.

The pharmacological investigation of a 2% freon extract of the leaves in sunflowerseed oil has shown its stimulating effect on repair processes [5].

The yields of hexane extracts and some of their chemical properties have been determined previously for samples of leaves of Central Asian forms of sea buckthorn [6].

We have investigated the epicuticular lipids in comparison with the intracellular lipids, using leaves of sea buckthorn growing in the Chimgan mountain area and gathered in the period of ripeness of the berries.

The main components of the epicuticular lipids were esters of long-chain alcohols and higher fatty acids (Table 1). By CC it was possible to isolate three fractions of these compounds, together making up more than half the weight of the whole extract. MS analysis of these fractions showed that some separation of the esters according to chain length and degree of unsaturation had taken place in the course of the experiment.

Although Aasen et al. [7] warn against the quantitative estimation of individual components of a mixture of esters from its MS because of the variation in the intensities of their molecular peaks with a variation in the volatility of their molecules, nevertheless, in the MSs of the three fractions it is possible to trace a clear uniformity of the increase in the intensities of the peaks of the fragmentary ions of the acyl and alcoholic moieties composing these types of ester molecules.

The peaks of the molecular and fragmentary ions of the aliphatic esters of the first two fractions (M^+M^+ 508-704) corresponded to the homologous series of compounds from $C_{34}H_{68}O_2$ to $C_{48}H_{96}O_2$ with a predominance of the even members. The set of probable species of saturated ester molecules is shown in Table 2.

GLC of the fatty acids (FAs) and the alcohols (Alcs) obtained after severe hydrolysis of the esters of the three fractions (Table 3) showed that the main esterifying acid of the esters of fraction I was behenic, $C_{22:0}$ (approximately half the mass of the FAs), while there was also a substantial contribution from arachidic, $C_{20:0}$, lignoceric, $C_{24:0}$, and palmitic, $C_{16:0}$, acids. Tetracosanol, $C_{24:0}$, and docosanol, $C_{22:0}$, were the main alcohols among those of fraction I.

In addition to higher aliphatic alcohols, the alcoholic part of fraction I contained about 12% of cyclic alcohols, among which, according to their RRTs in GLC and the peaks of the molecular ions revealed in MS, were: β-sitosterol [*m/z* (%): 414

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TABLE 1. Epicuticular Lipids of Sea Buckthorn Leaves

Class of lipids	Amount, % by weight	Composition of individual classes, % GLC
Hydrocarbons	9.8	C_{27}–1.8; C_{29}–71.5; C_{31}–26.7
Esters, fraction I	31.9	$C_{42}H_{84}O_2$–$C_{48}H_{96}O_2$ with a predominance of the even homologs; esters of β-sitosterol, of epifriedelanol, and of 24-methylenecycloartanol
Esters, fraction II	18.2	$C_{34}H_{68}O_2$–$C_{46}H_{92}O_2$ saturated series with a predominance of the even homologs
Esters, fraction III	8.6	$C_{32}H_{60}O_2$–$C_{48}H_{92}O_2$ unsaturated series
Free higher aliphatic alcohols	2.1	$C_{16:0}$–$C_{28:0}$ saturated series with a predominance of the even homologs
Free higher carboxylic acids	3.0	$C_{14:0}$–$C_{26:0}$ saturated series with a predominance of even homologs
Triterpenol and sterol acetates	2.4	Acetates of α-amyrin (48), of β-amyrin (28), 24-methylenecycloartenol (24), β-Sitosterol acetate (100)
Free triterpenols	4.1	α-Amyrin (46), β-amyrin (29), 24-methylenecycloartenol (21), epifriedelanol (4)
Free sterols	3.8	β-Sitosterol
Triterpene acids	16.1	Ursolic acid (75), oleanolic acid (10), unidentified acid (15)

(M^+, 45), 396 (10), 381 (5)]; epifriedelanol [*m/z* (%): 428 (M^+21), 413 (8), 395 (5), 341 (10), 304 (7), 275 (13), 207 (45)], and 24-methylenecycloartanol [*m/z* (%): 440 (M^+, 5), 425 (10), 422 (12), 407 (20)].

When subjected to Ag^+/TLC in system 1, ester fraction II revealed spots not only of saturated esters but also of unsaturated esters. In the acyl moiety obtained after hydrolysis of the esters of fraction II a decrease in the proportion of the main acids of fraction I was observed, with some rise in the amount of the 16:0 acid and of unsaturated acids of the 15:1-26:1 series. The alcoholic moiety was also characterized by the appearance of unsaturated compounds of the even series. This was reflected in the MS of the total esters of fraction II. It is known [8] that the main ion in the spectrum of saturated long-chain esters is that corresponding to a double hydrogen rearrangement (RCOO + $2H^+$), and, in addition, the spectra of saturated compounds are characterized by more intense molecular ions and fragments of the alcoholic moiety (R′ − 1). In the spectra of unsaturated esters, fragmentation takes place with the splitting out of a molecule of the alcohol, and also of the acid, from the molecular ion (M−R′OH and M−RCOOH).

According to IR spectroscopy, the monounsaturated acids of the esters from the epicuticular lipids were exclusively the *trans*acids.

In MS, the saturated esters of fraction II gave the peaks of molecular ions corresponding to the homologous series $C_{34}H_{68}O_2$–$C_{46}H_{92}O_2$, in which the even members predominated.

In fraction III the total unsaturation of the alcohols and acids composing the esters had risen even further, reaching 53.1 and 55.6%, respectively, according to GLC. The MS of the esters of this fraction showed, together with the peaks of the molecular ions of saturated esters, clear peaks of molecular ions corresponding to unsaturated esters (M^+504–M^+700). Molecules with such molecular masses could be composed both of a saturated/unsaturated pair of residues and of an unsaturated acid and an unsaturated alcohol residue.

A considerable contribution to the epicuticular lipids was made by hydrocarbons (HCs) (9.8%), represented mainly by three saturated homologs, more than 70% of which consisted of nonacosane, C_{29}.

Cyclic alcohols (triterpenols and sterols) were found in the epicuticular waxes, the triterpenols being present in both the free and the esterified states. Within these states they had similar compositions except for the fact that the GLC of the free alcohols, as compared with that of the natural acetates, showed an additional peak corresponding to epifriedelanol.

Free and bound sterols were represented solely by β-sitosterol.

The higher aliphatic alcohols and higher acids of the epicuticular lipids consisted of a series of saturated homologs, $C_{16:0}$-$C_{28:0}$, in the case of the alcohols and $C_{14:0}$-$C_{26:0}$ in the case of the acids.

The fraction of triterpene acids was converted into methyl esters (MEs) by the action of diazomethane, and these were investigated by GLC: the main peak (RRT relative to the ME of oleanolic acid 1.15) corresponded to ursolic acid, and a small amount of its isomer, oleanolic acid, was also present; there was also a peak of an unidentified acid (RRT 0.9) of the same type as ursolic, since the MS of the sum of the MEs of the acids showed only the peak of a common molecular ion, M^+470, and the fragmentation of an ursane skeleton.

TABLE 2. Probable Molecular Species of Higher Aliphatic Esters of the Epicuticular Lipids

Molecular species		Peaks of the molecular ions and of the main fragments, *m/z*			
Acyl moiety R	Alcoholic moiety R′	M^+	$(RCC_2H_2)^+$	$(RCO)^+$	$(R'-H)^+$
24:0	24:0	704	369	351	336
22:0	26:0	704	341	323	364
21:0	26:0	690	327	309	364
23:0	24:0	690	355	337	336
24:0	22:0	676	369	351	308
22:0	24:0	676	341	323	336
20:0	26:0	676	313	295	364
23:0	22:0	662	355	337	308
21:0	24:0	662	327	309	336
22:0	22:0	648	341	323	308
24:0	20:0	648	369	351	280
20:0	24:0	648	313	295	336
18:0	26:0	648	285	267	364
21:0	22:0	634	327	309	308
23:0	20:0	634	355	337	280
16:0	26:0	620	257	239	364
18:0	24:0	620	285	267	336
20:0	22:0	620	313	295	308
22:0	20:0	620	341	323	280
21:0	20:0	606	327	309	280
14:0	26:0	592	229	211	364
16:0	24:0	592	257	239	336
18:0	22:0	592	285	267	308
20:0	20:0	592	313	295	280
19:0	20:0	578	299	281	280
17:0	22:0	578	271	253	308
14:0	24:0	564	229	211	336
16:0	22:0	564	257	239	308
18:0	20:0	564	285	267	280
15:0	22:0	550	243	225	308
17:0	20:0	550	271	253	280
14:0	22:0	536	229	211	308
16:0	20:0	536	257	239	280
13:0	22:0	522	215	197	308
12:0	22:0	508	201	183	308
14:0	20:0	508	229	211	280

The composition of the HCs isolated from the cell lipids was more diverse and included eight components, among which, as in the case of the HCs, nonacosane predominated (Table 4).

According to their UV spectrum, the carotenoids were represented mainly by β-carotene.

As in the case of the surface lipids, a considerable part of the cell lipids consisted of esters, but in the cell lipids they had a different composition. The mixture of esters was also partially fractionated on a column. Hydrolysis products of the individual fractions were studied by PTLC, GLC, and MS.

On PTLC in system 2, the alcohols of ester fraction I′ were separated into four zones. The most polar consisted of terpene diols — uvaol and erythrodiol [MS, *m/z* (%): 442 (M^+, 2), 424 (4), 234 (15), 207 (13), 203 (100); GLC: RRTs relative to β-sitosterol 2.17 and 1.98, respectively]. The second zone corresponded to β-sitosterol, and the third to monomethylsterols — epifriedelanol and citrostadienol [MS, *m/z* (%): 426 (M^+, 54), 411 (59), 393 (10), 328 (60), 310 (14), 285 (100)], with a predominance of the latter by weight. The fourth zone proved to be inhomogeneous: it contained the trimethylsterols cycloartenol [MS, *m/z* (%): 426 (M^+, 15), 408 (24), 393 (5), 339 (13), 203 (80)] and 24-methylenecycloartanol and phytol (M^+296). Among the FAs participating in the esterification of these alcohols we found an unusually high level (20.8%) of myristic (14:0) acid. However, quantitatively the main esterifying acid was the 16:0 species, and, as a whole, the proportion of saturated acids in the esters of fraction I′ was 75.5%.

The alcohols from the esters of fraction II were also separated into four zones, but triterpene diols were absent. Here the main representatives, quantitatively, were isoprenoid alcohols (50%), represented by three homologs: decaisoprenol, M^+ 698 (33.4%); undecaisoprenol, M^+766 (58.1%); and dodecaisoprenol, M^+834 (8.5%). Aliphatic alcohols made up 20% by weight of the total alcohols, and among them the C_{29} species predominated (65%). It is interesting to note the coincidence of the maxima of the distribution curves of the alcohols and the HCs in the cell lipids, which was not observed for the surface lipids of the leaves.

The zone of methylsteroids contained citrostadienol and obtusifoliol [MS, *m/z* (%): 426 (M^+, 54), 408 (29), 245 (22), 227 (15)].

TABLE 3. Compositions of the Components of Individual Classes of Epicuticular Lipids, % GLC

Homologs	Ester fraction						Free higher carboxylic acids	Free higher alcohols
	I		II		III			
	FAs	Alcs	FAs	Alcs	FAs	Alcs		
14:0	0.6	–	2.1	–	–	–	1.5	–
15:0	0.5	–	2.6	2.5	–	–	–	–
15:1	–	–	0.4	0.5	–	1.2	–	–
16:0	11.3	7.0	13.5	3.7	10.2	6.3	13.0	4.0
16:1	2.9	–	5.1	1.1	15.6	4.5	–	–
17:0	–	5.7	–	2.2	–	–	–	2.9
17:1	–	–	0.5	0.6	1.4	7.2	–	–
18:0	1.2	6.8	2.3	2.5	5.3	–	8.2	6.3
18:1	1.9	–	2.3	1.1	11.7	5.8	–	–
19:0	–	–	–	–	–	–	–	2.5
19:1	–	–	0.5	–	–	–	–	–
20:0	14.9	9.6	11.9	11.5	10.3	6.1	15.5	12.4
20:1	–	–	–	4.2	2.2	9.9	–	–
21:0	3.3	–	2.7	–	–	–	1.2	3.3
21:1	–	–	–	–	1.8	–	–	–
22:0	49.9	24.7	40.2	42.5	21.1	25.3	41.0	29.5
22:1	–	–	0.8	10.3	2.5	16.2	–	–
23:0	1.1	–	0.7	–	–	–	1.3	3.6
23:1	–	–	0.6	–	1.2	–	–	–
24:0	12.4	28.2	3.3	10.0	–	6.7	12.8	18.5
24:1	–	–	9.1	5.1	13.4	10.4	–	–
25:1	–	–	0.6	–	2.3	0.4	–	–
26:0	–	6.0	–	–	–	–	5.5	9.3
26:1	–	–	0.8	2.2	1.0	–	–	–
27:0	–	–	–	–	–	–	–	2.6
28:0	–	–	–	–	–	–	–	5.1

*FAs — fatty acids; Alcs — alcohols.

The sterols consisted of β-sitosterol, with traces of campesterol (M^+400). The alcohols of this fraction were bound by acids possessing a lower degree of saturation than the preceding fraction (67%) through a decrease in the level of the 14:0-16:0 acids and an increase in the amount of the 16:1 acid. The acid fraction was also distinguished by the presence of long-chain acids.

In the following ester fraction (fraction III′) the total saturation had decreased still more (50.9%) because of a further increase in the amount of the 16:1 acid. In the alcoholic part, in addition to the methylsteroids, isoprenols, and fatty alcohols of the preceding fraction, we found α- and β-amyrins in a ratio of 1:2 [MS, *m/z* (%): 426 (M^+, 18), 411 (15), 218 (100); GLC, RRT relative to β-sitosterol: α-amyrin 1.15, and β-amyrin 1.05].

In ester fraction IV′ the level of the 16:1 acid was a maximum (40.7%) and the total saturation amounted to 46.8%. As before, these acids bound the bulk of the α- and β-amyrins and the residues of the isoprenols and methylsterols of the preceding fractions.

In a thin layer, the alcoholic part of ester fraction V′ revealed a single zone, corresponding to triterpenols (amyrins). The degree of saturation of the acids esterifying these residual triterpenols was the same as that of the acids of fraction IV′, but their qualitative composition differed by the appearance in the esters of the 18:3 acid, linolenic, not found in any of the preceding fractions. The 18:3 acid enriched other classes of cell lipids, amounting to one third of the weight of the acids in the triacylglycerols and to one half in the free fatty acids.

According to GLC and MS, in the triterpenol and sterol acetate fraction, the acetic acid esterified the amyrins and β-sitosterol selectively.

After the saponification of the triacylglycerols (TAGs), tocopherols were isolated from the neutral fraction. In the MS of the tocopherols we observed the peaks of the molecular ions of α-tocopherol (M^+430) and of γ- and δ-tocopherols (M^+ 416). The amount of this class in the total extract was not determined.

The free isoprenols, like the bound ones, were represented by three homologs: deca-, undeca-, and dodecaisoprenols in practically the same ratio, with a predominance of undecaisoprenol in the mixture.

TABLE 4. Intracellular Lipids of Sea Buckthorn Leaves

Lipid classes	Amount, % by weight	Compositions of the individual classes
Hydrocarbons	1.9	GLC, % by weight: C_{21}–0.7; C_{22}–1.0; C_{23}–2.4; C_{24}–1.5; C_{25}–2.8; C_{27}–5.2; C_{29}–66.2; C_{31}–20.2
Carotenoids	0.7	β-Carotene (UV spectrum, EtOH, λ_{max}, nm): 437, 462, 489
Esters fraction I′	12.9	Acids, GLC, % by weight 12:0–4.4; 14.0–20.8; 15:0–1.8; 16:0–43.6; 16:1–12.6; 18:0–4.2; 18:1–10.7; 18:2–1.2; 20:0–0.7
		Alcs, PTLC, % by weight: triterpene diols, 15; sterols, 25; monomethylsterols, 15; triterpenols, 45.
Esters fraction II′	9.4	Acids, GLC, % by weight 12:0–0.7; 14:0–3:9; 15:0–0.7; 16:0–33.6; 16:1–21.3; 18:0–15.1; 18:1–7.1; 18:2–4.4; 20:0–3.9; 22:0–1.0; 24:0–8.3
		Alcs, PTLC, % by weight: sterols, 15; methylsterols, 15; aliphatic alcs, 20; isoprenols, 50
Esters fraction III′	9.9	Acids, GLC, % by weight 12:0–0.4; 14:0–1.0; 16:0–34.4; 16:1–39.5; 18:0–15.1; 18:1–6.5; 18:2–3.0
		Alcs, PTLC, % by weight: methylsterols, 10; aliphatic alcs, 10; triterpenols, 55; isoprenols, 25
Esters fraction IV′	9.5	Acids, GLC, % by weight 12:0–0.4; 14:0–0.8; 16:0–30.8; 16:1–40.7; 18:0–14.8; 18:1–7.6; 18:2–4.9
		Alcs, PTLC, % by weight: methylsterols, 10; triterpenols, 70; isoprenols, 20
Esters fraction V′	2.7	Acids, GLC, % by weight 12:0–0.3; 14:0–0.4; 16:0–29.9; 16:1–29.1; 16:2–1.2; 18:0–10.8; 18:1–8.2; 18:2–10.7; 18:3–7.5
		Alcs: triterpenols
Triterpenol and sterol acetates	0.9	Acetates of α- and β-amyrins and of β-sitosterol
Triacylglycerols	5.9	Acids, GLC, % by weight 12:0–0.4; 14:0–2.1; 16:0–19.7; 16:1–23.7; 18:1–9.4; 18:2–15.5; 18:3–31.0
		Alcs: glycerol, α, δ, and γ-tocopherols
FFAs	6.2	GLC, % by weight: 14:0–1.6; 16:0–15.9; 16:1–4.7; 18:0–1.2; 18:1–10.4; 18:2–14.5; 18:3–51.7
Isoprenols	7.0	MS, *m/z* (%): $C_{50}H_{81}OH$ 698 (M^+,30.5), $C_{55}H_{89}OH$ 766(M^+,62), $C_{60}H_{97}OH$ 834 (M^+,7.5)
Fatty alcohols, triterpenols, pink pigment	8.7	GLC, % by weight: 19:0–7.4; 21:0–1.3; 22:0–4.5; 24:0–23.9; 25:0–2.3; 26:0–25:0–1.1; 26:0–11.9; 27:0–1.9; 28:0–3.7; α-amyrin, 14.4; β-amyrin, 27.5
Monomethylsterols, sterols	2.2	GLC, % by weight: citrostadienol, 18.5; obtusifoliol, 10.0; β-sitosterol, 72.5
Sterols	3.4	β-sitosterol
Triterpene aldehydes	1.2	MS, M^+440
Triterpene acids	10.0	GLC, % by weight: ursolic, 90; oleanolic, 10
Triterpene diols	3.9	GLC, % by weight: uvaol, 45; erythrodiol, 55
Pigments + unident.	0.6	Chlorophylls and pheophytins

Issuing from the column after the isoprenols was a mixture of α- and β-amyrins with aliphatic alcohols, the main ones being the 26:0 alcohol, as has also been observed for sea buckthorn pericarp oil [9]. The same fraction contained a pink pigment of unknown nature.

The monomethylsterols, both in the free state and in the form of esters, were represented by citrostadienol and obtusifoliol, and the sterols by β-sitosterol.

The mass spectrometry of the more polar fraction, likewise giving on TLC a positive Liebermann–Burchard reaction, permitted us to assign this fraction to the triterpene aldehydes [MS, *m/z* (%): 440 (3), 422 (4), 232 (45), 207 (25), 203 (100)]. Its composition was not further studied.

The fraction next in polarity, with an intense green color, was subjected to the action of diazomethane, after which PTLC led to the isolation of the MEs of triterpene acids, 90% of the weight of which, according to GLC consisted of ursolic acid and 10% of oleanolic acid.

In the more polar fraction we detected triterpene compounds which, by their chromatographic mobilities, the nature of their colorations with H_2SO_4, and the results of MS and GLC, were identified as uvaol and erythrodiol.

The pigments of the polar fractions were the usual ones for photosynthesizing tissues and were represented by chlorophylls and their oxidation products.

We have been unable to detect the nortriterpene acids previously identified in sea buckthorn leaves, which Kukina et al. consider to be products of the autoxidation of triterpene aldehydes taking place during the drying of the leaves. It is possible that the nature of the solvent plays a role here: we used a less polar solvent than in [1].

Thus, the epicuticular lipids, like the cell lipids are represented mainly by the class of esters, but in the lipids of the surface wax of the leaves more than half the weight of the total lipids (58.7%) consists of esters of long-chain alcohols and acids, while in the cell lipids 44.4% of the weight of the total extract consists of esters of fatty acids and various alcohols — mainly cyclic and isoprenoid. The main esterifying acids in the first case are saturated long-chain species, 20:0-24:0, and in the esters of the cell lipids they are the 16:0 and 16:1 species.

The epicuticular lipids contain no carotenoids whatever, these being substances of the plastid apparatus of the cell.

It is an interesting fact that the 18:3 acid that is characteristic for photosynthesizing tissues is not present in the esters of the cell lipids although there is a large amount of it in the TAGs and FFAs.

The leaf lipids have been studied previously [1, 2] by saponifying a total extract and investigating the acid and neutral fractions separately. According to our results it is quite obvious that in sea buckthorn leaves in the native state a large part of the alcohols are present in the bound, and not the free, form. The fractional compositions of the FAs esterifying particular alcohols have been established. Moreover, in the Central Asian form of sea buckthorn that we have investigated we have detected epifriedelanol, which has not been found in other forms.

There are very few publications devoted to the comparative study of surface and cell lipids [10, 11]. Thus, Svenningsson [11] considers that triterpene acids are present only in the intracuticular, and not in the epicuticular, wax. Other researchers [1] assume that these acids are autoxidation products, although it is known [12] that the extracellular membranes of fruit and leaves may contain triterpene compounds, including the above-mentioned acids, in the form of disseminated crystal grains. In apples and grapes, triterpene acids make up about half the cuticular wax [13].

Kolattukudy [14] has proposed a biosynthetic pathway for these acids from α- and β-amyrins. In a model experiment due to Delanghe [15], isolated surface lipids without ursolic acid were replaced by surface lipids with an ursolic acid content corresponding to that encountered in nature. It was found that the addition of ursolic acid had a structure-reinforcing action and enhanced the water-repellent capacity. All these facts permitted the triterpene acids to be to be assigned to the native compounds of the surface. Their nondetection in the cell lipids of sea buckthorn leaves can be explained by the experimental conditions — namely the use of the nonpolar solvent hexane and, as a consequence, the incomplete extraction of epicuticular waxes.

EXPERIMENTAL

The epicuticular lipids were extracted by immersing intact air-dry leaves in hexane for 1-2 min. Then, after filtration and the elimination of the solvent by drying, the same leaves were ground in an electric mill and were extracted repeatedly by steeping at room temperature with the same solvent until the neutral lipids had been extracted completely.

The yield of epicuticular waxes was 0.47% of the weight of the initial leaves, and the yield of cell lipids was 3.53%.

Solvent systems: 1) hexane–diethyl ether (9:1); 2) petroleum ether–ethyl acetate–benzene (80:15:5).

For the conditions of separating the resulting extracts by CC and ATLC/PTLC, see [8, 14], and for the severe hydrolysis of the wax esters, see [16].

The individual alcohol fractions were purified by PTLC in various solvent systems, and further purification was achieved by acetylating the fractions and rechromatographing in the same systems. For the conditions in the GLC of the free alcohols and of their acetates, see [14]; of the FAs in the form of their MEs, [8]; and of the MEs of the triterpene acids, [17].

The IR spectra of the higher aliphatic esters of the epicuticular lipids of fractions I-III were similar (KBr, cm^{-1}): 2920, 2850, 1470, 770 (CH_3 and CH_2 groups), 1740-1750, 1160-1170 (–COOR).

MS of the aliphatic esters of the epicuticular lipids, fraction I, *m/z* (%): 704(M^+, 30), 690 (M^+, 3.5), 676(M^+, 28), 662(M^+, 3), 648(M^+, 30), 634(M^+, 4), 620(M^+, 20), 410(6), 408(4), 396(1.5), 369(78), 364(40), 351(40), 341(100), 336(42), 323(50), 313(95), 309(15), 308(35), 295(43), 285(13), 280(27), 267(7), 257(60), 252(15), 239(30), 238(9), 224(5), 57(85), 43(100).

MS of the esters of the epicuticular lipids, fraction II, *m/z* (%): 676(M^+, 15), 672(M^+, 10), 662(M^+, 1), 648(M^+, 35), 634(M^+, 1), 620(M^+, 25), 606(M^+, 2), 592(M^+, 40), 578(M^+, 2), 564(M^+, 30), 550(M^+, 2.5), 536(M^+, 25), 522(M^+, 1), 508(M^+, 10), 348(40), 341(100), 336(25), 323(40), 308(60), 306(50), 313(60), 295(25), 280(40), 257(80), 239(30), 229(18), 211(12), 201(8), 183(5), 83(80), 69(80), 57(80), 55(100), 43(80).

MS of the esters of the epicuticular lipids, fraction III, *m/z* (%): 700(M^+, 8), 672(M^+, 6), 648(M^+, 12), 644(M^+, 3), 620(M^+, 9), 616(M^+, 2), 592(M^+, 7), 588(M^+, 4), 562(M^+, 3), 560(M^+, 4), 532(M^+, 1), 504(M^+, 1), 476(0.5), 348(35), 341(60), 334(28), 323(25), 313(30), 308(60), 306(40), 295(14), 278(36), 264(50), 257(28), 239(10), 236(35), 83(85), 69(90), 55(100).

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