UNUSUAL ALKANES PATTERN OF SOME PLANT CUTICULAR WAXES*

Karel STRÁNSKÝ, Milan STREIBL and Karel UBIK

Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague

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Dedicated to the memory of Professor František Šorm.

Eight gas chromatograms present an unusual composition of alkanes (including branched alkanes) from cuticular waxes. In plants of a lower phylogenetic class (liverwort, lichens), further in seeds and sprouts of tubers (*Solanaceae*) and especially in the anthers and pollens of some plants a high proportion of branched alkanes has been found (up to 72%).

In the course of our studies of cuticular waxes of plant origin, during which we have analysed about 150 different materials, we also met cases when the composition and the content of alkanes was quite unusual. Some such selected cases are described in this paper.

The composition and the proportion of alkanes in cuticular waxes has been studied by various authors; the results are published in the review articles by Hamilton[1], Nelson[2] and Kolattukudy[3]. In some of our earlier papers[4,5] we too have described the composition and the proportion of alkanes, but we have not studied branched hydrocarbons in greater detail.

RESULTS AND DISCUSSION

The samples analysed in this paper (see the gas chromatograms on Figs 1–4) were prepared so as to contain only saturated hydrocarbons, i.e. n-alkanes, 2-methylalkanes (iso-), 3-methylalkanes (anteiso-), or also alkanes branched in a different way. The presence of iso- and anteiso-alkanes was detected on the basis of retention times[6,7] in gas chromatography (GLC) and their structures were confirmed by means of gas chromatography-mass spectrometry (GC-MS)[8–11].

Fig. 1a shows the composition and the proportion of alkanes in the leaves of oak, *Quercus petraea* (MATTUSCH.) LIEBL. A clear predominance of odd n-alkanes is

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evident, with the maximum at n-C_{29}, while branched hydrocarbons are not visible on the record. This pattern is common in the majority of plant waxes, but usually the extent of the homologous series and/or the position of the maximum is variously changed.

We found a completely different pattern in the aquatic form of the liverwort *Fegatella conica* (L.) CORDA, a representative of more primitive plants. The ratio of odd and even homologues (maximum at n-C_{27}) is unusually level (Fig. 1b). Traces of branched alkanes (iso- and anteiso-) are discernible on this chromatogram (about 2·2% of the total content of hydrocarbons).

Alkanes from the lichen *Biatora lucida* (ACH.) FR. (Fig. 2a) display only an indistinct predominance of odd members. The amount of branched hydrocarbons is almost 11%, with predominating anteiso-alkanes (9%). However, in the case of anteiso-C_{27} and anteiso-C_{29} the known fact is invalidated[2,12], i.e. that in odd-numbered homologues iso-derivatives should predominate.

Anthers and pollens of some plants are also interesting material, the composition of which we had investigated earlier[5]. Fig. 2b represents alkanes from the anthers of the flowers of horse chestnut, *Aesculus hippocastanum* L. The amount of branched

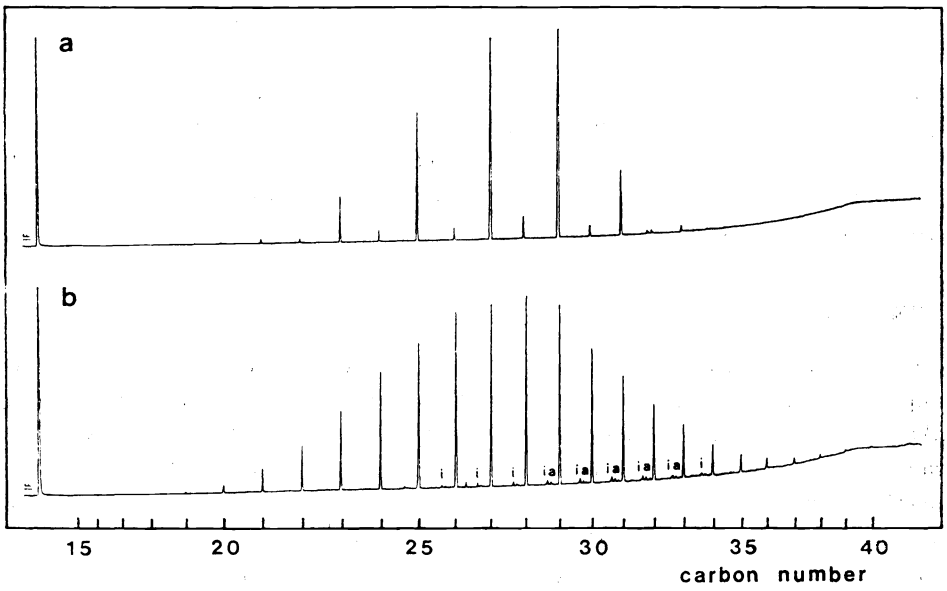

FIG. 1

Gas chromatogram of alkanes from: a *Quercus petraea* (MATTUSCH.) LIEBL. — leaves; b *Fegatella conica* (L.) CORDA — aquatic form

alkanes is up to 20% (16% of iso- and 4% of anteiso-). In agreement with earlier findings[2,12] the odd-numbered 2-methylalkanes and even-numbered 3-methylalkanes predominate.

Figure 3a shows a chromatogram of alkanes from anthers of corn poppy, *Papaver rhoeas* L. In the $C_{24}-C_{27}$ interval isoalkanes predominate (45%) over corresponding n-alkanes. The total content of branched hydrocarbons is 48%. The record of hydrocarbons isolated from the pollen of the same plant (not shown in the figure) again displays a considerable amount of branched alkanes (30% iso- and 2% anteiso-) in the C_{22} to C_{29} interval.

A prevalent content of iso- and anteiso-alkanes (57% and 3%) is evident from the chromatogram of alkanes from the pollen of *Lilium willmottiae* E. H. WILSON (Fig. 3b), where 2-methyltetracosane (iso-C_{25}) is even the dominant peak (40%). A similar predominance of isoalkanes has also been described for pollens of five other species of the genus *Lilium* and the pollen of *Tulipa gesneriana* earlier by Tsuda and co-workers[13].

Hydrocarbons isolated from the sprouts of tubers of the potato (*Solanum tuberosum* L.) contain significant amounts of iso- (13%) and anteiso-alkanes (15%)

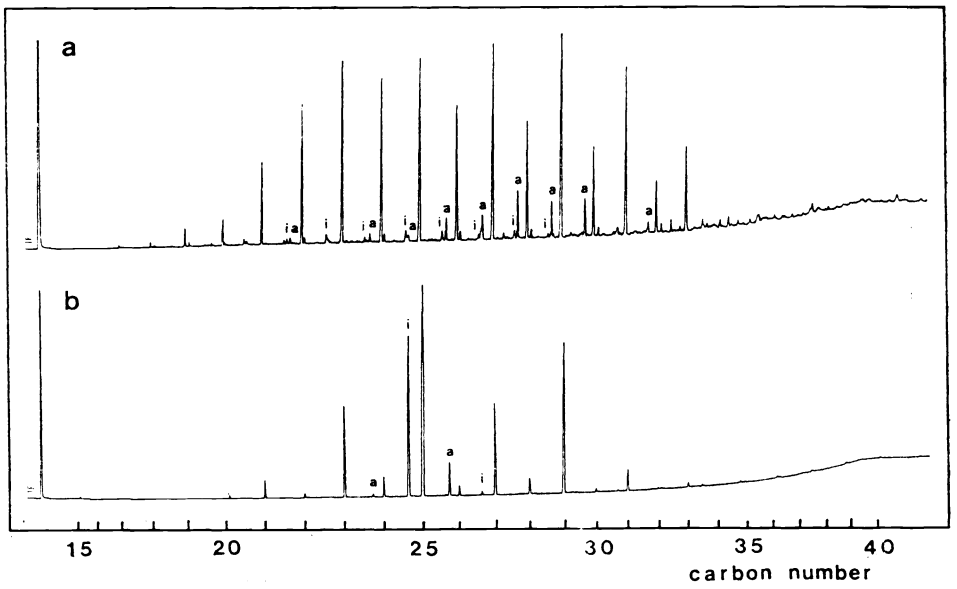

FIG. 2

Gas chromatogram of alkanes from: a *Biatora lucida* (ACH.)FR.; b *Aesculus hippocastanum* L. — anthers

(Fig. 4a). Similarly as in the lichen *Biatora lucida* (ACH.) FR., here too the content of anteiso-alkanes is higher than that of iso-alkanes. Relatively high amounts of branched alkanes have been described recently in other parts of this plant as well[14], and still earlier in the leaves of eggplant[15] *Solanum melongena* L.

Purely incidentally we also analysed hydrocarbons from the seeds of henbane *Hyoscyamus niger* L., also belonging to the *Solanaceae* family. Here the homologous series of alkanes of the $C_{16}-C_{35}$ interval was present, with a predominance of odd members (with a maximum at n-C_{31}). The content of iso-alkanes and anteiso-alkanes is 22% and 6%, respectively, with a maximum at 2-methyltriacontane (iso-C_{31}, 10%) (no chromatogram is shown).

The alkanes of the lichen *Umbilicaria pertusa* RASSAD. (Fig. 4) represent an extraordinary case. The content of n-alkanes is only 28%, while the rest (72%) is composed of iso-alkanes (24%), anteiso-alkanes (3%) and several homologous series of differently branched hydrocarbons (45%). Since some of the series are relatively extensive (from C_{15} to C_{40}), it is possible to find more than 160 peaks on the original chromatogram, more or less separated. As already found[2,16,17], every chromatographic peak of the more branched alkanes represents at least two to three aditional positional isomers, which cannot be separated even on a long capillary column.

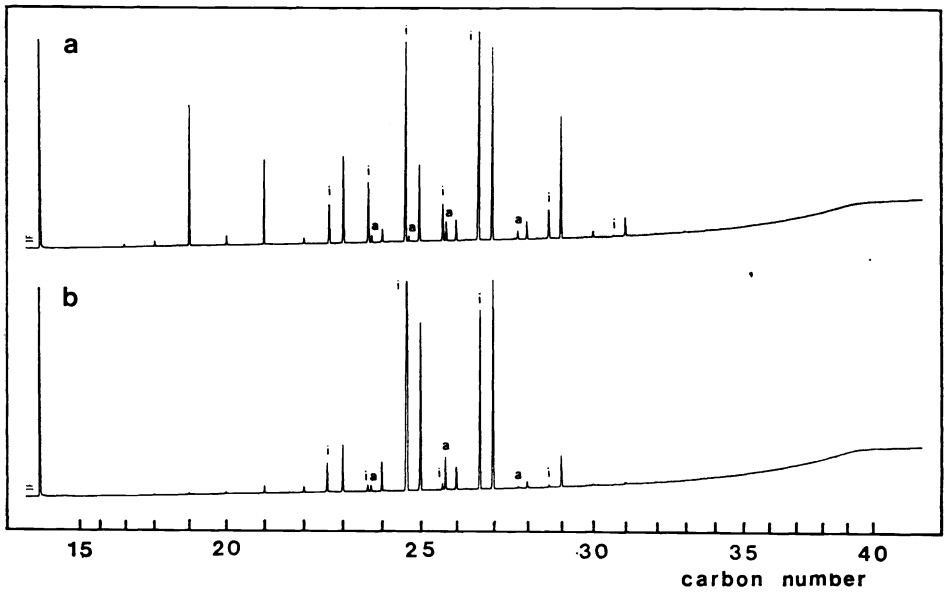

FIG. 3

Gas chromatogram of alkanes from: a *Papaver rhoeas* L. — anthers; b *Lilium willmottiae* E. H. WILSON — pollen

A considerable variety in the composition and quantitative representation of alkanes follows from the results of our analyses of more or less randomly selected plant samples, both in plants of different phylogenetic evolution and in various morphological forms. Hence, for a more thorough categorization of cuticular wax alkanes, with regard to their composition and biogenetical relationships, more kinds of plant material should be evaluated.

EXPERIMENTAL

Material and Extraction

The plant material was extracted at room temperature with chloroform, either by triple maceration or continuously in a column. In Table I only the total amount of solvent is given. If wet weight is given, fresh material was extracted.

Isolation of Alkanes

The extract was chromatographed in n-hexane on a column containing silica gel according to Pitra[18] (30—60 μm), deactivated with 12% of water. The fraction containing hydrocarbons (frequently a mixture of paraffins and olefins, $R_F = 1{\cdot}00$ in TLC on silica gel and n-hexane as solvent) were combined and further chromatographed either on a silica gel column (30—60 μm)

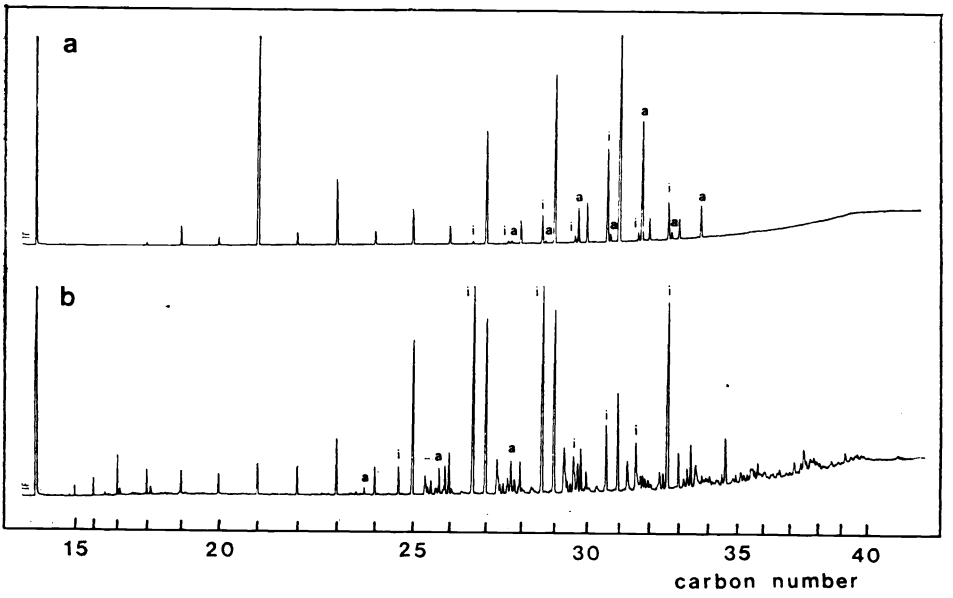

FIG. 4

Gas chromatogram of alkanes from: a *Solanum tuberosum* L. — sprouts of tubers; b *Umbilicaria pertusa* RASSAD

impregnated with 20% $AgNO_3$ and deactivated with 10% of water, or on a thin layer of silica gel (10—35 μm) impregnated with 20% of $AgNO_3$. For subsequent analysis by GLC only those fractions were taken which displayed an R_F value = 1·00 in n-hexane on thin layer chromatography on silica gel impregnated with 20% $AgNO_3$. In this way it was ensured that they contained saturated hydrocarbons only.

Gas Chromatography (GLC)

Gas chromatography was carried out on an HP 5890A (Hewlett–Packard, U.S.A.) instrument with a flame ionization detector and a split-splitless injection system, which was used in the split mode. For separation fused silica capillary column (J & W Scientific, U.S.A.) was used, column length 30 m, internal diameter 0·25 mm, with the non-polar phase DB-1, film thickness 0·25 μm. All samples were chromatographed under the following conditions: injector temperature 260°C, detector temperature 280°C, oven temperature 140°C, then 3°C/min to 320°C (5 min), carrier gas H_2, 90 kPa, 1·85 ml/min, $\bar{u}$ = 50 cm/sec at 140°C, split ratio 25 : 1. The samples were injected as a 1% solution in $CHCl_3$. For the recording and integration the integrator HP 3393A (Hewlett–Packard, U.S.A.) was used, chart speed 0·5 cm/min.

Gas chromatography–Mass spectrometry (GC-MS)

GC-MS was carried out on a mass spectrometer ZAB-EQ (VG Analytical Ltd., England) combined with a gas chromatograph HP 5890A, at electron energy of 70 eV and ion source temperature 150—300°C.

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TABLE I

Survey of the analysed plant materials

Plant	Weight		Extraction with $CHCl_3$, ml	Yield of alkanes, mg
	wet, g	dry, g		
Quercus petraea (MATTUSCH.) LIEBL. — leaves[a]	436		300	21
Fegatella conica (L.) CORDA — aquatic form[b]	75	6·5	480	1·5
Biatora lucida (ACH.) FR.[c]		123	6 000[j]	81
Aesculus hippocastanum L. — anthers[a]		8·4	500[j]	25
Papaver rhoeas L. — anthers[a,f]		52	2 200[j]	72
Papaver rhoeas L. — pollen[a,g]		7	250[j]	3·5
Lilium willmottiae E. H. WILSON — pollen[a,h]		3·3	100[j]	16
Solanum tuberosum L. — sprouts of tubers[a]	800		3 000	5·5
Hyosciamus niger L. — seeds[d,i]		70	600[j]	1·5
Umbilicaria pertusa RASSAD.[e]		155	3 200[j]	3

Location: [a] Prague, Czechoslovakia; [b] Sv. Jan pod Skalou, Czechoslovakia; [c] Czech Paradise, Dolánky, Czechoslovakia; [d] South Moravia, Czechoslovakia; [e] Area Chorgo, northwest Mongolia. The analysed material represented: [f] 7 500 buds; [g] 600 buds; [h] 160 buds; [i] 110 000 seeds. [j] Extracted with $CHCl_3$ in a column.

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