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PATTERNS OF VARIATION AND DEVELOPMENT IN LEAF WAX ALKANES*

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Abstract—When *n*-alkanes form only a small percentage of the leaf cuticular wax, the dominance of odd over even carbon number chain length tends to disappear. Similarly the alkanes from lipids extracted from within the leaf, as opposed to cuticular alkanes, lack this alternation in chain length. Alkane production in *Solandra grandiflora* continues throughout the life of the leaf, the chain length increasing with age. No relationship is apparent between the chain length of alkanes and alkanoic acids contained in the cuticular wax of a series of *Aloe* species. These results are discussed in the context of recent research on the biosynthesis and possible taxonomic use of leaf wax alkanes.

THE UNIVERSAL presence of normal alkanes as constituents of leaf cuticular waxes is well established and both their biogenesis and their value as a taxonomic criterion have been extensively discussed.[1] Variation in the importance of alkanes as constituents of the cuticular wax, obtainable by brief (up to 30 sec) washing of freshly collected leaves with chloroform, is considerable, and has been found to range from a fraction of 1 per cent in many *Eucalyptus*[2] and *Pinus*[3] species to greater than 90 per cent, as in *Solandra grandiflora* Sw. (Solanaceae) (see below). Homologous series of aliphatic hydrocarbons with branched chains occur sporadically as constituents of the cuticular wax hydrocarbon fraction but, with some exceptions,[4–8] form only a small proportion of the total hydrocarbon content. Members of some genera of the Solanaceae and Crassulaceae appear to be particularly rich sources of cuticular waxes with branched-chain alkanes as a significant component.

Herbin[9] has shown, in a survey of a large number of leaf cuticular waxes from a range of families in the Angiosperms (summarized in Ref. 2), that in the homologous series of *n*-alkanes present, nonacosane (C_{29}) and hentriacontane (C_{31}) are the most frequent major components among the predominating odd carbon number constituents and that C_{28} and C_{30} are the most frequent major components among the, usually, less significant even carbon number constituents. The dominance of n-C_{29} molecules among the constituents of the leaf wax of cabbage (*Brassica oleracea* L.) and its morphological variants[10,11] has aroused speculation

* Studies on Plant Cuticular Waxes, Part VI. For Part V see G. A. HERBIN and K. SHARMA, *Phytochem.* **8**, 151–160 (1969).

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on the mode of biosynthesis of this particular chain length in leaf cuticular waxes. Earlier hypotheses of Chibnall and his group and the more recent work on biosynthetic pathways using radioactively labelled precursors by Kolattukudy are summarized in the review cited.[1] The work of Kolattukudy[12,13] on the biosynthesis of C_{29} compounds in *B. oleracea* seems to have firmly established one of the early hypotheses of Chibnall[14] that the long-chain components of plant waxes, hydrocarbons, alcohols, ketones and fatty acids arise from a chain elongation process from fatty acids of medium chain length.

The implication of Kolattukudy's findings is the that C_{29} alkane of cabbage leaf wax must arise from the initial formation of a C_{30} chain followed by the loss of a terminal carbon atom by decarboxylation. Eglinton and Hamilton[15] had already suggested that a repetition of the malonyl–coenzyme A extension scheme on the common C_{14}–C_{18} range of fatty acids, to increase the chain length by six or seven two-carbon units, would lead to a predominance of chain lengths in the C_{26}–C_{32} range such as is frequently encountered in the plant wax acids and primary alcohols and which would lead to the production of alkane chain lengths C_{25}–C_{31} by loss of one carbon atom in a final stage.

Some evidence that not all long-chain alkanes are derived by a straightforward chain elongation process has been brought forward by Kaneda[16,17] who has claimed that a "head to head" type of condensation is operative during the incorporation of 8-^{14}C-caprylic acid into a branched-chain alkane of tobacco leaf wax. The recent identification by Brieskorn and Feilner[18] of 2,($\omega-1$)-dimethyl alkanes in *Marrubium vulgare* L. adds interest to this possibility of a "head to head" condensation mechanism.

Kolattukudy[19] has recently criticised the work of Kaneda on the grounds that long periods of incorporation were employed, thus allowing randomization of labels by degradation and resynthesis of some of the fatty acid precursors used. Subsequently, Kolattukudy[20] has shown that rapid incorporation of branched-chain precursors takes place both into branched-chain fatty acids and into branched-chain paraffins in the tobacco leaf. He has also shown that the chain elongation–decarboxylation hypothesis is valid for the production of the C_{31} hydrocarbon which predominates in *Senecio odoris* and has adduced further evidence against a "head to head" condensation mechanism for the formation of the C_{29} hydrocarbon of broccoli (*B. oleracea*).

In this paper we report a number of observations which are of relevance to the investigation of the biosynthetic pathways involved in the formation of plant cuticular waxes and in particular of the hydrocarbon fraction, and which are also of relevance to any attempts to construct a chemotaxonomy based on these products.

The very common high odd to even carbon number ratio in the normal alkane series of leaf cuticular waxes is by no means universal. In many cases, where alkanes form only a minor (<5 per cent) constituent of the leaf wax as a whole, as in the genera *Pinus*[3] and *Eucalyptus*,[2] this alternation between odd and even carbon number through the homologous series is almost indiscernible. We have already commented[2] on this variation in pattern and

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it is clear that a whole range of patterns exist from those in which one, odd number, constituent is the dominant component of the alkane fraction, through those in which several major, odd-number constituents are present, giving a flatter distribution with usually an increase in the relative proportion of the even-number members of the series, to the extreme pattern in which virtually no alternation exists and the pattern resembles that of the *n*-alkanes of a natural petroleum fraction. We have never encountered yet a leaf wax in which the even-number members of the series were dominant and the one example reported in the literature[21] must, we feel, be an error in identification of the chain length. A non-alternating type of pattern, coupled with a very low hydrocarbon content in the total lipid fraction, naturally raises the suspicion that the alkanes are present as the result of contamination of solvents or equipment by high-molecular weight natural petroleum products. However, several additional examples of alkane mixtures which lack the odd–even chain length alternation have been recently reported from living organisms. Clarke and Blumer[22] have found that, while recent marine sediments contain alkanes with predominantly odd carbon numbers, red and brown marine algae and mixed phyto-and zooplankton all contained alkanes in the C_{20}–C_{30} chain-length range with little or no alternation between the odd- and even-numbered members of the normal homologous series, although in the C_{14}–C_{18} region a strong dominance of C_{15} and C_{17} was observed. Similar results were obtained by Stransky *et al.*[23] for the freshwater alga *Scenedesmus quadricauda*, and on the basis of several other similar observations Stransky *et al.*[24] suggested that lack of alternation was perhaps diagnostic of a lower evolutionary level. In contrast, however, Laseter *et al.*[25] when examining the wax from chlamydospores of the Basidiomycete *Ustilago maydis*, found a strongly alternating sequence of alkanes, with both normal and branched chains. Further examples of non-alternating series of *n*-alkanes from higher plants have been reported from the heartwood of several species from the Guttiferae[26] and Myristicaceae.[27] However, all these cited cases were not samples of "cuticular wax" but were obtained from extractions of macerated or powdered plant material, so that intracellular lipids would have been included in the lipid fractions examined. We comment upon the differences between the constitution of "internal" and "external" lipid hydrocarbons later in this paper.

Discordant analyses of the hydrocarbon fraction of commercial samples of the leaf wax of *Copernicia cerifera* (Palmae), carnauba wax, as recorded by Downing *et al.*[28] and Mazliak[29] might have been due to contamination of the commercial product by petroleum-derived hydrocarbons. Carnauba wax contains only 0·16 per cent of alkanes in the total wax, according to Downing, who found a fairly strong dominance of C_{29} and C_{31} among the *n*-alkane fraction from a commercial sample. Mazliak, whose material was a similar commercial sample from a different source, obtained a non-alternating homologous series of *n*-alkanes with a maximum concentration in the C_{29}–C_{30} region. Both sets of results are given in Table 1, together with a further analysis which we have carried out on another sample of the commercial wax. Our results agree with Mazliak's in the non-alternation between odd

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TABLE 1. *n*-ALKANES FROM LEAF CUTICULAR WAXES OF THE FAMILY PALMAE (PALMALES). CONCENTRATIONS IN MOLE PERCENTAGE (EXCEPT FOR THOSE RESULTS QUOTED FROM OTHER WORKERS)

Species		C_{35}	C_{34}	C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}	C_{22}	C_{21}	C_{20}	C_{19}	C_{18}	C_{17}	C_{16}
1. *Copernicia cerifera*	a[26]			5·8	0·6	28·9	1·3	25·0	2·5	15·3	1·4	5·7	2·0	5·4	1·2	1·2	0·6	0·8	0·8	0·7	0·8
Mart. (commercial	b[27]	1·0	1·2	4·1	6·6	7·9	12·3	16·0	16·0	13·3	8·3	5·4	3·2	2·3	1·3	0·4	0·3				
carnauba wax)	c			3·3	4·5	8·1	8·7	11·2	10·5	13·1	12·9	11·6	7·9	5·6	2·2	0·4					
2. *Phoenix reclinata* Jacq.	a			8·3	4·6	46·5	19·6	7·3	4·9	3·9	2·4	1·3	0·8	0·3	0·1						
	b			8·5	4·5	46·5	19·9	7·1	4·5	3·9	2·5	1·5	0·7	0·3	0·1						
3. *P. canariensis* Hort.	a			20·2	8·7	36·7	14·8	9·4	3·8	3·2	1·6	1·1	0·4	0·1							
	b			20·1	11·2	34·7	16·3	8·5	3·5	2·5	1·6	1·0	0·3	0·2	0·1						
	c			15·9	13·5	33·3	18·3	8·3	4·0	3·4	1·5	1·2	0·4	0·2							
4. *Washingtonia filifera*	a					7·7	2·2	27·4	6·9	40·2	3·4	6·8	1·9	2·1	0·9	0·5					
H. Wendl.	b					8·1	5·4	17·8	6·4	32·6	6·4	11·5	3·6	7·0	1·0	0·2					
5. *Cocos plumosa* Hook.				48·7	2·9	37·0	1·0	4·3	0·8	2·2	0·6	1·2	0·2	1·0	0·1						
6. *Sabal* sp.	a			20·3	13·3	17·0	9·0	12·3	7·0	7·8	5·6	4·1	2·0	1·3	0·3						
	b			15·3	8·4	15·7	9·1	31·2	6·4	6·4	3·5	2·3	0·9	0·7	0·1						
	c			18·3	12·6	19·7	11·0	11·9	7·3	7·8	4·6	3·4	2·0	1·0	0·4						
	d			23·4	9·0	20·0	8·4	12·8	7·2	8·4	4·2	3·7	2·0	0·7	0·2						
	e			27·0	11·1	20·8	10·3	11·8	6·3	5·7	3·3	2·3	0·9	0·4	0·1						

and even carbon number in the chain length of the *n*-alkane series, but show a maximum concentration at C_{26}–C_{27}. The non-alternating pattern appears to be anomalous when compared with other members of the family Palmae which we have been able to examine (Table 1), but is not at variance with the observation that very low alkane contents of leaf waxes are often associated with non-alternating carbon-number patterns. An age variation (see below) could also be the source of these discrepant results, since Warth[30] notes that the best quality waxes come from young leaves (and may undergo a partial refining process), while cheaper grades are made from older leaves.

Variation in hydrocarbon pattern between different organs of an individual plant (even though these organs may be closely related morphologically) has already been recorded by us[31] for several species of the genus *Aloe*, by Stransky *et al.*[24] for *Robinia pseudacacia* L., by Wollrab[32] for several cultivars of apples and pears and by Radler[33] for the grape vine. Further results are presented in Table 2 which indicate that such variation is widespread in higher plants.

Leaf cuticular wax formation has been considered as taking place within the epidermal cells, followed by extrusion of the wax through pores in the cuticle, which is a non-cellular membrane. The existence of such pores in *B. oleracea* was demonstrated by electron micrography by Hall and Donaldson[34] and the variety of shapes in which the cuticular wax is extruded in different species has been illustrated by Juniper (in Ref. 1). A variation in the chemical content of the wax from green and glaucous variants in a number of species has been found by Hall *et al.*,[35] who showed that glaucous varieties, in which the wax is in rods or filaments growing outwards from the surface, have higher diketone contents (in *Eucalyptus urnigera* and *Poa colensoi*) or monoketone content (*B. oleracea*) than the green variety, in which the wax forms a smooth film or lies as platelets on the surface of the cuticle. In *E. urnigera*, the lower β-diketone content was paralleled by an increased alkane content (25–26 per cent in the green as opposed to 2–7 per cent in the glaucous variety), while the alkane pattern was also strikingly different, with C_{29} as the weakly dominant chain length in the glaucous variety and C_{27} as a strongly dominant chain length in the green variety, with, in addition, a more pronounced alternation between the odd- and even-numbered homologues in the latter. Lindqvist *et al.*[36] have observed a similar phenomenon in induced mutants in barley and have been able to connect variation in wax form and the associated chemical composition with specific genetic changes. The increased alkane content paralleling the reduced ketone content is not at variance with our suggestion[2] that an inverse biogenetic relationship between the two classes of compounds might exist.

The method of washing fresh leaves with chloroform for about 30 sec to obtain the cuticular wax would not be expected to remove all the wax that was contained in the wax-excreting pores, and Hall and Donaldson[34] showed that even after several minutes' washing small mounds of wax remained over the mouths of the pores in *B. oleracea*. Nor should such a washing extract any significant quantity of lipid material from within intact epidermal cells. Some workers have, however, used more drastic extraction procedures for obtaining plant

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TABLE 2. HYDROCARBON VARIATION IN WAXES OBTAINED FROM DIFFERENT ORGANS OF INDIVIDUAL PLANTS (HYDROCARBON FRACTION EXPRESSED AS A MOLE PERCENTAGE)

			C_{35}	C_{34}	C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}	C_{22}	C_{21}
Family Liliaceae																	
Aloe ferox Mill.	Leaf	Alkanes			5·3	2·6	53·6	13·6	14·2	5·9	3·4	1·2	0·3				
	Perianth	Alkanes					58·3	2·1	34·9	0·7	3·0	0·5	0·5				
	Style	Alkanes					5·5	0·6	8·2	1·2	16·4	1·1	3·6	1·4	0·2		
	Style	Alkenes					30·8	0·8	29·4		0·7						
	Filament	Alkanes					19·0	2·3	21·4	2·2	8·6	0·8	0·9	0·2	0·1		
	Filament	Alkenes					41·8	1·0	1·5		0·2						
Aloe marlothii Berger	Leaf	Alkanes					7·1	2·1	38·6	11·3	25·8	1·5	4·8	2·0	0·6	0·2	
	Perianth	Alkanes					64·7	4·0	37·8	0·7	1·7	0·3	0·5	0·1	0·2		
	Style	Alkanes					6·7	1·9	20·2	2·3	16·2	1·6	2·2				
	Style	Alkenes			2·5		32·6		13·6		0·2						
	Filament	Alkanes					7·8	1·7	9·7	1·9	18·4	1·7	6·9	0·3	0·3		
	Filament	Alkenes			8·0		28·1		15·3								
Hemerocallis aurantiaca (cultivar)	Leaf	Alkanes					2·4		55·4	0·9	40·1	0·2	0·4	0·2	0·4		
	Perianth	Alkanes					1·2	0·5	13·4	1·3	33·5	1·6	29·2	0·6	2·5		
	Perianth	Alkenes					8·4		5·0		2·6		0·2				
	Filament	Alkanes			0·2	0·1	1·2	0·3	3·7	0·8	17·8	0·9	14·4	0·4	1·2		
	Filament	Alkenes			2·4		18·7	0·1	19·9	0·1	16·6		1·2				
Family Solanaceae																	
Solandra grandiflora Sw.	Leaf	Alkanes			6·9	1·1	20·5	2·3		41·3	4·7	21·9	0·6	0·7			
	Petal	Alkanes					4·7	1·1	61·5	3·4	13·7	0·6	1·1	0·3	1·0	0·2	0·2
	Petal	Alkenes					0·7	1·4	6·7	1·2	1·0	0·3	0·3				
	Style + filament	Alkanes					6·5	2·3	22·0	5·9	20·0	2·3	6·0	0·5	0·9	0·2	0·4
	Style + filament	Alkenes					2·9	4·7	12·5	5·9	4·9	1·6	0·5				
	Pollen	Alkanes					8·0	2·6	21·5	6·7	18·9	3·0	1·5	1·9	0·7		
	Pollen	Alkenes					4·1	1·1	17·4	2·2	5·6	0·4	0·7				
Family Acanthaceae																	
Thunbergia laurifolia Lindl.	Leaf	Alkanes	6·6	2·1	36·4	7·3	32·1	2·7	6·3	1·2	2·4	0·9	2·0				
	Peduncle	Alkanes			14·9	3·7	65·3	2·6	11·2	0·6	1·1	0·3	0·3				
	Sepals	Alkanes			6·7	1·4	45·0	2·3	38·1	1·1	4·6	0·3	0·6				
	Petals	Alkanes			5·8	1·3	39·2	2·5	41·5	1·9	6·7	0·3	0·8				

TABLE 3. COMPARATIVE ANALYSIS OF ALKANE FRACTIONS FROM EXTERNAL AND INTERNAL LIPIDS OF LEAVES (EXPRESSED TO THE NEAREST MOLE PERCENTAGE)

Species		C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}	C_{22}	C_{21}
1. *Solandra grandiflora* Sw.	Ext.	8	2	52	2	31	2	3	1	1	1			
(Solanaceae)	Int.	2	3	6	7	11	11	12	13	11	9	7	5	3
2. *Allamanda cathartica* L.	Ext.	16	2	48	2	28	1	2	1	1	1	1		
(Apocynaceae)	Int.	2	4	14	7	13	11	12	11	9	7	5	3	2
3. *Brassica oleracea* L.	Ext.			9	1	89	1	1	1	1				
(Cruciferae)	Int.	2	3	6	7	17	12	13	13	10	7	6	3	1
4. *Monstera deliciosa* Liebm.	Ext.			54		32								
(Araceae)	Int.	1	3	6	6	21	9	11	10	9	9	8	5	2

waxes, including more or less prolonged solvent extraction of macerated fresh, or of ground, dried plant material (see examples quoted above), which would be expected to give a total lipid sample, containing both "external" (cuticular) and "internal" (cell content) components. In the case of many lower plants, where cuticular layers and cuticular waxes are absent or greatly reduced, the distinction between external and internal lipids becomes less meaningful, and selective extraction less possible.

In Table 3 we demonstrate that a profound difference in kind exists between the hydrocarbon patterns for external and internal leaf lipids, obtained respectively by brief washing of the fresh leaf with chloroform and subsequent extraction of the whole leaf by boiling chloroform under reflux. Isolation of the hydrocarbon fraction and analysis in the usual way shows that, for each of the species examined: (*a*) the internal lipid hydrocarbons have a different composition from the external wax hydrocarbons, (*b*) the internal hydrocarbon pattern has the flat distribution of the chain lengths and the lack of alternation between odd and even numbers of the homologous series that is characteristic of higher plants in which the leaf wax has a very low hydrocarbon content and, perhaps more significantly, of those lower plants whose "wax" has been obtained by extraction of total lipids, and in which internal lipids have probably formed the major part of the extractive (Refs. 22–24).

It would seem therefore that the results of Stransky *et al.*,[24] from which the authors believed that they could discern a difference in kind between the hydrocarbons of plants in the higher and lower evolutionary levels, may be more a reflection of the difference in kind between internal and external lipids, the latter being quantitatively more significant in higher plants.

Furthermore, our results on *B. oleracea* (Table 3), indicate that care is necessary in the interpretation of differential rates of uptake of radioactive precursors into components separated from internal and external lipids. Kolattukudy, in his earlier studies[12,13] had observed such a differential and had shown, by the use of enzyme inhibitors, that external wax components are synthesized at a different site from the internal lipids. Subsequently, Kolattukudy[20] showed in *Senecio odoris* that the site of synthesis of long-chain ($>C_{16}$) fatty acids and the related paraffins (predominantly C_{31}) takes place in the epidermal layer of the leaf and that, while 1-^{14}C-acetate was incorporated into fatty acids up to C_{16} in both the mesophyll and the epidermal layer, C_{18} acid only incorporated ^{14}C in significant proportion in the epidermal layer. Gülz[37] has also recently demonstrated quantitative differences between the alkanes derived from the lipids of whole leaves and those from isolated chloroplasts.

Variation in cuticular wax components with leaf age has already been demonstrated by us,[3] in several evergreen Gymnosperms, and by Stransky *et al.*[24] in the deciduous *Betula verrucosa* Ehrh., there being in each case an increase with age in the relative proportion of longer chain-length components in the class of compounds examined (ω-hydroxy acids and alkanes respectively). We now report a detailed examination of *Solandra grandiflora*, a species in which age variation seems particularly pronounced. *S. grandiflora* is commonly grown as an ornamental climber in East African gardens and was found to have one of the highest alkane contents of any leaf wax yet investigated, with more than 90 per cent of the cuticular wax being in the *n*-alkane homologous series: branched-chain alkanes, which seem to be a general and often highly significant component of leaf waxes of members of the Solanaceae,[4,5,8,9,16] are present only in traces in this species. *S. grandiflora* also has a very

[37] P.-G. GÜLZ, *Phytochem.* 7, 1009 (1968).

TABLE 4. *Solandra grandiflora*

Description of leaf colour and texture	Variation of the leaf alkane composition with age: Total area of the leaf (abaxial plus adaxial surfaces) (mm^2)	Leaf area used for analysis = A (mm^2)	Total area under alkane chromatogram peaks = B (mm^2)	Hydrocarbon concentration index = B/A	C_{33} (%)	C_{31} (%)	C_{29} (%)	C_{27} (%)
1. Mauve-brown, flexible	750	375	713	1·9	4	15	38	28
2. Mauve-brown, flexible	840	338	504	1·5	7	15	26	40
3. Mauve-brown, flexible	2010	1000	597	0·6	7	15	37	30
4. Green-brown, flexible	4370	1250	1328	1·1	7	15	33	38
5. Green-brown, tinged, flexible	6240	1250	1728	1·4	11	18	48	21
6. Bright green, flexible	9840	720	1786	2·5	9	24	43	17
7. Dark green, semi-rigid	11,230	313	1688	5·4	9	53	30	2
8. Dark green, rigid	11,250	200	2123	10·6	10	61	24	2
9. Faded green, rigid	12,500	63	1492	23·9	15	69	15	1
10. Faded yellow, rigid	10,440	113	2162	19·2	12	63	22	2

high alkane concentration per unit area of leaf, as is shown by its high position in Table 5 (see below). An investigation of the leaf wax alkanes has been made at different stages of leaf development from bud opening to leaf fall: these stages, which are readily recognized by leaf texture, colour and size, were examined by the usual alkane analysis procedure to give the results presented in Table 4.

As can be seen from Table 4, the development of the leaf can be divided into two phases, A and B. Phase A, represented by stages 1–5 (or 6), covers the period of growth of the leaf, when an approximately tenfold area expansion of the total leaf surface takes place. During this phase the wax alkane composition pattern remains relatively constant (a slight increase in the ratio C_{29}/C_{27} being the only trend) as does also the relative wax concentration on the surface of the leaf, as shown by the column headed B/A in Table 4, a ratio of wax alkane quantity to leaf unit area which we refer to hereafter as the hydrocarbon concentration index (HCI). Wax alkane production by the epidermal cells of the rapidly expanding leaf must be relatively uniform in type and regulated to a rate that is dependent upon the leaf area expansion. That this rate of hydrocarbon production may even fall short of the rate of leaf area expansion is indicated by a slight fall in the HCI at stage 3; Roberts *et al.*[38] have observed a similar fall in total wax per unit area on actively growing Bramley apple leaves.

In phase B, represented by stages 6–10 in Table 4, the leaf area remains effectively constant but wax alkane production continues, so that a tenfold rise in the HCI occurs, an observation in contrast to the opinion of Schieferstein and Loomis,[39] who considered that leaf production ceased when the cuticular layer hardened and became impermeable. Furthermore a change in the hydrocarbon pattern occurs, resulting in a final composition for the senescent leaf (stage 9) of a dominant C_{31} and a negligible C_{27} content. Evidently during phase B of the life of the leaf, the epidermal cell layer's production of alkanes changes to one in which C_{31} is the major and almost the sole product. These data suggest that the enzyme system of the leaf is capable at any stage of producing hydrocarbons of chain length up to C_{33} but that a limiting factor operates during the growth phase A, perhaps in the availability of precursors. The increase in availability of these precursors in phase B may be due to a reduced demand by some other epidermal cell activity, of which cuticle production would seem to be the most likely.

It has been noted earlier in this paper that a relationship appears to exist between the relative importance of alkanes in leaf cuticular waxes and the chain-length distribution of these alkanes. As a means of demonstrating this relationship, alkane distribution patterns for a number of species of higher plants were arranged in order of decreasing HCI in Table 5. The alkane distribution patterns have been simplified by the omission of components of less than 5 mole per cent concentration, while the major components are expressed to the nearest integral mole per cent. Mature leaves were not necessarily used for all determinations since the possible effect of leaf-age variation was not appreciated at the time of the survey.

Examination of Table 5 shows that those species (Nos. 1–15) with an HCI of 1000 or greater have simple alkane patterns consisting almost exclusively of C_{33}, C_{31} and C_{29} usually with C_{31} dominant and with a very low content of even carbon number alkanes, which only reach a level of 5% in example No. 15. In contrast, species with an HCI of 100 or less (Nos. 25–37) have a more complex pattern of chain-length distribution with even-number alkanes

[38] M. F. ROBERTS, J. T. MARTIN and O. S. PERIES, *Ann. Rep. Long Ashton Sta.* 102 (1960).
[39] R. H. SCHIEFERSTEIN and W. E. LOOMIS, *Plant. Physiol.* **31**, 240 (1956).

TABLE 5. THE ALKANE MOLECULAR CONCENTRATION PER UNIT AREA AS A FUNCTION OF THE ALKANE DISTRIBUTION PATTERN

Family	Species	H.C.I.*	C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}
1. Crassulaceae	*Kalanchoe dyeri* N. E. Br.	16,820	88		10								
2. Solanaceae	*Solandra grandiflora* Sw.	14,969	8		52		31						
3. Liliaceae	*Aloe sessiliflora* Pole Evans	9,226	22		67		8						
4. Crassulaceae	*Kalanchoe pumila* Baker	5,464	69		24								
5. Araceae	*Monstera deliciosa* Liebm.	4,101			54		32						
6. Rosaceae	*Rose* sp. (cultivar)	3,830	11		60		23						
7. Crassulaceae	*Escheveria runyonii* Rose et Walth.	3,578	29		54		8						
8. Crassulaceae	*Bryophyllum pinnatum* Kurz.	3,470	81		12								
9. Agavaceae	*Agave fourcroydes* Lem.	2,754	20		63		6						
10. Sapotaceae	*Chrysophyllum pruniforme* Engl.	1,924	10		57		26						
11. Bignoniaceae	*Tabebuia guiacum* Hemsl.	1,253	14		35		25						
12. Geraniaceae	*Pelargonium* sp. (cultivar)	1,170	34		52		8						
13. Sterculiaceae	*Sterculia incana* Benth.	1,109	28		42		20						
14. Cycadaceae	*Encephalartos hildebrandtii* A. Br. et Bouche	1,025	17	8	40	7	18						
15. Moraceae	*Ficus hochstetteri* A. Rich.	814			52	7	32						
16. Bignoniaceae	*Kigelia aethopica* Decne	709	15		44	7	20						
17. Crassulaceae	*Escheveria peacockii* Croucher	685	46	6	36								
18. Palmae	*Phoenix canariensis* Hort.	478	16	14	33	18	8						
19. Bignoniaceae	*Markhamia hildebrandtii* Sprague	230	11		37	9	22	7	5				
20. Plocospermaceae	*Allamanda cathartica* L.	135			37	5	29		7				
21. Palmae	*Washingtonia filifera* H. Wendl.	133	22	9	19	10	12	7	9	5			
22. Agavaceae	*Agave cantala* Roxb.	126	6		12	11	15	14	13	11	10		
23. Palmae	*Sabal* sp.	99	25	9	19	11	11	7	6				
24. Liliaceae	*Aloe tenuior* Haw.	75			16		32		33				
25. Sapindaceae	*Dodonaea viscosa* Jacq.	73			26	8	52						
26. Liliaceae	*Aloe wickeneii* Pole Evans	63			19		17		50	6			
27. Amaryllidaceae	*Agapanthus umbellatus* L'Herit	58	21		28	6	22	7	8				
28. Mimosiaceae	*Acacia prunosa* A. Cunn.	45			3		46	20	11	8	5		
29. Myrtaceae	*Melaleuca leucadendron* L.	32			6		21	11	37	12	6		
30. Myrtaceae	*Eucalyptus clocziana* F. Muell	27			3		12	7	22	15	21	11	6
31. Cannaceae	*Canna indica* (cultivar)	23			16	7	44		13				
32. Euphorbiaceae	*Jatropha curcas* L.	14	6	7	29	12	27	8	7				
33. Polygonaceae	*Ruprechtia viraru* Griseb.	9			13		69	6	7				
34. Musaceae	*Musa sapientum* L.	8	5		19	6	28	10	12	10	5		
35. Strelitziaceae	*Strelitzia regina* Banks	7			5		33	12	33	8			
36. Euphorbiaceae	*Croton megalocarpus* L.	5	8	5	24	11	15	8	9				

* Owing to altered instrumental conditions this value of H.C.I. includes a factor of approximately $\times 10^3$ when compared with Table 4.

forming a more significant proportion of the total and C_{29}, rather than C_{31}, tending to be the most frequent constituent of highest concentration.

Although, in some examples, low alkane content of the leaf wax is compensated for by the predominance of long-chain components of different types (e.g. the β-diketones of the *Eucalyptus* genus) which could be formed in a competitive manner from the same precursors, that is not always the case. In many Gymnosperms, the major leaf wax component units are the medium chain length C_{12}–C_{18} ω-hydroxy acids[3,40] which are however combined in the leaf wax as cyclic estolides with an average of four units per molecule,[41] so that the leaf wax still consists of relatively high molecular weight hydrophobic molecules.

To check whether age variation was connected with HCI, determination of alkane distribution pattern for young and old leaves of a number of species of high or low HCI was

TABLE 6. AGE VARIATION IN LEAF CUTICULAR ALKANES FOR SPECIES OF HIGH OR LOW HYDROCARBON CONCENTRATION INDEX (HCI) CONCENTRATIONS IN MOLE PERCENTAGE (COMPONENTS OF LESS THAN 5 MOLE PER CENT OMITTED)

Species	H.C.I (see Table 5)	Leaf age	C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}
1. *Kalanchoe dyeri* N. E. Br.	16,820	Young	81		17						
		Old	88		10						
2. *Solandra grandiflora* Sw.	14,969	Young	7		14		30	6	35		
		Old	10		60		25				
3. *Monstera deliciosa* Liebm.	4,101	Young			12		57		23		
		Old			38		53				
4. *Rosa* sp. (cultivar)	3,830	Young	37		51		9				
		Old	37		48		8				
5. *Pelargonium* sp. (cultivar)	1,170	Young	20		56		16				
		Old	37		51		6				
6. *Canna indica* L. (cultivar)	23	Young		5	16	7	44	5	13		
		Old			10	5	43	9	20		5
7. *Jatropha curcas* L.	14	Young			6	5	16	6	35	6	17
		Old			15	8	21	12	16	6	7
8. *Musa sapientum* L.	8	Young			5		70		17		
		Old			8		67		13		
9. *Strelitzia regina* Ait.	7	Young			8		38	5	37		6
		Old			5		33	12	33	8	

carried out. The results are recorded in Table 6, being simplified by the omission of components of less than 5 mole per cent concentration. Inspection of the data in Table 6 indicates that *Solandra grandiflora* is unusual in its high age variability and that, although a tendency for average chain length to increase with age is discernible in examples 1, 3, 5 and 7, this is small and in the case of *Canna indica* (example 6) a slight diminution in chain length appears to occur with age.

Kolattukudy's evidence[12,13] that synthesis of long-chain acids, primary alcohols and esters is independent (at least in *B. oleracea*) of the synthesis of long-chain alkanes, ketones and secondary alcohols, even though chain elongation of medium-chain fatty acids may be the main step in the formation of a precursor ("elongation complex") for each group of compounds, does not exclude the possibility of there being a relationship on a quantitative level between the patterns of long-chain fatty acids and long-chain alkanes in the leaf cuticular wax of a particular plant, though Kolattukudy[19] has commented that there is no reason to

[40] G. A. HERBIN and K. SHARMA, *Phytochem.* **8**, 151 (1969).
[41] E. VON RUDLOFF, *Can. J. Chem.* **37**, 1038 (1959).

expect such a relationship. If a common precursor was involved in the pathway to these two groups of compounds, one of two possible relationships might be expected: (*a*) an inverse relationship, i.e. a low relative content of fatty acid of chain length (*n*) due to the depletion of a common precursor of that chain length by the formation of a high content of an alkane of chain length ($n-1$), *or* (*b*) a direct relationship, i.e. a high relative content of fatty acid of chain length (*n*) because of the ready availability of a common precursor also involved in the formation of a high content of alkane of chain length ($n-1$).

Eglinton and Hamilton[15] noted that, in a number of diverse species from different families, no clear relationship of either type (*a*) *or* (*b*) could be discerned. In the expectation that species within a genus would have similar biosynthetic pathways and would therefore reveal a consistent pattern if one existed, we determined the alkane and alkanoic acid patterns for the leaf cuticular waxes of a number of *Aloe* species, the results being detailed in Table 7. In this investigation the alkanoic acid fraction was obtained by saponification of the whole wax and no distinction was made between combined and uncombined long-chain acids. The results show clearly that there is no consistent pattern of relationship between the alkane and alkanoic acid chain lengths. The examples in Table 7, which were chosen at random from among species reported previously[31] and which were readily available, include patterns consistent with relationships of type (*a*) (e.g. No. 6) and of type (*b*) (e.g. No. 20), together with a range of intermediates. Oro *et al.*[42] have similarly found a lack of relationship in a number of algae and bacteria, as did Radler[33] in the grape. If any relationship is discernible for the *Aloe* species, it is that in general the major alkane is *longer* than the major alkanoic acid by one carbon atom (e.g. Nos. 1, 7, 8, 12, 14, 15, 20), or three carbon atoms (e.g. Nos. 5, 6, 11, 13, 16).

Our principal interest in these comparative studies of patterns of variation and development in the alkanes of leaf cuticular waxes has been to determine whether, as a class of universally present and readily analysable plant products, they can be utilized in chemosystematics. Results to date from our own work and that of other workers only allow an equivocal answer. It is evident that there is a basic genetic control over the composition of the wax components, including the alkanes, but also that variable factors associated with age and environment can be superimposed upon the specific pattern in some cases, though in others the genetically controlled pattern appears to be very stable and unaffected by external influences. Elimination or control of these variables, and the recognition that infraspecific genetic variation may be detectable only by certain chemical features of a species and little or not at all by the gross morphology (cf. the chemical varieties of some *Eucalyptus* species as described by Penfold[43] or of *Cryptomeria japonica* as found by Appleton *et al.*[44]), enables leaf alkane patterns to be utilized as confirmatory taxonomic criteria with success within limited groupings of plants.[1,45] A further example where alkane patterns have clearly distinguished two related groupings has recently been reported by Martin Smith *et al.*,[46] when South American and New Zealand species of *Cortaderia* were compared.

Wax alkanes from the whole inflorescence[8] seems to have given more clear-cut correlations than many other examples from the leaf, and in our experience with *Aloe* species,[31] the perianth wax alkanes proved more suitable than the leaf wax alkanes in the confirmation of an

[42] J. Oró, T. G. Tornabene, D. W. Nooner and E. Gelpi, *J. Bacteriol.* **93**, 1811 (1967).
[43] A. R. Penfold and J. L. Willis, *The Eucalypts*, Leonard Hill, London (1961).
[44] R. A. Appleton, R. McCrindle and K. H. Overton, *Phytochem.* **7**, 135 (1968).
[45] J. Borges del Castillo, C. J. W. Brooks, R. C. Cambie, G. Eglinton, R. J. Hamilton and P. Pellitt, *Phytochem.* **6**, 391 (1967).
[46] M. Martin Smith, G. Subramanian and H. E. Connor, *Phytochem.* **6**, 559 (1967).

TABLE 7. ANALYSIS OF ALKANE AND ALKANOIC ACID FRACTIONS OBTAINED FROM INDIVIDUAL ALOE PLANTS. VALUES EXPRESSED AS A MOLE PERCENTAGE

Species		C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}	C_{20}
1. *Aloe kedongensis*	A*		5·9	0·3	43·2	0·7	35·2	0·3	10·2	0·1	4·1		
Reyn.	H*	2·5	1·3	70·1	4·1	14·1	1·8	4·3	0·4	1·1	0·2	0·2	
2. *A. kedongensis*	A		2·8		29·3	0·9	44·9	1·2	10·3	0·1	9·3		1·1
Reyn.	H	3·0	1·1	74·6	1·6	14·1	1·1	2·1	0·7	1·0	0·3	0·4	
3. *A. kedongensis*	A		4·6		39·7	0·2	45·7	0·2	7·7		1·5		0·4
Reyn.	H			80·9	2·3	10·1	1·3	2·3	0·8	1·2	0·4	0·8	
4. *A. kedongensis*	A		4·0		50·0	1·5	34·4	0·7	7·2	0·3	1·8		
Reyn.	H	2·1	3·4	72·4	3·2	8·4	1·9	3·6	1·5	1·9	0·6	0·8	0·2
5. Unidentified aloe	A		6·0	0·1	36·5	0·5	39·6	0·2	10·0	0·1	6·4		0·7
	H	1·3		80·0	1·8	13·5	0·8	1·5	0·3	0·6		0·1	
6. *A. ciliaris* Haw.	A				3·5	1·2	54·5	0·5	37·1	0·2	2·2		0·7
	H	1·6		54·5	3·2	16·0	1·5	8·0		0·6			
7. *A. secundiflora*	A				40·2	2·3	27·3	0·6	23·0	0·3	5·9		0·6
Engler	H	1·1	0·6	80·7	2·2	6·4	1·7	3·4	1·1	1·7	0·6	0·6	
8. *A. secundiflora*	A				34·6	2·9	25·5	1·0	29·8	0·5	5·3		0·3
Engler	H	3·3	1·5	75·7	3·5	9·3	1·8	2·4	0·7	1·1	0·4		
9. *A. eminens* Reyn.	A		4·1	0·5	48·2	1·5	36·8	1·5	3·6	0·4	2·5		0·8
et Bally	H	2·8	1·7	35·1	4·3	35·3	5·6	8·0	3·5	1·9	1·3	0·6	
10. *A. eminens* Reyn.	A				48·9	3·0	37·8	2·2	4·4	0·7	3·0		
et Bally	H	2·3	1·0	41·3	4·3	31·3	3·7	8·3	3·0	2·3	1·0	1·3	
11. *A. mutabilis*	A				21·4	2·2	53·7	4·9	7·7	2·2	6·6	0·5	0·8
Pillans	H	7·0	2·2	40·8	4·0	14·8	4·2	17·4	4·6	2·4	1·8	0·8	
12. *A. dawei*	A		7·6	3·2	49·7	1·6	33·2	0·3	3·2		1·3		
Berger	H	2·9	9·7	43·8	6·5	13·5	8·1	5·9	2·7	3·2	1·6	1·6	0·5
13. *A. rivae* Bak.	A				34·0	2·0	46·0	5·5	7·0	1·5	3·5	0·1	0·3
	H	4·0	2·8	28·4	5·6	18·2	7·4	16·8	6·3	5·6	2·8	1·7	
14. *A. ukambensis*	A				66·3	1·4	22·9	0·9	5·1	0·7	2·0	0·2	0·5
Reyn.	H	2·3	1·5	23·7	7·6	22·1	8·4	11·5	6·9	7·6	4·6	3·8	
15. *A. arborescens*	A			5·7	24·4	6·5	38·3	7·3	11·4	1·2	4·1	0·2	0·8
Mill.	H	1·0	0·8	26·9	3·0	53·5	3·4	6·1	2·7	1·6	0·8	0·6	
16. *A. crytopoda*	A				12·5	2·2	52·4	9·6	12·5	3·0	5·9	0·4	1·5
Bak.	H	1·6	2·2	23·8	6·4	12·1	9·2	21·7	13·5	6·7	2·2	1·6	
17. *A. tenuior* Haw.	A				13·0	0·8	45·3	1·2	29·1	1·0	9·4	0·1	0·2
	H			19·4	2·6	16·4	3·9	34·1	6·9	10·8	3·4	2·6	
18. *A. christianii*	A		0·7	0·9	43·3	4·5	33·6	3·1	9·3	0·9	3·1		0·5
Reyn.	H	2·3	0·8	16·0	3·2	23·9	15·6	17·9	13·1	3·9	1·9	1·1	0·3
19. *A. ballyi* Reyn.	A				14·0	1·5	47·2	3·6	12·2	2·0	18·4	0·3	0·8
	H	7·2	0·5	14·8	2·5	24·9	7·2	30·8	6·5	3·2	1·6	0·7	
20. *A. volkensii*	A		0·4	0·2	33·7	4·2	45·0	5·6	4·5	1·8	3·4	0·5	0·8
Engler	H	0·3		2·7	0·2	38·7	7·0	25·8	10·0	7·6	2·3	5·3	

A* = alkanoic acids; H* = alkanes.

existing taxonomic system. Other leaf wax components, when forming the major part of the total wax, as the β-diketones of *Eucalyptus* species[47] and the ω-hydroxy acids of *Pinus*,[40] have given satisfactory correlations.

EXPERIMENTAL

Experimental procedures for the isolation and analysis of leaf cuticular wax alkanes and alkanoic acids were as previously described in this series of papers.[31,3]

[47] D. H. S. HORN, Z. H. KRANZ and J. A. LAMBERTON, *Australian J. Chem.* **17**, 464 (1964).