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STUDIES ON PLANT CUTICULAR WAXES—II.

ALKANES FROM MEMBERS OF THE GENUS *AGAVE* (AGAVACEAE), THE GENERA *KALANCHOE*, *ECHEVERIA*, *CRASSULA* AND *SEDUM* (CRASSULACEAE) AND THE GENUS *EUCALYPTUS* (MYRTACEAE) WITH AN EXAMINATION OF HUTCHINSON'S SUB-DIVISION OF THE ANGIOSPERMS INTO HERBACEAE AND LIGNOSAE

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Abstract—Analyses of leaf wax alkanes from 19 *Agave* species are reported and compared with those previously obtained for *Aloe* species. Examples of leaf wax alkane patterns for 16 species from four further genera of the family Crassulaceae are recorded to extend published information on this family: branched chain alkanes are absent in all the species presently reported. Alkanes from 19 species of *Eucalyptus* are reported and discussed in conjunction with the occurrence of other leaf wax constituents in this genus. Hutchinson's sub-division of the Angiosperms into Herbaceae and Lignosae is examined on the basis of a large number of analyses of leaf wax alkanes from many orders.

THE family Agavaceae has been formed by Hutchinson[1] as an intermediate between the Liliaceae proper and the Palmae, and comprises groups of genera which have been placed by other taxonomists (cf. Rendle[2]) in either Liliaceae or Amaryllidaceae. We have previously reported[3] data on the alkanes obtained from the leaf cuticular waxes of a large number of *Aloe* species (Liliaceae) and now present comparable data for some members of the genus *Agave* (Agavaceae). Although some genera of Agavaceae are native to East Africa (e.g. *Dracaena*, *Sanseveria*), the genus *Agave* is confined to the Americas. Many members of the genus were, however, readily available to us in the collection of named species at the High Level Sisal Research Station, Thika, Kenya. *A. sisalana* Perr., in particular, is an important economic crop in East Africa for the production of sisal fibre. Hecogenin is produced as a byproduct of the industry[4] and unsuccessful commercial attempts have been made to exploit the leaf wax.[5]

The taxonomy of the genus is difficult since the flowers of some species are unknown and others flower rarely. Jacobsen,[6] following an earlier classification of Berger,[7] describes the subdivision of the genus and notes that Berger's main key is difficult to use owing to infrequent flowering, although leaf character affords a useful secondary method of identification.

Using techniques previously described,[3] we have analysed the alkane fraction of leaf cuticular waxes from 19 species and two varieties of *Agave*, the results being recorded in

[1] J. HUTCHINSON, *The Families of Flowering Plants* (2nd edition), Clarendon Press, Oxford (1959).
[2] A. B. RENDLE, *The Classification of Flowering Plants* (2nd edition), Cambridge University Press, Cambridge (1959).
[3] G. A. HERBIN and P. A. ROBINS, *Phytochem.* (preceding paper).
[4] R. K. CALLOW, J. M. CORNFORTH and P. C. SPENSLEY, *Chem. Ind.* **1951**, 699 (1951).
[5] W. POLITZER, *Chem. Ind.* 408 (1948).
[6] H. JACOBSEN, *A Handbook of Succulent Plants*, Vol. 1, Blandford Press, London (1960).
[7] A. BERGER, *Die Agaven*, Fischer, Jena (1915).

Table 1. Agave leaf wax alkanes. Alkane fraction expressed as a mole percentage

Plant No.	Botanical authority Jacobsen[6] based on Berger's classification[7]	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)	*A. heteracantha* Bgr. Sub-genus II Sect. 5 Pericamptagave	6·7	0·6	54·4	2·2	32·6	1·4	1·4	0·4	0·2	0·1			
(2)	*A. ghiesbreghtii* C. Koch Sub-genus II Sect. 6 Brachysolenagave	3·8	0·3	57·6	1·2	34·9	0·7	0·9	0·2	0·3		0·1		
(3)	*A. attenuata* Salm. Sub-genus II Sect. 7 Sub-sect. I Dracontagave	16·7	2·4	20·0	4·1	21·9	5·0	18·4	4·1	5·4	0·7	1·0	0·1	0·2
(4)	*A. ferox* C. Koch Sub-genus III Series I Salmianae	12·6	0·7	79·6	1·1	3·6	0·4	1·4	0·3	0·2	0·1			
(5)	*A. atrovirens* Karw. Sub-genus III Series I Salmianae	14·9	1·0	43·2	4·4	13·6	4·2	10·5	3·2	3·4	0·9	0·4	0·2	0·1
(6)	*A. americana* var. marginata aurea Trel. Sub-genus III Series 2 Americanae	67·3	2·7	17·3	2·9	4·8	1·7	1·9	0·6	0·5	0·2	0·1		
(7)	*A. ingens* var. picta Salm. Sub-genus III Series 2 Americanae	8·7	2·6	29·7	6·8	23·4	5·9	15·0	4·7	2·4	0·5	0·2		
(8)	*A. potatorum* Zucc. var. verschaffelti Bgr. Sub-genus III Series 7 Sub-series 2 Eu-Scolymoides	13·7	3·6	25·9	7·0	13·4	6·7	16·7	5·8	4·7	1·5	0·8	0·2	
(9)	*A. werklei* Weber Sub-genus III Series 7 Sub-series 5 Costaricenses	33·3	2·2	54·0	2·0	4·6	0·8	2·7	0·2	0·2				
(10)	*A. sisalana* Perr. Sub-genus III Series 13 Sub-series A1 Sisalanae	54·0	3·7	34·0	2·8	2·0	1·2	1·4	0·4	0·3	0·2			
(11)	*A. fourcroydes* Lem. Sub-genus III Series 13 Sub-series A1 Sisalanae	31·0	3·9	55·9	3·6	4·1	0·6	0·6	0·2	0·1				
(12)	*A. decipiens* Bak. Sub-genus III Series 13 Sub-series A1 Sisalanae	29·0	3·7	57·5	2·7	4·7	0·5	0·9	0·3	0·4	0·1	0·2		
(13)	*A. angustifolia* Haw. var. marginata Sub-genus III Series 13 Sub-series A1 Sisalanae	24·5	3·1	37·3	4·6	9·5	6·2	7·7	3·3	2·3	0·8	0·6	0·1	

(14)	*A. angustifolia* Haw. Sub-genus III Series 13 Sub-series A1 Sisalanae	20·3	3·8	35·4	4·4	18·4	4·4	8·9	1·9	1·3	0·6	0·6		
(15)	*A. miradorensis* Jacobi Sub-genus III Series 13 Sub-series A1 Sisalanae	23·2	3·7	26·9	7·8	20·7	5·1	10·5	1·0	1·0	0·9	0·2		
(16)	*A. cantala* Roxb. Sub-genus III Series 13 Sub-series A1 Sisalanae	14·6	2·3	19·3	5·9	17·9	6·3	19·1	5·4	6·6	1·1	1·5		
(17)	*A. amaniensis* Trel. et Nowell Sub-genus III Series 13 Sub-series A1 Sisalanae	8·0	2·7	23·6	9·4	25·7	9·4	14·4	4·1	4·8	0·6	0·3		
(18)	*A. lespinassei* Trel. Sub-genus III Series 13 Sub-series B2 Tequilanae	42·4	3·4	25·8	3·9	7·4	3·3	6·6	1·5	2·4	0·7	1·3	0·1	0·2
(19)	*A. marmorata* Roezl. Sub-genus III Series 17 Marmoratae	36·3	5·4	45·5	3·8	6·0	1·5	1·1	0·3	0·1				
(20)	*A. americana* nairobiensis. Classification not indicated	26·2	1·5	65·2	1·0	4·3	0·4	0·9	0·2	0·2	0·1			
(21)	*A. nirvana* Classification not indicated	14·7	2·7	23·8	9·7	14·4	10·7	11·7	7·0	2·7	1·4	0·6	0·4	0·2

Table 1 and in simplified form as histograms in Fig. 1. For Fig. 1, alkane constituents present in a molar percentage of less than 5 per cent are omitted: the serial numbers refer to the data in Table 1.

As can readily be seen from Fig. 1 a wide variety of leaf alkane patterns are present, ranging from the high tritriacontane (C_{33})-hentriacontane (C_{31}) content of *A. sisalana* and *A. fourcroydes* Lem. (Nos. 10 and 11) to the more equable distribution of chain lengths from C_{25} to C_{33}, including substantial quantities of the even carbon number alkanes, exemplified

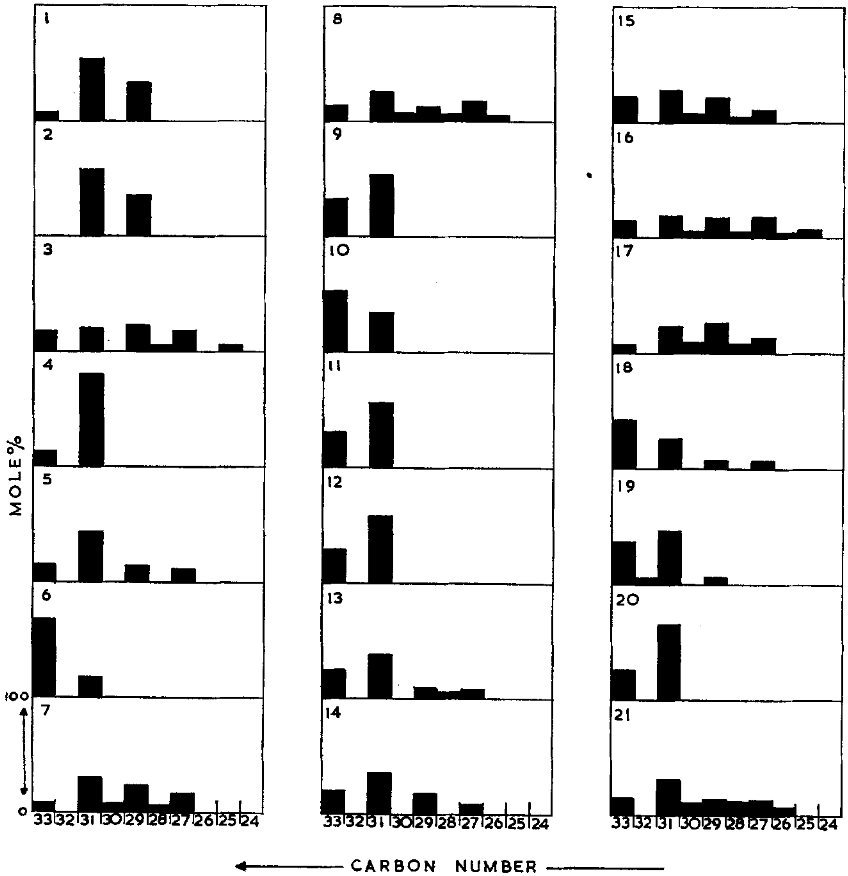

FIG. 1. LEAF ALKANE DISTRIBUTION PATTERNS OF SPECIES OF THE GENUS *Agave*. (THE HISTOGRAM NUMBER REFERS TO THE POSITION OF THE SPECIES IN TABLE 1).

by *A. cantala* Roxb. and *A. amaniensis* Trel. et Nowell (Nos. 16 and 17) which are placed in the same subgenus, series and subseries by Jacobsen as are Nos. 10 and 11. Differences between the allegedly related *A. americana* Trel. var. *marginata aurea* and *A. americana nairobiensis* (Nos. 6 and 20) are in contrast to the relatively small differences between *A. angustifolia* Haw. var. marginata and *A. angustifolia* Haw. (Nos. 13 and 14), suggesting that sub-specific and varietal rank can be confirmed by a close similarity of chemical composition. In this connection it is of interest to draw attention to the similar leaf alkane patterns of *A. amaniensis* (No. 17, blue sisal) and *A. cantala* (No. 16). *A. amaniensis* has an unusual history of discovery, having been found growing at the East African Agricultural Research

Station, Amani, Tanzania in the late 1920's. It was subsequently declared to be a new species by Trelease but is unknown in any natural habitat. The close similarity of its leaf alkane pattern to that of *A. cantala* suggests a possible relationship between the two.

Comparison of the *Agave* species leaf alkane patterns with those from *Aloe* species[3] indicates that in each genus a wide range of patterns is present which does not permit any close correlation with the subclassification. In the case of the genus *Aloe* a better correlation is given by the perianth rather than the leaf wax patterns: in the genus *Agave* lack of material prevented extension of analytical work to this part of the plant.

The family Crassulaceae is of particular interest in that it was used by Eglinton and co-workers[8] in their pioneer study of leaf wax alkane patterns as chemotaxonomic criteria. The species examined by Eglinton[8a] were all characterized by a high C_{31} and/or C_{33} normal alkane content, and in certain sub-genera by the presence of a significant content of branched chain alkanes. The latter are of a limited distribution in the plant world, having only been reported outside the Crassulaceae in two examples of leaf wax, *Nicotiana tabacum* (Solanaceae)[9,10] and *Aloe komatiensis* (Liliaceae).[3] Sixteen examples from four further genera of Crassulaceae have been examined and the leaf alkane analyses are reported in detail in Table 2 and in simplified form in Fig. 2. Most of the examples are from gardens and are exotics, though the genera *Kalanchoe* and *Crassula* are both well represented in the East African flora. No branched chain alkanes were detected in any of the leaf wax samples nor in the two perianth waxes examined; this absence is confirmed by a report of an isolated example of a further species, *Echeveria secunda*, by Horn.[11] It would seem then that the presence of branched chain alkanes is a characteristic feature of the leaf waxes of a limited number of sub-genera of the genus Aeonium from the Canary Islands (as reported by Eglinton[8a]) and otherwise is only observed at a significant level in isolated examples in other families. In a widespread survey of leaf wax alkanes from many families of dicotyledons (described below), branched chain alkanes were only encountered in detectable amounts in ten out of seventy-three species: of these ten, four were from the family Solanaceae.

Hutchinson[1] has suggested that a major phyletic dichotomy occurred early in the evolutionary history of flowering plants, resulting in a herbaceous offshoot (Herbaceae) which produced the monocotyledons and those dicotyledons which are of predominately herbaceous habit. The original woody stock is considered to have given rise to those dicotyledonous families which remain predominantly woody (Lignosae). Those families which we[3] and Eglinton[8a] have so far examined extensively, Crassulaceae, Liliaceae and Agavaceae, all fall within Hutchinson's herbaceous sub-division. Leaf wax alkane patterns in these families are characterized by the predominance of normal odd carbon number hydrocarbons in the range C_{31}–C_{33} for the Crassulaceae and C_{29}–C_{31} for the Liliaceae and the Agavaceae, although some notable exceptions to the general patterns in the latter families are apparent.

It seemed to us of value to carry out a parallel investigation of the leaf wax alkanes of an extensive genus from a family which had retained the woody habit in order to examine the detailed classification from a chemical standpoint and also to determine whether any evidence of an evolutionary dichotomy such as that suggested by Hutchinson could be discerned from this particular chemical characteristic. For this we were fortunate in having

[8a] G. Eglinton, A. G. Gonzalez, R. J. Hamilton and R. A. Raphael, *Phytochem.* **1**, 89 (1962).
[b] G. Eglinton, R. J. Hamilton and M. Martin-Smith, *Phytochem.* **1**, 137 (1962).
[9] W. Carruthers and R. A. W. Johnstone, *Nature* **184**, 1131 (1959); J. D. Mold, R. K. Stevens, R. E. Means and J. M. Ruth, *Biochemistry* **2**, 605 (1963).
[10] J. D. Waldron, D. S. Gowers, A. C. Chibnall and S. H. Piper, *Biochem. J.* **78**, 435 (1961).
[11] D. H. S. Horn, Z. H. Kranz and J. A. Lamberton, *Australian J. Chem.* **17**, 464 (1964).

TABLE 2. PLANT WAXES OF THE FAMILY CRASSULACEAE. THE HYDROCARBON FRACTION OF THE WAX EXPRESSED AS A MOLE PERCENTAGE

Plant No.	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}
(1)	*Kalanchoe pinnata* Cham Lam. Persoon	(L+)	3·0	0·8	81·0	2·0	12·3	0·2	0·4	0·1	0·2				
(2)	*K. pinnata* (a second plant)	(L)	4·3	1·5	77·3	3·2	12·2	0·2	0·8	0·1	0·3		0·1		
	K. pinnata	(P+)			49·3	2·8	31·2	0·8	11·9	0·6	2·5	0·6	0·3		
(3)	*K. grandiflora* Wright et Arn.	(L)	4·8	0·4	83·9	1·3	8·6	0·1	0·3	0·2	0·2	0·1	0·1		
(4)	*K. grandiflora* Wright et Arn.	(L)	1·5	0·2	86·6	1·6	9·9	0·2							
(5)	*K. tubiflora* (Harvey) Hamet	(L)	2·3	0·3	83·4	3·0	10·2	0·1	0·5		0·1		0·1		
(6)	*K. beharensis* virides Drake Del Castillo	(L)	1·0	0·3	71·6	4·7	17·1	0·5	1·0	0·6	1·0	0·9	0·9	0·3	0·1
(7)	*K. beharensis* virides Drake Del Castillo	(L)	1·2	0·8	73·5	4·1	16·3	0·7	0·9	0·8	0·8	0·4	0·5		
(8)	*K. pumila* Bak.	(L)	1·6	0·5	63·8	2·7	30·0	0·2	1·1		0·1				
	K. pumila	(P)	1·1	0·4	51·4	3·3	32·3	0·3	6·8	0·2	3·7	0·1	0·2		
(9)	*Echeveria* "set-oliver" E Walth (Hybrid)	(L)	3·3	0·6	72·3	1·5	20·1								
(10)	*Echeveria* Perle von Nuernberg (Hybrid)	(L)			57·2	2·7	37·4	0·5	1·4	0·2	0·4	0·1	0·1		
(11)	*E. peacockii* Croucher	(L)			46·2	4·3	44·6	0·9	3·0	0·5	0·4	0·1			
(12)	*E. peacockii* Croucher	(L)	1·0	0·2	38·8	3·4	43·6	1·4	8·6	1·3	1·2	0·3	0·2		
(13)	*E. runyonii* Rose et Walth	(L)	0·3	0·2	29·2	2·7	54·3	1·4	7·7	2·0	1·3	0·4	0·3	0·1	0·1
(14)	*E. elegans* Rose	(L)	0·8	0·1	38·2	3·6	55·1	1·0	1·2						
(15)	*Crassula perforata* Thunbg.	(L)	29·7	1·9	56·6	1·7	8·7	0·2	0·5	0·1	0·2	0·1	0·2		
(16)	*C. corymbulosa* Link et Otto	(L)	6·9	1·4	52·7	6·9	30·9	0·3	0·6	0·1	0·2				
(17)	*C. columella* Marl et Schoenl.	(L)	8·4	1·4	43·2	3·4	19·9	2·2	5·3	3·7	3·3	3·7	3·6	1·6	0·3
(18)	*C. portulacea* Lam.	(L)			1·5	0·4	77·3	3·4	11·7	2·4	1·9	0·4	0·9	0·1	
(19)	*Sedum hispanicum* L.	(L)	0·5	0·4	35·5	4·7	45·7	1·7	9·1	1·2	0·9	0·2	0·1		
(20)	*S. pachyphyllum* Rose	(L)			47·0	1·6	48·1	0·8	2·0	0·1	0·4				

L+=Leaf.
P+=Petal.

access to the extensive collection of named *Eucalyptus* species (Myrtaceae) at the arboretum of the East African Agriculture and Forestry Research Organization at Muguga, near Nairobi.

While this work was in progress, Horn, Kranz and Lamberton published[11] their extensive study of the leaf wax components of the genus *Eucalyptus* in which the occurrence of long chain β-diketones (e.g. *n*-tritriacontane-16, 18-dione) as the major components in many cases is reported, although in a few species this type of compound was entirely lacking. The same workers also reported analyses of alkane fractions from seven *Eucalyptus* leaf waxes in

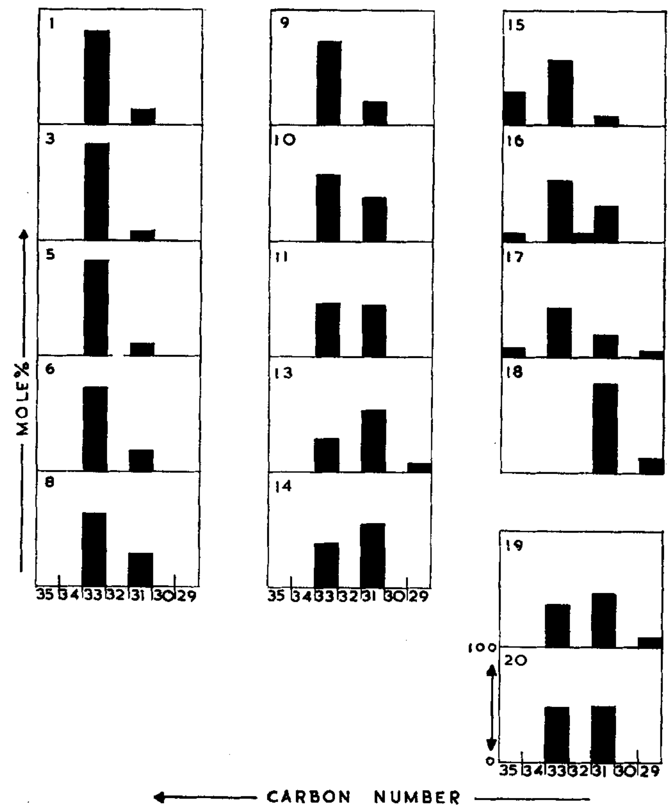

FIG. 2. ALKANE DISTRIBUTION PATTERNS OF SPECIES OF THE FAMILY *Crassulaceae*. (THE HISTOGRAM NUMBER REFERS TO THE POSITION OF THE SPECIES IN TABLE 2).

which hydrocarbons only comprise a small fraction (0·5–3 per cent, according to species). Horn *et al.* noted that no simple chain length relationship was apparent between the β-diketones and the *n*-alkane fractions. Certain correlations between chemical content of leaf cuticular wax and the sub-classification of the genus *Eucalyptus* (according to Blakely[12] vide also Penfold and Willis[13]) were observed by Horn *et al.*, as for example the existence of shorter chain length β-diketones in *E. risdoni* and *E. coccifera*, the only species examined in the Series Piperitales and the absence of β-diketones in four species from the Series Corymbosae

[12] W. F. BLAKELY, *A Key to the Eucalypts* (2nd edition), Commonwealth of Australia Forestry and Timber Bureau, Canberra (1955).

[13] A. R. PENFOLD and J. L. WILLIS, *The Eucalypts*, Leonard Hill, London (1961).

TABLE 3. EUCALYPTUS SPECIES LEAF WAXES. ALKANE FRACTION EXPRESSED AS A MOLE PERCENTAGE

Plant No.	Botanical classification according to Blakely[12]: Species	Series	C_{31}	C_{30}	C_{29}	C_{28}	C_{27}	C_{26}	C_{25}	C_{24}	C_{23}	C_{22}	C_{21}
(1)	*E. citriodora* Hook.	V Corymbosae peltatae	4·4	1·2	44·5	5·4	23·6	10·6	5·7	2·8	1·4	0·4	
(2)	*E. gummifera* (Gaertn.) Hochr.	V Corymbosae peltatae	3·4	0·4	70·3	3·7	21·1	0·7	0·3	0·1			
(3)	*E. botryoides* Sm.	VI Transversae	5·7	1·4	38·5	4·4	21·5	6·7	14·2	4·4	2·4	0·7	0·4
(4)	*E. robusta* Sm.	VI Transversae	9·9	1·8	28·1	3·2	17·1	9·2	15·4	10·0	3·7	1·3	0·3
(5)	*E. camaldulensis* Dehnh	XV Exsertae	1·5	1·1	5·2	3·0	29·6	11·9	32·6	11·1	3·0	0·7	0·3
(6)	*E. bicostata* Maiden, Blakely and Simmonds	XVIII Globulares			16·2	4·7	31·9	6·9	25·9	8·6	4·1	1·3	0·4
(7)	*E. globulus* Labill.	XVIII Globulares	1·7	0·9	64·0	6·3	22·4	1·9	2·5	0·2	0·1		
(8)	*E. macarthuri* Deane and Maiden	XX Viminales	1·2	0·9	20·7	4·7	24·9	6·6	19·9	10·3	8·4	1·7	0·7
(9)	*E. cloeziana* F. Muell.	XXII Paniculatae	2·6	2·0	11·6	6·6	22·1	15·0	20·6	11·0	6·5	1·7	0·3
(10)	*E. microcorys* F. Muell.	XXVIII Steatoxylon	1·2	0·5	43·5	5·1	23·2	12·0	9·4	4·1	0·7	0·3	
(11)	*E. baxteri* (Benth.) Maiden and Blakely	XXIX Pachyphloiae	0·7	0·4	36·9	1·5	52·3	1·7	5·9	0·4	0·2		
(12)	*E. fastigata* Deane and Maiden	XXIX Pachyphloiae	1·7	1·9	23·1	4·6	56·4	3·6	6·5	1·6	0·5	0·1	
(13)	*E. regnans* F. Muell.	XXIX Pachyphloiae	0·5	0·3	52·8	2·6	25·5	4·0	7·6	3·2	2·6	0·7	0·2
(14)	*E. dives* Schau.	XXXII Piperitales	1·1	0·6	53·1	3·0	35·0	2·9	3·1	0·8	0·3	0·1	
(15)	*E. largiflorens* F. Muell.	XXXVII Buxeales	4·0	2·3	7·2	3·3	16·6	14·0	28·1	18·9	3·5	1·7	0·4
(16)	*E. bosistoana* F. Muell.	XXXVII Buxeales	1·9	1·8	9·6	8·2	44·1	11·2	12·4	7·1	2·3	0·9	0·5
(17)	*E. crebra* F. Muell.	XXXVIII Siderophloiae	1·7	0·7	25·3	6·3	27·4	13·1	15·7	8·9	0·8	0·1	
(18)	*E. paniculata* Sm.	XXXVIII Siderophloiae	1·1	1·0	53·2	7·0	16·6	9·4	6·0	4·2	1·0	0·4	0·1
(19)	*E. melliodora* A. Cunn. ex Schau.	XXXIX Melliodorae	3·8	1·7	28·5	2·9	52·7	2·5	7·1	0·8			

peltatae. The alkane patterns of the seven species for which hydrocarbon contents were analysed did not show any correlation with Blakely's classification.

In Table 3 we show the detailed results of analyses of the hydrocarbon fractions from the leaf waxes of 19 species of *Eucalyptus*, while in Fig. 3 these are displayed in a simplified form as histograms. In agreement with Horn *et al.*[11] we have found the alkane content of the total leaf cuticular wax to be low, ranging from 0·2 per cent for *E. largiflorens* (No. 15) to 6 per cent for *E. gummifera* (No. 2). Two features of the alkane patterns of *Eucalyptus* leaf waxes apparent from an examination of Fig. 3 are:

(a) the occurrence of even carbon number alkanes, mainly C_{24} and C_{26}, as significant components of the alkane pattern (Nos. 5, 9, 15 and 17) and
(b) the virtual absence of any chain lengths greater than C_{29} in any of the species examined and the frequent dominance of C_{27} and, in a few cases of C_{25} in the patterns.

Only one species (*E. globulus* No. 7) appears both in Horn's[11] and our report: although the results are expressed in slightly different terms, it is clear for this species that they are very similar in the dominance of C_{29}, a strong indication that the wax composition is genetically rather than environmentally controlled.

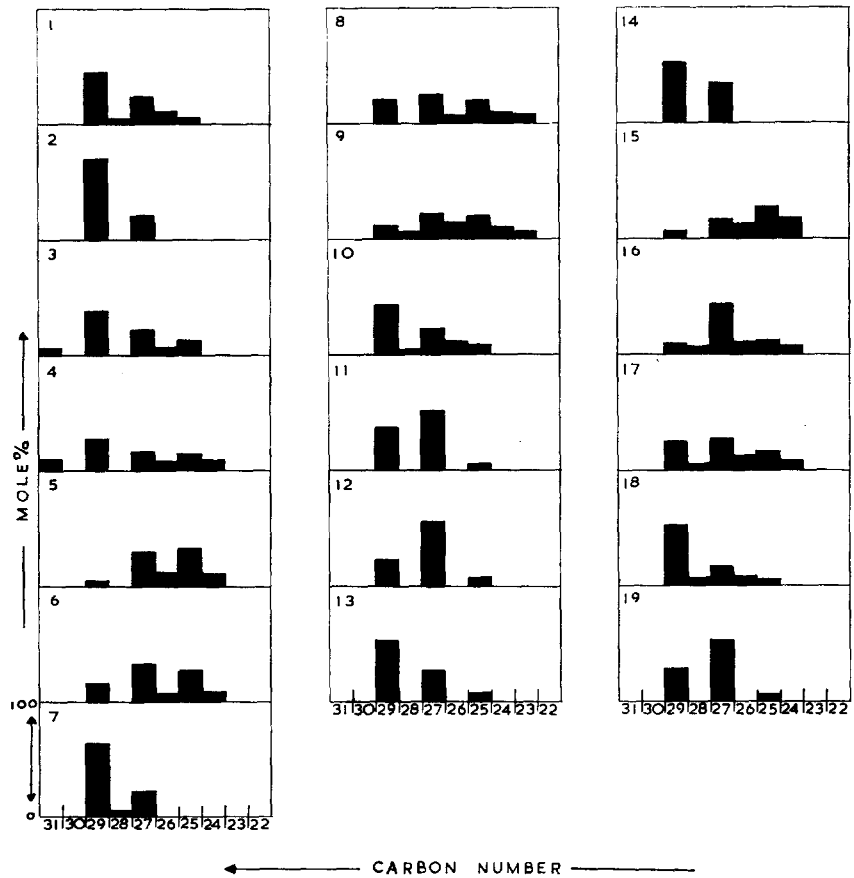

FIG. 3. LEAF ALKANE DISTRIBUTION PATTERNS OF SPECIES OF THE GENUS *Eucalyptus*. (THE HISTOGRAM NUMBER REFERS TO THE POSITION OF THE SPECIES IN TABLE 3).

Horn's work has shown that the commonest chain length in the β-diketones from *Eucalyptus* leaf wax is C_{33}, followed in diminishing order of importance by C_{31} and C_{29}. The virtual absence of C_{33} and the negligible importance of C_{31} in the alkane fractions, together with the very low hydrocarbon content of the leaf wax could be interpreted either as evidence that the two components arise from different biosynthetic pathways or alternatively that the inverse relationship in chain lengths is due to the plant's enzyme system being less able, with increasing chain length, to carry out the precursor to product reaction of reduction of the oxo-groups to methylenes.

The alkane patterns from the genus *Eucalyptus* show in their chain length distribution a clear contrast to the predominating C_{33}–C_{31} patterns of the Crassulaceae (Fig. 2) and the less marked dominance of C_{31}–C_{29} in the genera *Aloe*[3] and *Agave* (Fig. 1). A more widespread examination of examples from Hutchinson's Lignosae and Herbaceae seemed necessary to check whether this apparent distinction in chain length distribution was generally applicable. Accordingly 58 species drawn from 34 families (24 orders) of the subdivision Lignosae and 15 species from 9 families (9 orders) of the subdivision Herbaceae of the Dicotyledons, available from the local flora and readily identifiable, were analysed for leaf wax alkane pattern (mature leaves were used in each case). The detailed results are to be found in Herbin[14] but are summarized in Table 4. Table 4 Section 1 shows the frequency with which odd carbon number alkanes occur in order of magnitude down to the fifth position in the hydrocarbon fraction of the 58 Lignosae species, while Section 2 is similarly constructed for the even carbon number alkanes of the same 58 species. Sections 3 and 4 give the corresponding data for the 15 Herbaceae species. The results of the detailed work (reported above) on the Crassulaceae and on *Eucalyptus* are not included to avoid giving undue weight to a limited section within the general classification.

Inspection of the data in Table 4 makes it clear that, with the limited sample employed, there is no distinction apparent between the Lignosae and the Herbaceae on the basis of leaf alkane pattern.

In none of the 73 species examined in this survey, nor in any of the species previously reported upon after detailed gas-liquid chromatographic analysis were alkanes found to be entirely absent nor were alkenes found to be present in the leaf waxes. One species, *Santalum album* (Santalaceae) which Chibnall and co-workers[15] noted as containing no paraffins, was included in the present survey and found to contain less than 1 per cent of hydrocarbons in the total leaf wax. Another leaf wax, from *Annona senegalensis* (Annonaceae), reported by Mackie and Misra[16] as containing no hydrocarbons but only primary alcohols and *n*-hentriacontan-16-one (palmitone) was not re-examined. It is of interest that Chibnall found the low-hydrocarbon content leaf wax of *Santalum album* to contain mainly palmitone and 10-hydroxypalmitone, a parallel situation to that of the *Eucalyptus* leaf waxes, with their high β-diketone and low hydrocarbon content, suggesting that the inverse relationship of precursor-product tentatively put forward for the *Eucalyptus* species may be of a more general nature. Perhaps it is also significant that in *Santalum album* where the principal (ketonic) components of the whole leaf wax are C_{31} in chain length, hentriacontane forms only 6·1 per cent of the alkane fraction of which the main components are C_{29} (38·1 per cent) and C_{27} (27·6 per cent). At the opposite end of the scale, it has been found that *Solandra*

[14] G. A. HERBIN, Thesis for Doctor of Philosophy, University of London (1967).
[15] A. C. CHIBNALL, S. H. PIPER, H. A. EL MANGOURI, E. F. WILLIAMS and A. V. V. IYENGAR, *Biochem. J.* 31, 1981 (1937).
[16] A. MACKIE and A. L. MISRA, *J. Sci. Food Agri.* 7, 203 (1956).

TABLE 4. FREQUENCY OF OCCURRENCE* AND ORDER OF MAGNITUDE OF INDIVIDUAL ALKANES IN DICOTYLEDON LEAF WAX HYDROCARBON FRACTIONS

(1) Lignosae species odd-alkanes.
58 species represented.

Odd-Alkanes	1st	2nd	3rd	4th	5th
C_{35}					
C_{33}		2	23	9	3
C_{31}	28	20	6	3	1
C_{29}	27	28	3		
C_{27}	3	7	23	24	
C_{25}		1	3	21	31
C_{23}				1	15

(2) Lignosae species even-alkanes.
From the same 58 species represented in (1)

Even-Alkanes	1st	2nd	3rd	4th	5th
C_{34}				1	
C_{32}	3	16	12	3	1
C_{30}	38	12	4	4	
C_{28}	15	24	20		
C_{26}	2	6	18	25	
C_{24}			4	15	22
C_{22}					8

(3) Herbaceae species odd-alkanes.
15 species represented.

Odd-Alkanes	1st	2nd	3rd	4th	5th
C_{35}				1	
C_{33}		2	6		
C_{31}	8	4	3		
C_{29}	7	6	2		
C_{27}		3	4	7	1
C_{25}				7	7
C_{23}					5

(4) Herbaceae species even-alkanes.
From the same 15 species represented in (3)

Even-Alkanes	1st	2nd	3rd	4th	5th
C_{34}			1		1
C_{32}	4	3	1	1	1
C_{30}	7	5	2	1	
C_{28}	4	5	5	1	
C_{26}		2	4	6	1
C_{24}			1	4	5
C_{22}					2

* Numbers tabulated refer to the number of times a particular odd- or even-carbon number alkane occurs as the major, second, third, fourth or fifth order constituent in leaf wax hydrocarbon fractions.

grandiflora Sw. (Solanaceae) has a leaf wax consisting almost entirely (92 per cent) of alkanes.

The alkane distribution patterns in this extended survey as well as those reported in detail above are all in accord with Eglinton and Hamilton's generalisation[17] that for both odd carbon number and even carbon number alkanes considered separately, 'the plot of percentage of constituent against the number of carbon atoms is a simple distribution curve with a single maximum'. However another generalisation by the same authors (Ref.[17] p. 203) that 'the content of odd carbon number alkanes is usually greater than that of even carbon number alkanes by a factor of more than ten' requires modification. It does not hold for a number of *Aloe* species (Ref.[3] Table 3) or for many of the *Agave* species (Table 1, Nos. 3, 5, 7, 8, 13–19, 21). In the *Eucalyptus* species (Table 3), C_{26} and C_{24} form very high proportions of the total alkane content (e.g. *E. largiflorens* has 14 per cent C_{26} and 18·9 per cent C_{24}, but also has the lowest hydrocarbon content of any leaf wax in this genus).

The data in Table 4 confirm the overall preponderance of C_{31} and C_{29} as the dominant chain lengths of the odd carbon number normal alkane series in leaf cuticular waxes and shows the predominance of C_{30} and C_{28} in the even carbon number series. However, as already noted above, the *Eucalyptus* species have a shorter chain length, usually C_{27} or C_{25}, as the dominant alkane, while in the Cactaceae Waldron[10] found C_{35} dominant and C_{37} present to 10 per cent in the cuticular wax alkane fraction. Among the four species of Cactaceae investigated by Herbin[14] the leaf and stem cuticular wax alkane fraction of one (*Harrisia martini* Britton) had a dominant C_{37} (29·4 per cent) with C_{35} and C_{33} (14·6 per cent each) and C_{39} (10·9 per cent) as important constituents: it is thus evident that in this family the average chain length is higher than in most others examined up to now. Kranz *et al.*[18] have reported the presence of the normal alkane $C_{62}H_{126}$ in the stem wax of the grass *Leptochloa digitata*. Alkanes of this molecular size would not however be detected by the analytical techniques used in the present work.

EXPERIMENTAL

All experimental procedures employed in this work were as described in Part I.[3]

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[17] G. EGLINTON and R. J. HAMILTON in T. SWAIN (editor), *Chemical Plant Taxonomy*, Academic Press, London (1963).

[18] Z. H. KRANZ, J. A. LAMBERTON, K. E. MURRAY and A. H. REDCLIFFE, *Australian J. Chem.* **14**, 264 (1961).