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STUDIES ON PLANT CUTICULAR WAXES*—I.

THE CHEMOTAXONOMY OF ALKANES AND ALKENES OF THE GENUS *ALOE* (LILIACEAE)

G. A. HERBIN and P. A. ROBINS

Department of Chemistry, University College, Nairobi, Kenya

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Abstract—Gas–liquid chromatographic analyses of the alkane fraction of cuticular waxes from 63 species in the genus *Aloe* (Liliaceae) have been carried out. Species specificity in composition has been confirmed and a correlation between composition and the sub-classification of the genus according to Reynolds is discernible in the leaf wax alkanes and is more clearly revealed in the perianth wax alkanes. The presence of branched chain alkanes in one leaf wax and of alkenes in two perianth waxes and in all the style and filament waxes investigated is reported.

IN RECENT years considerable interest has been shown in the systematic distribution of chemical compounds throughout the plant kingdom and attention has been directed towards the possibility of using the distribution of such compounds as a means of establishing a system of taxonomy based on chemical characteristics. The field has been reviewed by various authors.[1]

Despite an early adverse prognostication by one of the pioneers in the field of chemotaxonomy (Erdtman[2]), the constituents of plant cuticular waxes have been shown by Eglinton and co-workers[3] to have significance in the study of interrelationships within a group of closely related genera in the sub-family Sempervivoideae (Crassulaceae). In Eglinton's study gas–liquid chromatographic analyses of the alkane fraction of leaf cuticular waxes gave distribution patterns of normal and branched chain alkanes which could be correlated with the accepted taxonomy based on morphological characters. It appeared from this work that the alkane distribution pattern was substantially constant within a species, while variation in pattern between closely related species was sufficiently great to show a distinction but at the same time to confirm the relationship. The evidence did not, however, indicate that differences between genera would always be sufficiently discriminating.

In a further study[4] of a number of New Zealand species drawn from different families, Eglinton and co-workers found that in four species of the genus *Hebe* (Scrophulariaceae) a much wider variation in alkane pattern existed within a single genus than had been found by the earlier work within a sub-family. This variation within a genus indicates that, while alkane distribution patterns may be valid criteria for distinguishing related plants, difficulties might be encountered in any attempt to correlate less closely related groupings.

* In this and subsequent papers of the series, "Cuticular Wax" refers to the lipid mixture obtained by brief dipping of the freshly collected plant material in chloroform, followed by filtration from insoluble fragments and removal of the solvent.

[1] T. SWAIN (editor), *Chemical Plant Taxonomy*, Academic Press, London (1963).
[2] H. ERDTMAN in A. R. TODD (editor), *Perspectives in Organic Chemistry*, Interscience, New York (1956).
[3] G. EGLINTON, A. G. GONZALES, R. J. HAMILTON and R. A. RAPHAEL, *Phytochem.* **1**, 89 (1962).
[4] G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochem.* **1**, 137 (1962).

The existence of normal alkanes in plant cuticular waxes had been clearly demonstrated by Chibnall and co-workers:[5] using fractional crystallization and X-ray diffraction methods, the presence of odd carbon number alkanes in the range C_{25} to C_{37} was established. The difficulties of experimental technique were such that little further advance could be made until the exploitation of gas–liquid chromatography and of mass spectrometry gave a means of rapidly analysing, both qualitatively and quantitatively, small samples of complex mixtures of hydrocarbons obtained from cuticular waxes. The presence of even carbon number normal alkanes as minor constituents of the hydrocarbon fraction of pyrethrum (*Chrysanthemum cinerariaefolium*) cuticle wax was established through mass spectrometry by Wanless *et al.*,[6] and confirmed in a number of other diverse species by Waldron *et al.*,[7] who re-examined many of the fractions previously isolated and examined by Chibnall.[5] Subsequent gas–liquid chromatographic analyses by numerous investigators[3, 4, 8–14] have amply demonstrated the common occurrence of even carbon number normal alkanes in cuticular waxes.

Branched chain alkanes were reported as constituents of plant waxes by Barbezat[15] and by Carruthers and Johnstone,[8] who demonstrated their presence by mass spectrometry. However Waldron *et al.*,[7] while confirming the presence of branched chain alkanes in tobacco leaf wax and rose petal wax, failed to detect them in other cuticular waxes from diverse species. Kosak and Swinehart[13] firmly identified both 2-methyl-and 3-methylalkanes in tobacco leaf wax, the former being, like the normal alkanes, in the odd carbon number series, the latter having even carbon numbers. Eglinton *et al.*,[3, 4] found high percentages of branched chain alkanes in some of the Sempervivoideae (Crassulaceae) species but little or no branched chain alkanes in those species investigated from other families.

Hallgren and Larsen[16] detected alkenes in high concentration in the hydrocarbon fraction of wax from the pollen of rye (*Secale cereale* L.) while Sôrm *et al.*[17] found alkenes in sugar cane wax and rose petal wax, the latter containing *cis*-alk-3-enes (predominantly C_{29} and C_{31}) and a second series of *trans*-alkenes (predominantly C_{27} and C_{29}) in which the position of the double bond was not established.

In the present study we have undertaken the investigation of cuticular waxes from a large number of species of the genus *Aloe* (Liliaceae) which were readily available to us in East Africa either as wild plants or as authenticated garden specimens. The genus *Aloe* is widespread throughout Africa including Madagascar and other island groups and occurs also in the Arabian peninsular and NW India, inhabiting a wide variety of ecological conditions. It has a complex taxonomic structure, being divided by Reynolds[18] (after Berger[19]) into a

[5] A. C. CHIBNALL and S. H. PIPER, *Biochem. J.* **28**, 2209 (1934).
[6] G. G. WANLESS, W. H. KING and J. J. RITTER, *Biochem. J.* **59**, 684 (1955).
[7] J. D. WALDRON, D. S. GOWERS, A. C. CHIBNALL and S. H. PIPER, *Biochem. J.* **78**, 435 (1961).
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[9] D. T. DOWNING, Z. H. KRANZ and K. E. MURRAY, *Australian J. Chem.* **13**, 80 (1960).
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[12] P. MAZLIAK, *Compt. Rend.* **251**, 2393 (1960); **252**, 1507 (1961).
[13] A. I. KOSAK and J. S. SWINEHART, *J. Org. Chem.* **25**, 222 (1960).
[14] J. D. MOLD, R. K. STEVENS, R. E. MEANS and J. M. RUTH, *Biochemistry* **2**, 605 (1963).
[15] S. BARBEZAT-DEBREUIL, *J. Rech. Centre Natl Rech. Sci. Lab. Bellevue (Paris)* **45**, 273 (1958).
[16] B. HALLGREN and S. LARSSON, *Acta Chem. Scand.* **17**, 1822 (1963).
[17] F. SÔRM, V. WOLLRAB, P. JAROLIMEK and M. STREIBL, *Chem. Ind.*, 1833 (1964).
[18a] G. W. REYNOLDS, *The Aloes of South Africa*, The Aloes of South Africa Book Fund, Johannesburg (1950).
[18b] G. W. REYNOLDS, *The Aloes of Tropical Africa and Madagascar*, The Aloes Book Fund, Mbabane, Swaziland (1966).
[19] A. BERGER in A. ENGLER (editor), *Das Pflanzenreich*, Vol. 38 (1908).

large number of sections, sub-sections and series on morphological criteria (Table 1). Such a complex genus, on which ample close botanical study has been carried out, appeared to be most suitable for the application of chemotaxonomic methods.

TABLE 1. THE GENUS *Aloe* (LILIACEAE)

Key to the Sections, Subsections and Series

(According to Reynolds [18a])

SECTION 1: ALOINELLA
SECTION 2: GRAMINIALOE
SECTION 3: LEPTOALOE
SECTION 4: EUALOE
  Subsection: A—PARVAE
    Series 1: Haemanthifoliae
    Series 2: Longistylae
    Series 3: Aristatae
  Subsection: B—HUMILES
    Series 4: Virentes
    Series 5: Echinatae
    Series 6: Proliferae
    Series 7: Madagascarienses
    Series 8: Rhodacanthae
    Series 9: Serrulatae
    Series 10: Saponariae
    Series 11: Paniculatae
    Series 12: Superpositae
    Series 13: Asperifoliae
    Series 14: Hereroenses
SECTION 4 (cont.)
  Subsection: C—GRANDES
    Series 15: Percrassae
    Series 16: Verae
    Series 17: Latebracteatae
    Series 18: Tropicales
    Series 19: Aethiopicae
    Series 20: Cernuae
  Subsection: D—PROLONGATAE
    Series 21: Macrifoliae
    Series 22: Monostachyae
    Series 23: Pleurostachyae
    Series 24: Fruiticosae
    Series 25: Mitriformes
  Subsection: E—MAGNAE
    Series 26: Comosae
    Series 27: Purpurascentes
    Series 28: Arborescentes
    Series 29: Principales
SECTION 5: ANGUIALOE
SECTION 6: PACHYDENDRON
SECTION 7: DRACOALOE
SECTION 8: ALOIDENDRON
SECTION 9: SABAEALOE
SECTION 10: KUMARA

It seemed necessary at the outset to ensure that species specificity in the wax hydrocarbon patterns could be relied upon. Accordingly replicate gas–liquid chromatographic analyses of the hydrocarbon fractions of cuticular waxes obtained from a number of individual plants were carried out on the species *Aloe kedongensis* Reynolds, *A. secundiflora* Engler and *A. ballyi* Reynolds. The results are shown in Table 2. The investigation was extended to include perianth waxes where specimens of the flowers were freely available; *A kedongensis* and *A. graminicola* Reynolds were at the time in flower and replicate analyses of the hydrocarbon fractions of the perianth waxes are included in Table 2.

Both *A. kedongensis* and *A. secundiflora* have similar leaf hydrocarbon patterns in that 70 per cent or more of the total fraction is composed of hentriacontane (C_{31}). The patterns are fairly consistent within the species but overlap too extensively to be of value in distinguishing one species from the other. *A. ballyi*, however, shows a very different leaf hydrocarbon pattern with heptacosane (C_{27}) as the major constituent but with nonacosane (C_{29}) also prominent; a greater "spread" in the magnitude of concentrations over a range of hydrocarbon chain lengths is discernible in this species. It is also noticeable that the "*A. ballyi* type" of leaf hydrocarbon pattern is associated with an increase in the relative proportion of even-number alkanes to odd-number alkanes when compared with the pattern from *A. kedongensis* and *A. secundiflora*. A comparison of the leaf and perianth hydrocarbon

TABLE 2. REPLICATE ANALYSES OF HYDROCARBON FRACTIONS OBTAINED FROM LEAVES AND PERIANTHS OF ALOE SPECIES

Species investigated and comments	Concentration (mole per cent)												
	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}
A. kedongensis Reynolds leaf analyses	1·8	0·6	80·1	2·0	10·7	1·2	2·0	0·4	0·8	0·1	0·3		
(All analyses listed are of alkane fractions obtained from			82·5	1·7	10·1	1·7	1·7	1·0	0·7	0·3	0·3		
individual plants collected in the type locality 30 miles	3·0		77·9	2.0	9·0	0·9	4·0	1·2	2·0				
north of Nairobi)			89·5	1·1	7·1	0·3	1·4	0·2	0·4				
	3·8	1·9	70·2	3·2	9·1	2·5	3·7	1·2	2·2	0·7	1·6		
	3·6	1·3	71·4	2·8	9·9	2·2	3·8	1·4	1·8	1·0	0·8		
	5·6	2·5	75·5	3·2	5·1	2·0	2·5	1·2	1·5	0·2	0·7		
	2·5	1·3	70·0	4·1	14·1	1·8	4·3	0·4	1·1	0·2	0·2		
	3·0	1·1	74·6	1·6	14·1	1·1	2·1	0·7	1·0	0·3	0·3		
	1·7	1·0	76·2	1·5	11·2	1·0	4·2	0·6	2·0	0·4	0·2		
	2·1	3·4	72·4	3·2	8·4	1·9	3·6	1·5	1·9	0·6	0·8	0·2	
A. secundiflora Engler leaf analyses	3·6	2·5	70·4	5·6	8·5	3·1	3·3	1·2	1·2	0·3	0·3		
One plant {abaxial leaf surface only analysed}	5·4	3·3	66·9	5·0	5·4	3·3	3·7	2·1	2·9	1·2	0·8		
One plant {adaxial leaf surface only analysed}	1·1	0·6	80·6	2·2	6·4	1·7	3·4	1·1	1·7	0·6	0·6		
One plant {young leaves only analysed}	3·3	1·5	75·8	3·5	9·3	1·8	2·4	0·7	1·1	0·2	0·4		
One plant {old leaves only analysed}	4·5	2·7	77·6	3·6	4·5	2·7	1·8	0·9	0·9	0·3	0·5		
This plant was obtained from Magadi—an arid region	1·8	2·9	76·6	5·7	4·1	3·5	2·7	1·8	0·6	0·3			
One plant {analysed after collection from Athi River area}	1·0	0·5	82·5	2·7	5·5	1·3	2·9	0·9	1·9	0·3	0·5		
One plant {transplanted in Nairobi}	3·9	1·3	78·1	2·6	5·1	1·8	3·2	1·7	1·1	0·8	0·4		
One plant {reanalysed a year later}	1·6	0·8	77·6	5·3	4·5	4·5	2·9	1·6	0·6	0·2	0·2		
A. ballyi Reynolds leaf analyses	6·8	0·7	12·5	2·8	24·8	5·9	31·2	7·7	5·3	1·7	0·6		
(Analyses of leaf wax hydrocarbons obtained from four	2·4	0·5	13·3	2·4	25·1	4·0	36·9	7·9	5·4	1·2	0·9		
plants growing in different geographical locations)	0·8	0·2	13·6	1·7	22·9	4·3	39·1	8·0	5·7	1·6	1·6	0·4	0·1
		0·8	9·4	2·2	22·6	7·3	37·3	6·9	6·2	3·8	2·7	0·2	0·6
A. graminicola Reyn. Perianth wax hydrocarbons obtained			97·8	1·2	1·0								
from 6 plants collected at intervals between Naivasha and			96·9	1·0	1·4	0·5	0·1	0·1					
Nakuru			97·5	1·1	1·0		0·3						
			96·8	1·0	1·4		0·3	0·5					
			98·2	1·0	0·8								
			97·2	1·0	1·4	0·2	0·1	0·1					
A. kedongensis Reyn. Perianth wax hydrocarbons obtained			7·0	1·8	88·5	0·6	2·0		0·1				
from 6 plants collected from different localities near			3·5	0·4	91·3	1·4	2·8	0·3	0·3				
Naivasha and the Rift Escarpment			4·5	1·7	85·9	1·4	5·9	0·2	0·4				
			3·2	0·9	90·7	1·3	3·4	0·1	0·4				
			4·1	1·5	81·4	0·8	11·2	0·1	0·8		0·1		
			4·6	2·8	87·8	1·0	3·4		0·3		0·1		

patterns of *A. kedongensis* shows that they are distinguished clearly by the presence as a major component of C_{31} in the former and of C_{29} in the latter.

These preliminary investigations lead to the conclusion that the hydrocarbon patterns are sufficiently consistent within each species to be considered as species specific. The variation in the patterns of hydrocarbons derived from different organs of the same plant indicates that the perianth hydrocarbon pattern might provide a second taxonomic character to be used in conjunction with the leaf pattern, as is clearly shown by the differences exhibited by *A. graminicola* and *A. kedongensis* perianth waxes.

Cuticular wax hydrocarbon analyses of all the *Aloe* species investigated are given in Table 3, being expressed in molar percentages. For ease of subsequent reference each species is given a serial number; leaf and perianth waxes are designated by L and P respectively. A simplified pictorial representation of the data contained in Table 3 is given in the form of histograms in Figs. 1, 2 and 3, where mole percentages of normal alkanes are plotted against carbon numbers (for the sake of clarity mole percentages of less than 5 per cent have been omitted); each histogram contains within its outline the serial number of the *Aloe* species as given in Table 3. Information on unpublished detailed classification of certain East African *Aloe* species was supplied by Reynolds (personal communications) and, in part, is included in his second monograph[18b] which was published subsequent to the completion of much of the work described herein.

The two Series that have been most intensively studied are Series 10: Saponariae and Series 24: Fruticosae, which fall within the Subsections B, Humiles and D, Prolongatae, respectively of Section 4 (Table 1). Series 10: Saponariae contains a large number of species, widely distributed throughout Africa in natural habitats ranging in altitude from sea-level to 9000 ft and from semi-arid desert to areas receiving 50 in. or more of annual rainfall. Of the species listed under Saponariae in Table 3, Nos. 6, 7, 8, 9 and 12 are indigenous to East Africa and the remaining four are derived from South Africa, where more than 32 species (approximately one quarter of all South African aloes) have been described.[18a] Hentriacontane is the dominant alkane in Series 10, comprising 94–98 per cent of the perianth wax alkanes and invariably forming the major constituent of the leaf wax alkanes.

With the exception of *A. komatiensis* Reynolds (No. 40), the hydrocarbon pattern is consistent within the series. The two East African species *A. lateritia* Engler and *A. graminicola* (Nos. 6 and 7) would be indistinguishable on the basis of either leaf or perianth wax patterns and are considered as very close by Reynolds[18b] (p. 80), who notes that in certain geographical locations in Kenya the two species appear to grade one into the other. No. 8, (formerly *A. angiensis* de Wild) is now considered by Reynolds[18b] (p. 99) as a variety of *A. lateritia*, (var. *kitaliensis*), from which its perianth wax pattern is indistinguishable, although the C_{31} content of the leaf wax is somewhat lower than in either Nos. 6 or 7. However as Reynolds remarks of the Saponariae (Ref.[18b] p. 76), "These species constitute a heterogeneous and frequently most exasperating group, and it is often impossible to know where one species ends and the other begins". Nevertheless, on chemical evidence, this Series is one of the most homogeneous of all those examined to date on the basis of both the perianth and leaf wax alkane patterns.

A. komatiensis (No. 40) is unique in this Series in that the leaf hydrocarbons include more than 20 per cent of branched chain alkanes. As indicated above, this appears to be an unusual feature in leaf waxes generally and only in this example out of 63 *Aloe* species investigated were branched chain alkanes encountered in detectable amounts: none were found in the perianth wax of this or any other species in the genus.

TABLE 3. DISTRIBUTION IN MOLE % OF THE HYDROCARBON CONSTITUENTS OF THE LEAF AND PERIANTH WAXES OF THE ALOE SPECIES INVESTIGATED

Plant No.	Species	Subdivision*		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. nubigena* Groenewald	L	3:2A	2·4	1·6	31·7	4·8	31·0	4·8	15·9	2·4	3·2	1·6	0·6		
		P				65·5	2·6	20·8	1·7	6·9	0·8	1·7				
(2)	*A. cooperi Bak.*	L	3:4A	2·1	3·4	72·4	3·2	8·4	1·8	3·6	1·5	1·9	0·6	0·8	0·2	
		P				88·6	1·3	5·7	0·6	2·9	0·3	0·6				
(3)	*A. deltoideodonta* Bak.	L	4:B7	3·4	2·2	47·1	6·1	12·9	5·4	9·5	4·4	4·1	2·2	2·7		
		P			0·2	93·3	2·1	3·4	0·2	0·5	0·1	0·2				
(4)	*A. komatiensis* Reynolds.	L	4:B10	11·3 (1·2)	1·5 (7·3)	40·2 (5·4)	2·6 (5·1)	7·8 (1·3)	3·1 (0·2)	6·7 (0·2)	1·8 (branched alkanes)	2·4	0·7	0·6	0·2	0·2
		P			1·6	95·4	1·9	0·7	0·2	0·1	0·1					
(5)	*A. saponaria* (Ait). Haw.	L	4:B10	1·8	2·9	76·6	5·7	4·1	3·5	2·7	1·8	0·6	0·3			
		P				96·9	1·0	1·4	0·5	0·1	0·1					
(6)	*A. lateritia* Engler	L	4:B10	1·5	2·4	57·9	4·4	11·0	5·5	6·5	3·3	3·0	1·5	1·1	1·3	0·6
		P		0·1	0·6	98·2	0·3	0·3	0·1	0·2	0·1	0·1				
(7)	*A. graminicola* Reynolds	L	4:10B	3·5	1·1	52·9	3·3	13·1	6·8	10·2	3·3	3·4	1·2	0·9	0·2	0·1
		P		0·8	0·6	96·9	0·8	0·4	0·2	0·3						
(8)	*A. angiensis* de Wild	L	4:B10	8·4	4·1	44·8	6·5	16·3	5·0	9·4	2·9	1·9	0·5	0·2		
		P				96·8	1·0	1·4		0·3	0·5					
(9)	*A. amudatensis* Reynolds	L	4:B10	7·3	2·5	33·9	6·4	10·1	5·9	12·9	5·6	6·7	3·4	3·3	1·1	0·9
		P			0·9	94·5	0·9	1·2	0·3	0·7	0·3	0·6	0·3	0·1	0·1	0·1
(10)	*A. davyana* Schonl.	L	4:B10	8·8	2·7	44·6	4·7	14·2	5·8	7·6	3·7	4·5	1·6	1·4	0·4	
		P			1·0	96·0	1·0	1·2	0·1	0·7						
(11)	*A. barbertoniae* Pole Evans	L	4:B10	10·8	2·2	36·5	4·8	12·3	7·9	9·2	5·5	5·9	1·8	2·0	0·4	0·7
		P		0·5	0·8	97·3	0·7	0·6		0·1						
(12)	*A. kilifiensis* Christian	L	4:B10	2·6	3·6	71·8	4·7	6·4	3·4	3·3	1·7	1·3	0·4	0·5	0·2	0·1
		P				96·8	0·8	1·3	0·1	0·6	0·1	0·3				

(13)	*A. suprafoliata* Pole Evans	L	4:B12		2·4	2·3	19·6	4·0	12·0	6·8	28·2	7·8	11·6	2·6	1·7	0·3	
		P				0·5	92·1	0·8	1·1	1·1	1·3	1·3	1·1	0·5	0·2		
(14)	*A. deserti* Berger	L	4:C16			2·0	51·3	9·2	10·5	8·2	6·5	5·6	2·9	1·3	1·6	0·5	0·2
		P				0·8	93·3	1·7	2·4	0·9	1·5		0·2				
(15)	*A. cryptopoda* Bak.	L	4:C17		1·6	2·2	23·8	5·4	12·1	9·2	21·7	13·5	6·7	2·2	1·6		
		P				1·4	85·9	3·1	4·0	1·1	2·7	0·4	0·7	0·3	0·2	0·1	0·1
(16)	*A. wickensii* Pole Evans	L	4:C17				18·6	1·9	17·2	4·1	50·1	6·2	1·7	0·2			
		P					79·2	3·8	9·2	0·4	4·7	0·4	2·0	0·1	0·2		
(17)	*A. tweediae* Reynolds	L	4:C18				19·9	10·0	16·2	12·8	14·7	6·6	6·6	5·2	4·7	1·9	1·4
		P															
(18)	*A. wilsonii* Reynolds	L	4:C18		0·9	0·5	77·2	4·4	6·3	2·2	3·0	1·5	1·8	1·3	0·7	0·2	
		P															
(19)	*A. rivae* Bak.	L	4:C18		4·0	2·8	28·4	5·6	18·2	7·4	16·8	6·3	5·6	2·8	2·1		
		P															
(20)	*A. ukambensis* Reynolds	L	4:C18		2·3	1·5	23·7	7·6	22·1	8·4	11·5	6·9	7·6	4·6	3·8		
		P															
(21)	*A. tororoana* Reynolds	L	4:C18		1·1	0·6	12·2	6·0	33·3	14·6	12·2	6·5	5·4	2·7	3·0	1·3	1·1
		P															
(22)	*A. bukobana* Reynolds	L	4:C19		1·2	0·8	30·3	7·3	35·6	7·2	7·7	2·6	2·8	1·3	1·4	0·8	1·0
		P															
(23)	*A. calidophila* Reynolds	L	4:C19		3·5	2·9	32·7	6·5	16·8	7·6	10·9	6·0	4·3	3·6	2·8	1·6	0·8
		P															
(24)	*A. schweinfurthii* Bak.	L	4:C19		1·5	0·7	36·6	8·9	18·8	7·6	12·3	5·2	4·5	1·6	1·0	0·8	0·5
		P					93·0	1·4	3·6	0·3	1·3		0·4				
(25)	*A. chabaudii* Schonl.	L	4:C19		1·4	1·8	62·7	5·7	16·5	5·0	4·3	1·4	0·8	0·4			
		P					95·5	1·7	1·8	0·2	0·4	0·2	0·2				

* The first number given is that of the Section; followed by, for Sections 3 and 6 the Group designation, or for Section 4 the Subsection letter and Series number. E.g. 4:B10 = Section 4, Subsection B, Series 10.

TABLE 3—*continued*

Plant No.	Species		Subdivision*	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}
(26)	*A. tidmarshii* (Schonl.) Muller	L	4:D21	33·4	3·3	59·1	1·8	1·4	0·3	0·3	0·1	0·1				
		P				94·1	1·7	2·5	0·3	1·0	0·2	0·2				
(27)	*A. ciliaris* Haw.	L	4:D21			49·6	4·1	13·5	5·5	7·4	5·0	6·1	2·5	0·8	0·7	0·2
		P			0·3	84·3	3·8	8·7	0·6	2·1	0·2					
(28)	*A. tenuior* Haw.	L	4:D21			4·0	1·6	11·9	4·3	34·4	8·0	25·2	5·1	4·2	1·0	0·3
		P		0·5	0·9	88·8	1·5	4·0	0·5	2·1	0·5	0·6	0·1	0·1		
(29)	*A. striatula* Haw.	L	4:D21		0·2	96·1	1·1	2·5	0·1							
		P				62·5	5·1	19·7	0·7	5·1	1·1	3·6	0·5	1·5	0·2	
(30)	*A. dorotheae* Berger	L	4:D22	4·7	4·0	26·7	7·6	13·2	8·7	17·3	7·3	6·0	2·6	1·9		
		P				96·6	1·1	1·8		0·4		0·1				
(31)	*A. yavellana* Reynolds	L	4:D24	1·1	0·9	92·2	1·3	1·8	0·7	0·8	0·6	0·6				
		P														
(32)	*A. kedongensis* Reynolds	L	4:D24	2·5	1·3	70·0	4·1	14·1	1·8	4·3	0·4	1·1	0·2	0·2		
		P				8·7	2·9	78·5	2·1	3·7	1·0	2·1	0·6	0·3	0·1	
(33)	*A. dawei* Berger	L	4:D24	1·2	0·6	68·2	2·4	11·7	2·7	4·8	2·1	2·4	0·9	2·1	0·3	0·6
		P				18·3	3·2	62·9	1·3	11·8	0·6	1·9				
(34)	*A. nyeriensis* Christian	L	4:D24	0·6	4·2	69·4	3·6	10·8	1·7	6·3	0·8	1·4	0·4	0·6	0·2	0·2
		P				6·5	1·0	85·8	0·5	5·7		0·5				
(35)	*A. ngobitensis* Reynolds	L	4:D24	1·1	0·9	15·9	4·8	28·6	10·4	14·0	8·1	5·2	3·7	4·6	1·5	1·2
		P				7·7	1·8	82·3	0·9	4·8	0·5	2·0				
(36)	*A. hildebrandtii* Bak.	L	4:D24	1·6	2·4	11·8	5·7	18·8	12·4	17·8	8·1	6·2	4·9	4·1	3·4	2·8
		P														
(37)	*A. megalacantha* Bak.	L	4:D24	1·9	1·3	50·3	4·7	19·3	6·0	6·3	2·8	4·4	1·4	1·6		
		P														

(38)	*A. confusa* Engler	L	4:D24		0·6	18·3	6·0	26·8	10·7	18·3	7·6	5·9	2·0	1·8	1·0	1·0	
		P				10·2	2·2	44·5	5·8	29·2	4·4	3·0	0·7				
(39)	*A. rabaiensis* Rendle	L	4:D24	1·4	1·0	34·5	3·8	38·4	8·3	8·6	3·8	3·8	1·9	2·3	1·0	1·1	
		P				1·9	0·8	80·5	2·0	14·0	0·3	0·5					
(40)	*A. volkensii* Engler	L	4:D24			2·8	1·4	24·8	5·5	46·4	8·7	5·6	2·9	1·2	0·5	0·2	
		P				4·8	0·7	57·0	3·9	30·8	0·7	2·1					
(41)	*A. mutabilis* Pillans	L	4:E28	16·3	3·1	52·9	3·3	14·8	3·1	3·9	1·4	0·8	0·4	0·1	0·1		
		P		1·1	0·4	90·8	1·2	4·9	0·2	1·0	0·2	0·2					
(42)	*A. vanbalenii* Pillans	L	4:E28	57·8	8·0	28·9	1·3	2·2	0·6	0·6	0·3	0·2	0·1				
		P		1·6	0·3	66·1	0·9	15·7	0·8	11·9	0·4	2·3					
(43)	*A. arborescens* Mill.	L	4:E28	1·0	0·8	26·9	3·0	53·1	3·4	6·1	2·7	1·6	0·8	0·6			
		P				92·8	1·5	4·9	0·2	0·5		0·1					
(44)	*A. recurvifolia* Groenewald	L	4			55·4	5·4	22·3	4·6	6·9	1·5	2·8	0·3	0·8			
		P				50·2	1·1	15·5	1·6	8·2	1·4	6·1	0·5	1·0	0·2	0·2	
						(8·6)	(1·2)	(2·6)		(2·5)		(0·2)		(alkenes)			
(45)	*A. sessiliflora* Pole Evans	L	5	21·9	1·6	67·0	1·3	7·8	0·1	0·2	0·1						
		P				55·6	1·9	18·4	1·2	10·9	0·8	4·5	0·1	0·2			
						(4·8)	(0·1)	(1·0)		(0·5)			(alkenes)				
(46)	*A. petricola* Pole Evans	L	6:4A	2·2	0·8	11·3	1·9	45·4	12·6	13·5	7·0	3·3	1·2	0·8			
		P				37·5	3·0	65·5	0·7	2·7	0·2	0·4					
(47)	*A. ferox* Mill.	L	6:4B	5·3	2·6	53·6	13·6	14·2	5·9	3·4	1·2	0·3					
		P				58·3	2·1	34·9	0·7	3·0	0·5	0·5					
(48)	*A. candelabrum* Berger	L	6:4B		2·0	8·9	6·5	17·2	15·1	22·0	11·1	10·5	3·7	2·3	0·6	0·1	
		P		2·8		58·4	1·8	32·4	0·5	2·9	0·3	0·6	0·1	0·2			
(49)	*A. marlothii* Berger	L	6:6			7·1	2·1	38·6	11·3	25·8	7·5	4·8	2·0	0·6	0·2		
		P				64·7	4·0	37·8	0·7	1·7	0·3	0·5	0·1	0·2			
(50)	*A. secundiflora* Engler	L	6:6	3·9	1·3	78·1	2·6	5·1	1·8	3·2	1·7	1·1	0·8	0·4			
		P			0·5	96·0	0·7	1·5	0·5	0·7		0·1					
(51)	*A. aculeata* Pole Evans	L	6:4A					35·8	8·1	12·6	8·1	8·9	7·5	7·6	6·7	3·9	C_{20} 0·8
		P				37·5	2·5	42·5	0·7	4·2	1·5	2·6	2·7	2·9	1·7	1·0	0·2

TABLE 3—*continued*

Plant No.	Species		Subdivision*	C_{33}	C_{32}	C_{31}	C_{30}	C_{29}	G_{28}	C_{37}	C_{26}	C_{25}	C_{24}	C_{23}	C_{22}	C_{21}
(52)	*A. bainesii* Th. Dyer	L	8		1·9	15·4	8·9	20·2	17·7	14·5	10·0	6·9	2·9	1·3	0·4	0·1
		P														
(53)	*A. eminens* Reynolds et Bally	L	8		0·8	42·2	4·3	36·9	3·1	5·4	2·3	1·6	1·3	1·0	0·5	0·6
		P														
(54)	*A. sinkatana* Reynolds	L	Unknown	1·9	2·1	84·5	2·5	5·0	1·1	1·8	0·5	0·5	0·1			
		P				97·9	0·5	1·1		0·5						
(55)	*A. bakeri* Scott Elliot	L	Unknown	1·5	3·0	67·2	9·0	6·0	3·7	4·5	1·5	2·2	0·7	0·7		
		P			1·4	91·5	2·5	2·2	0·3	1·8		0·3				
(56)	*A. mzimbana* Christian	L	Unknown	19·1	1·2	51·4	7·3	13·5	2·8	3·3	0·8	0·3	0·2	0·1		
		P		0·3	0·2	94·4	2·6	1·6	0·2	0·3	0·2	0·2				
(57)	*A. turkanensis* Christian	L	Unknown	2·3	1·7	32·7	8·2	14·6	7·9	13·4	4·7	10·2	2·6	1·7		
		P			0·3	96·9	0·7	1·9	0·1	0·1						
(58)	*A. jucunda* Reynolds	L	Unknown			24·5	2·3	40·8	4·1	25·9	1·2	0·6	0·1	0·3	0·1	0·1
		P				95·8	1·4	2·0		0·6		0·2				
(59)	*A. acutissima* Perr.	L	Unknown	1·0	1·0	82·6	4·8	7·4	1·4	1·2	0·4	0·2				
		P				89·7	2·5	6·2	0·2	1·4						
(60)	*A. andongensis* Bak.	L	Unknown			14·2	0·9	62·7	10·5	8·0	1·8	1·4	0·3	0·2		
		P		0·3	0·2	91·7	1·2	3·8	0·3	1·9	0·3	0·2	0·1			
(61)	*A. harlana* Reynolds	L	Unknown	4·0	4·6	14·8	9·5	20·0	15·4	18·2	6·8	4·6	1·5	0·6		
		P		0·7	0·2	59·6	1·8	19·2	0·6	12·3	0·3	3·9	0·1	1·3		
(62)	Not known—locality Kitui	P	Unknown	1·3		80·0	1·8	13·5	0·8	1·5	0·3	0·6		0·2		
		P				28·6	5·2	58·8	1·0	5·2	0·4	0·6	0·2			
(63)	*A. ballyi* Reynolds	L	8	2·4	0·5	13·3	2·4	35·1	4·0	36·9	7·9	5·4	1·2	0·9		
		P				95·0	1·4	1·7	0·3	0·9	0·2	0·3	0·2			

Series 24: Fruticosae is a group of very different external morphology from the Saponariae and of a much more restricted geographical range, being absent from South Africa. The ten species presently investigated are from East Africa, Somalia or Ethiopia. The leaf alkane patterns are more variable within the series than was the case for the Saponariae. Five species, (Nos. 31, 32, 33, 34 and 37) have a major C_{31}, while four (Nos. 35, 36, 38 and 39) have C_{29} as the major alkane. No. 40 (*A. volkensii* Engler) is unique in this series and unusual

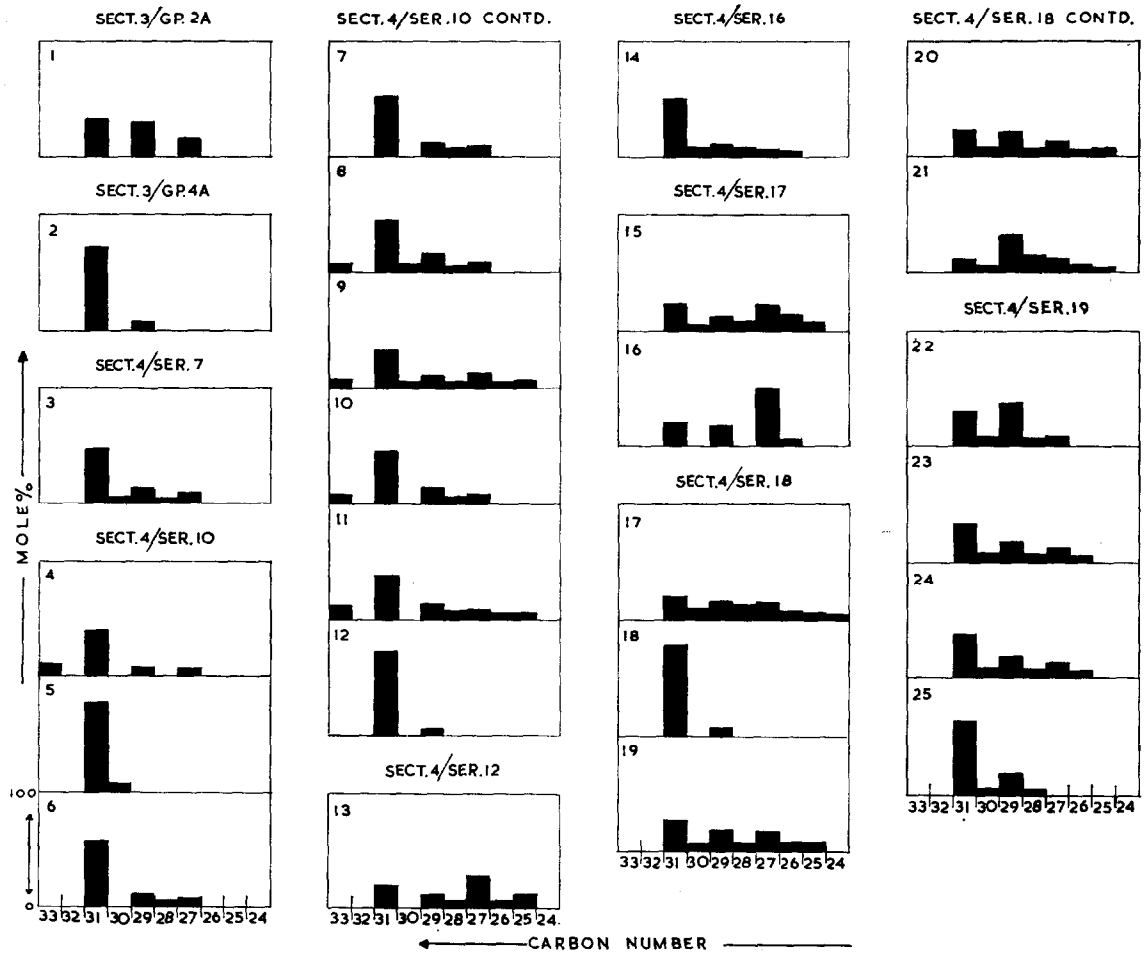

FIG. 1. CARBON-NUMBER DISTRIBUTION HISTOGRAMS (MOLES %) FOR ALOE SPECIES' LEAF WAX ALKANES.

among the *Aloe* species examined in having C_{27} as the major alkane of the leaf hydrocarbon fraction. The perianth alkane patterns are fully consistent within the series, however, and are sharply defined from those of the Saponariae, having C_{29} as the major alkane in every case and with C_{31} (5 out of 8) or C_{27} (3 out of 8) as the next most abundant alkane. In this series the perianth alkane patterns appear to be of greater value in indicating taxonomic relationships than the leaf alkane patterns, which by themselves are insufficiently discriminating.

For other series within Section 4 smaller numbers of specimens were available. In series 18: Tropicales, only leaf wax samples were obtained from the five species available. The leaf

alkane patterns from four of these (Nos. 17, 19, 20 and 21) are similar, with no alkane dominant, but with C_{31}, C_{29}, and C_{27} prominent and with relatively high proportions of the even carbon number alkanes. The leaf alkane pattern of No. 18 (*A. wilsonii* Reynolds) is dissimilar in showing a high concentration (77 per cent) of C_{31}. In the absence of data from the perianth waxes, which appear to offer more valuable criteria, it is not possible to make further comment on this apparent anomaly within Series 18.

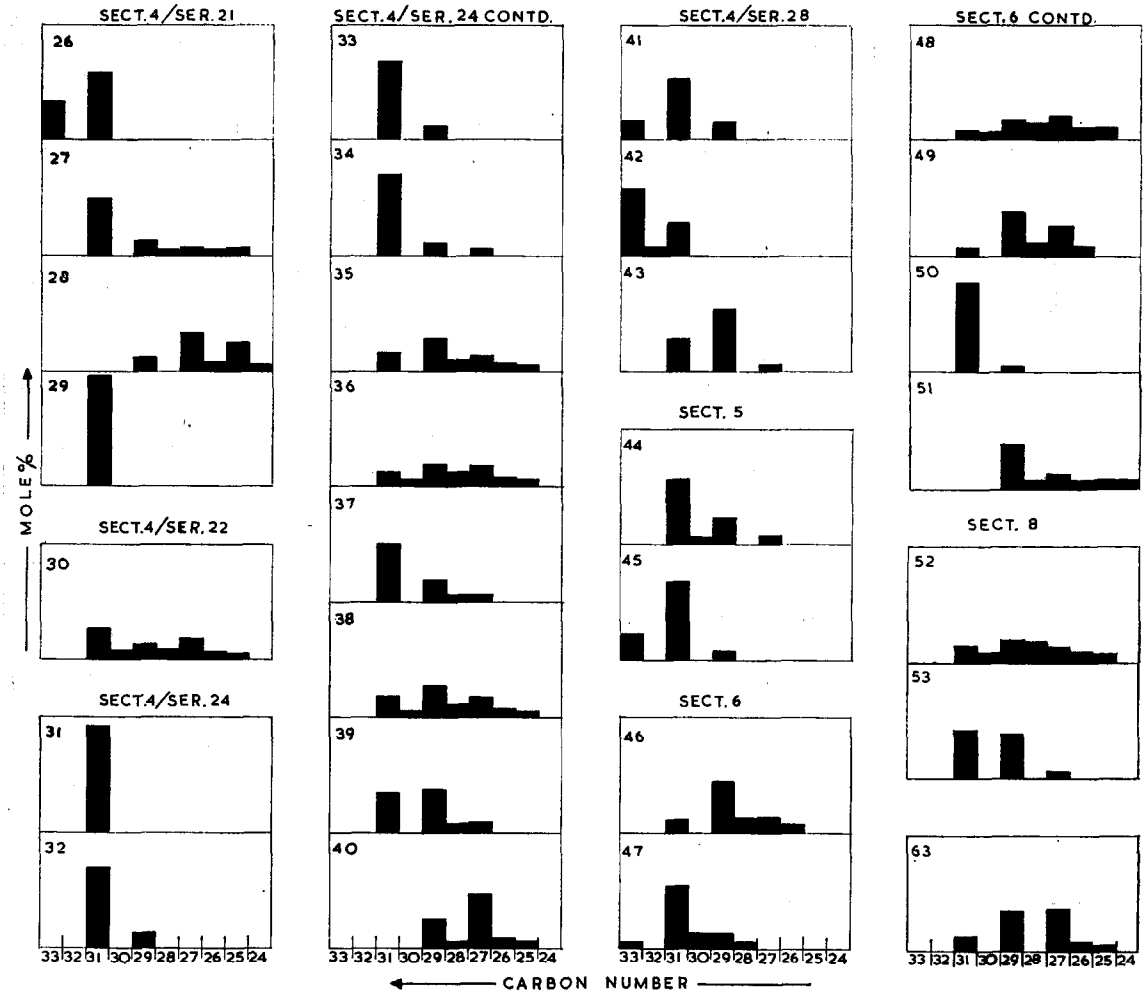

FIG. 2. CARBON-NUMBER DISTRIBUTION HISTOGRAMS (MOLES %) FOR ALOE SPECIES' LEAF WAX ALKANES.

In Series 19: Aethiopicae four species were accessible, but flowers could only be obtained from two. There is some variation in the leaf alkane pattern through the series, with either C_{29} or C_{31} dominant, but for the two perianth waxes the alkane patterns are consistent with very high C_{31} contents.

For Series 21: Macrifoliae four of the seven species in the series were available, all being of South African origin. Great variation of leaf alkane patterns within the series is evident and is clearly shown by examination of histograms Nos. 22–25. The perianth alkane patterns

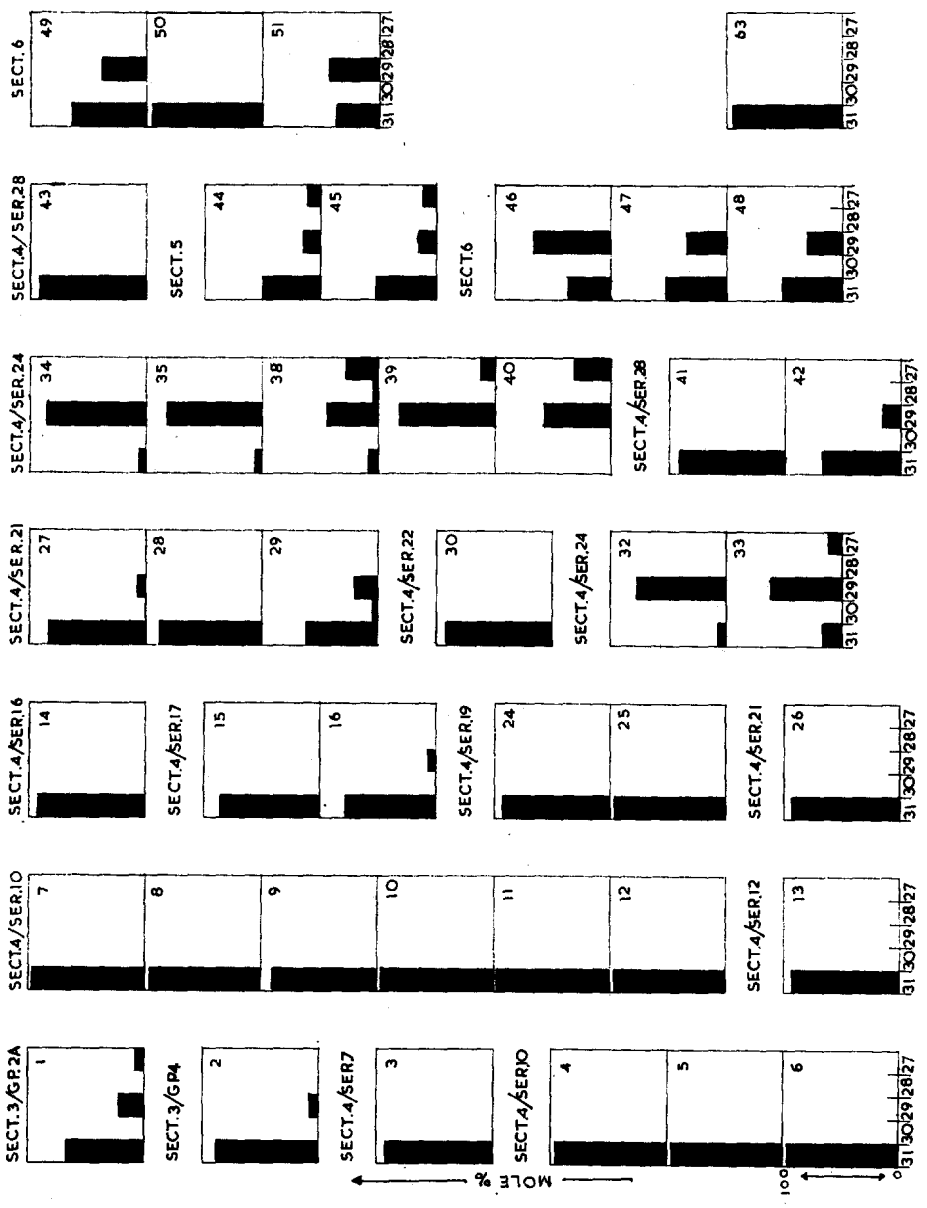

FIG. 3. CARBON-NUMBER DISTRIBUTION HISTOGRAMS (MOLES %) FOR ALOE SPECIES' PERIANTH WAX ALKANES.

are also less consistent than has been the case in other series so far described, No. 29. (*A. striatula* Haw.) having in particular a relatively higher C_{29} content (*ca.* 20 per cent) than other members of the series.

In Section 5: Anguialoe, of Reynolds' classification (Table 1) there are only five species listed, all of South African origin. Of these one, *A. recurvifolia* Groenewald, was available in Nairobi in a private collection, while a second *A. sessiliflora* Pole Evans, was supplied for examination by Reynolds from his collection at Mbabane, Swaziland. In both these species a densely multiflowered raceme is produced in which, in contrast to the majority of other aloes, the individual flowers mature and fade within 36 hr, flowering taking place progressively up the raceme from base to tip. In most other species, with more open or multibranched racemes, individual flowers persist for many days. Both the perianth wax hydrocarbon fractions from these two species contained measurable quantities of normal alkenes in addition to the normal alkanes, and were otherwise similar: no alkenes were detected in the leaf wax of either species.

Section 6: Pachydendron, of Reynolds' classification contains thirteen South African species which are admittedly "a heterogeneous group" for which it is intended that with further work "a revised grouping of relationships could be evolved" (Ref. [18a] p. 442). Inspection of the data obtained for Section 6 (Nos. 46–51) shows that there is broad agreement between the chemotaxonomic evidence, based on perianth alkane patterns, and the botanical grouping with the prominent exception of No. 50, (*A. secundiflora*) which, in contrast to the other members of the section, has an almost exclusive content of C_{31} in both leaf and perianth wax alkanes. Within Section 6, Reynolds places *A. secundiflora*, which is normally acaulescent, as closest to *A. marlothii* Berger, (No. 49), which has a tree-like form of growth, on the basis of the similar disposition of the inflorescence. It would seem that for *A. secundiflora* a strong case on chemical evidence can be made for its removal from Section 6 when consideration is given to the revision of the grouping. On the basis of the more diagnostic perianth alkane pattern, *A. secundiflora* would appear to be more closely related to the lower series of Section 4.

Of those *Aloe* species (Nos. 54–63) whose position in the detailed classification of Reynolds is not yet determined, all except Nos. 61 and 62 have high C_{31} perianth alkane patterns. No. 62, which has not hitherto been described in the botanical literature, would appear from its gross morphology to be akin to Section 4, Series 24 of the Reynolds' classification and in accord with this tentative placing shows the characteristic dominant C_{29} perianth alkane pattern of the other members of this Series.

In his second monograph[18b] Reynolds has included in Section 6: Pachydendron two species, *A. ballyi* (No. 63) and *A. volkensii* (No. 40) which latter he had previously considered (personal communication) to belong to Section 4, Series 24: Fruticosae. However, if perianth alkane pattern is to be considered as a valid taxonomic guide, these two species could hardly be further removed from one another: *A. volkensii* has a predominant C_{29}–C_{27} pattern which is in agreement with its former placing in the Fruticosae and is not in concert with the members of Section 6 (excluding *A. secundiflora*) while *A. ballyi* has an exclusively C_{31} perianth alkane content and, equally with *A. secundiflora*, is anomalous in Section 6, where a C_{29}–C_{31} pattern is dominant. We have therefore in Table 3 left *A. volkensii* with other members of the Fruticosae and have placed *A. ballyi* in the group of unclassified species.

To summarize it may be said that for the genus *Aloe* there is broad agreement of the chemical evidence with the botanical classification although the alkane patterns of leaf and perianth cuticular wax of a single species may not be sufficiently discriminating either to

TABLE 4. HYDROCARBON FRACTIONS OBTAINED FROM STYLE AND, OR, FILAMENT WAXES OF ALOE SPECIES. CONCENTRATION (MOLE %)

Plant No.	Aloe 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}	C_{20}
(7)	*A. graminicola* Reynolds	SF*	alkanes			21·5		11·7	2·3	13·8	3·1	7·6	2·8	3·4	2·2	1·1	
			alkenes			20·8		9·7									
(13)	*A. suprafoliata* Pole Evans	SF	alkanes			18·5	1·2	12·4	1·5	19·3	1·5	15·8	0·8	2·7			
			alkenes			17·0	0·4	8·5		0·4							
(29)	*A. striatula* Haw.	SF	alkanes			4·5	0·7	17·9	1·4	16·3	1·6	4·1	0·1	0·2			
			alkenes			41·0	0·7	11·3		0·2							
(40)	*A. volkensii* Engler	SF	alkanes			0·7	0·6	12·9	1·5	24·0	2·7	26·9	1·0	4·4		0·2	
			alkenes			22·2	0·3	2·1		0·6							
(44)	*A. recurvifolia* Groenewald	SF	alkanes			0·4		4·2	0·5	9·7	1·0	13·8	0·6	3·5	0·2	0·4	
			alkenes			32·3	0·6	11·1	0·1	21·1		0·5					
(47)	*A. ferox* Mill.	S*	alkanes			5·5	0·6	8·2	1·2	16·4	1·1	3·6	1·4	0·2		0·1	
			alkenes			30·8	0·8	29·4		0·7							
(47)	*A. ferox* Mill.	F*	alkanes			19·0	2·3	21·4	2·2	8·6	0·8	0·9	0·2	0·1			
			alkenes			41·8	1·0	1·5		0·2							
(48)	*A. candelabrum* Berger	SF	alkanes			7·7	0·8	13·7	1·2	12·9	1·6	3·2	0·3	0·7	0·2	0·3	
			alkenes			39·8	0·7	16·5		0·4							
(49)	*A. marlothii* Berger	S	alkanes			6·7	1·9	20·2	2·3	16·2	1·6	2·2					
			alkenes			32·6		13·6		0·2							
(49)	*A. marlothii* Berger	F	alkanes			7·8	1·7	9·7	1·9	18·4	1·7	6·9	0·3	0·3			
			alkenes	8·0		28·1		15·3									
(50)	*A. secundiflora* Engler	SF	alkanes			21·9	0·8	12·2	1·1	24·8	1·6	16·8	0·6	1·1	0·1	0·3	
			alkenes			15·7	0·6	2·1		0·3							
(51)	*A. aculeata* Pole Evans	SF	alkanes			3·9	0·8	10·0	1·5	13·0	2·7	7·0	3·0	4·4	3·3	1·2	0·4
			alkenes			30·0		18·8									
(64)	*A. capitata* Bak.	SF	alkanes			9·2	1·7	13·2	1·4	21·4	1·7	8·5	0·7	1·3			
			alkenes			39·3	0·3	1·0		0·3							
(64)	*A. capitata* Bak. (Wax from a second plant)	SF	alkanes		0·1	7·3	0·8	11·9	1·6	18·9	2·0	9·2	0·7	1·6	0·3		
			alkenes	6·2	0·2	36·8		2·0		0·1							

SF* = wax obtained from style and filament.
S* = wax obtained from style only.
F* = was obtained from filament only.

distinguish it from other related species or to place it unambiguously within the framework of the present botanical classification. However, in a limited number of cases, strong chemical evidence can be adduced to indicate the desirability of further examination of the detailed classification.

Since differences in the hydrocarbon patterns of leaf and perianth cuticular wax within an individual plant had been observed, it seemed likely that a similar variation in composition might be found for cuticular wax secreted by other organs of the same plant. Such a difference would be a reflection of subtle changes in the biochemical mechanism elaborating the hydrocarbon and might be dependent on the particular function of the organ. Variation from one part of a plant to the other offers an extension of the use of chemical characters as taxonomic criteria.

Analyses of the hydrocarbon fractions from style and/or filament cuticular waxes of a number of *Aloe* species from different Sections and Series of the genus are listed in Table 4. For most species examined the combined wax, removed from both style and filament, was analysed but in the cases of *A. ferox* Mill. and *A. marlothii* separate analyses of style and filament cuticular waxes were undertaken. Without exception, alkenes were present in all the style and filament waxes of the species examined, although considerable variation in the ratio of alkene to alkane is apparent. It is perhaps significant that *A. recurvifolia* (No. 44), which was one of the two aloes found to contain alkenes in the perianth wax, contains the highest concentration of alkenes (65·7 per cent) in the hydrocarbon fraction of the style and filament wax.

Further evidence of the apparent anomalous position of *A. secundiflora* in the classification of Reynolds is given by a consideration of the hydrocarbon fraction of the style and filament wax, which contains the lowest proportion of alkenes (18·7 per cent) of any of those examined, whereas all other examples from Section 6 have an alkene content of 50 per cent or more of the total hydrocarbon fraction.

It is of interest that the major alkenes of the style and filament waxes are of chain lengths C_{31}, C_{29} and C_{27} in decreasing order of importance as is frequently the case for the alkanes, and the same alternation of concentration with odd and even carbon number is evident. This observation leads to the supposition that the biochemical pathways of formation must be closely related and to the possibility that the alkenes may be precursors of the alkanes. The presence of alkenes in the two perianth waxes (Nos. 44, 45), reported above, which are derived from short lived flowers would also tend to support this possibility.

EXPERIMENTAL

Isolation of the Cuticular Waxes

The relative efficiencies of several solvents in extracting cuticular waxes by immersing the plant material at room temperature was determined by Martin,[20] and chloroform was found to be the most effective. This solvent was used by Eglinton and co-workers[3,4] in their study of leaf wax alkanes and a similar procedure is followed here. Except for a few cases in which the plant material was obtained from outside Kenya, plant specimens were extracted within 12 hr of removal from the living plant.

The specimen was dipped in chloroform at room temperature for approximately 15 secs. with gentle agitation. The resultant extract was filtered to remove earthy and other insoluble materials and distilled to afford a crude wax residue.

[20] J. T. MARTIN, *J. Sci. Food Agri.* **11**, 635 (1960).

Isolation of the Wax Hydrocarbon Fraction

The crude wax (50–100 mg) was treated with 10 ml of boiling light petroleum (b.p. 80–100°) and the solvent concentrated to a volume of about 3 ml. After cooling to room temperature, the solution was decanted from any insoluble matter (which was washed with a further 1–2 ml of light petroleum) onto a dry column of alumina (25 × 1 cm, dry packed with Peter Spence, Type H, alumina, 100/200 mesh, previously activated for 4 hr at 280° and stored at 65° until required). The column was developed with light petroleum and the first 4 ml of eluate collected. Distillation of the solvent gave a hydrocarbon residue which was checked by i.r. spectroscopy (KBr disc) for the absence of absorption bands due to oxygenated functional groups in the wave number range 600–3500 cm^{-1}.

Identification of the Hydrocarbons Present

Each wax hydrocarbon fraction was analysed by isothermal gas–liquid chromatography (Pye Argon Chromatograph) on a 100-cm column of celite (80–100 mesh) coated with 1·25 per cent Apiezon L grease. The column temperature was 235°, argon gas flow rate 54 ml/min, detector voltage 1500 V. A portion of the sample was introduced as a dry solid absorbed onto a few milligrams of celite by evaporation from a volatile solvent. Normal alkane peaks were identified from their log retention times, a paraffin wax sample with added dotriacontane being used as a standard for determining and checking retention times at intervals during the course of the work. Under these working conditions hentriacontane had a retention time of 84 min.

Branched chain alkanes were tentatively identified from their log retention data by comparison with peaks in a sample of tobacco leaf wax alkanes. Alkenes were identified by their shorter retention time as compared with the normal alkane of the same carbon number (Hallgren and Larsson[16]), by the reversal of this retention time relationship on a 10 per cent polyethyleneglycol adipate column and by the disappearance of the relevant peaks in the chromatogram after catalytic hydrogenation using palladized charcoal, with concurrent increases in the area of corresponding peaks.

Quantitative Determination of the Hydrocarbons

Relative areas of peaks on the chromatogram records were determined by multiplying peak heights by width at half height, unless broad or asymmetrical peaks were obtained. In the latter cases relative areas were determined by cutting out the peaks and weighing the paper. All results are expressed as mole percentages.

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* We learnt with regret of the death of Dr. Reynolds when this paper was in an advanced stage of preparation.