Phytochemistry, Vol. 28, No. 11, pp. 3027–3030, 1989.
Printed in Great Britain.

0031 9422/89 \$3.00+0.00

ALKANES OF FOLIAR EPICUTICULAR WAXES OF THE GENUS *ENCEPHALARTOS*

R. OSBORNE*, M. L. F. SALATINO† and A. SALATINO†

Department of Chemistry, University of Natal, King George V Avenue Durban 4001, South Africa; †Instituto de Biociências, Universidade de São Paulo, Caixa Postal 11461, 05499, São Paulo, Brazil

(*Received* 28 *February* 1989)

Key Word Index—*Encephalartos*; Zamiaceae; Cycadales; cycads; epicuticular waxes; alkanes; chemosystematics.

Abstract—The *n*-alkane distribution patterns in the epicuticular leaf wax of 42 taxa of *Encephalartos*, a major genus of Cycadales, are reported from GC analyses. Three chemical groups are identified. Group A has well-defined maxima for the odd carbon atom numbered alkanes n-C_{29}, n-C_{31} and n-C_{33}, a distribution commonly reported from higher plants. Group B shows a skewed normal *n*-alkane profile centred around n-C_{20}, while Group C has a bimodal n-C_{20}/n-C_{25-31} pattern. The latter two groupings are atypical for higher plants and are tentatively explained in terms of an α-oxidation process in the usual elongation-decarboxylation biosynthetic route. The chemical groups are not obviously correlated with the existing taxonomy of the genus. Mature leaves from seedling plants show similar *n*-alkane profiles to those of older plants of the same species.

INTRODUCTION

The large number of reports on the chemical composition of plant epicuticular leaf waxes, particularly with respect to the distribution of *n*-alkanes, testifies to the usefulness of such data to the chemotaxonomist. The common occurrence in Angiosperms of *n*-alkane distribution with predominantly high levels of nonacosane (n-C_{29}) and hentriacontane (n-C_{31}) documented first by Chibnall [1], remains consistent with the elongation-decarboxylation biosynthetic route proposed by Eglinton and Hamilton [2] and confirmed by the use of radiolabelled precursors by Kolattukudy [3]. However, this pattern is not ubiquitous in higher plants and is much less common in those at a lower evolutionary level [4]. For this reason we have commenced an investigation into the *n*-alkane distribution in the Cycadales. The extant cycads comprise the diverse, modified remnants of a much larger Gymnosperm group which is thought to have originated from the Palaeozoic pteridosperms and which reached their zenith in the Mesozoic Era, and is considered to occupy a key position in botanical hierarchy [5]. No such survey has hitherto been performed although it is clear that the order shows many unusual and some unique features [6].

We report here on the *n*-alkane distribution in epicuticular leaf waxes of *Encephalartos* Lehm. (Zamiaceae), one of the major genera of cycads. This genus is fairly well-defined taxonomically and is endemic in Africa with 52 species currently recognized [Osborne, unpublished data].

RESULTS AND DISCUSSION

The *n*-alkane distribution data from the epicuticular leaf waxes of 42 *Encephalartos* taxa, representing the mean results from 111 analysis of habitat and garden-collected material, are shown in Table 1. The alkanes all occur in the range from n-C_{17} to n-C_{35}. Figures for any branched-chain isomers have not been included as these occur sporadically in the C_{18} to C_{28} range generally at much lower concentrations than those of the corresponding straight chain hydrocarbons.

We consider the *n*-alkane profiles to comprise three main groups. Group A, typified by *E. ferox* and *E. villosus*, has well-defined maxima for the odd carbon atom numbered alkanes n-C_{29}, n-C_{31} and n-C_{33}, collectively accounting for between 40 and 60% of the total alkanes recovered. This pattern is similar to that observed in most higher plants. The group includes all samples tested from *E. delucae*, *E. ferox*, *E. gratus*, *E. hildebrandtii* var. *dentatus*, *E. villosus* and one of two samples each from *E. heenanii*, *E. tegulaneus* and *E. umbeluziensis*. The group also includes *E. hildebrandtii* on the basis of the only other published data on *Encephalartos* leaf waxes [4]. The only other species examined which shows a pattern of alternation with respect to chain length is *E. cupidus* which has maxima at n-C_{23} and n-C_{25}. There is no particular botanical coherence to these taxa on the basis of vegetative and reproductive morphology nor are they related phytogeographically.

Group B, typified by *E. ghellinckii* and *E. ngoyanus*, shows a skewed-normal *n*-alkane distribution centred unimodally at *ca* n-C_{20} with no significant alternation of alkane levels for even or odd carbon numbers. This pattern is unusual in higher plants and the validity of the few cases where it has been documented has been questioned [4]. However, *n*-alkane distribution profiles with-

---

*Author to whom reprint requests should be addressed.

icephalartos taxon	Number in sample	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
altensteinii Lehm.	4	0.1	2.5	6.2	9.5	3.5	3	4	5.5	5.5	6	5.8	9.8	7.2	6	2.3	0.8	0.8	—	—
arenarius R. A. Dyer	3	0.4	1	1.4	11	5	2.7	6.3	3.7	4.7	1.7	1.7	6.7	2.7	9	6.3	11.7	4.3	1.3	1.7
caffer (Thunb.) Lehm.	3	0.1	4	4	7.3	3.7	5.3	6	4.3	7.3	2.3	3	9.7	4.7	3	8.3	2.3	3	2.7	0.4
concinnus Dyer & Verdoorn	2	1	3.5	6	11	5	3	2.5	3	4	4.5	3.5	5.5	4	12.5	6.5	4	2	—	—
cupidus R. A. Dyer	2	—	—	0.1	1	2.5	4	14.5	6.5	32.5	5	4.5	5	4	4	4.5	1	2	—	—
cycadifolius (Jacq.) Lehm.	1	—	3	14	5	5	4	4	3	6	4	5	5	8	4	5	1	1	—	—
*delucae**	1	0.1	1	2	3	2	2	3	3	4	6	4	4	8	6	22	9	16	1	1
dolomiticus Lavranos & Goode	2	—	2	4	12.5	3.5	2.5	6	3	5	4	5.5	6	5.5	8	7	3	1.5	0.5	0.5
dyerianus Lavranos & Goode	1	—	2	8	11	15	16	18	12	7	0.1	0.1	0.1	0.1	3	3	0.1	0.1	—	—
eugene-maraisii Verdoorn	1	—	1	4	5	7	4	20	4	10	1	1	12	2	2	3	1	3	2	5
ferox Bertol. f.	4	0.5	1.5	1.3	3.5	1.5	1.8	3	2.2	1.8	2.2	3	2.8	8.2	8	26.5	11	18.8	0.3	0.5
friderici-guilielmi Lehm.	4	0.3	1.8	6.8	9.8	5	2.8	3.5	2.8	6.2	2.8	3	8.5	5	6.2	7.2	1.5	2.8	0.5	0.8
ghellinckii Lem.	4	0.8	4.8	13.2	16.8	6.5	2.5	1.8	2.2	2.2	2.5	2	3	2.5	1.5	1.8	0.1	0.1	—	—
gratus Prain	2	—	2	3.5	11.5	3	1	1.5	1.5	1.5	0.6	1	0.6	3	4	19	12.5	21.5	0.6	0.6
hildebrandtii var. *hildebranditii* A.Br. & Bouché	1	—	4	16	29	7	2	3	3	3	3	4	2	2	1	1	0.1	0.1	—	—
hildebrandtii var. *dentatus* Melville	1	1	0.1	0.1	0.1	0.1	1	1	1	2	1	1	1	36	5	43	3	4	0.1	0.1
heenanii R. A. Dyer	2	0.5	4.5	2.5	2.5	2	4	4	8	6	3	1.5	2	3.6	1	17.6	2	12.5	—	—
horridus (Jacq.) Lehm.	3	0.7	2.1	6.3	9	5.7	5.3	5	8	7.3	4	2.3	4	5.3	7	6	5	1.7	1.7	0.1
humilis Verdoorn	2	0.1	8	9.5	13	5.5	3.5	3.5	3.5	7	4	2.5	2.5	3	2.5	3.5	—	—	—	—
inopinus R. A. Dyer	3	1.4	2.4	5	8.3	11.3	3.7	2.4	4.3	2.7	4.3	4.3	4.7	7.7	7	7	3.7	2.4	0.1	—
kisambo Faden & Beentje	1	—	1	2	4	3	5	7	8	7	5	3	2	3	3	2	2	1	0.1	0.1
laevifolius Stapf & Burtt Davy	3	2	1.7	4.4	10.7	5.3	4.3	3.3	4.3	5	4	4.3	6.7	8.3	5.7	8	0.4	0.4	—	—
lanatus Stapf & Burtt Davy	2	—	14.5	17	15	4	2.5	4	2	4.5	1	1.5	5	2	1	2	0.6	1	0.5	1
latifrons Lehm.	3	3.3	7.4	9	7.7	2.7	4.7	9.7	4.7	6	4	2.3	6.7	3.3	3.3	3.4	0.7	1.7	0.3	—
lebomboensis Verdoorn	2	4	7	7	13.5	8.5	5.5	3.5	4.5	3.5	5	4	5	5	6	5.5	3.5	2.5	—	—
lebomboensis var. Piet Retiefii	3	—	—	1.7	10	6.7	5	3.7	4.7	3.3	4.3	4	7	6	8.7	8.7	7.7	8.7	1.7	2
lehmannii Lehm.	2	1	3	10.5	21	8.5	3	3.5	4	5	4.5	3	1.5	1	1.5	1	1	0.6	—	—
longifolius (Jacq.) Lehm.	2	1	4	13	14.5	6	2.5	10.5	3.5	4.5	3	4	4.5	0.6	0.6	0.6	0.1	0.6	—	
manikensis (Gilliland) Gilliland	1	—	1	4	6	2	1	1	1	2	3	4	10	10	21	18	7	6		—
middelburgensis Vorster *et al.*	3	0.1	3	7	11.7	5	4	6.3	5.7	12	5	2	4.3	3.7	4	4.7	2.7	3	1	0.4
munchii Dyer & Verdoorn	2		3.5	4.5	13.5	4	2	2.5	4	5	5	5.5	5.5	7	8	10	3	2.5	0.1	—
natalensis Dyer & Verdoorn	11	0.1	0.6	2.4	5.5	3.6	2.6	7.2	4.7	6.5	5.4	5.5	11	8.8	9.7	7.5	4.1	2.2	0.5	0.7
ngoyanus Verdoorn	4	—	5.3	6.2	16.8	12.8	6	4.2	3.8	5.3	3.2	3.5	3.8	3.3	2.5	2.3	1	1	—	—
paucidentatus Stapf & Burtt Davy	3	0.4	0.7	3.7	10	5	2.7	4	4.7	7.3	5	6	6.3	6	7.7	8.7	3	1.7	0.1	—
princeps R. A. Dyer	3	0.4	1.7	7	11.3	5	4.3	5.7	6.7	9	4.7	5	3.7	4.3	5.7	5	1.7	1.3	0.1	—
pterogonus Dyer & Verdoorn	2	1.6	2.5	4	13	4	4.5	2.5	4.5	3.5	3.5	3.5	4.5	6	7	6.5	3	2	—	—
tegulaneus Melville	2	2.6	2.5	10.5	17	5	3.5	5	6	3	3	2	1	3.5	2	15	2.5	2.6	—	—
transvenosus Stapf & Burtt Davy	2	—	1	4	10.5	6.5	5	14	6	7.5	3	4	6.5	6	6	7.5	1.5	2.5	—	—
trispinosus (Hook.) R. A. Dyer	5	0.4	3.6	6.4	13.6	5.8	5	4.6	4.6	5.2	3.6	2.4	3.2	3	5.2	5.8	2.8	3	0.7	—
umbeluziensis R. A. Dyer	2	5	4	9.6	4.5	3	3	3.5	3	3	2	2.5	1.5	19	2.6	16	1	1	—	—
villosus Lem.	4	—	0.3	0.8	3.8	1.8	1	1	1	1	1	2	1.8	8.3	5.3	31.8	9	27.5	0.8	0.5
woodii Sander	3	1	2.3	7.3	10.3	4.7	2.3	4.3	3.7	4.7	4.7	5.7	11.3	13.7	3.3	4.3	0.7	1	0.4	—

out alternation with carbon numbers have been reported from non-epicuticular waxes of some trees [4] and from certain marine and freshwater algae [7–9]. The suggestion that this pattern may be diagnostic of plants of a lower evolutionary level [9] however, has been challenged [4].

Group B includes all samples tested from *E. ghellinckii*, *E. hildebrandtii* var. *hildebrantii*, *E. lebomboensis*, *E. lehmannii*, *E. ngoyanus*; one of two samples from *E. concinnus*, *E. humilis*, *E. lanatus*, *E. pterogonus*, *E. tegulaneus*, *E. umbeluziensis*; two of three samples from *E. latifrons*; one of three samples from *E. laevifolius*, *E. middelburgensis* and one of four samples from *E. trispinosus*. Possibly added to this group are single samples from *E. heenanii*, *E. princeps* and *E. kisambo* which have similar unimodal profiles but which are centred at n-C_{24} or n-C_{25}. As with group A, there is little or no evidence of common ground amongst the taxa in our group B on the basis of morphological characters or phytogeography.

Group C, typified by *E. altensteinii* and *E. woodii*, is numerically the largest in our series. It is characterized by a bimodal n-alkane distribution with one maximum at or near n-C_{20} and the other varying between n-C_{25} and n-C_{31}, again with no bias towards oddness or evenness in carbon atom number, and also with no evidence of botanical coherence. All taxa not referred to above fall into this group. The pattern is uncommon in higher plants.

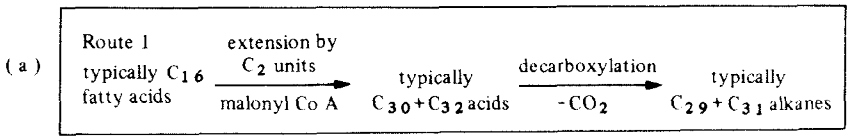
In general, the degree of species specificity of the alkane profiles is variable. A relatively consistent pattern was obtained from six of the eight species where four or more samples were analysed; these were *E. ferox* and *E. villosus* (Group A), *E. ghellinckii* and *E. ngoyanus* (Group B) and *E. altensteinii* and *E. friderici-guilielmi* (Group C). By contrast, considerable sample-to-sample variation was seen in the profiles from *E. natalensis* and *E. trispinosus*. It is significant that a high degree of phenotypic plasticity has been observed in the latter two species [10, 11] but it is not known if any genetic polymorphism is present. Enzymological and immunological surveys may prove rewarding avenues for future research in this connection. From our results none of the infraspecific variation could be ascribed to either the sex or the locality of the plants sampled.

In an analysis of the leaf waxes of mature leaves from 2-year-old seedling plants from eight, *Encephalartos* species we found a general correlation in n-alkane distribution with that from individual older plants of the same species. (Table 2). This is particularly evident in *E. ferox* and *E. villosus* where maxima at n-C_{31} and n-C_{33} were clearly apparent. These species thus again fall into our Group A. Analyses from seedling leaves of *E. caffer*, *E. lanatus*, *E. longifolius*, *E. natalensis*, *E. ngoyanus* and *E. transvenosus* give the bimodal n-alkane profiles of our Group C. From these results there was no evidence of a trend towards increasing alkane chain length with plant age. The latter trend is well-documented for higher plants with respect to age of individual leaves [4], an aspect which we have not investigated.

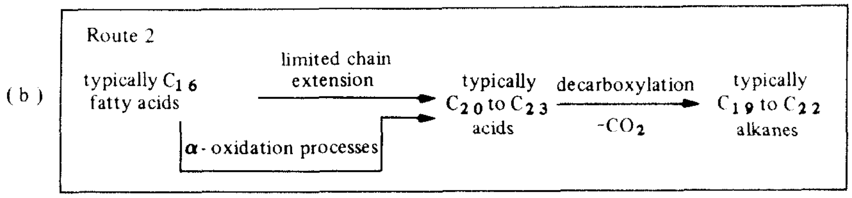
In attempting to relate our findings to alkane biogenesis it is necessary to postulate at least two biosynthetic routes (Fig. 1). The elongation of fatty acids such as palmitic (n-C_{16}) and stearic (n-C_{18}) by successive C_2 unit additions followed by a final decarboxylation step (Fig. 1a) is the process by which high proportions of odd carbon atom numbered n-alkanes are formed in higher

Table 2. The percentage abundances of individual n-alkanes obtained from single samples of epicuticular waxes from mature leaves of two-year-old *Encephalartos* seedlings

Encephalartos species	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
E. caffer	—	0.1	1	1	3	2	24	5	12	2	2	28	4	3	4	1	4	—	—
E. ferox	—	2	6	6	4	2	10	2	5	1	2	5	11	5	25	2	8	—	—
E. lanatus	—	—	1	3	2	—	11	7	9	7	7	11	4	2	3	0.1	5	1	2
E. longifolius	1	6	13	12	7	4	9	1	3	1	1	3	2	2	2	1	2	—	—
E. natalensis	—	—	3	4	3	2	16	2	22	2	4	7	10	7	9	2	3	—	—
E. ngoyanus	—	—	1	1	3	3	21	5	15	4	4	16	2	1	3	1	5	2	3
E. transvenosus	—	—	3	3	2	1	9	2	8	2	2	6	3	2	3	18	4	3	4
E. villosus	—	—	0.1	1	0.1	0.1	1	0.1	1	1	1	2	5	3	33	5	43	1	2



This route leads to predominance of higher alkane homologues with odd numbers of carbon atoms, i.e. the 'Group A' class.



This route leads to a predominance of lower alkane homologues with both odd and even numbers of carbon atoms, i.e. the 'Group B' class

Combinations of routes 1 and 2 lead to bimodal and 'normal' distributions

Fig. 1. Possible biosynthetic routes for *n*-alkanes found in epicuticular leaf waxes from *Encephalartos* species.

plants [2, 3] and accounts satisfactorily for the *n*-alkane profiles in our Group A. The occurrence of significant quantities of even carbon atom numbered alkanes has invoked the possibility of an α-oxidation process at some stage in the biosynthetic sequence (Fig. 1b) [3]. It is possible that this process is well-developed in the *Encephalartos* specimens which exhibit our Group B and C profiles.

EXPERIMENTAL

Fresh leaf material was collected from 111 habitat and garden grown plants of 42 *Encephalartos* taxa. Samples were taken from mature unblemished leaves of adult plants where no doubt existed as to their taxonomic identity. Voucher specimens of leaf samples are deposited at the herbarium of the University of Durban-Westville. Only the median leaflets were used in the analysis. Samples were also collected from 2-year-old seedling plants of eight *Encephalartos* species grown by one of the authors (RO). Selected leaf portions were oven-dried at 50° for *ca* 72 hr.

Epicuticular waxes were obtained from the leaf material by three successive immersions of 30 sec in $CHCl_3$. The crude residue was fractionated by CC on silica gel with petrol and then analysed by GC following procedures described elsewhere [12].

REFERENCES

1. Chibnall, A. C. and Piper, S. H. (1934) *Biochem. J.* **28**, 2209.
2. Eglinton, G. and Hamilton, R. J. (1963) in *Chemical Plant Taxonomy* (Swain T., ed.). Academic Press, London.
3. Kolattukudy, P. E. (1967) *Phytochemistry* **6**, 1963.
4. Herbin, G. A. and Robins, P. A. (1969) *Phytochemistry* **8**, 1985.
5. Pant, D. D. (1987) *Geophytology* **17**, 125.
6. Osborne, R., Grobbelaar, N. and Vorster, P. (1988) *S. Afr. J. Sci.* **84**, 891.
7. Clarke, R. C. and Blumer, M. (1967) *Limnol. Oceanog.* **12**, 79.
8. Stránský, K. Streibl, M. and Herout, V. (1967) *Coll. Czech. Chem. Commun.* **32**, 3213.
9. Stránský, K., Streibl, M. and Šorm, F. (1969) *Coll. Czech. Chem. Commun.* **33**, 416.
10. Dyer, R. A. (1965) *Bothalia* **8**, 405.
11. Giddy, C. (1984) *Cycads of South Africa*. 2nd Revised Edn. C. Struik, Cape Town.
12. Salatino, M. L. F., Salatino, A., Menezes, N. L. and Mello-Silva, R. (1989) *Phytochemistry* **28**, 1105.