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HYDROCARBONS IN LEAF WAXES OF *SACCHARUM* AND RELATED GENERA

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Key Word Index—*Saccharum*; Saccharinae; Gramineae; sugar cane; chemotaxonomy; C_{27}–C_{35} *n*-alkanes.

Abstract—The composition of the *n*-alkanes in the leaf waxes of over 80 clones of *Saccharum officinarum*, *S. edule*, *S. robustum*, *S. spontaneum* and from a number of related species have been compared by GLC. The waxes contain predominantly odd alkanes, C_{27}–C_{35}, the major components being C_{29} and C_{31}. In a number of clones, particularly of *S. edule*, a homologous series of alkenes was also present. No chemotaxonomic relationship could be derived from the compositions as the intraspecific variation was greater than the interspecific variations.

INTRODUCTION

n-Alkanes are frequently found as constituents of leaf surface waxes and there has been considerable interest in their role [1, 2] and biosynthesis [3]. Alkane composition has been used in chemotaxonomy but often wide variations occur within a species and its use is limited [4]. In some plants the wax composition varies with changes in soil and climate [5]. Previous studies on grasses have used alkanes to compare *Cortederia* grasses from New Zealand and South America [6] and have examined the wax from wheat [7].

The present work reports the composition of *n*-alkanes in a number of clones of the genus *Saccharum* and species in related genera. Previous work was carried on *S. officinarum* L. clone Pindar by Lamberton *et al.*, who identified *n*-alkanes and long chain alcohols and aldehydes [8, 9]. In other reports, Cuban wax from unspecified clones of *S. officinarum* has been reported to yield iso- and anteiso-alkanes [10] and Δ^{10}-alkenes [11].

RESULTS AND DISCUSSION

The leaf waxes were obtained by extraction and chromatographed to give a hydrocarbon fraction [12] which was analysed by GLC (Table 1). The results were tested for consistency by repeating the analysis of Badila at intervals throughout the year and from different locations and although the total amount of hydrocarbon varied considerably there was negligible variation in its composition. In some clones a second homologous series of compounds was also present with a shorter R_t. These peaks in the sample from Fiji 76 (*S. edule*) were lost on hydrogenation or on bromination and are thus assigned to alkenes. The alkene series usually had a shorter chain length than the alkane series and again odd numbered chains predominated. Eight sub-clones of Pindar gave identical results with the major *n*-alkane component being C_{33} compared to the earlier report of C_{27} [9].

One clone SES 341 (*S. spontaneum*) was very unusual as it gave only a complex mixture of $C_{<27}$ components, including alkenes (PMR δ, 5.28, *t*, $J = 5$ Hz).

Comparison of the *Saccharum* species (Table 2) shows that the cultivated species *S. officinarum* and *S. edule* and the wild ancester *S. robustum* have similar average compositions with the major component at C_{33}. *S. spontaneum*, considered to be the original *Saccharum* species, gave on average shorter *n*-alkanes (major component C_{31}). Unlike the triterpene methyl ether composition [12] there was no significant difference between clones with chromosome numbers $2n < 80$ and $2n \geqslant 80$. The intraspecies variations in Table 1 are much greater than the interspecific variations and thus the *n*-alkanes have no chemotoxonomic value.

The distribution of the alkenes is interesting as they are particularly characteristic of *S. edule* $2n = 70$, where C_{27}, C_{29} and C_{31} components are present in similar proportions to the *n*-alkanes. Occasional *S. officinarum* clones contain minor quantitites of alkenes whereas alkenes occur only rarely in *S. robustum* and *S. spontaneum* [10].

It has been suggested that evolutionary old genera show a reduced predominance of odd over even alkanes [14]. However, in the present study there is no significant difference between the species.

Table 1 also includes results from some related grasses which are considered to have been involved in the evolution of *Saccharum*, but the number of clones studied are limited and no conclusions can be drawn.

EXPERIMENTAL

The samples and hydrocarbons were obtained as described previously [12].

Analysis of hydrocarbons. Hydrocarbons were analysed by GLC at 250° on a 2 m × 3 mm column packed with 0.5% Apiezon L using N_2 as carrier gas (30 ml/min) and a FID detector. A plot of log R_t against *n*-alkane chain length was linear and peaks were compared with authentic even number alkanes. The positions of odd number alkanes was confirmed using a soln of Parafilm [15]. Alkenes were detected by their absence

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Table 1. Composition of *n*-alkanes C_{27}–C_{35} in the leaf waxes of *Saccharum* and related species

Species, clone	Chromosome No.† 2n	Leaf composition‡ ppm alkane	Relative peak areas on GLC for *n*-alkanes§ C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
S. officinarum											
(Noble clones)											
Badila	80	123(25–450)	2	2	6	6	19	10	29	8	20
Fiji 19	80	26	10**	2*	11*	4	28	2	30	6	7
Fiji 20	80	15	12*	1	13	17	34	—	23	—	—
Fiji 21	80	25	8*	3	11*	5	28	5	34	—	6
Fiji 23	80	17	12*	3	14*	6	25	3	37	—	—
Fiji 24	80	12	6*	4	9*	10	30*	6	20	6	10
Fiji 25	80	13	12*	4	15*	12	29*	4	27	—	—
Fiji 27	80	150	2	2	5	6	25	7	25	4	22
Fiji 28	80	68	2	2	4	9	20	9	28	4	22
Fiji 30	80	81	1*	2	4*	6	23	7	32	4	21
Fiji 40	80	100	2**	2	4*	5	19	8	32	3	24
Korpi	80	20	6	5	10	8	26	7	22	3	11
Mahona	80	21	4	5	7	9	20	11	26	7	13
Oramboo	80	48	3	2	5	5	27	6	31	4	17
(Hybrid clones)											
Homer	80	96	2	2	5	4	15	9	36	8	20
Mali	80	250	4*	3	5	8	27	9	35	—	9
Pindar	80	1–80	7*	4	9*	8	22	10	24	5	10
Ragnar	80	108	7*	4	9	8	22	9	30	3	9
Waya	80	110	2	2	7	11	25	11	27	5	9
S. edule											
SE 97	60	28	22	12	20	9	37	—	—	—	—
Fiji 72	70	115	2**	3	6*	8	19*	15	30	6	11
Fiji 73	70	85	2**	3	6**	10	17**	14	31	4	12
Fiji 74	70	30	3**	2	5**	9	21**	12	33	6	9
Fiji 75	70	57	3**	2	7**	10	21**	12	29	6	10
Fiji 76	70	29	3*	3	7*	12	22*	12	29	7	7
28 NG 201	80	40	3	2	3	5	16	9	34	5	23
SE 15	80	43	5	4	5	5	15	6	25	18	15
SE 34	80	105	13	4	12	6	28	14	16	10	6
SE 78	80	38	10*	6	13*	7	29*	5	18	3	10
S. robustum											
(a) Red Fleshed group *S. sanguineum*											
28 NG 219	60	58	1	2	3	5*	23	5	35	5	20
28 NG 219A	60	12	5	4	13	8	21	8	25	6	9
US-57-159-9	60	115	36	18	12	6	8	5	15	—	—
(b) Teboe Salah group											
Tangangee	60	26	35	12	23	3	15	—	12	—	—
57 NG 170	60	8	8	5	11	6	44	—	25	—	—
US 57 142-4	60	400	6	5	9	9	27	8	26	1	9
MOL 6121	60	110	5*	4	6*	5	21	9	34	2	14
MOL 6125	60	44	5*	4	7*	6	33*	3	36	1	5
Teboe Titioewa Teniggaron	60	169	6*	3	7*	4	27*	2	38	—	14
(c) Wau–Bulolo group											
57 NG 11	60	148	6	4	9	6	23	3	32	1	15
MOL 4503	60	37	10	4	12	7	22	8	27	2	6
(d) Goroka group											
57 NG 208	80	460	6	6	11	8	32	4	26	—	7
MOL 4357	80	63	7	3	7	6	19	9	32	5	13
(e) Port Moresby group											
MOL 4861	80	42	5*	3	6*	6	15*	12	31*	6	15
MOL 4972	80	92	4	1*	5	5	12	6	26	8	31
51 NG 140	80	36	2	3	5	7	17	10	29	8	20
NH 1	80	185	9	5	9	5	25	3	36	1	5
NH 70–10	80	35	6	4	7	4	20	2	42	—	15
(f) Others											
57 NG 134	140	220	22	4	20	4	24	2	20	—	5
51 NG 28	156	100	28	4	20	4	27	1	14	—	4

Table 1.—*Continued.*

Species, clone	Chromosome No.† 2n	Leaf composition‡ ppm alkane	Relative peak areas on GLC for *n*-alkanes§ C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
S. spontaneum											
SES 184A	40	10	6	4	24	5	48	3	10	—	—
SES 184B	40	60	8	2	10	2	46	2	32	—	—
SES 106B	48	10	7	5	27	3	31	1	22	—	3
SES 189	50	39	36	5	40	3	9	—	—	—	—
SES 352	54	7	15*	14	37	5	20	2	7	—	—
SES 317	56	59	18	16	40	23	3	—	—	—	—
SES 351	56	15	11	7	32	5	35	2	6	1	—
SES 197A	60	225	3	2	5	1	15	1	44	1	28
SES 356	60	18	12	5	20	4	26	4	22	1	5
SES 205A	64	80	3	2	8	5	31	5	30	1	15
SES 205B	64	80	4	3	9	6	28	6	31	3	11
DACCA	80	250	26*	9	21*	2	14	2	12	6	5
SES 297B	80	63	4	3	20	3	34	2	31	—	3
SES 341	80	112	—	—	—	—	—	—	—	—	—
MOL 5801	80	80	9*	6	19*	3	44	1	19	—	2
MOL 5903	80	70	7*	3	12*	4	32	4	28	2	8
MOL 5904	80	55	5*	4	13	4	33	2	31	1	8
28 NG 101	80	156	9	4	14	4	33	3	24	3	6
51 NG 2	80	43	4*	2	15	2	40	2	28	1	7
US 56-4-1	96	81	9	2	27	2	25	2	28	—	5
HASUDA	112	108	4	1	19	4	48	3	21	—	1
TOKYO	112	179	1	2	25	1	48	—	21	—	3
PASOEROEN	112	356	10	3	15	3	27	2	33	—	6
PANGANI	?	151	31	9	26	3	22	—	8	—	—
US 46–28	?	102	9	1	12	1	23	2	41	1	10
Related species											
Erianthus bengalense		35	5	7	17	14	31	8	13	3	3
E. maximus											
Raitea		55	6*	6	10*	7	26	5	29	2	9
Fiji 15		17	4	2	8	2	25	2	40	2	16
Fiji 35		42	7*	5	7*	6	19	8	26	11	10
Ripidium arundinaceum											
Mindinao		40	7	8	25	7	28	5	15	2	3
R. elephantinum											
SES 305		10	10	6	17	7	23	6	21	4	7
Miscanthus floridulus											
Fiji 2		210	11	6	17	9	26	6	22	—	3
Fiji		715	11	6	21	7	25	7	17	1	5
Miscanthus sinensis¶		47	12	14	17	4	22	2	14	1	8
Imperata conferta											
Fiji 71		70	2	16	6	4	31	4	35	6	11

† For details of chromosome numbers and origin see preceding paper [12].
‡ For total yield of wax see previous paper [12].
§ Peaks sometimes also present at shorter retention times indicated by * if small or ** if comparable in size to the *n*-alkane.
¶ Also C_{13}–C_{24}, 6.8; C_{25}, 4; C_{26}, 4; C_{37}, 2% [13].

Table 2. Average composition of C_{27}–C_{35} *n*-alkanes in *Saccharum* species

Species	No. of clones examined	Percentage composition of *n*-alkane C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}
S. officinarum	19	5	3	8	8	24	7	29	4	10
S. robustum	21	11	5	10	6	23	5	28	2	10
S. edule	10	7	4	8	8	22	10	24	6	10
S. spontaneum (all)	25	11	4	24	4	31	2	21	1	7
2n < 80	14	11	3	20	3	30	2	23	1	9
2n ⩾ 80	11	11	6	29	6	32	2	18	1	6

from chromatograms following either (a) prior hydrogenation of the mixture over 10% Pd/C in $CHCl_3$/EtOAc or (b) addition of Br_2 in CCl_4 and standing for 30 min.

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