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TRITERPENE METHYL ETHERS IN LEAF WAXES OF *SACCHARUM* AND RELATED GENERA*

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Key Word Index—*Saccharum*; Saccharinae; Gramineae; sugarcane; chemotaxonomy; triterpene methyl ethers; arundoin; cylindrin; crusgallin.

Abstract—The triterpene methyl ethers in the leaf waxes of over 80 clones of *Saccharum officinarum, S. edule, S. robustum, S. spontaneum* and a limited number of related species were compared as possible chemotaxonomic markers by GLC. The principal components were arundoin, crusgallin and cylindrin. The overall interspecific variation was small, but arundoin was particularly characteristic of *S. officinarum*. However, each species showed marked interclonal variation, which was related to chromosome numbers and geographical origin. Most *S. spontaneum* clones from India were atypical containing no triterpene methyl ethers.

INTRODUCTION

The genus *Saccharum* includes sugarcane *S. officinarum* L., which has been cultivated as a source of sucrose since primitive times and is now an important export crop in many tropical countries. The genus is considered to have originated in the India–China–Burma area as *S. spontaneum* L., which spread westwards to Africa and eastwards to Japan, the Malayan Archipelago and New Guinea. In these last two areas a second wild species *S. robustum* Brandes and Jeswiet ex. Grassl is present, which is believed to have evolved into *S. officinarum* under selection by man for high sucrose content [1].

Three other cultivated species are known, *S. sinense* Roxb. and *S. barbari* Jesw. in China and India respectively and *S. edule* Hassk., which is grown for its edible inflorescence in New Guinea and the Pacific Islands [2].

Because of long cultivation and ready hybridization between species, sugarcane taxonomists suspect that some of these cultivated species may be of hybrid origin but proof is difficult purely on morphological grounds. Attempts have been made to relate *Saccharum* species and related sympatric Andropogonae grasses which have been suggested as being involved in the evolution of *Saccharum*, such as *Miscanthus*, *Erianthus*, *Ripidium* and *Imperata* using their leaf flavonoids [3, 4] or β-amylase isoenzymes [5, 6] and a number of characteristic markers have been noted for each species.

In the present work, the compositions of the triterpene methyl ethers in the leaf waxes are reported and compared from a number of clones of 4 *Saccharum* species and related grasses. Triterpene alcohols are common plant constituents and their occurrence in a number of clones of *Saccharum* species has been reported previously [7]. However, the distribution in plants of triterpene methyl ethers is limited and they are found characteristically in the Gramineae [8]. Previous studies of the ethers in *Saccharum* species have usually examined only one clone or have used material of unspecified clonal origin [8–11].

The number of triterpene methyl ethers known is limited but they have proved useful chemotaxonomic tools in the study of *Cortaderia* [12–14] and *Chionochloa* [15, 16] grasses of New Zealand, particularly at the interspecific level.

RESULTS

The leaf waxes from individual clones were fractionated to give a triterpene methyl ether fraction, which was analysed by GLC. Wherever possible the GLC identifications were supported by TLC analysis and by IR and PMR comparison of isolated components with authentic samples.

The distributions of methyl ethers in clones of *S. officinarum*, *S. edule*, *S. robustum*, and *S. spontaneum* are given in Table 1 and are summarized by species in Table 2.

Results from the current study on a number of related grasses, *Ripidium* (*Erianthus*), *Miscanthus* and *Imperata*, together with reports by other workers are shown in Table 3.

The principal triterpene methyl ethers present in *Saccharum* species are arundoin, crusgallin and cylindrin. Lupeol methyl ether and β-amyrin methyl ether were thought to be present in a few cases but were only identified by chromatography. A number of the minor components were unidentified, but could include miliacin, parkeol methyl ether or cycloartenol methyl ether, which have been found in *Chionochloa* [15].

The validity of triterpene methyl ethers as chemotaxonomic markers in *Saccharum* was checked by repeating analyses on selected clones. The relative composition in the complex mixture from Badila (*S. officinarum*) was constant during the growing season and when grown on different soils, although the total yields of wax and methyl ether fractions varied. Eight sub-clones, from single cell

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Table 1 Distribution of triterpene Me ethers in leaf waxes of *Saccharum* species

Species, clones	Origin*	Chromosome No. (2n)	Yield from leaf: Wax ppm	Yield from leaf: Triterpene OMe ppm	Relative composition of triterpene Me ethers from GLC† (relative retention time): Cylindrin (1.78)	Arundoin (1.52)	Lupeol OMe (1.45)	Lupeol OMe (1.25)	Lupeol OMe (1.17)	Crusgallin (1.00)	Source
S. officinarum (Noble canes)											
Badila	PNG	80	1419	355 (250–680)	52	17	17	—	t	18	FSC
Fiji 19	Fiji	80	1100	13	49	15	—	—	—	36	FSC
Fiji 20	Fiji	80	671	136	50	25	—	—	—	25	FSC
Fiji 21	Fiji	80	1277	100	38	32	—	—	—	29	FSC
Fiji 23	Fiji	80	1050	124	45	29	—	—	—	26	FSC
Fiji 24	Fiji	80	725	236	54	t	7	—	—	39	FSC
Fiji 25	Fiji	80	762	16	57	8	4	—	—	33	FSC
Fiji 27	Fiji	80	1440	280	73	—	—	—	—	27	FSC
Fiji 28	Fiji	80	1090	36	—	64	—	—	—	36	FSC
Fiji 30	Fiji	80	1610	68	52	30	9	—	—	9	FSC
Fiji 40	Fiji	80	1540	90	—	—	4	—	—	96	FSC
Korpi	PNG	80	1055	250	37	44	t	—	t	18	FSC
Mahona	PNG	80	695	205	75	t	—	—	—	25	FSC
Oramboo	PNG	80	1625	317	39	35	8	—	—	17	FSC
S. officinarum (Hybrids)											
Homer		80	1190	0	—	—	—	—	—	—	FSC
Mali		80	1920	8	85	—	—	—	—	15	FSC
Pindar (8 subclones)		80	448	90 (60–150)	4	53	7	1	2	33	FSC
Ragnar		80	1200	100	—	—	—	—	15	85	FSC
Waya		80	820	24	—	67	—	—	5	28	FSC
S. edule											
SE 97	PNG	60	447	0	—	—	—	—	—	—	CSR
Fiji 72 duruka damu	Fiji	70	1025	356	75	—	—	—	—	25	FSC
Fiji 73 duruka leka	Fiji	70	925	370	60	—	—	—	—	40	FSC
Fiji 74 duruka vuaka	Fiji	70	755	235	69	—	—	—	—	31	FSC
Fiji 75 duruka kibo	Fiji	70	1116	320	72	—	—	—	—	28	FSC
Fiji 76 duruka vicowa	Fiji	70	501	180	71	—	—	—	—	29	FSC
28 NG 201	PNG	70	3375	21	36	—	24	—	6	33	FSC
SE 15	PNG	70	1280	460	47	—	t	14	—	39	FSC
SE 34	PNG	80	1695	820	—	95	—	5	—	—	FSC
SE 78	PNG	80	1595	390	—	100	—	—	—	—	FSC
S. robustum											
(a) Subgroup Red Fleshed (*S sanguineum*)											
28 NG 219	PNG-N	60	1676	0	—	—	—	—	—	—	FSC
28 NG 219A	PNG-N	60	300	0	—	—	—	—	—	—	FSC
US 57-159-9	PNG-N	60	655	0	—	—	—	—	—	—	USA
(b) Subgroup Wau—Bulolo											
57 NG 11	PNG	60	2586	0	—	—	—	—	—	—	USA
MOL 4503	NG	60	910	2	49	—	—	—	—	51	FSC
(c) Subgroup Teboe Salah											
Tanangge	Sabah	60?	725	0	—	—	—	—	—	—	USA
57 NG 170	Irian Java	60?	1020	0	—	—	—	—	—	—	CSR
US 57 142-4	Irian Java	60?	3200	420	71	—	2	—	—	26	USA
Mol 6121	PNG	60	2200	170	71	4	—	—	—	23	USA
Mol 6125	PNG	60	2422	163	52	42	—	—	—	5	USA
Teboe Titioewa Teniggaron		60	1567	85	30	64	—	—	—	6	CSR
(d) Subgroup Goroka											
57 NG 208	PNG	80	3300	276	81	5	—	—	—	12	USA
Mol 4357	PNG	80	1325	104	—	—	20 (1 5)	26	—	54	CSR
(e) Subgroup Port Moresby											
Mol 4861	NB	80	1215	239	51	—	5	—	4	40	FSC
Mol 4972	NB	80	450	9	37	11	12	—	2	40	FSC
51 NG 140	PNG-S	80	835	0	—	—	—	—	—	—	FSC
NH 1	NH	80	2211	450	74	—	—	—	—	26	CSR
NH 70-10	NH	80	1415	378	83	6	—	—	—	12	CSR
(f) Others											
57 NG 134	PNG	140	2385	409	23	—	—	—	—	77	CSR
51 NG 28	PNG	156	2820	278	92	1	—	—	1	6	CSR
S. spontaneum											
SES 184A	India	40	300	0	—	—	—	—	—	—	FSC
SES 184B	India	40	1411	0	—	—	—	—	—	—	CSR

Table 1. Distribution of triterpene Me ethers in leaf waxes of *Saccharum* species

Species, clones	Origin*	Chromosome No. (2n)	Yield from leaf		Relative composition of triterpene Me ethers from GLC† (relative retention time)						Source
			Wax ppm	Triterpene OMe ppm	Cylindrin (1.78)	Arundoin (1.52)	(1.45)	Lupeol OMe (1.25)	(1.17)	Crusgallin (1.00)	
SES 106B	India	48	237	0	—	—	—	—	—	—	FSC
SES 189	India	50	2830	0	—	—	—	—	—	—	CSR
SES 352	India	54	176	0	—	—	—	—	—	—	FSC
SES 317	India	56	2164	0	—	—	—	—	—	—	CSR
SES 351	India	56	680	0	—	—	—	—	—	—	CSR
SES 197A	India	60	1480	82	72	13	16	—	—	—	CSR
SES 356	Nepal	60	512	33	100	—	—	—	—	—	FSC
SES 205A	India	64	2420	0	—	—	—	—	—	—	FSC
SES 205B	India	64	1020	0	—	—	—	—	—	—	FSC
Dacca	Bengal	80	3216	0	—	—	—	—	—	—	USA
SES 297B	India	80	1875	0	—	—	—	—	—	—	CSR
SES 341	India	80	2450	0	—	—	—	—	—	—	FSC
Mol 5801	PNG-S	80	895	190	82	—	—	—	—	18	FSC
Mol 5903	PNG-S	80	3430	355	77	—	—	—	—	23	FSC
Mol 5904	PNG-S	80	2030	110	69	—	—	—	—	31	FSC
28 NG 101	PNG	80	3210	930	39	—	—	25	—	35	FSC
51 NG 2	PNG-S	80	1290	215	73	—	—	—	—	27	FSC
US 56-4-1	SE Asia	96	769	0	—	—	—	—	—	—	CSR
Hasuda	Japan	112	3675	430	98	1	—	—	—	—	CSR
Tokyo	Japan	112	4474	760	99	1	—	—	—	—	CSR
Pasoeroen	Indonesia	112	3260	0	—	—	—	—	—	—	CSR
Pangani	Tanzania	?	1711	0	—	—	—	—	—	—	CSR
US 46-28		?	2445	165	93	3	5	—	—	—	CSR

*. PNG(-S)(-N) Papua New Guinea (-S South) (-N North)
NB New Britain
NH New Hebrides.
†. t trace.
‡. OV210 column 190° (Crusgallin 10 min).
§. FSC Fiji Sugar Corporation, Fiji
CSR CSR Sydney or Macknade, Australia
USA Hawaii Sugar Planters Association, Hawaii.
¶. Subclones 70-2, 70-3, 70-26, 70-37, 70-5, 70-6, 70-7, 70-31.

cultures of Pindar (*S. officinarum*) [22], which have different morphological characters and different disease resistance, yielded identical patterns of triterpene methyl ethers.

As well as the leaf wax, *S. officinarum* has a heavy stem wax. Previous studies on this wax from Pindar found alkanes, alkanols, esters and aldehydes, but did not examine methyl ethers [23]. Kreger had previously identified the principal component of the stem wax as octacosanol [24]. These reports were confirmed by examining the stem wax from Badila which yielded alkanes and alkanols (ν_{max} 3350 cm^{-1}) but no ethers were detected despite the complex leaf mixture of this clone.

In contrast previous work in India on mill mud, which is a by-product of the crushing of stems, yielded crusgallin, β-amyrin methyl ether, arundoin and cylindrin [9]. However, when a sample of mill mud from the Lautoka Mill in Fiji was extracted with petrol the extract on IR spectroscopy gave no band at 1100 cm^{-1} characteristic of methyl ethers.

DISCUSSION

Triterpene methyl ethers are present in the waxes of all 4 *Saccharum* species studied. Comparison of the relative frequency of occurrence of each principal component between species shows only a limited variation (Table 2). Arundoin in particular is apparently more characteristic of the cultivated species *S. officinarum* and *S. edule* than of the wild species. Especially noticeable are the wild clones with 2n < 80 from India, which often lacked any triterpene methyl ethers. The overall results mirror the earlier study on the presence of phytosterols in which *S. robustum* (2 out of 21) *S. edule* (0 out of 4) and *S. officinarum* (1 out of 314) were contrasted with *S. spontaneum* (S.E. Asia 6 out of 67 and India 118 out of 261) [7].

Table 2. Frequency of distribution between *Saccharum* species of the major triterpene Me ethers

	No. of clones examined	Cylindrin	Arundoin	Crusgallin
S. officinaum	19	14	14	18
(Noble)	(14)	(12)	(12)	(14)
(Hybrid)	(5)	(2)	(2)	(4)
S. edule	10	7	2	7
S. robustum	19	11	5	12
S. spontaneum	25	10	4	5
(Indian)	(14)	(2)	(1)	(0)
(others)	(11)	(8)	(3)	(5)

Table 3. Distribution of triterpene Me ethers in leaf waxes of *Erianthus*, *Miscanthus Ripidium* and Imperata species

Species, clones	Origin	Chromosome No 2n (plant part)	Yield from leaf Wax ppm	Triterpene OMe ppm	Relative composition of triterpene Me ethers from GLC Cylindrin (1.78)	Arundoin (1.52)	(1.45)	Lupeol OMe (1.25)	Crusgallin (1.00)	Others	Source or reference
Erianthus bengalense		—	630	160	72	3	—	4	—	21 β-amyrin-OMe (1.00)	FSC
E. maximus											
Raiatea	Society I.	60	2000	400	97	—	—	—	3	—	FSC
Fiji 15	Fiji	90	1325	0		-	—	—	—	—	FSC
Fiji 35	Fiji	90	1695	130	28	32	23	—	17	—	FSC
Ripidium arundinaceum											
Mindinao	Phillipines	60	3210	235	100	—	—	—	—	—	FSC
R. elephantinum											
SES 305		20	730	0	—	—	—	—	—	—	FSC
Miscanthus condensatus Hack		Culms and blades	—	—	—	+	—	—	—	—	17
M. floridulus Warb (Tokiwasusuki)) (Japan)			—	—	+	+	—	—	—	Arborinol-OMe	18
M. floridulus (Labill)											
Fiji 2	Fiji	38	1650	t	33	55	—	—	11	—	FSC
Fiji (Tawakula)	Fiji	38	4938	0	—	—	—	—	—	—	FSC
M. sacchariflorus											
Berth (Ogi)		76 culms and blades			+	—	—	—	+	—	11
M. sinensis		38 rhizomes			—	—	—	—	-	—	19
Anderss. (Susuki)		38 culms and blades			—	—	—	—	—	—	11
Imperata conferta											
Fiji 71	Fiji	20	628	210	57	41	—	—	2	—	FSC
Imperata cylindrica (L.) P. Beauv var *koenigii* Durand et Schinz		Leaf culms and blades			+	+	—	—	—	Arborinol OMe	20
		Rhizomes			+	+	—	—	—	—	21

Detailed examination of the results shows correlations with intraspecific changes in chromosome number and geographical origin of the clones. In the *Chionochloa* studies [16], the main interest again lay in intraspecific variations because of a similar problem in relating species largely because of the limited number of marker compounds.

S. officinarum

Triterpene methyl ethers were present in reasonable quantities in all the noble (naturally occurring) sugarcanes. Considerable variation occurred between clones but generally cylindrin (12 out of 14) crusgallin (all) and arundoin (12 out of 14) were present. The commercial hybrid canes were more erratic in composition and from Homer no triterpene methyl ethers could be isolated. In an earlier study the leaf wax from a Cuban sugarcane (but unspecified clone) yielded arundoin, crusgallin and a minor unidentified component [10].

S. edule

Three chromosome numbers have been reported for *S. edule* 2n = 70, 2n = 80 and in one isolated case 2n = 60 [25]. The triterpene ethers in the two main groups are distinctly different, 2n = 80 yields only arundoin, and 2n = 70 yields no arundoin but crusgallin and cylindrin. The 2n = 60 clone yielded no methyl ethers. A similar clear distinction was also found between the β-amylase isoenzymes of the 2 main groups [5].

The origin of *S. edule* is unclear and a number of theories have been put forward, mostly based on its derivation from *S. robustum* [25]. In a recent study Grassl suggests that it is a hybrid between *S. robustum* and *Miscanthus floridulus* and thus is not a valid species [26]. Its lack of inflorescence and sterility, preventing further hybridization is possibly the cause of the uniformity in the triterpene compositions.

S. robustum

This is a complex species and has been divided by Price into 5 subgroups based on chromosome numbers, morphology and origin [27] (used in Table 1). It has subsequently been suggested that one of these groups 'Red Fleshed' deserves species status as *S. sanguineum* Grassl (Grassl) [28].

A recent study based on flavonoid data confirms this change and suggests that the Port Moresby group is the typical *S. robustum*. The Teboe Salah and Wau-Bulolo groups with revision are also considered to deserve species status. The remaining group Goroka is either an isolated form of the Port Moresby group or a hybrid of *S. officinarum* and *S. spontaneum* [4].

In the present study, the Red Fleshed group is characterized by a lack of methyl ethers. A second group, Wau-Bulolo also with chromosome number 2n = 60 gave only a trace of methyl ethers.

The third 2n = 60 subgroup Teboe Salah is further divided into 3 sections based on Indonesia (Sabah), Irian Java, and Papua New Guinea, the first of these now being suggested as the typical group [4]. The samples in this study of the Irian Java group are incompletely characterized and may have 2n = 80, and thus be more correctly in the Port Moresby group. In the flavonoid study the Papua New Guinea clones are placed in the Wau Bulolo subgroup, however in the present study these clones are characterized by triterpene methyl ethers in contrast to the Wau Bulolo group.

The clones with 2n = 80 are of particular interest as it has been proposed that they are the direct progenitors of sugarcane. Typically both the Goroka and Port Moresby subgroups yielded methyl ethers but the frequency of the arundoin marker was low (3 out of 9) compared to *S. officinarum*. The results would agree with the proposal that the Goroka group is a geographical isolate of the Port Moresby group [4].

Clearly further clones would need to be studied by both these techniques before the chemotaxonomy of the *robustum* subgroups is clear. The remaining two clones 57 NG 134 and 51 NG 28 are of an unusual high chromosome type and are thought to have originated from *Saccharum* × *Miscanthus* [4].

S. spontaneum

A wide range of chromosome numbers from 40 to 128 are known for this species based in 3 main groups: India (40–80), Africa (104–128) and S.E. Asia and the Pacific (80–112) [29]. The composition of methyl ethers also shows a marked variation.

All the clones from India except SES 197A and SES 356 (both 2n = 60) lack triterpene methyl ethers. As N.W. India is regarded as the centre of origin of the genus this finding suggests that methyl ethers are an added character. It is particularly notable that no methyl ethers were found in Indian clones with 2n = 80, whereas clones from Papua New Guinea with 2n = 80 yielded cylindrin and crusgallin. A similar marked distinction was found for β-amylase isoenzymes [5] but was less evident in the flavonoid studies [3]. In other work it has been reported that on the 2n = 80 level the breeding of *S. spontaneum* is atypical [30].

The higher chromosome number clones are a mixture, those from Africa (Pangani), Java (Pasoeroen) and SE Asia (US 54-4-1) lacking methyl ethers but those from Japan (Tokyo and Hasuda) yielded cylindrin. A previous study from Japan on *S. spontaneum* var. *arenicola* (Ohwi) (Waseobana) reported cylindrin [11].

Related species

The majority of the species related to sugarcane that were studied contained triterpene methyl ethers characteristic of the Andropogenae (Table 3). However, insufficient clones of each species were examined to enable conclusions to be drawn. *Erianthus bengalense* is notable as the only clone to yield β-amyrin methyl ether this study, although it has previously been reported from other grasses [8].

CONCLUSIONS

Triterpene methyl ethers have been found in all 4 *Saccharum* species studied, in agreement with their presence as a typical character of the Gramineae. However, there is considerable variation within each species emphasizing the need when studying chemical compositions in species that undergo hybridization, of reporting the clone under investigation.

The build-up of methyl ethers with chromosome number and evolution of the genus suggests that it could be an acquired character. It is not known whether this change represents a development of a terpene alcohol formation route or of a methylation mechanism. From the early work on phytosterols it appears that the alcohols are most frequently present in cases when methyl ethers are absent and vice versa.

EXPERIMENTAL

Fresh leaves were studied from a number of breeding collections (see Tables 1 and 3). For samples from outside Fiji, the extraction to give the leaf wax was carried out in Sydney and the crude total wax was sent to Fiji.

Isolation of triterpene Me ethers. Leaves (200 g) were cut into 10–15 cm lengths and immersed in 60–80° petrol for 6–8 hr at room temp. The solvent was filtered and evaporated to leave the crude wax, which was examined by IR for Me ethers (1100 cm^{-1}). The wax was suspended in 40–60° petrol and was chromatographed on neutral Al_2O_3 (Activity I) using petrol as solvent. Fractions were analysed by TLC (Si gel, petrol) and combined to give a hydrocarbon fraction (R_f 0.9) and the triterpene Me ether fraction (R_f 0.4).

Analysis. The Me ether fraction was analysed on a 2 m × 3 mm column packed with 1.5% QF 1 or OV 210 (with identical results) at 190° using N_2 carrier gas at 30 ml/min and a FID detector. The peak areas were determined by triangulation and the R_t values were compared with standards (see Table 4).

Table 4. Relative retention times and R_f values for triterpene Me ethers

Compound	RR_t*	R_f†
Crusgallin	1.00	0.73
β-Amyrin Me ether	1.02	0.76
Miliacin	1.03	0.46
Cycloartenol Me ether	1.16	—
Lupeol Me ether	1.25	0.35
Arundoin	1.52	0.83
Cylindrin	1.78	0.61

* Relative to crusgallin (R_t 10 min) conditions as in Experimental.

† $AgNO_3$–Si gel (15:85) and 10% toluene: 90% 40–60° petrol run × 3 and detected with anisaldehyde–H_2SO_4 and charring.

Wherever possible the identification of the Me ethers was confirmed by comparison with authentic samples using TLC (see Table 4) and after crystallization by mp, IR and PMR spectroscopy.

Attempts to isolate individual components from mixtures of crusgallin and cylindrin by crystallization were unsuccessful as the components co-crystallized in a 3:1 mixture, mp 238–242°, a value close to that reported for cylindrin crystallized from mill mud [9]. Fractional sublimation of the mixed crystals gave pure cylindrin mp 263–264° (lit. 269–270° [21]). A similar problem arose with mixtures of crusgallin and arundoin, but these compounds could be separated by TLC (Table 4).

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