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THE TAXONOMIC SIGNIFICANCE OF LEAF FLAVONOIDS IN *SACCHARUM* AND RELATED GENERA

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Key Word Index—*Saccharum*; Saccharinae; Gramineae; sugar cane; tricin 7-glucoside-KSO_3; tricin 7-neohesperidoside; tricin 7-diglucoside; flavone *C*-glycosides; biochemical systematics.

Abstract—A survey of 120 plants of the genera *Saccharum, Erianthus, Ripidium, Miscanthus, Narenga, Sclerostachya, Imperata*, of intergeneric and interspecific hybrids and of commercial cane varieties revealed that some leaf flavonoids were useful systematic markers. *Saccharum officinarum, S. edule, S. robustum* and *Erianthus maximus* were clearly distinguished from *S. spontaneum, Narenga, Miscanthus, Imperata, Sclerostachya, Ripidium* and other *Erianthus* species by the presence of the potassium bisulphate salt of tricin 7-glucoside and of tricin 7-neohesperidoside and 7-diglucoside. In *Erianthus*, all Old World Species were characterized by the presence of a luteolin di-*C*-glycoside which clearly distinguished them from the New World species, which have recently been separated off into a separate genus, *Ripidium* by Grassl. Some other flavone *C*-glycosides were present in all genera: iso-orientin, iso-orientin *O*-rhamnosylglucoside, iso-orientin 7-glucoside, a possible iso-orientin *O*-triglycoside and three luteolin di-*C*-glycosides. In *Saccharum*, some F_1 hybrids showed a clear additive inheritance of the parental leaf flavonoids (mainly iso-orientin derivatives) but also contained two flavone *C*-glycosides based on vitexin. The *de novo* production of apigenin *C*-glycosides in the hybrid plants suggests that some chromosomal disturbance occurs, affecting the enzymes controlling flavone hydroxylation. The flavonoid evidence supports the view that the cultivated sugar canes originated from *S. robustum* and that *Erianthus maximus* is a hybrid involving *Saccharum* and *Miscanthus*.

INTRODUCTION

PLANTS of the genus *Saccharum* have been selected and used as a source of sweetness since the days of primitive man. Cultivated sugar canes are thought to have originated in New Guinea and associated islands of the Malayan Archipelago, where the two wild species, *S. robustum* and *S. spontaneum* are sympatric. These wild species, however, do not accumulate sucrose so that the origin of the sweet sugar canes is still a matter of some dispute. It has been suggested by Warner[1] that the sweet *S. officinarum* arose from chance mutations from *S. robustum* selected and preserved by natives in New Guinea. Present day commercial canes are largely the result of successful cross-breeding of *S. officinarum* with *S. robustum* and *S. spontaneum*, with the *S. officinarum/S. spontaneum* hybrids being the most predominant. Three other cultivated species are recognized: *S. sinense* and *S. barberi* grown for centuries in China and India, respectively and *S. edule* grown as a vegetable in New Guinea for its edible inflorescence. However, the long history of cultivation and

[1] WARNER, J. N. (1962) *Ethnology* 1, 405.

the ready hybridization between species has led sugar cane taxonomists to suspect that all four cultivated "species" may be of hybrid origin,[2] but absolute proof is lacking because of the difficulty of identifying hybrids purely on morphological grounds. A preliminary leaf flavonoid survey showed differences in chromatographic pattern between the two wild species *S. spontaneum* and *S. robustum*. In view of the taxonomic difficulties mentioned above, it was decided to extend the survey to include cultivated *Saccharum* species, F_1 hybrids, commercial cane varieties and closely related genera in the tribe Andropogonae. Although Verma *et al.*[3] have compared stem flavonoid patterns in four sugar cane varieties, no leaf flavonoids have been completely identified in any of these genera. Other phenolic constituents reported in *Saccharum* include lignins[4] and ferulic acid in the leaf[5] and tannins, anthocyanins[6] and syringic acid[7] in cane juice. Farber and Carpenter[8] have found a number of hydroxycinnamic acids, kaempferol and umbelliferone and Smith[9] predicted the presence of *C*-glycosylflavones from the remarkable stability of cane sugar colourants to acid hydrolysis.

RESULTS

The results of the leaf flavonoid survey are presented in Table 1. Fresh leaf material was received by air from Australia and Fiji and sampled on arrival. This was supplemented by herbarium material in the case of some genera. As many cultivars and samples of each species as possible were included in the survey. The data in Table 1 refer to flavone *O*- and *C*-glycosides found on 2-D PCs of 80% methanol leaf extracts. Individual glycosides were identified from detailed examination of a few selected *Saccharum* and *Ripidium* species and the presence of potassium bisulphate salts confirmed by paper electrophoresis of direct leaf extracts.

Tricin glycosides

Tricin 5-glucoside, a common leaf constituent of grasses, occurs in 99% of the present sample. Three other tricin glycosides: tricin 7-neohesperidoside, a tricin 7-diglucoside and the unusual tricin 7-glucoside-bisulphate, reported here for the first time, occur together in *S. officinarum* (90% of clones), *S. robustum* (82% of clones), *S. edule* (87% of clones) and *E. maximus* (all three clones studied). The three compounds, however, are rare in *S. spontaneum* (17% of clones) and absent from *Narenga*, *Sclerostachya*, *Imperata*, *Miscanthus*, *Ripidium* and all true *Erianthus* species. Tricin 7-neohesperidoside and the tricin 7-diglucoside are present in all *S. sinense* and *S. barberi* clones but tricin 7-glucoside-bisulphate is absent from all but one clone of *S. sinense* and *S. barberi*, subgroup Nagori. Eighty per cent of commercial cane varieties examined contained all three tricin compounds, and in *S. spontaneum* a different tricin diglucoside is present.

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TABLE 1. DISTRIBUTION OF FLAVONE *O*- AND *C*-GLYCOSIDES IN LEAVES OF THE SACCHARINAE

	Tricin				Iso-orientin																			
Genus, species, clone	7GS	7GG	7RG	5G	I	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
S. officinarum L.																								
HQ 409	+	+	+	+	+	+	−	−	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−
Oramboo	+	+	+	+	+	−	−	−	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−
Chittan	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−	−	−
NG 57–225	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−	−	−
Badila	−	+	+	+	+	+	+	−	+	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−
Korpi	+	+	+	+	+	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Mahona*	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−
SC 1214*	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	−	−	−	−	−
EK 2*	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	+	−	−	−	−	+	−
NG 51–142*	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	−	−	−	+	−
S. edule Hassk.																								
NG 57–28	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−	−	−
SE 97	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	+	−	−	−	−	−	−
SE 34	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−
Fiji 1*	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	+	−	−	−	−	−	+
NG 28–201*	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−
NG 57–19*	+	+	+	+	+	+	+	−	+	+	−	−	−	+	+	−	−	+	−	−	−	−	−	−
NG 57–40*	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	+
SE 15	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	−	−	−	−	−
S. robustum																								
Brandes & Jeswiet ex Grassl																								
NG 57–208	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−
NG 57–11	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
US 57–159–9	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+	−	−	+	−	−	−	−	+	−
MOL 4972	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	−	−	+	−	−	−	−	+	−
NG 28–219	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+	−	−	+	−	−	−	−	+	−
MOL 4357*	+	+	+	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
NG 28–219A*	+	+	+	+	−	+	−	−	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−
NG 28–251*	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	+	−	−	−	−	−	−
NG 51–140	−	+	+	+	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	−	−	−	−	+
NG 51–63	+	−	+	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
NH 1	−	−	+	−	+	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−
S. sinense Roxb.																								
China	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−
UBA	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−
Gandi Cheni	−	+	+	+	+	+	+	−	+	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+
S. barberi Jesw.																								
Subgroup Nagori																								
Nagori	+	+	+	+	+	+	−	−	+	+	+	+	−	−	+	−	−	+	−	−	−	−	−	+
Mango Sic	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−
Subgroup Saretha																								
Saretha	−	+	+	+	+	+	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−	−	−	+
Chunnee	−	+	+	+	+	+	−	+	−	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−
Subgroup Mungo																								
Mungo	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	−	−	−	−	−	−	−	+	−
Agoule	−	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S. spontaneum L.																								
M'beya	−	−	−	−	+	−	−	−	+	+	−	+	+	+	+	−	−	+	−	−	−	−	−	−
MOL 1032	−	−	−	+	+	−	+	−	+	+	−	+	+	−	+	−	−	+	−	−	+	−	−	−
SES 341	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−
Moentai	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−
SES 182	−	−	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	+	−	−	−
SES 365	+	−	+	+	+	−	−	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
SES 154B	−	−	−	+	+	−	+	−	+	+	+	+	+	+	−	−	−	−	+	−	+	−	+	−
SES 106B	=	−	−	+	+	+	−	−	+	+	−	+	+	+	+	−	−	−	−	−	+	−	+	−
Tabongo	−	−	−	−	+	+	−	−	+	+	−	+	+	−	+	−	−	−	+	−	−	−	+	−
SES 351	−	−	−	−	+	+	+	−	+	+	−	+	+	−	−	−	−	−	−	−	+	−	−	−
SES 184A	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	−
SES 208	−	−	−	+	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
SES 356	−	−	−	−	+	+	+	−	+	+	−	+	+	−	+	−	−	−	−	−	+	−	−	−
NG 28–101	−	−	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	+	+	−	+	−
Glagah 1286	−	−	−	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−
Coimbatore	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	+	−
Tainan	−	−	−	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−
Saudi Arabia	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	−	−	−
Okinawa	+	−	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−
NG 51–2	+	−	+	+	+	+	−	−	+	+	−	+	+	+	+	−	−	+	−	−	−	−	−	−
Mandalay	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
US 4515*	−	−	−	+	+	+	−	−	+	+	+	−	+	+	+	−	−	+	−	−	−	−	−	+
Krakatau*	−	−	−	+	+	+	−	−	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−

TABLE 1. (continued)

Genus, species, clone	Tricin 7GS	7GG	7RG	5G	Iso-orientin I	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
S. officinarum × *S. spontaneum* *F₁ Hybrids*																								
BM 638 Badila × Mandalay	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+	−	−
BN 288 Badila × NG 51-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
BT 281 Badila × Tabongo	+	−	+	+	+	+	−	+	−	+	+	+	−	+	+	−	−	−	−	−	−	−	+	−
KM 301 Korpi × Mandalay	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+	+	−
KN 535 Korpi × NG 51-2	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−
KO 431 Korpi × MOL 1032	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−
KN 768 Korpi × NG 51-2	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	+	−
KT 226 Korpi × Tabongo	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	+	−
BO 783 Badila × MOL 1032	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	+	−
BM 599 Badila × NG 51-2	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−
E. maximus Brongn.																								
Fiji 10	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Fiji 15	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	+
Raiatea*	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	−	−	−	−	−
E. coarctatus Fernold† (coll. Josiah Hale, N. America)	−	−	−	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−
E. contortus Baldwin ex Ell. (H. E. Moore Jr. 1053, N. America)†	−	−	−	−	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−
E. divaricatus (L.) Hitch. (P. Dusén, Brazil)†	−	−	−	+	+	+	+	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−
E. saccharoides Michx.	−	−	−	+	+	+	+	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−
E. strictus Baldwin† (Frank W. Gould 12005, Texas)	−	−	−	+	+	−	−	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−	+	−
E. trinii Hack.† (Luiza Thereza Dombrowski 2741, Brazil)	−	−	−	−	+	−	−	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−
E. trinni Hack. var *glabrinodes* Hack.‡	−	−	−	+	+	+	+	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−
R. arundinaceum (Retz.) Grassl																								
Mindinao	−	−	−	+	+	+	+	−	+	+	−	+	+	−	+	−	−	−	−	−	−	+	−	+
NG 28-7	−	−	−	+	+	+	+	−	+	+	−	+	+	−	+	−	−	−	−	−	−	+	−	−
SES 181	−	−	−	+	+	+	+	−	−	−	−	+	+	−	+	−	−	−	−	−	−	+	−	+
R. bengalense (Retz.) Grassl																								
US 47-5	−	−	−	+	−	+	−	−	−	+	+	+	−	+	−	−	−	+	−	−	−	+	−	+
R. elephantinum (Hook. f.) Grassl																								
SES 372	−	−	−	+	+	−	+	−	+	−	+	+	−	+	+	−	+	−	−	−	−	+	−	−
SES 305	−	−	−	+	+	−	−	−	+	−	+	+	−	+	+	−	+	−	−	−	−	+	−	−
R. kanashiroi (Ohwi) Grassl																								
IMP 2384	−	−	−	+	+	−	+	−	+	+	−	+	+	+	−	+	−	−	−	−	−	+	−	−
R. procerum (Roxb.) Grassl																								
IMP 1251	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	+	+	−	−	−	+	−	−
R. ravennae (L.) Trin.																								
US 59-271-1	−	−	−	+	+	+	+	−	+	+	+	+	−	+	+	−	−	+	−	−	−	+	−	+
M. floridulus (Labill) K. Sch. et Lant.																								
Fiji 37*	−	−	−	+	−	+	+	+	+	+	+	+	−	+	+	−	−	+	−	−	−	−	−	−
Fiji 65*	−	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−
Fiji 66*	−	−	−	+	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
NG 51-24*	−	−	−	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
M. floridulus† (Labill) K. Sch. et Lant. (Coll. R. Schodde 2003, New Guinea)	−	−	−	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+	−
M. floridulus† (Labill) K. Sch. et Lant. Coll. J. R. Flenley ANU 2057, New Guinea)	−	−	−	+	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
M. japonicus† Anderss. (A. Henry, Formosa No. 1823A)	−	−	−	−	+	−	−	+	+	+	+	+	−	−	−	−	−	(+)	−	−	−	−	−	−

TABLE 1. (continued)

Genus, species, clone	Tricin 7GS	7GG	7RG	5G	Iso-orientin I	RG	TG	IV	OG	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
M. sinensis† Anderss. (Tsant W. T20750 China)	−	−	−	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	+	−
Narenga porphyrocoma (Hance) Bor	−	−	−	+	+	−	−	−	+	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−
Sclerostachya fusca A. Camus US 56–73	−	−	−	+	+	+	−	−	+	+	−	+	+	+	−	−	−	−	−	−	+	−	−	−
Imperata conferta (Presl) Ohwi Fiji 71*	−	−	−	+	+	+	+	−	+	+	+	+	+	−	+	−	−	+	−	−	−	−	+	−
Imperata cylindrica (L.) P. Beauv.	−	−	−	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−

5G = 5-Glucoside; 7G = 7-Glucoside; 7GG = 7-diglucoside; 7RG = 7-neohesperidoside; 7GS = 7-glucoside bisulphate; RG = *O*-rhamnoglucoside; TG = *O*-triglucoside; IV = isoviterin; OG = iso-orientin 7-glucoside F denotes unidentified flavonoid glycoside. The R_fs of the unidentified compounds in BAW and 15% HOAc are as follows: F1 25/58; F2 37/23; F3 18/39; F4 17/46; F5 28/19; F6 35/12; F7 18/13; F8 61/16; F9 47/45; F10 47/10; F11 20/16; F12 28/30; F13 55/79; F14 62/51; F15 64/58. The colours of the unknown flavonoids in UV without and with NH_3 are as follows: F1, F2, F3, F4, F8, F9, F10, F13, F15, dark to reluctant yellow; F6, F12, dark to orange–yellow; F5, F7, dark to yellow; F11, F14, dark to dark.

* Plants grown in Fiji by Fiji Sugar Corporation Ltd.

† From the Herbarium, The Royal Botanic Gardens, Kew (K).

‡ From Reading University Herbarium (RNG).

× From the World Collection, Canal Point, Florida.

All other plants were sent from the C.S.R. Research Laboratories, Roseville, New South Wales and their Field Experimental Station, Macknade, North Queensland, Australia. The Fiji sample of *Imperata conferta* was formerly called *I. cylindrica* but this was altered on the advice of the U.S. National Herbarium.

Flavone C-glycosides

Flavone *C*-glycosides were found in all genera. Iso-orientin, iso-orientin *O*-rhamnosylglucoside, iso-orientin 7-*O*-glucoside and isovitexin were identified and a possible orientin tri-*O*-glycoside isolated. Four luteolin di-*C*-glycosides were also isolated but difficulty in the identification of the *C*-sugars on a microscale prevented their complete elucidation. *Ripidium* species were characterized by the presence of one luteolin di-*C*-glycoside (F_{13}) not present in the *Erianthus* species. *S. officinarum* × *S. spontaneum* F_1 hybrids contained isovitexin (100% of sample) and spot F_{14}, (80% of sample) not present in the parent clones, which accumulate luteolin *C*-glycosides only. Thus, there is apparently some chromosomal disturbance in the development of the hybrid plants, which affects the enzymes controlling the hydroxylation pattern of the flavone *C*-glycosides. It is interesting that a similar phenomenon has been observed in *Briza media*,[10] where doubling of the chromosomes affects the hydroxylation pattern so that vitexin (and isovitexin) accumulate in diploids, whereas orientin (and iso-orientin) accumulate in the tetraploid.

DISCUSSION

The flavonoid data discovered during this survey appear to be relevant to the taxonomy of the sugar cane plants in a number of ways. The data will be discussed in turn in relation to the taxonomy of *Saccharum*, *Erianthus*, *Narenga* and *Sclerostochya*, and of the tribe generally.

[10] WILLIAMS, C. A. and MURRAY, B. G. (1972) *Phytochemistry* **11**, 2507.

Saccharum

Saccharum robustum is clearly distinguished from the other wild species *S. spontaneum* by the presence of the three unusual tricin glycosides: the 7-glucoside bisulphate, the 7-neohesperidoside and a 7-diglucoside. All clones of *S. officinarum* examined, with the exception of Badila (Table 1), have a leaf flavonoid pattern similar to that of *S. robustum*, thus supporting the view of most sugar cane breeders[11] that *S. officinarum* arose from this species. However a suggestion[12] that *Ripidium arundinaceum* might be involved can be disputed on chemical grounds, because the characteristic "*Ripidium*" compound (F_{13}, Table 1) is absent from all *S. officinarum* clones studied.

Saccharum sinense and *S. barberi* form a more complex group of canes and explanations for their origin include hybridization and selection within *S. spontaneum*,[11] intergeneric hybridization involving *S. spontaneum*[11] and selection within *S. officinarum* × *S. spontaneum*.[13] Chemically, the group is not uniform but the clones UBA, Nagori and Mango Sic (Table 1) do show a *S. robustum* leaf flavonoid pattern; all other clones lack the negatively charged tricin compound. This evidence therefore suggests that the group has a diverse origin.

Saccharum edule, which is similar in gross morphology to *S. robustum*, is suggested by Lennox[14] and Brandes *et al.*[12] to be a mutant form of that species. Grassl,[15] however, thinks that there are two types of *S. edule*, one evolved from *S. robustum* × *Miscanthus* in New Guinea and the other from *S. officinarum* × *Miscanthus* in Fiji. All clones of *S. edule* examined, except SE 34, had the same leaf flavonoid pattern and the one clone from Fiji was quite indistinguishable from the New Guinea clones. Thus the data indicate that *S. edule* most probably arose from *S. officinarum* or *S. robustum*.

There is much dispute as to the species status of *S. sinense* and *S. barberi*. Barber[16] in 1918 recognized five groups of *S. barberi* in North Indian canes, namely Mungo, Nagori, Saretha, Sunnabile and Panashi. The Panashi clones, which are also found in Indo-China, S. China and Taiwan are included by Jeswiet[17] in his species *S. sinense* (Chinese canes) leaving Barber's remaining four groups in *S. barberi*. Most cane breeders now agree with Artschwager[18] and Price[19,20] that *S. sinense* and *S. barberi* should be included under *S. sinense*. The present results support this but do show Nagori as a distinct group.

Erianthus

The taxon referred to as "*Erianthus maximus*" differs markedly in its leaf flavonoid pattern from all other *Erianthus* species surveyed but closely resembles those of *S. robustum*, *S. officinarum* and *S. edule*. This supports the morphological evidence of Grassl,[21] which indicates that *E. maximus* is in fact a hybrid group involving *Miscanthus* and *Saccharum*. Grassl,[21] in a recent revision of *Erianthus*, has transferred the following species: *E. arundinaceus*, *E. bengalense*, *E. elephantinus*, *E. kanashiroi*, *E. procerum* and *E. ravennae* to the

[11] GRASSL, C. O. (1969) *Proc. ISSCT* **13**, 868.
[12] BRANDES, E. W., SARTORIS, G. B. and GRASSL, C. O. (1938) *Proc. ISSCT* **6**, 128.
[13] PARTHASARTHY, N. (1946) M.O.P. Iyengar commem. vol., *J. Ind. Bot. Soc.* 133.
[14] LENNOX, C. G. (1938) *Proc. ISSCT* **6**, 171.
[15] GRASSL, C. O. (1967) *Proc. ISSCT* **12**, 995.
[16] BARBER, C. A. (1918) *Mem. Dept. Agric. India Bot. Ser.* **9**, 135.
[17] JESWIET, J. (1925) *Archief. Suikerindustrie in Ned. Indië.* 33ste Jaarg. 3E Deel, 391.
[18] ARTSCHWAGER, E. (1954) *USDA Tech. Bull.* 1089.
[19] PRICE, S. (1957) *Bot. Gaz.* **118**, 146.
[20] PRICE, S. (1968) *Econ. Botany* **22**, 155.
[21] GRASSL, C. O. (1971) *Proc. ISSCT 14th Cong.*, New Orleans.

genus *Ripidium*. All these *Ripidium* species contain a distinctive flavone di-*C*-glycoside (F_{13}, Table 1) absent from the New World *Erianthus* species and the chemistry thus clearly supports this separation.

Narenga *and* Sclerostachya

Grassl[21] suggests that, from their morphological similarities, these two genera should be combined. Only one clone of *N. porphyrocoma* and *S. fusca* were examined for leaf flavonoids and although these were very similar, there is insufficient evidence to support or dispute their combination as one genus.

TABLE 2. CHROMATOGRAPHIC DATA FOR TRICIN *O*-GLUCOSIDES AND FLAVONE *C*-GLYCOSIDES

Flavone glycoside	BAW	BEW	R_f (× 100) in PhOH	H_2O	15% HOAc
Tricin					
7-*β*-D-glucoside*	36	35	89	02	08
7-glucoside†	42	29	57	03	12
7-rutinoside‡	34	37	84	07	24
7-neohesperidoside	43	41	84	06	20
7-glucoside KSO_3 a	22	17	19	11	26
7-glucoside KSO_3 b§	09	10	45	04	18
7-glucoside KSO_3 c‖	18	23	24	08	39
7-diglucoside	32	21	44	05	25
Flavone *C*-glycosides					
Iso-orientin	43	44	60	17	34
Iso-orientin 7-*O*-rutinoside	37	44	60	66	53
Iso-orientin *O*-triglycoside	35	47	43	84	70
Orientin 7-*O*-glucoside	22	41	68	13	32

* By partial hydrolysis with 2 N HCl of tricin 7-neohesperidoside.
† By partial hydrolysis with 2 N HCl of both tricin 7-diglucoside and tricin 7-glucoside KSO_3.
‡ From *Chamaerops humilis*.
§ From *Phoenix roebilinii*.
‖ From *Rhopaloblaste singaporensis* and *Phoenix tomentosa*.
R_f data in this table are given for pure compounds and there is some discrepancy with those values given for BAW and 15% HOAc in Table 1, which are recorded from 2-D PC of crude plant extracts.

The Andropogoneae

Since negatively charged flavonoids, present in some *Saccharum* species, proved taxonomically useful within the Saccharinae it was decided to extend the survey for these compounds to the Andropogoneae and the Gramineae as a whole. These results will be presented more fully at a later date but it is worth mentioning a few of the present findings. Thus flavonoid conjugates have been found in two other genera of the Andropogoneae, *Bothriochloa* and *Dimeria* but are absent from the *Andropogon*, *Heteropogon*, *Hyparrhenia*, *Sorghum*, *Themeda*, *Ischaemum* and *Rottboellia* species studied. Within the Gramineae these compounds were detected in 17% of the 120 species surveyed and it is interesting that they are confined mainly to tropical and subtropical genera in the tribes Andropogoneae, Arundinoideae and Paniceae since negatively charged flavonoids have recently been found in 50% of 125 species in the tropical–subtropical family the Palmae.[22] While in the Palmae a number of flavone *O*- and flavone *C*-glycosides and flavonol *O*-glycosides

[22] WILLIAMS, C. A., HARBORNE, J. B. and CLIFFORD, H. T. (1973) *Phytochemistry* **12**, 2417.

occur as the bisulphate salts, in the Gramineae the only charged flavonoid so far detected is tricin 7-glucoside–KSO_3. Two other tricin 7-glucoside bisulphates have been partially characterized in three palm species[22] but they are all chromatographically distinct (Table 2) from the tricin 7-glucoside-KSO_3 found in the present survey of the Saccharinae. Finally, a further chemical difference between the palms and the Saccharinae of the Gramineae is that isomeric forms of tricin 7-rhamnosylglucoside occur: the 7-rutinoside has been identified in the former group (e.g. in *Chamaerops humilis*) while the 7-neohesperidoside is present in *Saccharum*.

EXPERIMENTAL

Plant material. The sources of plant material are given in Table 1. Fresh leaf material was received by air and sampled as soon as possible on arrival. Leaf material from Australia and Fiji has been verified by Roach and Daniels respectively.

Flavonoid identification. The solvents used for 2D PC of direct 80% MeOH leaf extracts on Whatman No. 1 paper were (1) BAW and (2) 15% HOAc. Individual glycosides of *S. spontaneum*, of a cultivated sugar cane variety "Tate & Lyle D14148" and of *Ripidium* species were isolated and purified on Whatman No. 3MM paper using standard solvents. Known glycosides were identified on the basis of R_f, UV spectral analysis, acid hydrolysis to aglycone and sugar and by direct comparison with authentic samples. Flavone *C*-glycosides were acid hydrolysed for 4 hr and flavone *C*-glycoside-*O*-glycosides further identified by means of partial acid hydrolysis.

Identification of Tricin 7-glucoside KSO_3. Chromatographic data are presented in Table 2. Electrophoretic mobility on Whatman No. 3 paper in 2·5% formic acid–7·5% HOAc (1:1) pH 2·2 buffer for 3 hr at 400 mV was 0·30 when compared with luteolin 7-KSO_3-3'-*O*-glucoside.[18] Five-minute acid hydrolysis gave an intermediate compound, dark absorbing in UV and changing to bright yellow with NH_3. K^+ and HSO_4^- were detected after acid hydrolysis by TLC using the method previously described.[18] The intermediate compound both on acid and enzyme hydrolysis with β-glucosidase gave tricin and glucose only and remained unchanged with α-glucosidase. Spectral date (λ_{max}) for the intermediate are as follows: MeOH 248, 269, 354; + NaOAc 248, 269, 354; + alk. 260, 412; + $AlCl_3$ 276, 363, 386; + H_3BO_3 248, 269, 354 nm. The lack of NaOAc shift indicates that there is a sugar in the 7 position. However, this compound did not co-chromatograph with tricin 7-glucoside obtained from partial acid hydrolysis of tricin 7-neohesperidoside (Table 2). Since the 7-glucoside formed from the 7-neohesperidoside is almost certainly (from R_f and other data) the expected 7-β-D-glucopyranoside, then this new 7-glucoside from the bisulphate salt must differ in some subtle, and as yet unexplained, way from the common structure.

Tricin 7-diglucosides. Chromatographic data are shown in Table 2. Partial hydrolysis of tricin 7-diglucoside 1 gave an intermediate compound identical with the tricin 7-glucoside from tricin 7-glucoside KSO_3. This evidence and chromatographic behaviour indicate the presence of a diglucoside. Tricin 7-diglucoside 2 gave tricin and glucose on acid hydrolysis and chromatographic data suggest a diglucoside; however, there was insufficient amount for further characterization.

Tricin 7-rhamnosylglucosides. The tricin 7-rhamnosylglucoside in *Saccharum*, identified as such by standard procedures, was found to be chromatographically distinct (see Table 2) from a 7-rhamnosylglucoside isolated earlier from several members of the Palmae.[22] The two isomers were identified as the 7-neohesperidoside (Rha α 1 → 2 Glc) and the 7-rutinoside (Rha α 1 → 6 Glc) respectively, largely by direct analogy in R_f and properties with authentic synthetic samples of apigenin 7-neohesperidoside and 7-rutinoside. These were kindly provided by Prof. Wagner of Munich. Thus, in alcoholic solvents (see Table 2) and also in electrophoresis (pH 8·8 for 3 hr at 3 V/cm) the neohesperidosides always travelled slightly ahead of the rutinosides; in aq. solvents, the reverse was true. Furthermore, differences in R_f between the rutinosides, neohesperidosides and the corresponding aglycones were almost identical in both series (e.g. ΔR_f in BEW were 0·28 and 0·36 for apigenin 7-neohesperiodoside and 7-rutinoside respectively, 0·26 and 0·32 for the tricin derivatives). All samples were rapidly hydrolysed by α-rhamnosidase, indicating an α-linkage in all cases. Alkaline treatment (in 0·5% aq. KOH at 100° for 120 min)[23] decomposed the 7-rutinosides to tricin or apigenin, but left the 7-neohesperidosides largely unaffected. Finally, H_2O_2 oxidation of both 7-neohesperidosides failed to give a disaccharide fragment, while that of the 7-rutinosides gave rutinose in low yield.

Iso-orientin O-glycosides. Two iso-orientin *O*-glycosides were found and both gave glucose, rhamnose and iso-orientin on acid hydrolysis. Chromatographic data (Table 2) suggest that one is an *O*-rhamnosylglucoside and the other a tri-*O*-glycoside from their relative mobilities in aq. solvents. Partial acid hydrolysis of both glycosides gave iso-orientin in 2 min and positive NaOAc, $AlCl_3$ and borate shifts suggest that the *O*-sugars are probably attached via the *C*-sugar.

[23] LITVINENKO, V. I. and MAKAROV, V. A. (1969) *Khim. Prir. Soedin.* **5**, 366.

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