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NEGATIVELY CHARGED FLAVONES AND TRICIN AS CHEMOSYSTEMATIC MARKERS IN THE PALMAE

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Key Word Index—Palmae; negatively charged flavonoids; flavone *C*-glycosides; potassium bisulphate flavone salts; tricin glycosides; chemotaxonomy; computer analysis.

Abstract—A survey of 125 species of the Palmae revealed a complex pattern of flavonoids in the leaf. *C*-Glycosylflavones, leucoanthocyanins and tricin, luteolin and quercetin glycosides were common, being present in 84, 66, 51, 30 and 24% of the species respectively. Apigenin and kaempferol were recorded in only a few species and isorhamnetin only once. Eighteen flavonoids were identified: the 7-glucoside, 7-diglucoside and 7-rutinoside of both luteolin and tricin, tricin 5-glucoside, apigenin 7-rutinoside, quercetin 3-rutinoside-7-galactoside, isorhamnetin 7-rutinoside, orientin, iso-orientin, vitexin, isovitexin and vitexin 7-*O*-glucoside. Many of the *C*- and *O*-flavonoid glycosides were present as the potassium bisulphate salts and negatively charged compounds were detected in 50% of the species. The distribution patterns are correlated with the taxonomy of the family in several ways. Thus, the Phoenicoideae and Caryotoideae have distinctive flavonoid patterns, there is evidence to support the separation of the subfamilies Phytelephantoideae and Nypoideae, and tricin is a useful marker at tribal level. At the generic level, *Cocos* is clearly separated from *Butia*, and other Cocoseae and *Mascarena* and *Chamaedorea* form well defined groups within the Arecoideae. A numerical analysis of these biochemical data, together with morphological characters, produces a new classification which suggests that the flavonoid data may have more systematic value than is indicated when they are applied to the traditional classification.

INTRODUCTION

THE Palmae, an ancient family of some 3400 species, is taxonomically difficult, classification being based largely on morphological and anatomical characters of the leaf and fruit. There is as yet no complete agreement on the relationships within the palms. Bentham and Hooker[1] and Drude,[2] in two of the earliest classifications, divide the family into 6 tribes and 18 subtribes and 5 subfamilies and many tribes, respectively. A later treatment by Beccari and Pichi-Sermolli[3] describe 8 subfamilies without further subdivision. Moore[4] supports this treatment but suggests a grouping of the subfamilies into two principal groups depending on whether the leaf segments are induplicate or reduplicate in the bud. Burret[5] and Burret and Potztal[6] have also provided a taxonomic treatment of the family. The flavonoid results in this paper are arranged according to a later classification by Potztal[7] in Engler's *Syllabus*, in which 8 subfamilies are recognised, further divided into tribes.

[1] BENTHAM, G. and HOOKER, J. D. (1883) *Genera Plant.* **3** (ii), 870.
[2] DRUDE, O. (1889) in *Die Natürlichen Pflanzenfamilien* (ENGLER, A. and PRANTL, K., eds.), Vol. 3, p. 1,
[3] BECCARI, O. and PICHI-SERMOLLI, R. E. G. (1955) *Webbia* **9**, 1.
[4] MOORE, H. E. (1961) *Am. Hort. Magazine*, **40** (i), 17.
[5] BURRET, M. (1953) *Willdenowia* **1**, 59.
[6] BURRET, M. and POTZTAL, E. (1956) *Willdenowia* **1**, 350.
[7] POTZTAL, E. L. (1964) in Engler-Diels, *Syllabus der Pflanzenfamilien* 579.

Chemically the family has been neglected, probably because of the difficulty of collecting fresh material and getting it authenticated. Most work has been carried out on economically important plants such as *Phoenix dactylifera*, *Cocos nucifera* and other palms cultivated for their oils. Litchfield,[8] in surveying 10 genera, found some correlation of fat content, fatty acid composition and triglyceride composition at the subfamily level. Bennett *et al.*[9] isolated the steroidal estrogen, estrone and cholesterol from the seeds and pollen of *Phoenix dactylifera*, the fruit of which contains the unusual *p*-coumaroyl, caffeoyl and feruloyl esters of shikimic acid.[10,11] Little is known, however, of the flavonoids present in the family. Bate-Smith[12] reports leucoanthocyanins in 13 and flavonols in 2 of 17 taxa. In the course of a chemotaxonomic survey of monocotyledonous families related to the grasses, the flavonoids in 5 palm species were identified.[13] Examination of further taxa revealed a new class of flavone pigments[14] which, both as *O*- and *C*-glycosides, occur like the flavonol persicarin, in conjugation with potassium bisulphate. This discovery stimulated a more complete flavonoid survey which is presented in this paper.

RESULTS

The results of the leaf flavonoid survey are presented in Table 1. Fresh leaf material was collected from plants at the Royal Botanic Gardens, Kew and both fresh and herbarium material was received from abroad. Wherever possible, at least two samples of any one species from different sources were examined. The data in Table 1 refer to flavonoid aglycones detected in the leaf tissue after acid hydrolysis. The aglycones were identified by means of R_f and colour reaction in UV light when compared with standard markers, after removal of interfering flavone *C*-glycosides. These results were confirmed by means of 2-D PC of alcoholic leaf extracts and, where possible, by more detailed identification of individual glycosides (Table 2). Flavone *C*-glycosides were confirmed by their resistance to 4-hr acid hydrolysis and potassium bisulphate salts detected by electrophoresis of direct leaf extracts.

Flavone C-*Glycosides*

These are remarkably common (in 84% of the sample) and add greatly to the complexity of the flavonoid mixtures by their frequent occurrence in two isomeric forms and also in combination with potassium bisulphate. Vitexin, vitexin 7-*O*-glucoside and 7-*O*-rutinoside, orientin and iso-orientin have been identified, together with their potassium bisulphate salts (Table 2). There are also partly characterised flavone *C*-diglycosides present in some taxa. Taxonomically, the *C*-glycosides are of little value except at the family level. Their consistent presence in the palms indicates a relationship with the Gramineae and Cyperaceae, the only related families which also have *C*-glycosides as regular constituents.[13]

Flavone O-*Glycosides*

Tricin, which is known to occur frequently in only two plant families, the grasses and the sedges, is now found to be a common leaf constituent in the Palmae (in 50% of

[8] LITCHFIELD, C. (1970) *Chem. Phys. Lipids* **4**, 96.
[9] BENNETT, R. D., KO, S. and HEFTMANN, E. (1961) *Phytochemistry* **5**, 231.
[10] GOLDSCHMID, O. and HERGERT, H. L. (1961) *TAPPI* **44**, 858.
[11] MAIER, V. P., METZLER, D. M. and HUBER, A. F. (1963) *Biochem. Biophys. Res. Commun.* **14**, 124.
[12] BATE-SMITH, E. C. (1968) *J. Linn. Soc.* **60**, 383.
[13] WILLIAMS, C. A., HARBORNE, J. B. and CLIFFORD, H. T. (1971) *Phytochemistry* **10**, 1059.
[14] HARBORNE, J. B. and WILLIAMS, C. A. (1971) *Z. Naturforsch.* **26b**, 5.

TABLE 1. DISTRIBUTION OF FLAVONES, FLAVONOLS, LEUCOANTHOCYANINS AND NEGATIVELY CHARGED FLAVONOIDS IN THE LEAVES OF THE PALMAE

Palmae* subfamily, tribe, species	Negatively charged flavonoids	Flavone *C*-glycosides	Tricin	Luteolin	Apigenin	Quercetin	Kaempferol	Leuco-anthocyanins	Plant source
Subfamily Cocosoideae									
1. Attaleae									
Attalea speciosa Mart.	–	–	–	–	–	–	–	Cy	K
2. Bactrideae									
Acrocomia mexicana Karw. ex Mart.	–	+	+	+	–	–	–	–	UCSB m2113
Aiphanes caryotaefolia (H. B. K.) Wendl.	–	–	+	+	+	–	–	–	K533-58
Astrocaryum mexicanum Liebm. ex Mart.	–	+	+	+	–	–	–	–	LY
Bactris guineensis Steud.	+	+	–	–	–	–	–	Cy	KG 60-66
3. Elaeideae									
Corozo oleifera Bailey	–	+	–	–	–	–	–	–	K
Elaeis guineensis L.	–	–	–	–	–	–	–	–	K699-58
4. Cocoseae									
Arecastrum romanzoffianum† var *australe* × *Butia capitata*	+	+	–	–	–	+	+	–	M
Arecastrum romanzoffianum (Cham.) Becc.	+	+	+	+	–	+	+	–	K, U
Arecastrum romanzoffianum var *australe* Becc.	+	+	–	–	–	–	–	–	K
Butia bonnetii Becc.	+	+	+	+	–	+	+	Cy	K
Butia capitata Becc.	+	+	+	+	–	+	–	Cy	K661-58, UCSB m2125, U
Butia capitata var *pulposa* Becc.	+	+	+	+	–	–	–	Cy	K 239-62
Butia eriospatha Becc.	+	+	+	+	–	+	–	Cy	K, UCSB 2127
Butia yatay (Mart.) Becc.	+	+	+	–	–	+	–	Cy	K, UCSB m2126
Cocos nucifera L.	–	+	+	–	–	–	–	Cy Pg	LY
Cocos plumosa Lodd. ex Loud.	–	–	–	+	–	+	–	–	K
Jubaea chilensis Baill.	+	+	+	–	–	+	–	Cy	K
Microcoelum weddelianum (Wendl.) H. E. Moore	+	+	+	–	–	–	–	–	CAIRNS
Subfamily Nypoideae									
Nypoideae									
Nypeae									
Nypa fruticans Wurmb.	+	+	+	+	–	–	–	Cy	SING
Subfamily Borassoideae									
Borasseae									
Latania loddigesii Mart.	–	–	+	–	–	–	–	Cy	K
Subfamily Lepidocaryoideae									
1. Lepidocaryeae									
Mauritia flexuosa L.	+	+	–	+	–	–	–	Cy	K

Table 1.—*continued*

Palmae* subfamily, tribe, species	Negatively charged flavonoids	Flavone C-glycosides	Tricin	Luteolin	Apigenin	Quercetin	Kaempferol	Leuco-anthocyanins	Plant source
Subfamily Lepidocaryoideae—*continued*									
2. Calameae									
Calamus ciliaris Blume	−	+	+	+	+	−	−	−	BO
Calamus mulleri H. Wendl. & Drude	−	+	−	+	−	+	+	−	K
Calamus polystachys Becc.	+	+	+	−	+	−	−	Cy	BO
Calamus reinwardtii Mart.	−	−	+	−	−	−	−	−	BO
Daemonorops jenkinsiana Mart.	+	+	−	−	−	−	−	Pg	K
3. Metroxyleae									
Kothalsia echinanetra Becc.	+	+	+	+	−	−	−	Pg	BO
Kothalsia scaphigera Griff.	−	+	+	−	−	−	−	Cy	BO
Kothalsia teysmannii Mig.	−	+	+	−	+	−	−	Pg	BO
Metroxylon sagu Roxb.	−	+	+	−	−	−	−	Cy Pg	SING
Raphia pedunculata Beauv.	nd	+	−	−	−	−	−	Pg	K
Subfamily Coryphoideae									
1. Corypheae									
Brahea calcarea Liebm.	−	−	+	−	−	+	+	Cy Pg	K 189-54
Coccothrinax dussiana L. H. Bailey	+	+	−	−	−	−	−	Cy	K 288-66
Copernicia macroglossa Wendl.	+	+	−	−	−	−	−	Cy	K 495-66
Corypha umbraculifolia L.	−	+	−	+	−	−	−	−	K
Erythea armata Wats.	−	+	+	+	−	+	−	Cy	K, UCSB m23061
Erythea brandegeei Purpus	−	+	+	+	−	+	−	Cy	UCSB m2116+ B65200
Erythea elegans Franceschi ex Becc.	−	+	+	+	−	+	−	Cy	UCSB B65209
Erythea edulis Wats.	−	+	+	+	−	+	−	Cy	UCSB B65188, m2112
Licuala grandis H. Wendl.	−	+	−	−	−	−	−	−	CAIRNS
Licuala muelleri Wendl. & Drude	+	+	+	−	−	−	−	Pg	CAIRNS
Livistona altissima Zoll.	+	+	+	+	−	−	−	−	SING
Livistona australis Mart.	+	+	+	−	−	+	−	Cy Pg	K, UCSB B651240
Livistona chinensis R. Br.	+	+	+	−	−	−	−	Pg	SING UCSB m2296
Livistona kingiana Becc.	−	−	+	−	−	−	−	Pg	SING
Livistona mariae Muell.	+	+	+	−	−	+	−	Cy	K, UCSB m2303
Livistona robinsoniana Becc.	−	+	+	−	−	−	−	Pg	SING
Nannorhops ritchiana Wendl.	+	+	−	−	−	−	−	−	K
Pritchardia affinis Becc.	+	+	−	−	+	−	−	−	K140-56
Pritchardia martii Wendl.	+	+	−	−	+	−	−	−	K
Raphidophyllum hystrix (Pursh) Wendl. & Drude	−	+	+	−	−	−	−	−	UCSB m2129
Sabal beccariana L. H. Bailey	+	+	+	−	−	−	−	−	K454-64
Sabal causiarum Becc.	+	+	+	−	−	−	−	−	UCSB m2122 UCSB m2123
Thrinax parviflora Swartz.	+	+	+	−	−	+	−	Cy	K473-33
Trithrinax acanthocoma Drude	+	−	+	−	−	−	−	Cy	UCSB m2305

Palmae* subfamily, tribe, species	Negatively charged flavonoids	Flavone *C*-glycosides	Tricin	Luteolin	Apigenin	Quercetin	Kaempferol	Leuco-anthocyanins	Plant source
Subfamily Coryphoideae—*continued*									
1. Corypheae—*continued*									
Washingtonia filifera Wendl.	+	+	+	+	−	−	−	Cy	K 35166
Washingtonia robusta Wendl.	+	+	−	−	−	−	+	Cy	K 472-65, UCSB
2. Trachycarpeae									
Acoelorrhape wrightii Wendl.	−	+	−	+	−	−	−	Pg	K45-58
Chamaerops humilis L.	−	+	+	−	−	−	−	Cy	LY, UCSB B65908
Rhapis excelsa Henry ex. Rehder	+	+	+	−	−	−	−	Cy	BRI, UCSB
Rhapis flabelliformis L'Hérit	+	+	+	−	−	−	−	Cy	K, UCSB
Rhapis humilis Bl.	nd	+	+	−	−	−	−	Cy	K
Trachycarpus fortunei (Hook.) Wendl.	−	−	−	−	−	−	−	Cy	K237-66
Subfamily Phoenicoideae									
Phoeniceae									
Phoenix canariensis Hort. ex Chabaud	+	+	+	+	−	+	+	Cy	U, BRI
Phoenix canariensis var *porphyrococca* Vasconcelos et Franco	+	+	+	−	−	−	−	Cy	K
Phoenix dactylifera L.	+	+	+	+	−	−	−	Cy	K
Phoenix farinifera Roxb.	+	+	+	−	−	−	−	Cy	K
Phoenix humilis Royle	−	+	+	+	−	+	−	Cy	UCSB m2302, m2307
Phoenix hanceana Naudin	−	+	+	−	−	−	−	Cy	K
Phoenix loureiri Kunth	+	+	+	−	−	−	−	Cy	K
Phoenix pusilla Gaertn.	+	+	+	−	−	−	−	Cy	K
Phoenix reclinata Jacq.	+	+	+	−	−	−	−	Cy	K, UCSB m1258 B1261
Phoenix roebelenii O'Brien	+	+	+	+	−	−	−	Cy	K
Phoenix rupicola T. Anders.	−	+	+	−	−	−	−	Cy	K
Phoenix sylvestris (L.) Roxb.	+	+	+	−	−	−	−	Cy	K
Phoenix tomentosa Hort. ex. Gentil	+	+	+	−	−	−	−	Cy	K
Subfamily Arecoideae									
1. Areceae									
Areca alicae W. Hill, ex. F. Muell.	+	+	+	+	−	−	−	−	SING
Areca triandra Roxb.	−	+	−	+	−	−	−	Cy	SING
Areca sp.	−	+	−	−	−	+	+	Cy	K
Dictyosperma album (Bory) Wendl.	−	+	−	−	−	−	−	Cy	K
Dictyosperma aureum Wendl. & Drude	−	+	+	−	−	−	−	Cy	K
Dictyosperma furfurceum Wendl. & Drude	−	+	+	−	−	−	−	Cy	K556-64
Gigliolia insignis Becc.	+	+	−	−	−	−	−	−	S
Howeia balmoreana (Moore & Muell.) Becc.	−	+	+	−	−	−	−	Cy	LY, UCSB B651243
Howeia forsteriana (Moore & Muell.) Becc.	−	+	+	+	−	−	−	Cy	LY, UCSB B651244

Table 1.—*continued*

Palmae* subfamily, tribe, species	Negatively charged flavonoids	Flavone C-glycosides	Tricin	Luteolin	Apigenin	Quercetin	Kaempferol	Leuco-anthocyanins	Plant source
Subfamily Arecoideae—*continued*									
1. Areceae—*continued*									
Mascarena lagenicaulis Bailey	+	+	−	+	−	−	−	Cy	K
Mascarena revaughanii Bailey	+	+	−	+	−	−	−	Cy	K
Mascarena verschaffeltii (Wendl.) Bailey	+	+	−	+	−	−	−	Cy	K
Opsiandra maya Cook	+	+	+	+	−	−	−	−	K
Pinanga disticha Blume	−	−	−	−	−	−	−	Cy	GFR
Veitchia merrillii (Beccari) H. E. Moore	+	+	+	−	−	+	+	Cy	CAIRNS
2. Kentieae									
Hydriastele douglasiana F. M. Bail.	−	+	−	−	−	+	−	Cy	CAIRNS
Kentia forsteriana Muell. ex. Wendl.	−	+	−	+	−	−	−	Cy	BRI
3. Linospadiceae									
Linospadix monostachya (Mart.) Wendl.	+	+	−	−	−	+	+	Cy	BRI
Linospadix sp.	−	+	−	−	−	−	−	−	CAIRNS
4. Ptychospermeae									
Archontophoenix cunninghamiana Wendl. & Drude	−	+	−	−	−	−	−	−	K241-66
Ptychosperma macarthurii Wendl.	+	+	−	−	−	+	−	−	K
Ptychosperma propinquum (Becc.) Moore	+	+	−	−	−	−	−	Cy	K
Rhopalostylis baueri Wendl. & Drude	+	+	−	−	−	−	−	Cy	K
Rhopaloblaste singaporensis Benth. & Hook.	+	+	+	−	−	−	−	Cy	SING
Strongylocarpum latius Burret	−	+	−	−	−	+	−	−	CAIRNS
5. Clinostigmeae									
Clinostigma samoënse Wendl.	+	+	−	−	−	+	−	Cy	K
6. Iguanureae									
Iguanura geonomaeformis Mart.	+	−	−	−	−	+	+	Cy	GFR
7. Dypsideae									
Chrysalidocarpus lutescens Wendl.	−	+	−	−	−	+	−	Cy	K
Neodypsis decaryi Jum.	+	+	−	+	−	−	−	Cy	K
Roystonea regia (H.B.K.) Cook	nd	+	−	−	−	+	+	Cy	BRI
8. Ceroxyleae									
Ceroxylon andicola H.B.K.	−	+	−	+	−	−	−	−	K
Gaussia attenuata Becc.	+	+	−	+	−	−	−	−	K569-08
Pseudophoenix sargentii Wendl.	−	−	−	−	−	−	−	−	K502-58
9. Cyrtostachyeae									
Cyrostachys sp.	+	+	−	−	−	−	−	Cy	K486-66
10. Oncospermeae									
11. Oranieae									
Ravenea hildebrandti Wendl. ex. Bouché	−	+	−	−	−	−	−	−	K9028
12. Geonomeae									
13. Iriarteae									
14. Chamaedoreae									
Chamaedorea elatior Mart.	−	−	−	−	−	−	−	−	LY
Chamaedorea elegans Mart.	−	+	−	−	−	−	−	−	CAIRNS
Chamaedorea ernesti-augusti Wendl.	−	−	−	−	−	−	−	−	LY, K
Chamaedorea oblongata Mart.	−	−	−	−	−	−	−	−	LY, K
Chamaedorea tepijilote Liebm.	−	−	−	−	−	−	−	−	LY, K

Palmae* subfamily, tribe, species	Negatively charged/flavonoids	Flavone C-glycosides	Tricin	Luteolin	Apigenin	Quercetid	Kaapferol	Leuco-onthocyanins	Plant source
Subfamily Arecoideae—*continued*									
14. Chamaedoreae—*continued*									
Chamaedorea microspadix Burret	–	–	–	–	–	–	–	–	CAIRNS
Chamaedorea tenella H. Wendl.	–	–	–	–	–	–	–	–	CAIRNS
Subfamily Caryotoideae									
Caryoteae									
Caryota mitis Lour.	–	+	–	–	–	–	–	Cy Pg	LY
Caryota plumosa Hort. ex. Voll. & Brade	–	+	–	–	–	–	–	–	K
Caryota urens L.	–	+	–	–	–	–	–	–	LY
Didymosperma porphyrocarpon Wendl. & Drude	–	–	–	–	–	–	–	–	K
Wallichia densiflora Mart.	–	+	–	+	–	–	–	Cy	K
Subfamily Phytelephantoides									
Phytelephanteae									
Phytelephas macrocarpa Ruis. & Pav.	–	+	–	–	–	+	–	Cy	K

* Classification according to Potztal.[7]
† Also contains isorhamnetin.

Key: Cy—leucocyanidin; Pg—leucopelargonidin; K—The Royal Botanic Gardens, Kew; UCSB—The Herbarium, Botany Dept., The University of California, Santa Barbara, California, U.S.A.; U—material collected and verified by one of us (H.T.C.); BRI—Botanic Garden, Brisbane, Australia; CAIRNS—North Queensland Herbarium, Cairns, Queensland, Australia; LY—Jardin Botanique de la Ville de Lyon, Lyon, France, verified by Professor Berthet; BO—Herbarium Bogoriense, Bogor, Indonesia; SING—Botanic Gardens, Singapore; GFR—material collected and verified by Miss Ruth Evans, of the Botany Dept., Cambridge, at the Gombak Forest Reserve, Singapore; S—material collected by Miss Ruth Evans at Bako National Park, Sarawak; M—material collected and verified by Dr. P. Moyna of the Chemistry Dept., The University, Montevideo, Uruguay; nd—not determined.

the sample) mostly as the 5-glucoside. Other glucosides found (Table 2) include tricin 7-*O*-glucoside, 7-*O*-diglucoside, 7-*O*-rutinoside and 7-*O*-glucoside-KSO_3. Tricin is universal within the Phoenicoideae, quite absent from the Caryotoideae, Borassoideae and Phytelephantoideae and infrequent in the large subfamily Arecoideae (in only 8 of 41 species). Luteolin, present in 30% of the species surveyed, has been found as the 7-*O*-glucoside, 7-*O*-rutinoside and 7-*O*-diglucoside. The complex potassium bisulphate salt, luteolin 7-KSO_3-3′-*O*-glucoside, previously identified in *Mascarena verschaffeltii*[14] was found in two other *Mascarena* species and luteolin 7-KSO_3-3′-*O*-rutinoside was tentatively identified in *Opsiandra maya*, another member of the Areceae. Apigenin occurs in only 5% of palms surveyed and was identified as the 7-*O*-rutinoside in *Rhopaloblaste singaporensis* (Table 2).

Flavonol O-*Glycosides*

Both quercetin and kaempferol are infrequent in the family (in 24 and 10% of species respectively). They were found as the complex 3-*O*-rutinoside-7-*O*-galactosides in *Oreodoxa* *regia.*[13] Quercetin 3-*O*-rutinoside and isorhamnetin 3-*O*-rutinoside were found in the hybrid, *Arecastrum romanzoffianum* vao *australe* × *Butia capitata*, where they co-occur with their potassium bisulphate salts. This is the only record of isorhamnetin in the family, but as it is difficult to distinguish from kaempferol in a general survey it may well be present in other species.

TABLE 2. FLAVONOIDS IDENTIFIED IN LEAVES OF SOME PALMAE SPECIES

Species	Leaf flavonoids identified
Arecastrum romanzoffianum var. *australe* × *Butia capitata*	Quercetin and isorhamnetin 3-rutinosides, quercetin and isorhamnetin 3-rutinoside-KSO_3 Orientin 7-glucoside-KSO_3 Orientin with positive charge
Chamaerops humilis	Tricin 5-glucoside + unidentified flavones
Kentia forsteriana	Luteolin 7-glucoside, flavone *C*-glycosides
Mascarena verschaffeltii	Luteolin 7-rutinoside, luteolin 7-diglucoside, luteolin 7-KSO_3-3′-*O*-glucoside*
Opsiandra maya	Luteolin 7-KSO_3-3′-*O*-rutinoside,* tricin 5-glucoside + other unidentified flavone glycosides
Oreodoxa regia	Kaempferol 3-glucoside, Kaempferol and quercetin 3-rutinoside-7-galactoside, flavone *C*-glycoside
Phoenix dactylifera	Luteolin 7-glucoside, flavone *C*-glycosides
Phoenix roebelenii	Luteolin 7-rutinoside, tricin 7-glucoside-KSO_3, vitexin 7-KSO_3†, isovitexin 7-KSO_3†, iso-orientin 7-KSO_3†, vitexin 7-rutinoside-KSO_3†, Orientin-KSO_3. Luteolin 7-glucoside-KSO_3
Phoenix rupicola	Tricin 7-diglucoside, tricin 7-rutinoside, vitexin, vitexin 7-rutinoside, vitexin 7-glucoside
Phoenix tomentosa	Tricin 7-rutinoside, tricin 7-diglucoside, tricin 5-glucoside, tricin 7-glucoside-KSO_3, vitexin 7-glucoside, vitexin 7-rutinoside Isovitexin 7-KSO_3
Rhopaloblaste singaporensis	Tricin 7-glucoside, tricin 7-rutinoside, tricin 7-glucoside-KSO_3. Apigenin 7-rutinoside, flavone *C*-glycosides
Washingtonia robusta	Luteolin 7-rutinoside-KSO_3†, orientin, orientin 7-KSO_3, orientin 7-glucoside-KSO_3, iso-orientin, vitexin, vitexin 7-glucoside-KSO_3, vitexin 7-KSO_3

* The potassium bisulphate in this pigment was completely determined. In other charged flavones the presence of potassium bisulphate was assumed from their electrophoretic mobility.
† K^+ and HSO_4^- ions detected by TLC (see Experimental).

Potassium Bisulphate Salts

These distinctive compounds occur in half the species studied. Negatively charged flavone *O*- and *C*-glycosides and flavonol *O*-glycosides have all been found. The compound in *Mascarena verschaffeltii*[14] has been identified as the 7-potassium bisulphate of luteolin 3′-glucoside. Most of the other compounds characterised, though, appear to have the bisulphate group attached to the flavone glycoside through the sugar residue (see Experimental). These compounds are notably absent from the Caryotoideae, Phytelephantoideae and Borassoideae and are most common in the Phoenicoideae (in 10 of 13 species). They are present in all the other subfamilies. At the generic level it is interesting that they occur in all the genera of the Cocoseae, except in *Cocos* itself (see below). All the *Erythea* and *Chamaedorea* species examined are also negative.

Leucoanthocyanins

Leucoanthocyanins are present in 66% of the sample. Leucocyanidin is most frequent but leucopelargonidin is also present in the Lepidocaryoideae, Coryphoideae and in a single species of both the Cocosoideae and Caryotoideae.

DISCUSSION

Phytochemical Data and its Bearing on Traditional Classification

The present survey shows that the Palmae have a relatively complex pattern of leaf flavonoids: *C*-glycosylflavones, tricin and luteolin glycosides and their potassium bisulphate salts. Such chemical complexity can be compared with that found in other 'advanced' monocotyledonous families such as the Gramineae and the Cyperaceae,[15] in which *C*-glycosylflavones and tricin glycosides are also common leaf constituents. These compounds are absent from the Typhales, Restionales and the Arales,[13] although tricin has been reported as a rare constituent in the Iridaceae[16] (in three *Crocus* species). In a recent survey Williams *et al.*[13] suggest a grouping together of the Palmae, Cyperaceae and Gramineae on chemical grounds, which is further supported by the present results. In a numerical taxonomic study of the monocotyledons, using a widely representative sample of genera, Clifford[17] also concluded that these three families were closely related. However, it is now clear that the Palmae can be separated from the other two families by the regular presence of flavones as their potassium bisulphate salts. A survey for these compounds in other families is now underway; so far they have been found in 7 species of the Gramineae, in 5 *Saccharum* species and in 2 species of the related genera *Erianthus* and *Miscanthus.*[18] However, some 93 other grass species (23 genera) all proved to be negative. Leucoanthocyanins are common to both the Palmae and Cyperaceae but, as Bate-Smith suggests, the phyletic significance of this character in the Monocotyledons may differ from its role as a 'woody' indicator in the Dicotyledons.

The present results are potentially useful for solving taxonomic problems within the Palmae. For example, the positions of the unusual genera *Nypa* and *Phytelephas* have long been in dispute. Hooker[1] and Burret and Potztal[6] place them both in the Arecoideae, whilst Engler[2] and Hutchinson[19] include them in the separate Phytelephantoideae. The flavonoid data provide some support their separation from the Arecoideae into the two subfamilies Nypoideae and Phytelephantoideae, as in the classifications of Beccari,[3] Potztal[7] and Corner.[20] The flavone, tricin, present in all subfamilies except the Caryotoideae, Borassoideae and Phytelephantoideae may also be a useful marker. Within the Arecoideae, for example, it is common in only one tribe, the Areceae (7 of 15 spp.), being almost completely absent from the others (one record only in *Rhopaloblaste*, Ptychospermeae).

The subfamilies Phoenicoideae, Coryphoideae and Borassoideae each have a relatively uniform flavonoid pattern, while the Arecoideae, a morphologically diverse group, is not

[15] Harborne, J. B. (1971) *Phytochemistry* **10**, 1569.
[16] Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, Academic Press, London; and unpublished results.
[17] Clifford, H. T. (1970) *Bot. J. Linn. Soc.* **63**, suppl. 1, 25.
[18] Williams, C. A. unpublished results.
[19] Hutchinson, J. (1959) *The Families of Flowering Plants*, Vol. 2, Clarendon Press, Oxford.
[20] Corner, E. J. H. (1966) *The Natural History of the Palms*, Weidenfield & Nicolson, London.

surprisingly chemically variable. However, within the Arecoideae there are two genera with distinctive patterns: *Mascarena*, characterized by having luteolin 7-KSO_3-3′-*O*-glucoside, *C*-glycosylflavones and leucoanthocyanins; and *Chamaedorea*, with almost complete lack of flavonoids, except for *C*-glycosylflavones in *Chamaedorea elegans*. Within the Cocosoideae there is chemical evidence to support the separation of the tribe Cocoseae into several genera. All the taxa, which have been split off from *Cocos*, differ from this genus in having negatively charged flavonoids and the newly created genus *Arecastrum* is further distinguished by the absence of leucoanthocyanins.

TABLE 3. CHROMATOGRAPHIC DATA FOR FLAVONOID *O*- AND *C*-GLYCOSIDES AND THEIR POTASSIUM BISULPHATE SALTS

	R_f ($\times$100) in				
Glycoside	BAW	BEW	PhOH	H_2O	15% HOAc
Tricin					
7-Glucoside	34	33	90	0	09
7-Rutinoside	34	44	84	03	27
7-Diglucoside	32	33	34	03	32
* Glucoside-KSO_3(i)	09	10	45	04	18
† Glucoside-KSO_3(ii)	18	23	24	08	39
Luteolin					
7-Glucoside	41	42	59	01	11
7-Glucoside-KSO_3	23	30	21	18	31
7-KSO_3-3′-glucoside	06	18	03	71	69
7-KSO_3-3′-rutinoside	14	—	16	45	61
7-Rutinoside	37	42	60	05	31
7-Rutinoside-KSO_3	21	25	40	25	49
Vitexin	43	38	72	07	24
7-Glucoside	38	45	53	—	52
7-KSO_3	23	31	20	42	53
7-Glucoside-KSO_3	33	45	40	57	70
7-Rutinoside	49	58	66	58	67
7-Rutinoside-KSO_3	17	24	09	75	84
Isovitexin	62	42	89	19	46
7-KSO_3	29	35	44	66	73
Orientin	27	26	30	03	17
7-KSO_3	10	17	07	20	38
7-Glucoside-KSO_3	25	30	23	23	40
Iso-orientin	46	46	62	07	26
7-KSO_3	23	31	20	42	53
Isorhamnetin					
3-Rutinoside	38	53	67	22	49
3-Rutinoside-KSO_3	21	20	23	87	73
Quercetin					
3-Rutinoside	42	47	41	22	48
3-Rutinoside-KSO_3	20	19	16	87	66

* From *Phoenix roebelinii*.
† From *Rhopaloblaste singaporensis* and *Phoenix tomentosa*.

The glycosides of the well defined Phoenicoideae have been studied in sufficient detail (Table 2) to show that within *Phoenix*, different species can be clearly distinguished by their leaf flavonoid patterns. The full extent of infraspecific variation in flavonoids in the Palmae

TABLE 4. COMBINED CLUSTER ANALYSES OF A SAMPLE OF PALM GENERA AND THEIR CHEMICAL AND MORPHOLOGICAL ATTRIBUTES

Taxonomic group I	II	III	IV	V	VI		Attribute group
+*	+	−	+	+*	+*		+* Inflorescence–interfoliar, + = infrafoliar
−	−	−	+*	−	−		+* Seed: Endosperm non-ruminate, + = ruminate
+*	+	+*	+*	+*	+*	1	+* Seedling: 'Ochre' not scaly, + = scaly
+	+	−	+*	+*	−		+* Seed: Raphe branches absent, + = present
+*	+	−	+*	+*	−		+* Ovary: 3-locules, + = 1-locule
+	+	+	−	−	+*		+* Inflorescence: Spathe incomplete; + = complete
+	−	+	+	−	+*		+* Seed: free, + = adnate to endocarp
+	−	+	−	+*	+*	2	+* Germination: remotive; + = admotive
+	−	+	−	−	+*		+* Embryonic axis: straight; + = curved
+	+	+	−	−	+*		+* Stem with central vas. bundle: metaxylem 2 cells wide. + metaxylem 1 cell wide
−	−	+	−	−	−		+ Petiole unarmed
+	−	−	−	−	−	3	+ Tricin present
−	−	−	−	−	−		+ Luteolin
+	+	−	−	−	−		+ Flower unisexual
+	−	−	−	−	−		+ Anther. versatile
−	−	−	−	−	−	4	+ Leaf epidermal cells
+	−	−	−	−	−		+ Flower solitary
−	−	−	+*	−	+		+* ♂ flower, assymmetrical
−	+	+	+	+	+		+ Leaf vessel element: end wall oblique and scalariform
−	+	+	−	+	+		+ Apigenin absent
−	−	−	−	−	−	5	+ Quercetin
−	+	−	−	−	−		+ Kaempferol absent
+	+	+	−	−	+		+ Inflorescence: spathe texture—coriaceous
+	+	+	+	−	+	6	+ Lamina: dorsiventral
+	+	−	−	−	+		+ Seed: endocarp, not woody
+	+*	+*	−	−	−		+* Leaf base: not persistent, + = persistent
+	−	−	−	+	−		+ Stamen: filaments connate
+*	−	−	−	+	−	7	+* Anther: not sagittate, + = sagittate
+	−	+	−	−	−		+ Negatively charged flavonoid present
+	+	−	−	−	+		+ Ovary free
−	−	−	−	+	−	8	+ Flower not sunken in rachis
+*	+	−	−	−	−		+* Lamina: hair: absent, + = present
+	+	+	−	+	−		+ Polycarpic
+	+	+	−	+	+		+ Root, vessel element, end wall: simple perforation, transverse
+	+	+	−	+	+*	9	+* Flower: pedicelled, + = sessile
+	−	+	−	−	−		+ Leucoanthocyanin present
+*	−	−	−	−	+		+* Sex: Dioecious, + = Monoecious
+*	+	−	−	+	+	10	+* Leaf: hypodermis absent, + = present
+	+	−	−	−	+		+ Glycoflavones present

Key: +*, + Members of taxonomic group homogeneous for attribute (presence of asterisk indicates attribute in the first state, absence of asterisk in the second state); − Members of taxonomic group heterogeneous for attribute.

remains to be investigated, since only two or three samples of individual species were normally available for study. However, in the case of *Chamaerops humilis*, 14 samples collected from different habitats in Morocco and South Spain were examined and found to be chemically uniform.

Phytochemical Data and a Computer Analysis of the Palms

One approach to using new phytochemical data for classification is to incorporate the new attributes, together with traditional morphological features, into a new system of classification and then see whether the new data have any significance or not. This has been done with a fairly intense clustering information-gain programme,[21] employing 8 flavonoid characters and 31 morphological features (see Experimental for list). This has been applied to some 40 representative genera from the 70 surveyed for flavonoids (Table 1).

TABLE 5. GENERIC COMPOSITION OF SIX TAXONOMIC GROUPS AND THE FLAVONOID ATTRIBUTES OF EACH GROUP

Group	Genera	Flavonoid attributes
I	*Calamus, Daemonorops*	Negatively charged flavonoids, leucoanthocyanins, glycoflavones present
II	*Acanthophoenix, Archontophoenix, Dictyosperma, Howeia, Hydriastele, Kentia, Rhopalostylis*	Glycoflavones present; apigenin absent
III	*Areca, Mascarena, Pinanga, Ptychosperma, Rhapis, Roystonea*	Negatively charged flavonoids and leucoanthocyanins present; apigenin absent
IV	*Arecastrum, Attalea, Bactris, Chaemadorea, Cocos, Elaeis, Jubaea, Linospadix, Nypa, Phytelephas, Trachycarpus*	No constancy in flavonoid composition
V	*Chamacrops, Copernicia, Latania, Licuala, Livistona, Nannorhops, Phoenix, Sabal*	Apigenin absent
VI	*Caryota, Coccothrinax, Corypha, Raphia, Thrinax, Washingtonia*	Glycoflavones present; apigenin absent

Six groups of palm genera emerged on the basis of the resulting dendrogram. The various attributes were then classified in terms of the palms and here 10 groups emerged from the dendrogram. The two analyses were then combined and the results are shown in Table 4. The six palm groups of genera are listed in Table 5, together with an indication of the constancy of the various flavonoid characters.

The results show, as before, that some biochemical characters (glycoflavones, negatively charged flavones) are much more useful than others (luteolin, quercetin). They also indicate that some of the 6 taxonomic groups can be clearly separated on phytochemical grounds, whereas others are not.

One novel feature of this approach to classification is that it indicates associations between chemical and morphological features that are not otherwise apparent (Table 6). Some of these associations may have no real significance, but others may well reflect ecological correlations and indicate that some of the flavonoid characters have some adaptive value within the palm family. The 6 taxonomic groupings proposed (Table 5)

[21] LANCE, G. N. and WILLIAMS, W. T. (1967) *Australian Comp. J.* **1,** 15.

are only provisional and their validity will have to be tested by palm taxonomists. Only then, will it be apparent whether they are of utility in providing a revision of the traditional classification.

TABLE 6. ASSOCIATION OF ATTRIBUTES IN THE PALMAE

1. Tricin and luteolin are associated with each other and with the armature of the petiole
2. The absence of apigenin and kaempferol and the presence of quercetin are interassociated with one another and with the form of the vessel elements
3. Negatively charged flavonoids are associated with anther structure
4. Leucoanthocyanin is associated with root vessel elements and floral structure
5. Glycoflavones are associated with the sexuality of the plants and their leaf anatomy

EXPERIMENTAL

Verified plant material was received from various sources, details of which are given in Table 1. All the leaf samples used were fresh except those from the Herbarium, University of California, Santa Barbara (U.C.S.B.). Fresh material from abroad was sent by air and analysed immediately on arrival. Voucher specimens are available of most plants examined in the respective herbaria of the donors (see footnote to Table 1); population samples of *Chamaerops humilis* are deposited at R.D.G.

Identification of flavonoids. Flavonoid aglycones were identified in acid hydrolysed leaf extracts using standard procedures and by comparison with authentic markers after first overunning all chromatograms in H_2O to remove interfering flavone *C*-glycosides. Solvents used for chromatography on Whatman No. 1 paper were: BAW, Forestal, 50% HOAc and Phenol. Direct 95% EtOH extracts of all tissues were chromatographed 2-D in BAW and 15% HOAc. Known glycosides, isolated and purified by standard procedures, 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 detected by 4-hr acid hydrolysis, extraction in amyl alcohol and PC against authentic markers in BAW and H_2O.

Potassium bisulphate salts were detected by electrophoresis of direct leaf extracts on Whatman No. 3 paper in 2·5% HCO_2H 7–8% HOAc (1:1) pH 2·2 buffer for 3 hr at 400 mV. Electrophoretic mobilities on the purified salts compared with luteolin 7-KSO_3-3′-*O*-glucoside as 100 are: Vitexin 7-KSO_3 42; Isovitexin 7-KSO_3 55; Orientin 7-KSO_3 14; Iso-orientin 7-KSO_3 41; Luteolin 7-rutinoside-KSO_3 29; Tricin glucoside-KSO_3 (i) 11; Tricin glucoside-KSO_3 (ii) 28. Luteolin 7-KSO_3-3′-*O*-glucoside was previously identified:[12] the potassium ion was determined by flame spectrophotometry and the sulphate by precipitation as the insoluble magnesium salt. The ions were present in a 1:1 ratio. In the present work, individual salts were hydrolysed for 0·5 hr with 2N HCl and the K^+ and HSO_4^- ions detected qualitatively by TLC. The flavone aglycones were first removed by extraction into EtOAc and the aqueous residue evaporated to dryness until all the HCl was driven off and then dissolved in 0·1 ml H_2O. This extract was then co-chromatographed with $KHSO_4$ on cellulose plates, solvent 20% 0·1 NHCl in EtOH,[22] and sprayed with sodium cobaltous hexanitrite. K^+ (as the Cl) appears as a yellow green spot and HSO_4^- as a white spot on a pale yellow background, R_fs 16 and 72 respectively. Chromatographic data for the potassium bisulphate salts of flavone *O*- and *C*-glycosides are given in Table 3. In general, the effect on R_f of potassium bisulphate substitution in the flavone glycosides is to decrease mobility in non-aqueous solvents. There are also some minor, but variable effects on the UV spectra in MeOH; there may be an increase in absorption at *ca.* 265–270 and a slight hypsochromic effect on the longwave band was noted in a few cases. This may be due to the effect of the charge properties of the salt and spectral measurement in buffer may be necessary to avoid such anomalous behaviour.

Morphological and chemical characters used for the computer analysis. The morphological and anatomical data were based on original observations on living plants. The various attributes used in the computer analysis were as follows: Sex of plant—monoecious/dioecious; spadix—interfoliar/infrafoliar, polycarpic/monocarpic; flowers—unisexual/bisexual, solitary/clustered, sessile/pedicelled, sunken in cavity of spike/not sunken; male flower—calyx connate/free, petal connate/free, symmetrical/asymmetrical, stamens—filaments connate to very short/free, anthers sagittate/not, anthers versatile/not; female flower—ovary free/fused, ovary 1-celled/3-celled; Spadix—interfoliar/infrafoliar; spathe—complete/incomplete; spathe texture—woody/coriaceous; seed—endocarp woody/not woody, seed—adnate to endocarp/free, raphe branches present/absent. Embryonic axis—curved/straight; embryo position—(a) lateral, (b) apical, (c) basal; germination—remotive/admotive; seedling—cataphylls scaly/smooth; seed—ruminate/nonruminate; seedling 1st green leaf (a) simple/lanceolate, (b) bipid, (c) compound.

[22] BEERSTRECHER, E. (1950) *Anal. Chem.* 22 (9), 1200.

Adult plant. Stem—annulate/not too vaguely. Stem anatomy—vascular bundle with (a) one metaxylem, (b) or more; stem—solitary/clustered, vessel element end wall transverse/oblique and scalariform. Leaf lamina—epidermal cells—sinuous/not (adaxial), isolateral/dorsiventral, hypodermis present/absent, hair present/absent. Leaf anatomy—vessel element simple, transverse/oblique scalariform; leaf bases—persistent on stem/not (half-way on trunk); leaf petiole—armed/not armed; root anatomy: vessel element end wall simple transverse/oblique, scalariform. Biochemistry—negatively charged flavones p/a; glycoflavones p/a; tricin p/a; luteolin p/a; apigenin p/a; quercetin p/a; kaempferol p/a; leucoanthocyanin p/a (p—present/a—absent).

Adult plant. Leaf—pinnate/palmate, leaflet or segment induplicate/reduplicate; fruit—scaly/not; inflorescence—branched/not.

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