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ENANTIOMERISM: A CHARACTERISTIC OF THE PROANTHOCYANIDIN CHEMISTRY OF THE MONOCOTYLEDONAE

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Key Word Index—Monocotyledonae; proanthocyanidins; *enantio*-2,3-*cis*-procyanidin units; distribution.

Abstract—Proanthocyanidin polymers containing 2,3-*cis*-procyanidin units with a partly racemic mixture of 2*R* (the normal configuration) and 2*S* units are widespread in the Monocotyledonae, being present in several families in the Arecidae, Commelinidae and Lilliidae.

INTRODUCTION

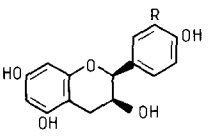
Proanthocyanidins consist of chains of flavan-3-ol units linked by acid-labile carbon to carbon bonds [1]. They are widely distributed in the plant kingdom, being generally associated with plants of a woody habit [2]. However, it is evident from earlier surveys by Bate-Smith [3] and the Reading group [4–12] that they are also common constituents of the Monocotyledonae, in spite of their generally herbaceous character.

Proanthocyanidins are very widespread leaf constituents in the Palmae [4]. However, of particular interest is the fact that ent-epicatechin (**1**)* occurs as a natural product in several palm species [13]. Additionally, ent-procyanidin B2 [(**2**), ent-epicatechin-(4α → 8)-ent-epicatechin] was also isolated [15]. These observations were of outstanding interest being the first authenticated examples of procyanidins and catechins with a 2*S* configuration, in contrast to the 2*R* configuration observed in most plants [2]. This prompted us to reinvestigate the proanthocyanidins of palm species to determine if the 2*S* units were also a feature of the polymers [16] and subsequently to extend the study to the proanthocyanidins of other monocotyledonous plants.

RESULTS AND DISCUSSION

Extraction of the tannins from the leaves of *Phoenix canariensis* and fruits of *Cocos nucifera* and *Rhopalostylis sapida* yielded procyanidin (PC) polymers which ^{13}C NMR [1] showed to consist entirely of 2,3-*cis* PC units. Optical rotation, and resolution of the diastereomeric dimers **3** and **4**, formed by reaction of epicatechin (**5**) with the *Phoenix* polymer in acid solution, showed that it consisted of both 2*S* and 2*R* 2,3-*cis* PC units, in a ratio of 3:1 [16]. The palms, therefore, synthesize polymers containing PC units with both enantiomeric forms.

Further work by Delle Monache *et al.* [13] showed that ent-epicatechin (**1**) occurred in the leaves or fruit of six palm species, and in one case, *Livinstona chinensis*, ent-epiafzelechin (**6**) was also isolated. In contrast, recent work on the procyanidins of betel nuts (*Areca catechu* fruit) showed that the 2,3-*cis* PC units of the tri- and tetrameric flavan-3-ols all possessed the normal 2*R* con-



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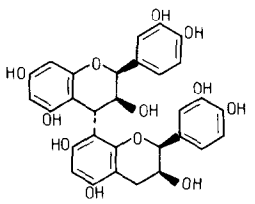

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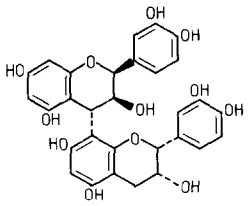

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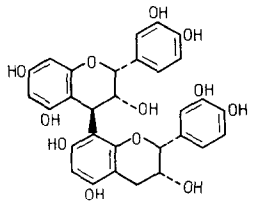

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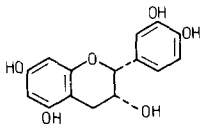

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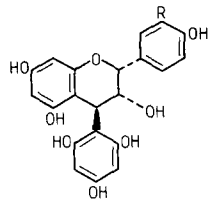

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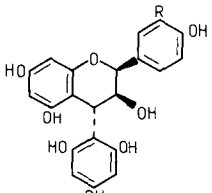
7b R = OH



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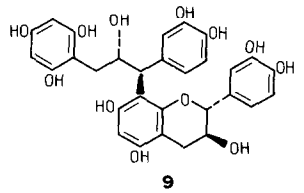

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*The system of nomenclature used for proanthocyanidins in this paper is described in detail elsewhere [14].

figuration [17]. Therefore, enantiomerism is not a universal feature of the chemistry of PC units in palm tannins.

The above observations prompted us to study further the proanthocyanidins of the Monocotyledonae, especially as only a few species had previously been investigated [15, 17–19]. This class represents thousands of species and, therefore, the aims of this survey were, of necessity, severely narrowed, as follows.

The Monocotyledonae may be divided into four groups: the Alismatidae, Commelinidae, Arecidae and Lilliidae, which are further divided into 20 orders. Our aim was to survey at least one species from each order and, ideally, one species from each of the 50 families constituting these orders,* to establish whether the enantiomerism observed in Palmae procyanidins is unique to this family, or a more widespread phenomenon. A secondary aim was to obtain some idea of the range of proanthocyanidin structural types in the Monocotyledonae. Regrettably it was not possible to survey all families because of the difficulty of obtaining suitable plant material.

The plants were surveyed initially by detecting the presence of proanthocyanidins by the vanillin–hydrochloric acid test [21]. If proanthocyanidins were present the polymer was isolated using methods outlined previously [1]. The purified tannin was then analysed by polarimetry [1], and IR spectroscopy [22], and the ratio of proanthocyanidin units with differing oxidation pattern was determined by acid hydrolysis [1]. Where possible these data were corroborated by ^{13}C NMR [1], especially if enantiomerism was indicated by a strong negative rotation [16] coupled with IR evidence that the polymer consisted of 2,3-*cis* units. The data obtained for the various polymers are given in Table 1.

Table 1. Proanthocyanidins of the Monocotyledonae

Group	Order	Family	Species	Organ	Oxidation pattern (PC:PD:PP)	Stereochemistry (*cis*:*trans*)	Optical rotation $([\alpha]_{578}^{30})$	Enantiomerism
Arecidae	Arecales	Palmae	*Phoenix canariensis* Hort. ex Chabaud	frond	100:0:0	100:0	− 91	+
			Rhopalostylis sapida Wendl. et Drude	fruit	100:0:0	100:0	− 77	+
			Cocos nucifera L.	fruit	100:0:0	100:0	− 75	+
	Pandanales	Pandanaceae	*Freycinetia baueriana* Endl. ssp. *banksii* (A. Cunn.) B. L. Stone	frond	42:58:0	90:10	+ 125	−
		Lemnaceae	*Lemna minor* L.	whole plant	100:0:0	72:28	+ 38	−
		Araceae	*Zantedeschia aethiopica** (L.) Sarengel	fruit	44:0:56	100:0	+ 164	−
			Zantedeschia aethiopica	fruit	15:85:0	25:75	− 195	−
			Zantedeschia rehmannii Engl.	fruit	100:0:0	87:13	+ 110	−
Alismatidae	Alismatales	Alismataceae	*Alisma lanceolatum* With.	leaf, fruit		no tannin		
	Hydrocharitales	Hydrocharitaceae	*Elodea canadensis* Mich.	leaf		no tannin		
	Najadales	Aponogetonaceae	*Aponogeton distachyus* L. f.	leaf	54:0:46	80:20	+ 74	−
		Juncaginaceae	*Triglochin striatum* Ruiz et Pav	inflorescence		no tannin		
		Zosteriaceae	*Zostera muelleri* Aschers.	leaf		no tannin		

Occurrence of proanthocyanidins in the Monocotyledonae

In agreement with earlier surveys of leaf flavonoids [3, 12] it may be seen that proanthocyanidins, while present in most families of the Monocotyledonae, are absent, or rare, in a few families. This is especially true of the Alismatidae, which contains largely aquatic or aquatic-associated plants. Proanthocyanidins were isolated from only one species, *Aponogeton distachyus*, and were absent from plants surveyed in the Alismataceae, Hydrocharitaceae, Juncaginaceae and Zosteraceae. Of those families not surveyed earlier work had shown that proanthocyanidins are apparently absent from the Najadaceae, Zannichelliaceae, Potamogetonaceae and Ruppiaceae, and present in the Posidoniaceae and Cymodoceaceae [7].

Proanthocyanidins were present in all species surveyed in the Arecidae. No species were available from the Cyclanthaceae, but according to Bate-Smith [3] tannins are absent from *Cyclanthus* species.

All families in the Commelinidae contained proanthocyanidins except the Commelinaceae and Bromeliaceae. This latter observation agrees with an earlier very extensive survey of this family by Williams [23]. It was earlier considered that proanthocyanidins were very rare in the Juncaceae [5]. However, whereas this appears to be true for the vegetative tissue, the inflorescences were a rich

*We have used the classification of the Monocotyledonae outlined in ref. [20].

Commelinidae	Commelinales	Commelinaceae	*Tradescantia fluminensis* Vell.	leaf	no tannin			
	Restionales	Restionaceae	*Leptocarpus similis* Edgar	inflorescence	100:0:0	74:26	+ 45	−
	Poales	Graminaceae	*Hordeum vulgare* L. cv Gwylan	ears	40:60:0	12:78	−259	−
			Sorghum vulgare L. cv Sudax	seed	92:8:0	77:23	+ 76	−
	Juncales	Juncaceae	*Juncus bufonius* L.	inflorescence	61:39:0	94:6	+142	−
	Cyperales	Cyperaceae	*Cyperus eragrostis* Lam.	inflorescence	100:0:0	88:12	+116	−
	Typhales	Typhaceae	*Typha orientalis* C. B. Presl.	inflorescence	83:0:17	100:0	−113	+
	Bromeliales	Bromeliaceae	*Ananas comosus* (L.) Merr.	fruit, leaf	no tannin			
	Zingiberales	Musaceae	*Musa sapientum* L.	fruit skin	78:22:0	100:0	− 69	+
		Strelitziaceae	*Strelitzia reginae* (Banks) Thunb.	leaf	75:0:22	100:0	− 81	+
		Zingiberaceae	*Hedychium flavescens* Carey ex Roscoe	leaf	67:33:0	80:20	+ 77	−
			Hedychium gardnerianum Ker-Gawl	leaf	53:47:0	80:20	+ 76	−
		Cannaceae	*Canna indica* L.	leaf	76:24:0	70:30	+ 27	−
		Marantaceae	*Ctenanthe oppenheimiana* K. Schum. cv *tricolor*	leaf	67:33:0	77:23	+ 59	−
Lilliidae	Liliales	Ponditeriaceae	*Eichhornia crassipes*	leaf	56:19:25	40:60	−120	−
			(mart.) Solms-Lamb	root	61:0:39	50:50	− 73	−
		Iridaceae	*Gladiolus* cv	leaf	43:57:0	16:84	−238	−
			Iris germanica L.	fruit	89:11:0	65:35	0	−
			Iris pseudacorus L.	fruit	20:80:0	40:60	−125	−
				leaf	100:0:0	65:35	0	−
			Watsonia pyramidata Klatt.	leaf	0:100:0	5:95	−295	−
			Watsonia ardernei F. Sander	leaf	0:100:0	1:99	−315	−
		Liliaceae	*Astelia fragrans* Col.	inflorescence	93:7:0	45:55	− 98	−
		Amaryllidaceae	*Amaryllis belladona* L.	leaf	no tannin			
			Narcissus (various)	leaf	no tannin			
		Agavaceae	*Phormium cookianum* Le Jolis	leaf	100:0:0	78:22	+ 66	−
		Haemodoraceae	*Anigozanthos flavidus* Steud.	leaf	20:0:80	100:0	− 15	+
		Smilacaceae	*Ripogonum scandens* J. R. et G. Forst.	leaf	100:0:0	100:0	− 73	+

*Variegated variety.

source of proanthocyanidins in several species we tested. This was a most interesting observation which should be pursued further to see if it is generally true for the family.

Proanthocyanidins were present in all families tested in the Lilliidae except the Amaryllidaceae and Orchidaceae. This is consistent with earlier observations [3, 24]. Unfortunately many of the Lilliidae families contain species which are exceedingly difficult to obtain and, consequently, species in the Philydraceae, Velloziaceae, Taccaceae, Stemnomaceae, Cyanastraceae and Burmanniaceae were not surveyed.

Proanthocyanidin structures

The polymers isolated from Monocotyledonae species were similar to those earlier isolated from ferns, pines and the Dicotyledonae [25]. As was observed in these earlier surveys, polymers containing mostly 2,3-*cis* PC units are by far the commonest type. Units with a 2,3-*trans* stereochemistry predominated in only six of the 33 polymers isolated, and four of these were in the Iridaceae. As had been noted for the Dicotyledonae, there is a strong correlation between 2,3-*trans* stereochemistry and polymers containing prodelphinidin (PD) units. The PD polymers isolated in this study from *Hordeum*, *Zantedeschia*, *Gladiolus*, *Iris* and *Watsonia* were, therefore, very similar to PD polymers isolated from *Ribes* [25].

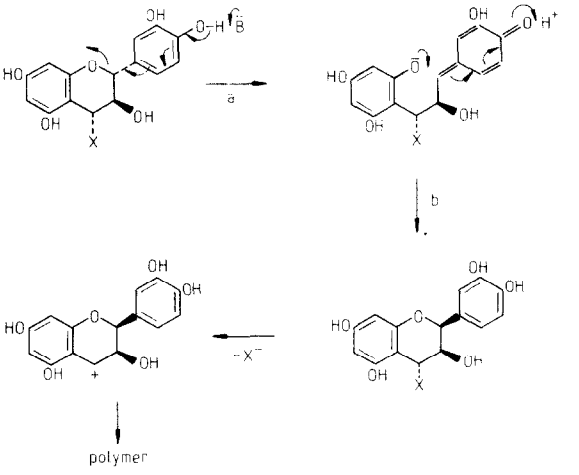
The presence of propelargonidin (PP) units, varying from significant to major components of the polymers from species of *Zantedeschia*, *Aponogeton*, *Typha*, *Strelitzia* and *Anigozanthos* is of interest. It may indicate that PP units are a much more common feature of tannins of the Monocotyledonae than those of the Dicotyledonae. Literature reports of propelargonidins are largely confined to tropical species in the latter group. PP units may be readily recognized by the high chromatographic mobility of the acid hydrolysis pigment and a characteristic resonance at δ128.9 in the ^{13}C NMR spectrum, due to C-2′ and C-6′ of the B-ring. Very small amounts of PP units may be detected in the ^{13}C NMR spectrum using the δ128.9 signal; thus PP units were found to be constituents of polymers from *Cyathea dealbata* (a fern), *Eucalyptus diversicolor*, *Lotus tenuifolius* and *Vitis vinifera* (Dicotyledonae) [Foo, L. Y. and Porter, L. J., unpublished results].

Enantiomerism

The survey showed clearly that enantiomerism of the proanthocyanidin polymers is not confined to the Palmae. The phenomenon was detected in two of the three other groups: the Commelinidae and Lilliidae, and was observed in five families: Typhaceae, Musaceae, Strelitziaceae, Haemodoraceae and Smilacaceae. The structure of each polymer manifesting enantiomerism was basically similar to that observed in the palms, i.e. it contained only 2,3-*cis* units in which 2*S* predominated over 2*R* units. In contrast to the palm tannins, four of the five polymers contained units with two different oxidation patterns: *Musa sapientum* PC and PD units; *Typha orientalis*, *Strelitzia regina* and *Anigozanthos flavidus* PC plus the less common PP units.

The polymer from *Typha orientalis* was available in larger amounts and was degraded with acid and phloroglucinol to form the enantiomeric pairs epiafzelechin-(4β → 2)-phloroglucinol (**7a**), ent-epiafzelechin-(4α → 2)-phloroglucinol (**8a**) and epicatechin-(4β → 2)-phloroglucinol (**7b**), ent-epicatechin-(4α → 2)-phloroglucinol (**8b**). Measurement of the specific rotation of the partly racemic mixtures of the products **7a**/**8a**, **7b**/**8b** showed that the 2*S* unit greatly predominates in each case. This result implies that the biosynthesis of the PP and PC units must be under similar control and the mechanism leading to enantiomerism must be largely independent of B-ring oxidation pattern.

The mechanism by which both 2*S* and 2*R* isomers are formed is of great interest. Perhaps of some significance is the fact that ent-epicatechin and catechin were isolated from the same palm species [13]. The plant obviously had the capability of producing both 2,3-*cis* and 2,3-*trans* units, albeit at the most reduced level. Another interesting recent discovery is that of Nonaka and Nishioka [26] who isolated chalcan–flavan dimers from *Uncaria gambir* in which the upper catechin unit has been reductively ring-opened (compound **9**). These dimers co-exist in the plant with normal proanthocyanidin dimers. These observations make enzyme-mediated ring-opening of the pyran ring of a proanthocyanidin unit a feasible proposition, and also imply that the palms are capable of producing 2*R* units with a 2,3-*trans* configuration. We, therefore, propose that the 2*S* units with a 2,3-*cis* configuration arise from 2*R* units with a 2,3-*trans* configuration by the action of an epimerase enzyme complex (see Scheme 1).



Scheme 1. The formation of 2*S* 2,3-*cis* procyanidin units. Steps (a) and (b) are epimerase enzyme mediated. The 4-substituent *X* implies a suitable binding to the enzyme surface.

The above mechanism also implies that *Areca catechu* procyanidins may not be an exception to palm species processing polymers with both 2*R* and 2*S* procyanidin units. Rather, this species may not be capable of producing 2*R* 2,3-*trans* procyanidin units which provide the substrate for the epimerase enzyme complex and, hence, they possess only 2*R* 2,3-*cis* procyanidin units.

Phylogenetic significance

The outstanding feature of the distribution of enantiomerism in proanthocyanidins of the Monocotyledonae is its highly disjunct nature. This suggests that the origin of the enzyme system responsible for the

formation of 2*S* units lies very early in the evolution of the group. The presence of proanthocyanidin enantiomerism in plants could have significance at the order or family level, and *may* imply closer phylogenetic relationships between those species possessing it, than between those without it. However, the current survey includes too few species to give a strong lead, one way or the other. The current survey clearly shows, however, that the phenomenon is a feature of the Monocotyledonae as a whole, rather than a group or order, and on current evidence is not paralleled in the procyanidin chemistry of the Dicotyledonae.*

EXPERIMENTAL

Plant material was generally collected from local Wellington commercial, forest, or garden sources and identified by Mr. A. P. Druce; except for *Cocos nucifera* supplied from the Cook Islands by Dr. R. A. Fullerton; *Hordeum vulgare* and *Sorghum vulgare* were local crops from the Canterbury and Waikato areas, respectively; *Hedychium* species were supplied by Mr. A. E. Elser from the Auckland area; and *Eichhornia crassipes* by Dr. M. H. Timperley from the Waikato.

Tannin extractions (from 500–1000 g of fresh plant material) and purifications were as described previously [1]. IR spectra were run as KBr disks [22]. Specific rotations were obtained in H_2O at 30° on 0.1–0.6% w/v solns. ^{13}C NMR spectra were run on 15% w/v solns in Me_2CO-d_6–H_2O (1:1, v/v) at 30° using a TMS external standard [1]. HPLC was performed on Waters μBondapak C18 columns using MeOH–1% HOAc (20:80 v/v) as eluant. TLC was on Schleicher and Schull cellulose using *t*-BuOH–HOAc–H_2O (3:1:1, solvent A) and HOAc–H_2O (6:94, solvent B).

Phloroglucinol derivatives. Tannin from *Typha orientalis* (1 g) was heated under N_2 for 18 hr with HOAc (0.2 ml) and phloroglucinol (1 g) in absolute EtOH (20 ml) in a sealed vial at 95°. The solvent was evaporated at 20° *in vacuo*, and the solid product was dissolved in H_2O (100 ml) and extracted with EtOAc (4 × 100 ml). The EtOAc-soluble fraction (0.8 g) was dissolved in EtOH (2 ml) and chromatographed on Sephadex LH-20 in EtOH. The order of elution was phloroglucinol, a mixture of catechin and epicatechin (ratio 3:1, measured by HPLC, rotations not determined), and then a mixture of epiafzelechin-(4β → 2)-phloroglucinol (**7a**) and ent-epiafzelechin-(4α → 2)-phloroglucinol (**8a**). [R_f(A) 0.75, R_f(B) 0.45, purified by HPLC, retention vol. 11.6 ml, $[\alpha]_{578}^{30}$ −54° (MeOH; *c* 0.35), optical purity of ent isomer 44% (assuming that $[\alpha]_{578}^{30}$ for the pure diastereomer is the same as that of the epicatechin derivative [14])]. It produced pelargonidin chloride and phloroglucinol on heating with *t*-BuOH–HCl.

The next fraction from LH-20 yielded a mixture of epicatechin-(4β → 2)-phloroglucinol (**7b**) and ent-epicatechin-(4α → 2)-phloroglucinol (**8b**). [R_f(A) 0.51 R_f (B) 0.58, purified by HPLC, retention vol. 8.2 ml, $[\alpha]_{578}^{30}$ −87° (MeOH; *c* 0.27), optical purity of ent isomer 71%]. It co-chromatographed with authentic (**7b**) on TLC and HPLC, and produced cyanidin chloride and phloroglucinol on heating with *t*-BuOH–HCl.

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*The phenomenon is paralleled, however, in the leucofisetinidin chemistry of the Leguminosae, where *Acacia mearnsii* contains 2*R*, 3*S*, 4*R*, and *Schinopsis lorentzii* the 2*S*, 3*R*, 4*S* isomers of leucofisetinidin.

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