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VARIATIONS IN FLAVONOID PATTERNS WITHIN THE GENUS *CHONDROPETALUM* (RESTIONACEAE)

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Key Word Index—*Chondropetalum*; Restionaceae; culms; flavonoids; myricetin derivatives; syringetin 3-arabinoside; gossypetin 7-methyl ether; herbacetin 4′-methyl ether; chemotaxonomy.

Abstract—HPLC and chemical analyses of the flavonoids in culms of 11 *Chondropetalum* species divide the genus into two groups: seven, with glycosides of myricetin, larycitrin and syringetin; and four, with glycosides of kaempferol, quercetin, gossypetin, gossypetin 7-methyl ether and herbacetin 4′-methyl ether. This chemical dichotomy is correlated with anatomical differences and confirms the view that the genus requires taxonomic revision. HPLC measurements on those species with myricetin derivatives show that taxa with a qualitatively similar pattern of glycosides can be readily separated on quantitative grounds. Syringetin 3-arabinoside and a glycoside of herbacetin 4′-methyl ether are reported for the first time from the genus.

INTRODUCTION

The Restionaceae, a rush-like group of mainly dioecious xeromorphic plants, grow almost exclusively either in South Africa or in Australasia [1]. The plants from the two continents can be separated anatomically [2] and palynologically [3], while earlier studies of the flavonoids present showed excellent correlations in their patterns with both geography and anatomy. The Australasian species generally lacked proanthocyanidins and contained characteristically either 8-hydroxyflavones or 8-hydroxyflavonols [4] while the South African species were rich in proanthocyanidins and also had common flavonols, flavones and glycosylflavones [5]. In addition, two of the South African genera, namely *Chondropetalum* and *Elegia*, were marked off from the rest of the family by the presence of myricetin, its 3′-monomethyl ether larycitrin, and its 3′,5′-dimethyl ether syringetin. These methyl ethers are particularly rare substances and their discovery in these plants constituted only the first and third reports, respectively, of their occurrence in the monocotyledons [5].

In view of the distinctive nature of the flavonoids of these two South African genera, more extensive studies have now been developed. This seemed particularly appropriate, since the taxonomy of the plants is difficult and new characters were needed to assist with systematic revision [6]. The present paper describes a detailed analysis of the flavonoids in 11 species of *Chondropetalum*, only three of which were examined in the earlier survey [5]. This genus was once included in *Restio* and has had a complex systematic history. According to Cutler [2], it now consists of 18 species, which divide anatomically into two groups, according to whether the epidermal cells are 1-layered or 2–4-layered. As many species as could be obtained, including members of both anatomical groups, were compared chemically during the present study. Recent developments in reversed-phase HPLC on C_8 columns [7] allowed us to measure the flavonoids quantitatively and these data are also recorded here.

RESULTS

Flavonoid aglycones

The plants are leafless; therefore all chemical analyses were confined to the aerial culm (or stem) tissues, after separation from the inflorescences. The flavonol aglycones detected after acid hydrolysis of culm tissue of the 11 *Chondropetalum* species available are shown in Table 1, with an indication of the relative quantities. As can be seen, the plants fall into two groups. Group A species contain myricetin, larycitrin and syringetin in varying amounts. This pattern is identical to that found earlier in three of these species and also in six *Elegia* species [5] and this finding confirms the close morphological relationship known to exist between these two genera. Group B species, by contrast, completely lack myricetin derivatives and have instead quercetin and kaempferol, together with gossypetin (in two spp.), gossypetin 7-methyl ether (in three spp.) and herbacetin 4′-methyl ether (in three spp.).

As indicated earlier, 8-hydroxyflavonols such as gossypetin are much more characteristic of Australasian Restionaceae [4] and the only earlier finding of gossypetin in South African taxa was of the 7-methyl ether in *Staberoha cernua* [5]. The present discovery of gossypetin and herbacetin (8-hydroxykaempferol) derivatives in three further African taxa indicates that there is possibly a closer similarity in chemical pattern between the plants of the two continents than at first appeared. It also appears from these results that the synthesis of 8-hydroxyflavonols is basic to the family Restionaceae. While being retained widely within the Australasian taxa, they have generally been lost from the African plants. Their occasional occurrences in the latter taxa would then represent the retention of a chemically primitive feature and this agrees very well with the morphological evidence.

Both the gossypetin and herbacetin methyl ethers are more labile than other flavonols and they can be lost by oxidation during normal acid hydrolysis of plants ex-

Table 1. Flavonol aglycones of *Chondropetalum* species

Species	My	La	Sy	Qu	Km	Go	G7	H4′
Group A								
C. mucronatum (Nees) Pillans	+++	−	−	−	−	−	−	−
C. nudum Rottb.	++	+++	+	−	−	−	−	−
C. hookerianum (Mast.) Pillans	++	++	+	−	−	−	−	−
C. deustum Rottb.	++	+	++	−	−	−	−	−
C. rectum (Mast.) Pillans	++	++	++	−	−	−	−	−
C. microcarpum (Kunth) Pillans	+	++	+++	−	−	−	−	−
C. tectorum (L.f.) Pillans	−	−	+++	−	−	−	−	−
Group B								
C. andreaeanum Pillans	−	−	−	+	+	+	+	+
C. chartaceum (Pillans) Pillans	−	−	−	+	+	+	+	+
C. macrocarpum (Kunth) Pillans	−	−	−	+++	+	−	−	−
C. paniculatum (Mast.) Pillans	−	−	−	+	+	−	+	+

Key: My, myricetin; La, larycitrin; Sy, syringetin; Qu, quercetin; Km, kaempferol; Go, gossypetin; G7, gossypetin 7-methyl ether; H4′, herbacetin 4′-methyl ether; +++, > 50%; ++, 25–50%; +, 0–25%.

tracts. Hydrolysis under nitrogen is therefore necessary and such precautions were carried out routinely. Gossypetin 7-methyl ether has been described before in other Restionaceae and it was readily identified from its properties (see Experimental) and by direct comparison with an authentic specimen [5, 8]. Herbacetin 4′-methyl ether appears to be a new flavonol derivative and was identified by spectral and chromatographic procedures. It was quite distinct from the two known monomethyl ethers of herbacetin (8-hydroxykaempferol), namely the 7- and 8-methyl ethers, pollenitin and sexangularetin [9, 10], samples of which were available for comparison (see Experimental). This is the first discovery of a herbacetin derivative in the family.

Proanthocyanidins were found to be present as well in all 11 *Chondropetalum* species. Interestingly enough, the patterns were different in the two groups of plants, i.e. species of group A contained procyanidin and prodelphinidin, whereas species of group B contained procyanidin alone. Thus prodelphinidin is restricted in its occurrence to those species in which the flavonol, myricetin, with the same hydroxylation pattern, is present.

Glycosidic patterns

Previous analyses of three species of group A, namely *C. hookerianum*, *C. mucronatum* and *C. tectorum*, showed that the three myricetin derivatives are present in glycosidic combination mainly as the respective 3-monogalactosides [5]. More detailed examination of the glycosides present in seven species showed that the corresponding 3-monorhamnosides are also present (Table 2). The different glycosides were best separated by paper chromatography in chloroform–acetic acid–water or by semi-preparative HPLC on a Spherisorb 5 μ C_8 column (25 cm × 10 mm) using methanol–acetic acid–water mixtures for elution, relative retention times being indicated in Table 3.

All three 3-rhamnosides have been reported before in other plants, but the larycitrin and syringetin 3-rhamnosides are still relatively rare. Larycitrin 3-rhamnoside was difficult to separate from syringetin 3-galactoside in a variety of chromatographic procedures and the only system which gave some resolution was HPLC on a Partisil C_8 column (Table 3). One new glycoside of syringetin, the 3-arabinoside, was also detected in three species (Table 2). It was readily identified by standard procedures (see Experimental). The R_fs in six solvents and relative retention times on two HPLC columns of the seven 3-monoglycosides are compared in Table 3.

As can be seen from Table 2, the different species of group A *Chondropetalum* do vary in their glycosides so that it is possible to distinguish some of them from the

Table 2. Flavonol glycosides of group A *Chondropetalum* species

	Myricetin		Larycitrin		Syringetin		
Species	3-Gal	3-Rha	3-Gal	3-Rha	3-Gal	3-Rha	3-Ara
C. deustum	+	+	+	+	+	+	−
C. hookerianum	+	+	+	+	+	−	+
C. microcarpum	+	−	+	+	+	−	+
C. mucronatum	+	−	−	−	−	−	−
C. nudum	+	+	+	+	+	−	−
C. rectum	+	+	+	+	+	−	+
C. tectorum	−	−	+	−	+	−	−

Key: 3-Gal, 3-galactoside; 3-Rha, 3-rhamnoside; 3-Ara, 3-arabinoside.

Table 3. R_fs and retention times of *Chondropetalum* group A flavonol glycosides

	R_f (×100) in*					RR_t (Sy 3 Gal)†	
	BAW	H_2O	15% HOAc	PhOH	CAW	C_8	Phenyl
Myricetin 3-galactoside	51	4	29	36	3	0.69	0.62
Myricetin 3-rhamnoside	68	14	45	25	10	0.81	0.69
Larycitrin 3-galactoside	53	7	30	69	10	0.85	0.82
Larycitrin 3-rhamnoside	53	24	50	69	12	1.02	1.00
Syringetin 3-galactoside	67	12	43	87	51	1.00	1.00
Syringetin 3-rhamnoside	83	32	65	89	80	1.20	1.26
Syringetin 3-arabinoside	72	4	22	90	70	1.13	1.21

*Measured on microcrystalline cellulose TLC plates; for solvent key see Experimental.

†Retention times, relative to syringetin 3-galactoside, on reversed-phase HPLC on a C_8 Partisil 5 CCS and on a Phenyl Hypersil column. Absolute retention times of syringetin 3-galactoside were 15.56 and 10.34 min, respectively. For solvents used for gradient elution, see Experimental.

glycosidic pattern. However, this is not always the case; for example, *C. hookerianum*, *C. rectum*, *C. microcarpum* and *C. nudum* are very similar to each other. It was, therefore, of interest to measure the relative amounts of the different glycosides, since visual inspection of two-dimensional chromatograms and comparison of aglycone yields (Table 1) indicated that there were significant quantitative variations between species.

The results of quantitative analyses on five of the seven species are indicated in Table 4. A phenyl Hypersil column gave the best separations using gradient elution with aqueous methanol mixtures. Larycitrin 3-rhamnoside did not separate completely from syringetin 3-galactoside, appearing as a shoulder on the main peak of the latter, and for convenience, the values for these two compounds are combined in the table. Minor peaks on the HPLC profile with absorption at 365 nm were present in most species (see Figs. 1 and 2), due to trace amounts of other

Table 4. Quantitative flavonol glycoside composition of group A *Chondropetalum* species

Plant collection No.	Wt (mg)	% Total flavonols (dry wt)	Normalized % (of total % dry wt) My 3-Gal	My 3-Rha	La 3-Gal	La 3-Rha* Sy 3-Gal	Sy 3-Ara	Normalized total
C. microcarpum								
805A	2290	0.5	3.0	0.5	15.8	46.6	30.9	96.8
805B	860	0.7	1.8	0.7	13.2	54.5	27.2	97.4
805B	860	0.7	2.0	0.9	14.1	56.0	25.5	98.5
805B	860	0.8	1.7	0.6	12.1	50.3	24.3	89.0
805Av.	—	0.6	2.1	0.7	13.8	51.9	27.0	95.4
8246A	2200	0.7	3.1	3.9	24.1	38.6	18.6	88.3
8246B	2040	0.7	3.3	4.4	23.3	38.3	18.3	84.3
8246Av.	—	0.7	3.2	4.2	23.7	38.5	18.5	86.3
8248A	1650	0.6	6.2	2.2	24.7	37.2	15.0	85.3
8248B	1340	0.5	5.8	2.4	25.1	40.4	13.6	81.5
8248B	1340	0.6	6.0	2.5	24.8	39.3	15.3	87.9
8248Av.	—	0.6	6.0	2.4	24.9	39.0	14.6	84.9
C. nudum								
8383	760	0.5	13.4	16.3	46.3	13.8	3.3	93.1
8380	330	0.7	17.8	15.9	47.7	11.4	1.7	94.5
C. deustum								
0002	440	0.7	24.5	13.8	21.0	28.0	12.4†	99.7
C. rectum								
0009	100	0.9	14.0	3.2	22.7	30.7	6.7	77.3
C. hookerianum								
0011	460	0.5	12.3	26.1	38.5	8.0	8.1	93.0

*Value is for the mixture of two glycosides.

†In this species, syringetin 3-rhamnoside replaces the 3-arabinoside.

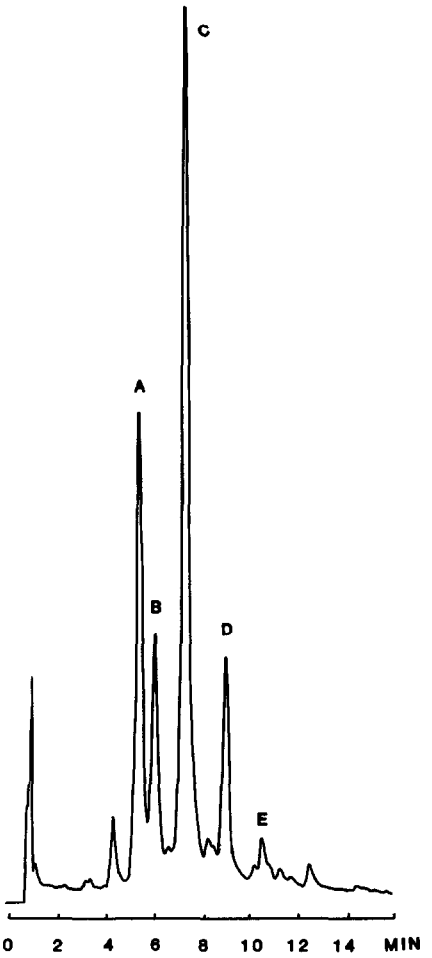

Fig. 1. Analytical HPLC separation of *C. nudum* on phenyl Hypersil column, monitored at 365 nm. For conditions, see Experimental. Key: A = myricetin 3-galactoside, B = myricetin 3-rhamnoside; C = larycitrin 3-galactoside, D = syringetin 3-galactoside/larycitrin 3-rhamnoside, E = syringetin 3-arabinoside.

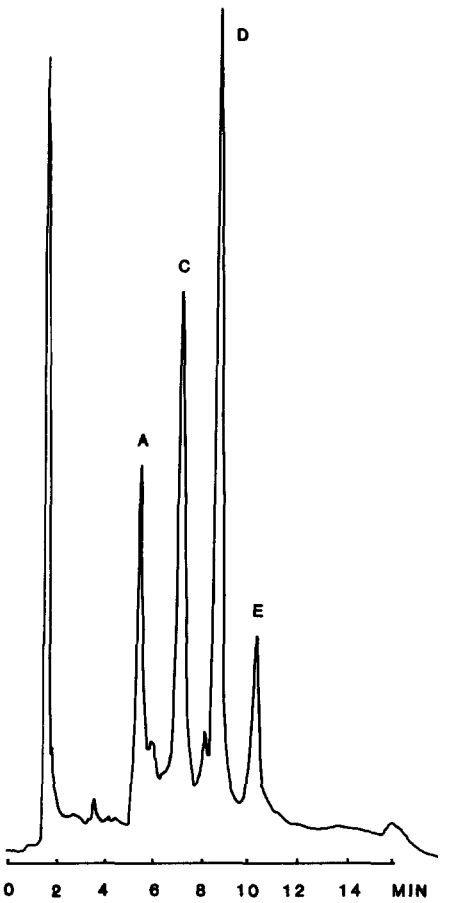

Fig. 2. Analytical HPLC separation of *C. rectum*; conditions as for Fig. 1.

glycosides besides the seven major constituents.

All five species studied do differ in the amounts of flavonol glycosides present and HPLC could be used as a taxonomic procedure to separate two otherwise indistinguishable plant samples in this genus. For example, *C. nudum* (Fig. 1) and *C. rectum* (Fig. 2) are well separated on the basis of large amounts of larycitrin 3-galactoside (47% as against 23%) in the former and of large amounts of syringetin 3-galactoside (31% as against 12%) in the latter. Differences at the specific level could be obscured by infraspecific variation and this was examined in more detail in the case of *C. microcarpum*, for which three accessions were available for analysis. As can be seen (Table 4), there are appreciable variations between accessions and between measurements on different samples of the same accession, but these are generally much less than the variations that exist between different species. Clearly, wider sampling of populations would be essential before these data could be employed directly for plant identification.

One possible application of the data of Table 4 is in comparing the degree of *O*-methylation which myricetin undergoes in the different species. Since both monomethylation (to larycitrin) and dimethylation (to syringetin) can occur, the percentage values for syringetin glycosides need to be doubled. If this is done, then the seven species fall into a series of increasing *O*-methylation: *C. mucronatum* (0%), *C. hookerianum* (66%), *C. nudum* (69%), *C. deustum* (73%), *C. rectum* (83%), *C. microcarpum* (92%) and *C. tectorum* (100%). If *O*-methylation of flavonols is of selective advantage in plants, then these species could be regarded as phylogenetically advancing in chemical terms in the above order. Other characters could be similarly analysed (e.g. the extent of the three different glycosidic patterns) using these results. Another remarkable feature of these data is the relative similarity in total flavonoid content (0.5–0.9% dry wt), suggesting that the flavonols may be localized in certain epidermal layers in the stem and may serve a similar function (e.g. in protection from UV-B radiation [11]) in all species.

Unfortunately, it was not possible to examine the flavonol glycosides of group B species of *Chondropetalum* (see Table 1) in the same detail because of the large amounts of interfering substances present in the crude stem extracts. However, three quercetin glycosides were isolated successfully from *C. macrocarpum* and identified

as the 3-galactoside, 3-gentiobioside and 7-glucoside, respectively. There is thus some evidence that glycosidic patterns differ between group A and group B species, the differences complementing those already observed in aglycone patterns (Table 1).

DISCUSSION

The genus *Chondropetalum*, as delimited by Pillans [12], includes all species with deciduous sheaths and three-locular capsular fruits. However, our flavonoid studies have revealed a remarkably clear dichotomy in the genus. A whole series of differences in flavonoid chemistry (in methylation, glycosylation and hydroxylation patterns) separate the plants into two groups. Furthermore, the two chemical groups correspond precisely with the anatomical divisions of Cutler [2], with the exception of *C. macrocarpum*. In addition, plants of chemical group A are more closely related to the genus *Elegia*, from which they are only separated with difficulty [6], than to *Chondropetalum* group B or to *C. macrocarpum*.

Gilg-Benedict [13] as early as 1930 separated *Chondropetalum capitatum* Pill., a potential group B species, as *Askidiosperma* on anatomical grounds, although this decision was later reversed [2, 12]. However, the very strong chemical data presented here suggest that this genus should be resurrected and enlarged to include all group B species of *Chondropetalum* (Table 1). Linder [6] shows elsewhere that such a grouping is perfectly acceptable on the basis of both bract and testa morphology. This leaves only group A species with myricetin derivatives present in *Chondropetalum* proper.

Finally, there is the anomalous single taxon, *C. macrocarpum*. Phytochemically, it is allied to group B (although it lacks the characteristic 8-hydroxyflavonols), while anatomically it has the epidermal characters of group A. Morphologically, it is also anomalous in its peculiar branching culms and in having a unique seed-coat [6]. The combined evidence suggests that it should have generic status and it has now been reclassified as *Dovea macrocarpa* [6].

The chemical diversity revealed in the 11 species of *Chondropetalum* by flavonoid analyses has thus been unusually helpful for arriving at a more satisfactory classification of these anatomically and morphologically distinctive plants. Detailed flavonoid studies of *Elegia* and other related taxa are being continued.

EXPERIMENTAL

Plant material. Chondropetalum species were collected in known habitats in South Africa, dried and airmailed to England. All collections were identified by one of us (P.J.L.) and herbarium vouchers have been deposited at Kew or Reading.

Chromatographic procedures. Flavonoids were separated by PC or TLC on micro-crystalline cellulose in one or other of the following solvents: BAW, *n*-BuOH–HOAc–H_2O (4:1:5); H_2O; 15% HOAc; PhOH, PhOH–H_2O (3:1); CAW, $CHCl_3$–HOAc–H_2O (90:45:6). Analytical HPLC was carried out with (a) a Partisil 5 CCS C_8 column (250 × 4 mm) with gradient elution with H_2O (A) and MeOH–H_2O–HOAc (90:5:5) (B), with an initial concn of 30% B in A, increasing the amount of B in A by 2% per min with a flow rate of 1.8 ml/min and (b) a phenyl Hypersil column (250 × 4 mm) with gradient elution with H_2O (A) and MeOH (B), with an initial concn of 20% B in A, increasing the amount of B in A by 2% per min with a flow rate of 2 ml/min. Eluants were monitored by UV detection at 365 nm. Semi-prep. HPLC was carried out on a wider C_8 column (250 × 8 mm) with similar gradient elution, except that the solvents used excluded HOAc. For the determination of flavonol glycoside concns, calibration was based on syringetin 3-galactoside since a pure crystalline sample of this was available.

Isolation and characterization. The various flavonoids were extracted from powdered stem tissue with 80% MeOH, which was then concd and the components were separated by PC, TLC or semi-prep. HPLC. Known compounds were characterized by spectral and chromatographic comparison with authentic markers.

Syringetin 3-arabinoside. This new glycoside was isolated from *C. microcarpum* and identified on the basis of its chromatographic properties (Table 3), its spectral properties (λ_{max}^{MeOH} 253, 267 sh, 358 nm; $\Delta\lambda + AlCl_3 + 47$ nm, $\Delta\lambda + NaOAc + 22$ nm, $\Delta\lambda + NaOAc + H_3BO_3$ 0 nm and $\Delta\lambda + NaOH + 62$ nm) and its hydrolysis to yield syringetin and arabinose. Its formulation as the 3-monoarabinoside followed from its colour reactions on paper and TLC, the spectral shifts indicated above and its mobility on TLC, and HPLC relative to other syringetin 3-monosides (Table 3).

Gossypetin 7-methyl ether. This was isolated after acid hydrolysis (2 M HCl, 100°) under N_2 of culms of *C. chartaceum* and *C. andreaeanum*, and purified by TLC on cellulose in CAW. Its spectral properties and R_f values were identical to lit. values [5] and it co-chromatographed without separation with an authentic marker in six solvents. On complete methylation, it gave gossypetin hexamethyl ether (R_f 0.30 in 15% HOAc; λ_{max}^{MeOH} 252,

Table 5. R_f data for herbacetin monomethyl ethers

Herbacetin derivative	R_f (× 100) in						
	BAW*	50% HOAc*	Forestal*	PhOH*	CAW*	TKM†	BPF‡
7-Methyl ether	85	39	65	94	76	50	58
8-Methyl ether	87	42	65	96	87	49	60
4′-Methyl ether	94	46	70	86	78	45	64

*On microcrystalline cellulose.

†On polyamide; TKM = toluene–MeCOEt–MeOH (4:3:3).

‡On silica gel; BPF = *n*-BuOH–pyridine–HCO_2H (36:9:5).

Colours in UV light: dark grey brown (7- and 8-methyl ethers) and dark yellow brown (4′-methyl ether) unchanged by ammonia.

271, 352 nm), identical to a product obtained by methylation of an authentic sample.

Herbacetin 4'-methyl ether. This new aglycone was isolated in the free state and as a 3-glycoside from culms of *C. paniculatum*. The purified 3-glycoside had λ_{max}^{MeOH} 274, 327 and 355 nm; +NaOH 408 nm; +NaOAc 278 nm; +NaOAc–H_3BO_3 355 nm; and R_fs of 0.76 (BAW), 0.35 (H_2O), 0.62 (15% HOAc) and 0.42 (CAW). On hydrolysis, it gave herbacetin 4'-methyl ether, λ_{max}^{MeOH} 274, 324, 372, 438 nm; +$AlCl_3$ 358, 438 nm; +NaOH 434 nm; +NaOAc 278 nm; MS: $[M]^+$ 316, 301 $[M-15]^+$. On demethylation, it gave herbacetin and it could be formulated as a monomethyl ether from MS ($C_{16}H_{12}O_7$ requires 316). It separated when co-chromatographed with either the 7- or 8-methyl ether (see Table 5). Since its spectral and colour properties rule out methyl substitution at positions 3 or 5, it can only be the so far undescribed 4'-methyl ether.

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REFERENCES

1. Pillans, N. S. (1950) in *Flora of the Cape Peninsula* (Adamson, R. S. and Saltere, T. M., eds.). Cape Town, South Africa.
2. Cutler, D. F. (1969) in *Anatomy of the Monocotyledons* (Metcalfe, C. R., ed.), Vol. IV. Juncales. Clarendon Press, Oxford.
3. Chanda, S. (1966) *Grana Palynol.* **6**, 355.
4. Harborne, J. B. and Clifford, H. T. (1969) *Phytochemistry* **8**, 2071.
5. Harborne, J. B. (1979) *Phytochemistry* **18**, 1323.
6. Linder, P. J., *Bothalia* (in press).
7. Harborne, J. B. and Boardley, M. (1984) *J. Chromatogr.* **299**, 377.
8. Harborne, J. B. (1980) *Phytochemistry* **19**, 1117.
9. Seigler, D. S. and Wollenweber, E. (1983) *Am. J. Botany* **70**, 790.
10. Combier, H., Markham, K., Audier, H., Lebreton, P., Mabry, T. and Jay, M. (1968) *C. R. Acad. Sci. Ser. D* **266**, 2495.
11. Caldwell, M. M. (1981) *Encyclopedia Plant Physiol. N.S.* **12A**, 169.
12. Pillans, N. S. (1928) *Trans. R. Soc. S. Afr.* **16**, 207.
13. Gilg-Benedict, C. (1930) in *Die Natürlichen Pflanzenfamilien* (Engler, A. and Prantl, F., eds.), 2nd edn, Vol. 15a, pp. 8–27. Borntraeger, Berlin.