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APIGENIN DI-*C*-GLYCOSYLFLAVONES OF *ANGIOPTERIS* (MARATTIALES)

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Key Word Index—*Angiopteris evecta*; *A. lygodiifolia*; *A. hypoleuca*; *A. palmaformis*; Marattiales; vicenins-1,2,3; schaftoside–isoschaftoside; violanthin–isoviolanthin; 6,8-di-*C*-arabinopyranosylapigenin.

Abstract—A survey of the flavonoids of four species of *Angiopteris* indicates that di-*C*-glycosylflavones and flavone-*O*-glycosides may be characteristic of this distinct group of eusporangiate ferns. Derivatives of flavonols, which are typical of leptosporangiate ferns and *Ophioglossum*, or biflavones, which are characteristic of the Psilotaceae, were not detected in *Angiopteris*.

INTRODUCTION

The Marattiales represent a small tropical to subtropical order of ferns dating from the Palaeozoic. Seven genera are known: *Marattia* is distributed pantropically; *Angiopteris*, *Archangiopteris*, *Macroglossum*, *Protomarattia* and *Christensenia* are Old World genera; while *Danaea* is a New World genus. The classification of these plants into families has varied from one to four [1]. Very little chemical information has been published concerning the group. Swain and Cooper-Driver [2] identified proanthocyanidins in *Marattia*; Voirin [3] identified kaempferol and/or quercetin in species of *Marattia* and quercetin as the flavonoid type in *Angiopteris evecta*; the latter could not be verified [4]. Wallace and Story [4] partially characterized two kaempferol-*O*-rhamnoglucosides in *M. salicina* but only detected flavone-*O*- and -*C*-glycosides in *A. evecta*; two of the latter were subsequently identified as violanthin (6-*C*-glucosyl-8-*C*-rhamnosylapigenin) and isoviolanthin [5]. The present report concerns the characterization of vicenin-1 (6-*C*-xylosyl-8-*C*-glucosylapigenin), vicenin-3 (6-*C*-glucosyl-8-*C*-xylosylapigenin), vicenin-2 (6,8-di-*C*-glucosylapigenin), schaftoside (6-*C*-glucosyl-8-*C*-arabinosylapigenin), isoschaftoside (6-*C*-arabinosyl-8-*C*-glucosylapigenin) and 6,8-di-*C*-arabinopyranosylapigenin in species of *Angiopteris*. Except for vicenin-2 [6], this is the first report of the occurrence of these compounds in pteridophytes.

RESULTS AND DISCUSSION

Standard PC procedures [7] of the aqueous acetone extracts of *Angiopteris evecta*, *A. lygodiifolia*, *A. hypoleuca* and *A. palmaformis* produced flavonoid bands spectrally (UV) and chromatographically (including work-up of 2 N HCl refluxing) characteristic of apigenin-di-*C*-glycosides. Further identifications were essentially based on MS of the bands obtained from TLC of the crude permethylation (PM) products [8], followed by co-chromatography with standard free compounds or their PM ethers. Except for violanthin and isoviolanthin, the di-*C*-glycosylapigenins usually occurred as mixtures. This was not an unexpected result owing to their similar behavior on PC, first disclosed by the study of synthetic compounds [9], and is a problem in chemotaxonomic surveys. When PM schaftoside and PM isoschaftoside were separated on TLC from PM vicenins, the PM vicenins-1,-2, and -3 could not be distinguished from each other. However, MS allowed differentiation of PM vicenin-2 from PM vicenin-1 or -3 by their M, M − 15 and M − 31 peaks. Superimposition of M − 131 and M − 175 peaks from vicenins-1 or -3 with M − 175 and M − 219 peaks from vicenin-2 may hinder the possible distinction between vicenin-1 and vicenin-3 on the basis of the relative importance of M − 131 and M − 175 peaks. In these instances the slightly lower migration of vicenin-1 in 2% HOAc (PC) may afford a solution. Acid isomerization of 6,8-di-*C*-arabinopyranosylapigenin was observed chromatographically and can be accounted for by α–β or pyranose–furanose isomerization on one or both of the sugars [10].

Two samples of *A. evecta* were studied. Both contained violanthin and isoviolanthin and vicenin-2; the latter was accompanied in one sample by vicenin-1 and perhaps vicenin-3, in the other by vicenin-3 and schaftoside. In *A. lygodiifolia*, vicenin-2 and schaftoside were found in one band, isoschaftoside and 6,8-di-*C*-arabinopyranosylapigenin in another band, violanthin and isoviolanthin being absent. In *A. hypoleuca*, violanthin was shown to be present by co-PC, 6,8-di-*C*-arabinopyranosylapigenin was characterized in one band and a mixture of vicenin-2, vicenin-3 and schaftoside in another band. *A. palmaformis* produced a similar flavonoid profile (PC). However, adequate material could not be accumulated for further analysis.

The present survey, representing four species of *Angiopteris*, indicates that di-*C*-glycosylapigenins are the dominant flavonoids for the genus. In addition to the apparent absence of mono-*C*-glycosylflavones, stress should be made that derivatives of neither biflavones nor flavonols were detectable using the standard procedures of Mabry *et al.* [7]. However, it is noteworthy that apigenin-*O*-glycosides have been partially characterized from each studied species. These *O*-glycosides were inseparable from the di-*C*-glycosides when the solvents TBA (3:1:1), 15% HOAc, H_2O, PhOH saturated/H_2O, and 3% NaCl were used. The flavone-*O*-glycosides were detected during the chromatographic work-up of the isomers of the di-*C*-glycosylflavones after acidic refluxing. In addition to the expected *C*-glycosylflavone isomers, the products included apigenin; when an adequate sample existed glucose and rhamnose were also identified (as their trimethylsilyl ether derivatives) [11]. Subsequent reisomerization of the above isomers did not produce additional aglycone, reaffirming the lack of *C*-glycosyl degradation. In *A. hypoleuca* an apigenin-*O*-glycoside (R_f 0.18, 0.22, 0.03, 0.82, 0.03) appeared free from *C*-glycosyl contamination; the aglycone was identified by cochromatography as described for other ferns [6]. UV analyses [7] before hydrolysis demonstrated only 7-*O*-substitution; further characterization could not be made due to a lack of material. None of the apigenin-*O*-glycoside R_f values correspond to literature values. Also occurring in relatively low concentrations in all species (except possibly *A. palmaformis* where an adequate sample was not available) were *C*-glycosides based on luteolin and/or chrysoeriol (interpreted from UV analysis according to Mabry *et al.* [7] and on acidic isomerization). Regrettably there was not enough material to characterize these compounds by MS.

All of the flavonoid analyses were hindered by relatively large amounts of tannins (which interfered at relatively low R_fs in TBA and either 15% HOAc or H_2O) and 'syrupy' yellow-green fluorescent material (PC viewed under UV) which ran high in 15% HOAc (TBA R_f *ca* 0.2–0.4). This fluorescent material could usually be separated from the flavonoids by chromatographic development in H_2O, the uncharacterized mixture migrating from the flavonoids.

With few exceptions, the accumulation of flavone-*C* and/or -*O*-glycosides at the expense of flavonol *O*-glycosides is not characteristic of flavonoid profiles of leptosporangiate ferns. These include species in the following primitive and advanced genera: *Schizaea, Stromatopteris, Gleichenia, Cardiomanes, Hymenophyllum* [6,12]; *Loxsoma, Loxsomopsis* [13]; *Adiantum* [14–16]; *Athyrium, Diplazium, Lunathrium, Matteuccia, Onoclea, Woodsia, Crytomium, Polystichum, Leptorumohra* [17]; *Dryopteris* [17,18]; *Pteridium* [19]; *Dennstaedtia* [20]; *Pityrogramma* [21]; *Hemionitis, Gymnopteris* [22]; *Bommeria* [23]; *Asplenium* [24]; *Osmunda, Lygodium* and *Anemia* (J. W. Wallace and G. F. Morris, unpublished results). However, *C*-glycosylflavones have been documented along with flavonol-*O*-glycosides in several species of the Athyriaceae and Dryopteridaceae [17]; a few species of *Dryopteris, Arachniodes* [12] and *Cyathea* (J. W. Wallace and G. F. Morris, unpublished results; [25,26]) apparently only accumulate *C*-glycosylflavones. In addition to *C*-glycosylflavones, flavone-*O*-glycosides have been identified in three species of *Dryopteris* [17], and in one species of both *Arachniodes* [17] and *Trichomanes* [6]. With the exception of vicenin-2 [6], the flavone-*C* and/or -*O*-glycosides of these ferns were not chromatographically similar to those detected in *Angiopteris* during the present study. It may be that these di-*C*-glycosylflavones and 'unique' flavone-*O*-glycosides will be characteristic of *Angiopteris* and hence chemically delimit these morphologically distinct eusporangiate ferns.

EXPERIMENTAL

Leaf material. Pinnae of *A. evecta* (G. Forst.) Hoffm. (50 g dry wt) was furnished by the Fairchild Tropical Gardens, Miami, Florida, U.S.A. (sample I; Acc. No. FG-68-156) and by Longwood Gardens, Kennet Square, PA. (sample II; Acc. No. 58156); the latter also furnished *A. lygodiifolia* (Acc. No. 571598; 26 g dry wt), *A. palmaformis* (Acc. No. 74583; 50 g dry wt) and *A. hypoleuca* (Acc. No. 60596; 50 g dry wt).

Extraction and chromatography. The dried material was pulverized in a blender, exhaustively extracted with CH_2Cl_2 and re-extracted with Me_2CO–H_2O (1:1). The latter extract was concd *in vacuo* and taken up in MeOH (or MeOH–H_2O) for prep. PC (Whatman 3 MM) in H_2O. The flavonoid bands were rechromatographed using standard procedures ([7] TBA and 15% HOAc). The isomers, produced by refluxing (2 N HCl 2 hr) the parent molecule, were individually re-isomerized to form the mixture, which was cochromatographed with the parent molecules (TLC-cellulose: TBA, 15% HOAc, BzAW; Si gel: EPWM). $R_f \times 100$ values were determined by PC on Whatman 3 MM using rutin as an internal standard (R_f rutin: TBA, 0.44; 15% HOAc, 0.55; H_2O, 0.23; phenol satd/H_2O, 0.52, and 3% NaCl, 0.20), the values presented below are in identical sequence. Permethylation and MS were according to ref. [8]. Cochromatography of the PM derivatives was on Si gel TLC $CHCl_3$–EtOAc–Me_2CO (5:4:1). The UV spectra for all of the following di-*C*-glycosylapigenins were similar to those published for violanthin and isoviolanthin [5].

A. evecta (sample I) bands: *Ae*-1: violanthin [5]; *Ae*-2: isoviolanthin [5]; *Ae*-3: vicenins-1 and -2. PC, R_f: 21, 49, 24, 65, 19. Permethylation of Ae-3 and subsequent TLC led to three bands: PM 1 (no definite MS), PM 2 (too low) and PM 3 which gave the MS of a mixture of a PM-6,8-di-*C*-hexosylapigenin (M^+ 748) and a PM *C*-pentosyl-*C*-hexosylapigenin (M^+ 704) in approximately equal amounts. MS of Ae-3 PM 3: *m/z* (rel. int.): 748 (M_I^+, 25), 733 ($M_I - 15$, 37), 717 ($M_I - 31$, 100), 704 (M_{II}^+, 25), 703 ($M_I - 45$, 31), 689 ($M_{II} - 15$, 25), 673 ($M_{II} - 31$, 94), 659 (19), 645 (19), 626 (25), 617 (19), 615 (19), 601 (15), 585 ($M_I - 163$, $M_{II} - 119$, 56), 573 ($M_I - 175$, $M_{II} - 131$, 69), 559 ($M_I - 189$, $M_{II} - 145$, 40), 541 ($M_{II} - 163$, 37), 529 ($M_I - 219$, $M_{II} - 175$, 37), 515 ($M_{II} - 189$, 15). Ae-3 PM 3 co-chromatographed with PM vicenin-2; underivatized Ae-3 (TLC: Si gel, EPWM) gave two main spots corresponding to vicenin-2 and vicenin-1. PC (2% HOAc) confirmed vicenin-1.

A. evecta (sample II) bands: *Ae*-4: violanthin (by PC); *Ae*-2*a* and *Ae*-5*a*: isoviolanthin (by PC); *Ae*-1*a*: schaftoside and vicenins-2 and -3. PC, R_f: 26, 52, 24, 57 (no 3% NaCl value). Permethylation and TLC gave two bands, PM 1 and PM 2. The MS of PM 1 showed it to be a mixture of a PM 6,8-di-*C*-hexosylapigenin (M^+ 748) and a PM 6-*C*-hexosyl-8-*C*-pentosylapigenin (M^+ 704, $M - 175 > M - 131$) in a ratio 1:3. MS of Ae-1a PM 1: *m/z* (rel. int.): 748 (M_I^+, 14), 733 (18), 717 ($M_I - 31$, 32), 704 (M_{II}^+, 36), 689 (36), 673 ($M_{II} - 31$, 100), 585 (18), 573 ($M_I - 175$, $M_{II} - 131$, 28), 559 (25), 541 (32), 529 ($M_I - 219$, $M_{II} - 175$, 43), 515 ($M_{II} - 189$, 21). The MS of PM 2 showed it to be a mixture of a PM 6,8-di-*C*-hexosylapigenin (M^+ 748) and a PM *C*-pentosyl-*C*-hexosylapigenin (M^+ 704) in a ratio 2:1. MS of Ae-1a PM 2: *m/z* (rel. int.): 748 (M_I^+, 35), 733 (33), 717 ($M_I - 31$, 100), 704 (M_{II}^+, 21), 689 (15), 673 ($M_{II} - 31$, 55), 585 (38), 573 ($M_I - 175$, $M_{II} - 131$, 55), 559 (16), 541 (20), 529 ($M_I - 219$, $M_{II} - 175$, 21), 515 ($M_{II} - 189$, 9). Ae-1a PM 1 cochromatographed with PM schaftoside, Ae-1a PM 2 with PM vicenins-1, -2 and -3. Underivatized Ae-1a (TLC, Si gel, EPWM) gave two spots corresponding to vicenin-2 and schaftoside.

A. hypoleuca bands: *Ah*-1*d*: 6,8-di-*C*-arabinopyranosylapigenin. PC, R_f: 15, 46, 18, 81, 16.

Permethylation and TLC led to one main band which gave the MS of a PM 6,8-di-*C*-pentosylapigenin (M^+ 660) containing a 6-*C*-arabinosyl group ($M - 131 > M - 119 > M - 145$). MS of PM Ah-1d: *m/z* (rel. int.): 660 (M^+, 27) 645 (31), 629 ($M - 31$, 100), 615 (7), 613 (11), 599 (19), 571 (11), 559 (11), 541 ($M - 119$, 31), 529 ($M - 131$, 42), 515 ($M - 145$, 23). PM Ah-1d cochromatographed with PM 6,8-di-*C*-arabinopyranosylapigenin and underivatized Ah-1d cochromatographed with synthetic 6,8-di-*C*-arabinopyranosylapigenin.

Ah-3*a*: violanthin (by PC); *Ah*-3*b*: schaftoside and vicenin-2. PC, R_f: 19, 55 (no H_2O value), 61, 33. Permethylation and TLC led to two equal bands, PM 1 and PM 2. The MS of PM 2 showed it to be a mixture of a PM 6,8-di-*C*-hexosylapigenin (M^+ 748) and a PM *C*-pentosyl-*C*-hexosylapigenin (M^+ 704) in a ratio 5:2. MS of Ah-3b PM 2: *m/z* (rel. int.): 748 (M_I^+ 30), 733 (35), 717 ($M_I - 31$, 100), 704 (M_{II}^+, 17), 703 (22), 701 (13), 689 (17), 687 (13), 673 ($M_{II} - 31$, 39), 645 (22), 617 (35), 603 (22), 585 (40), 573 ($M_I - 175$, $M_{II} - 131$, 65), 559 (26), 541 (26), 529 ($M_I - 219$, $M_{II} - 175$, 26), 515 ($M_{II} - 189$, 17). Ah-3b PM 2 cochromatographed with PM vicenins-1, -2 and -3. The MS of Ah-3b PM 1 was similar, but the ratio PM 6,8-di-*C*-hexosylapigenin (M^+ 748) to PM *C*-pentosyl-*C*-hexosylapigenin (M^+ 704) was 1:4. MS of Ah-3b PM 1: *m/z* (rel. int.): 748 (3), 733 (5), 717 (7), 704 (10), 689 (15), 673 (37), 659 (8), 657 (7), 643 (8), 626 (11), 603 (11), 601 (8), 585 (10), 573 (41), 559 (20), 541 (25), 529 (23), 515 (17), 371 (100). Ah-3b PM 1 cochromatographed with PM schaftoside and differs from PM vicenin-1 and PM isoschaftoside. Underivatized Ah-3b (TLC, Si-gel, EPWM) gave two main spots corresponding to vicenin-2 and schaftoside (or vicenin 1). On PC (2% HOAc), vicenin-1 appears to be absent.

A. lygodiifolia bands: *Al*-1*a*: schaftoside and vicenin-2. PC, R_f: 15, 54, 25, 76 (no 3% NaCl value). Permethylation and TLC led to two main bands, PM 1 and PM 3. The MS of PM 1 was that of a PM 6-*C*-hexosyl-8-*C*-pentosylapigenin (M^+ 704, $M - 175 > M - 13$). MS of Al-1a PM 1: *m/z* (rel. int.): 704 (M^+, 26), 689 (28), 673 ($M - 31$, 100), 601 (15), 573 ($M - 131$, 13), 541 (28), 529 ($M - 175$, 35), 515 (9). Al-1a PM 1 cochromatographed with PM schaftoside. The MS of PM 3 was that of a PM 6,8-di-*C*-hexosylapigenin (M^+ 748). MS of Al-1a PM 3: *m/z* (rel. int.): 748 (M^+, 33), 733 (27), 717 ($M - 31$, 100), 645 (13), 585 (31), 573 ($M - 175$, 40), 559 (8). Al-1a PM 3 cochromatographed with PM vicenin-2. Underivatized Al-1a (TLC, Si-gel, EPWM) showed two spots corresponding to vicenin-2 and schaftoside. *Al*-2*b*: isoschaftoside and 6,8-di-*C*-arabinopyranosylapigenin. PC, R_f: 16, 46, 18, 81 (no 3% NaCl value). Permethylation and TLC led to two bands, PM 1 and PM 2. The MS of PM 1 was that of a PM 6,8-di-*C*-pentosylapigenin (M^+ 660) containing a 6-*C*-arabinosyl group ($M - 131 > M - 119 > M - 145$). MS of Al-2b PM 1: *m/z* (rel. int.): 660 (M^+ 31), 645 (31), 629 ($M - 31$, 100), 615 (15), 613 (15), 599 (26), 541 ($M - 119$, 36), 529 ($M - 131$, 69), 515 ($M - 145$, 31). Al-2b PM 1 cochromatographed with PM 6,8-di-*C*-arabinopyranosylapigenin. The MS of PM 2 was that of a PM 6-*C*-pentosyl-8-*C*-hexosylapigenin (M^+ 704, $M - 131 > M - 175$) containing a 6-*C*-arabinosyl group ($M - 131 > M - 119 > M - 145$). MS of Al-2b PM 2: *m/z* (%) 704 (M^+, 22), 689 (16), 673 ($M - 31$, 100), 585 ($M - 119$, 17), 573 ($M - 131$, 23), 559 ($M - 145$, 11), 529 ($M - 175$, 12). Al-2b PM 2 cochromatographed with PM isoschaftoside.

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REFERENCES

1. Copeland, E. B. (1947) *Genera Filicum.* Chronica Botanica Co., Waltham, MA.
2. Swain, T. and Cooper-Driver, G. (1973) in *The Phylogeny and Classification of the Ferns* (Jermy, A. C., Crabbe, J. A. and Thomas, B. A., eds.). Academic Press, London.
3. Voirin, B. (1970) Ph.D. Dissertation, University of Lyon.
4. Wallace, J. W. and Story, D. T. (1978) *J. Nat. Prod.* **41**, 651.
5. Wallace, J. W., Story, D. T., Besson, E. and Chopin, J. (1979) *Phytochemistry* **18**, 1077.
6. Markham, K. R. and Wallace, J. W. (1980) *Phytochemistry* **19**, 415.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, New York.
8. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
9. Chopin, J. and Bouillant, M. L. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.). Academic Press, New York.
10. Besson, E. (1977) Thése de Doctorat de 3ème cycle, University of Lyon 1, No. 655.
11. Wallace, J. W. (1981) *Phytochem. Bull. Bot. Soc. Am.* **13**, 36.
12. Wallace, J. W. and Markham, K. R. (1978) *Am. J. Botany* **65**, 965.
13. Markham, K. R. and Given, D. (1979) *Biochem. Syst. Ecol.* **7**, 91.
14. Cooper-Driver, G. and Swain, T. (1977) *Bot. J. Lim. Soc.* **74**, 1.
15. Hasegawa, M. and Akabori, Y. (1968) *Bot. Mag. Tokyo* **81**, 469.
16. Akabori, Y. and Hasegawa, M. (1969) *Bot. Mag. Tokyo* **82**, 294.
17. Hiraoka, A. (1978) *Biochem. Syst. Ecol.* **6**, 171.
18. Kobayasi, K. and Hayashi, K. (1952) *Pharm. Soc. Jpn* **72**, 3.
19. Cooper-Driver, G. (1976) *Bot. J. Linn. Soc.* **73**, 35.
20. Akabori, Y. and Hasegawa, M. (1970) *Bot. Mag. Tokyo* **83**, 263.
21. Star, A. E., Seigler, D. S., Mabry, T. J. and Smith, D. M. (1975) *Biochem. Syst. Ecol.* **2**, 109.
22. Gianassi, D. E. and Mickel, J. T. (1979) *Brittonia* **31**, 405.
23. Haufler, C. (1979) *J. Arnold Arbor. Harv. Univ.* **60**, 445.
24. Harborne, J. B., Williams, C. A. and Smith, D. (1973) *Biochem. Syst.* **1**, 51.
25. Hiraoka, A. and Hasegawa, M. (1975) *Bot. Mag. Tokyo* **88**, 127.
26. Soeder, R. W. and Bass, M. S. (1972) *Phytochemistry* **11**, 3079.