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PHENOLIC COMPOUNDS IN FERNS—III.

AN EXAMINATION OF SOME FERNS FOR CAFFEIC ACID DERIVATIVES

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Abstract—A study was made of caffeic acid derivatives in forty species of ferns. Identifiable or partially identifiable compounds were observed in fourteen species, unidentified catechol derivatives in twenty-one species. The presence of rosmarinic acid in *Blechnum brasiliense* was confirmed. Chlorogenic acid and 1-caffeoylglucose were found to occur, although not together, in several species. Some data are presented concerning the structure of blechnic acid, a new caffeic acid ester isolated from *B. spicant* and detected chromatographically in *Lastrea globuliferum*.

INTRODUCTION

DURING an examination of the anthocyanin content of some ferns, Harborne[1] detected rosmarinic acid in *Blechnum brasiliense*. Rosmarinic acid, a caffeic acid depside of 3,4-dihydroxyphenyllactic acid, was first reported as a naturally occurring compound by Scarpatti and Orienti[2] who isolated it from *Rosmarinus officinalis*. Although rosmarinic acid appears to be a useful chemotaxonomic marker in certain groups of flowering plants[3] its discovery in *B. brasiliense* represents the first report of this compound in the Pteridophyta. This present paper describes a survey of ferns for rosmarinic acid and certain other caffeic acid derivatives. In an earlier paper[4] we reported the widespread occurrence of caffeic acid in hydrolysed extracts of a number of ferns.

RESULTS AND DISCUSSION

Results from a thin-layer chromatographic examination of forty species of ferns for caffeic acid esters are presented in Table 1. The fern families represented as well as the tribes within the Polypodiaceae are presented in evolutionary sequence (see Ref. 4). The presence or absence is recorded of chlorogenic acid, rosmarinic acid, blechnic acid, 4-glucosidocaffeic acid, and 1-caffeoylglucose. If a plant yielded a positive test for a catechol but the spot did not correspond in position of any of the standards a positive test for "unknown catechol derivative" was recorded. It should be appreciated that this represents an indiscriminate lumping, in many instances, of common flavonoids possessing a catechol substitution pattern (e.g. quercetin derivatives) and compounds which may be hitherto unknown catechol derivatives.

[1] J. B. HARBORNE, *Nature* **207**, 984 (1965).
[2] M. L. SCARPATTI and G. ORIENTI, *Ric. Sci.* **28**, 2329 (1958).
[3] J. B. HARBORNE, *Z. Naturforsch.* **21b**, 604 (1966).
[4] B. A. BOHM and R. M. TRYON, *Can. J. Botany* **45**, 585 (1967).

TABLE 1. CAFFEIC ACID DERIVATIVES IN FERNS

	Source*	CHL†	ROS	BLE	CFG	GLC	UCD
Eusporangiate							
Marattiaceae							
1 *Angiopteris evecta* (Forst.) Hoffm.	UBC	–	–	–	–	–	–
Leptosporangiate							
Gleicheniaceae							
2 *Dicranopteris emarginata* (Brack.) Robinson	HI	–	–	–	–	–	+
Hymenophyllaceae							
3 *Gonocomus minutus* (Blume) v.d. Bosch.	HI	–	–	–	–	–	–
4 *Mecodium recurvum* (Gaud.) Copel.	HI	–	–	–	–	–	–
5 *Vandenboschia davalliodes* (Gaud.) Copel.	HI	–	–	–	–	–	+
Dicksoniaceae							
6 *Dicksonia fibrosa* Col.	UBC	–	–	–	–	–	+
Cyatheaceae							
7 *Cyathea dealbata* Swartz	UBC	–	–	–	–	–	+
8 *C. fauriei* (Christ.) Copel.	TOK	–	–	–	+	–	+
9 *C. hancockii* Copel.	TOK	–	–	–	+	–	+
10 *C. metteniana* (Hance)	TOK	–	–	–	+	–	+
11 *C. podophylla* Hook.	TOK	–	–	–	+	–	+
Polypodiaceae							
Dryopterideae							
12 *Ctenitis decomposita* (R. Br.) Copel.	UBC	–	–	–	–	–	–
13 *Dryopteris parvula* Rob.	HI	–	–	–	–	–	–
14 *D. felix-mas* (L.) Schott.	BC	+	–	–	–	–	–
15 *D. austriaca* (Jacq.) Woynar.	BC	+	–	–	–	–	–
16 *Lastrea globuliferum* (Brack.) Mann	HI	–	–	+	–	–	–
17 *Polystichum munitum* (Kaulf.) Presl.	BC	–	–	–	–	–	–
Asplenieae							
18 *Asplenium adiantum-nigrum* L.	UBC	–	–	–	–	–	+
19 *A. trichomanes* L.	BC	–	–	–	–	–	–
20 *A. unilaterale* Lamarck	HI	+	–	–	–	–	+
21 *Athyrium felix-femina* (L.) Roth.	BC	+	–	–	–	–	–
Blechneae							
22 *Blechnum brasiliense* Desv. var. *crispum* hort.	TOK	+	+	–	–	–	–
23 *B. discolor* (Forsk) Keys	TOK	+	–	–	–	–	–
24 *B. orientalis* L.	TOK	+	–	–	–	–	–
25 *B. spicant* (L.) Roth.	BC	+	–	+	–	–	–
26 *Doodia dives* Kunze	TOK	–	–	–	–	–	+
27 *Sadleria cyatheoides* Kaulf.	HI	–	–	–	?‡	–	+
28 *S. hillebrandii* Robinson	HI	–	–	–	–	–	+
29 *Woodwardia orientalis* Sw. var. *formosana* Ros.	TOK	–	–	–	–	–	+
30 *W. unigemmata* (Makino) Nakei	TOK	–	–	–	–	–	+
Dennstaedteiae							
31 *Dennstaedtia wilfordii* (Moore) Koidz.	UBC	–	–	–	–	–	–
32 *Microlepia setosa* (Smith) Alston	HI	–	–	–	–	–	+
Gymnogrammeae							
33 *Coniogramma pilosa* (Brack.) Hier	UBC	–	–	–	¶	–	–
34 *Pityrogramma calomelanos* (L.) Link	UBC	–	–	–	–	–	+

TABLE 1—*continued*

	Source*	CHL†	ROS	BLE	CFG	GLC	UCD
35 *Pelleae rotundifolia* Hook. Pterideae	UBC	–	–	–	–	–	–
36 *Pteridium aquilinum* Kuhn var. *pubescens* Underw. Davallieae	BC	–	–	–	–	–	+
37 *Nephrolepis cordifolia* (L.) Presl Polypodieae	HI	–	–	–	–	–	+
38 *Platycerium bifurcatum* (Cav.) C. Christ.	UBC	+	–	–	–	–	–
39 *Pleopeltis thunbergiana* Kaulf.	HI	–	–	–	–	–	+
40 *Polypodium vulgare* L.	BC	–	–	–	–	–	+

* University of British Columbia Greenhouses (UBC), collected locally from the wild (BC), The University of Tokyo Botanical Gardens (TOK), Hawaiian Islands (HI).

† Chlorogenic acid (CHL), rosmarinic acid (ROS), blechnic acid (BLE), 1-caffeoylglucose (CFG), 4-glucosidocaffeic acid (GLC), unidentified catechol derivative (UCD).

‡ Large catechol-positive area overlapping 1-caffeoylglucose running position.

¶ Insufficient sample for completion of this test.

The chromatographic standards used in this study included four caffeic acid esters and one glucoside. The glucoside used was the 4-glucoside of caffeic acid. The esters used were: chlorogenic acid, a compound well known in flowering plants and one that might easily be predicted as a likely substance to be found in ferns; rosmarinic acid; blechnic acid, a newly isolated compound from *Blechnum spicant* (see below); and 1-caffeoylglucose, whose natural occurrence has been discussed by Harborne and Corner.[5] Pertinent characteristics of these compounds as well as caffeic acid are presented in Tables 2, 3, and 4. The thin-layer chromatographic behaviour of the compounds, in four solvent systems, is presented in Table 2. The four systems, two organic and two aqueous, allow reasonably conclusive decisions to be made concerning the presence or absence of the compounds under study. The aqueous systems appeared to give excellent resolution of the phenolic constituents of the plants but

TABLE 2. THIN-LAYER CHROMATOGRAPHIC CHARACTERISTICS OF SOME CAFFEIC ACID DERIVATIVES

Compounds	R_fs (× 100) in solvents*					
	A		B		C	D
Chlorogenic acid	58	75	68	81	71	38
Rosmarinic acid	32	51	51	65	94	72
Blechnic acid	45	65	61	74	92	58
Caffeic acid	24	56	41	69	88	84
4-Glucosidocaffeic acid	56	79	71	86	68	41
1-Caffeoylglucose	57	67	71	—	64	77

* A = 2% formic acid; B = 10% acetic acid; C = *n*-butanol:acetic acid:water (4:1:2·2); D = *n*-butanol:pyridine:water (14:3:4). Solvents C and D are single-phase systems. R_f values in solvents A and B are for the *trans* and *cis* isomers.

[5] J. B. HARBORNE and J. CORNER, *Biochem. J.* **81**, 242 (1961).

TABLE 3. COLOUR CHARACTERISTICS OF SOME CAFFEIC ACID DERIVATIVES

Compound	Treatment			
	U.v. light (3660 Å)	U.v. + NH_3	Visible + $FeCl_3$*	Visible + DPNA†
Chlorogenic acid	Blue	Yellow-green	Blue-grey	Yellow-brown
Rosmarinic acid	Blue	Yellow-green	Blue-grey	Blue-grey
Blechnic acid	Blue	Yellow-green	Blue-grey	Yellow-brown
Caffeic acid	Blue	Blue-white	Blue-grey	Grey-green
4-Glucosidocaffeic acid	Purple	Cream-yellow	Brown	Magenta
1-Caffeoylglucose	Blue	Lime-green	Blue-grey	Yellow

* All gave varying shades of blue-green when fresh but the colours changed to a uniform blue-grey on standing.

† Diazotized *p*-nitroaniline oversprayed with 5 per cent sodium hydroxide sol'n.

TABLE 4. ULTRAVIOLET SPECTRAL CHARACTERISTICS OF SOME CAFFEIC ACID DERIVATIVES

	λ_{max} (nm)* in							
	EtOH		EtOH/NaAc		EtOH/NaAc/H_3BO_3		$NaOH/NaBH_4$	
Chlorogenic acid	300s	332	300	330	310s	354	†	378
Rosmarinic acid	286	330	286	328	292	350	295	367
Blechnic acid	300s	328	295s	328	296s	350	†	387
Caffeic acid	300s	327	286	313	294	331	305	344
4-Glucosidocaffeic acid	285	315s	276	308	276	308	286	339
1-Caffeoylglucose	305s	336	305s	336	312s	360	†	386

* s = shoulder.

† band unstable.

initial results were misleading. Using only these systems several plants appeared to possess chlorogenic acid and blechnic acid. However, when these extracts were run in the two organic systems it became clear that the catechol-positive compounds present were not chlorogenic acid and blechnic acid in most instances. The R_f values presented are averages of at least two chromatographic runs; reproducibility is within 5%.

Table 3 records the colours of the standards employed in the study. The compounds do not appear in visible light but give very pronounced colours in u.v. light (3660 Å) both as such and after fuming with NH_3. Chromatograms were sprayed with ferric chloride, which is useful for detecting unbound *o*-dihydroxyphenols, and diazotized *p*-nitroaniline oversprayed with dilute sodium hydroxide, which is a general reagent for phenols.

The u.v. spectral characteristics of the compounds are listed in Table 4. Addition of anhydrous sodium acetate to the sample in EtOH serves to detect the presence of an ionizable carboxylic acid function while the further addition of boric acid allows detection of unsubstituted catechol structures.[6] Due to the rapid oxidative decomposition of catechol derivatives in the presence of strong base, stabilization was necessary during the course of the spectral measurements of alkaline shifts. This was accomplished by the addition of $NaBH_4$ to the sample prior to the addition of strong base.[7]

[6] J. B. HARBORNE, *Methods in Polyphenol Chemistry*, pp. 13–36, Pergamon Press, Oxford (1964).

[7] H. A. SCHROEDER, *Phytochem.* 6, 1589 (1967).

This study was initiated to examine the distribution of rosmarinic acid and related caffeic acid derivatives in plants related to *B. brasiliense* wherein rosmarinic acid has been reported[1] to occur naturally. Members of this group (Polypodiaceae, tribe Blechneae) examined included four *Blechnum* species, two *Sadleria* species, two *Woodwardia* species, and a single species of *Doodia*. Rosmarinic acid was detected only in *B. brasiliense*. Expansion of the survey to an additional thirty-two species of ferns from several families and other tribes within the Polypodiaceae failed to reveal another source of this compound. However, initial chromatographic studies, later substantiated by isolation and preliminary structural studies, disclosed the presence of a new caffeic acid ester in *B. spicant*. This new compound, termed blechnic acid, was also detected in *Lastrea globuliferum* (Polypodiaceae, tribe Dryopterideae). Blechnic acid was found in both fertile and sterile leaves of *B. spicant*. Chlorogenic acid was present in nine species tested: *B. spicant* (again, both fertile and sterile leaves), *B. orientale*, *B. discolor*, *B. brasiliense*, *Athyrium felix-femina*, *Asplenium trichomanes*, *Dryopteris austriaca*, *D. felix-mas*, and *Platycerium bifurcatum*. Although further, detailed examination is necessary due to a good deal of phenolic smearing on the chromatograms, extracts of four of five species of *Cyathea* tested appear to have 1-caffeoylglucose. *Sadleria cyatheoides* may also have this ester; the thirty-five other ferns tested appear not to. None of the forty species examined possessed 4-glucosidocaffeic acid. As shown in Table 1 a number of ferns have been recorded as possessing unidentified catechol derivatives; some of these appeared as distinct spots while others were little more than catechol-positive areas of poor definition.

Because large amounts of *B. spicant* are readily available some information on the structure of blechnic acid has been obtained. Blechnic acid was isolated as a tan-coloured, amorphous solid, containing a number of minor contaminants (TLC). It sintered above 200° but did not melt at 285°, was soluble in water, less so in ethanol, and insoluble in diethyl ether. Hydrolysis with warm, 3 N HCl produced a number of unidentified substances (TLC) whereas warm, 3 N NaOH yielded caffeic acid and another compound which gave colour reactions identical with 3,4-dihydroxyphenyllactic acid (from rosmarinic acid hydrolysates) or DOPA. The i.r. spectrum, taken on the amorphous product, consisted of a number of broad bands of medium intensity. A detailed analysis was not possible although it was clear that the compound possessed a carbonyl function and hydroxyl groups. The u.v. spectrum was similar to those of the other caffeic esters (Table 4). Addition of sodium acetate failed to produce an hypsochromic shift indicating the absence of a free carboxyl function. Borate produced a bathochromic shift of about 20 nm which substantiated the existence of an *o*-dihydroxybenzene substitution in the molecule. Addition of sodium hydroxide solution to the sample (stabilized by borohydride) produced a bathochromic shift of 59 nm which indicated the presence of a strongly ionizable phenolic group (or groups).

On the basis of the foregoing data, it is clear that blechnic acid is an ester of caffeic acid. However, to what the caffeic acid is esterified, i.e. the nature of the second catechol derivative or polyhydroxy compound present in this compound, remains unknown.

MATERIALS AND METHODS

Source of Plants

Fresh plant material was obtained from the University of British Columbia greenhouses, the countryside surrounding the University, The University of Tokyo Botanical Gardens, and the Island of Hawaii. The Hawaiian plants were collected by the author and by Dr. T. M. C. Taylor of this Department. Ferns from the vicinity of the University were collected

by the author. Local plants were extracted, at the latest, within 2 hr of collection and usually within half an hour. Plants from Tokyo and Hawaii were sent by air-mail packed in plastic bags.

Extraction and Analytical Procedure

Plant material was extracted repeatedly with boiling 80% EtOH, the extracts pooled, and evaporated to dryness under an air jet. The residue was extracted with boiling water and filtered through a bed of Celite. The aqueous extract was subjected to continuous extraction with ethyl acetate for 8 hr. Evaporation of the ethyl acetate solution to a small volume was accomplished under an air jet. This solution was used directly for chromatographic analysis.

Thin-layer chromatographic analyses were run on 200 × 200 mm plates of Cellulose MN of 0·5 mm thickness. Plates were dried at least 12 hr in the air before use. Solvent systems and the methods of detections of caffeic acid derivatives are given in Tables 2 and 3.

Source of Standard Compounds

Chlorogenic acid was purchased from Nutritional Biochemical Corp. Rosmarinic acid was isolated from *Mentha arvense* by Mr. B. Ellis of this Department. 4-Glucosidocaffeic acid and 1-caffeoylglucose were generously provided by Dr. W. Steck, Prairie Regional Laboratory, Saskatoon.

Isolation of Blechnic Acid

Blechnic acid was extracted from *Blechnum spicant* by the general procedure described above. Purification was achieved by repeated banding on Whatman 3MM paper and chromatography using solvents A and C. Some degree of purification was also achieved by repeated solution of the compound in either water or dimethylsulfoxide and precipitation by addition of acetone. The dried solid was stored in a desiccator although it also appears to be stable under normal conditions.

Degradation of Blechnic Acid

Acidic and basic hydrolyses were done at about 80° using 3 N reagents in water. The alkaline hydrolysis was done in the presence of $NaBH_4$ to prevent oxidation of the *o*-diphenoxide ions formed.[7] Hydrolysis products were extracted continuously with ether for several hours. These ether extracts were reduced in volume and used for TLC analysis. Chromatography an Avicel plates[8] was employed for the detection of caffeic acid.

Spectral Analyses

U.v. spectral measurements were taken with a Unicam SP-800 recording spectrophotometer. The i.r. spectrum was taken as a KBr disc using a Unicam SP-200G recording spectrophotometer.

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[8] R. K. IBRAHIM and G. H. N. TOWERS, *Arch. Biochem. Biophys.* **87**, 125 (1960).