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ACYLPHLOROGLUCINOLS AND FLAVONOID AGLYCONES PRODUCED BY EXTERNAL GLANDS ON THE LEAVES OF TWO *DRYOPTERIS* FERNS AND *CURRANIA ROBERTIANA*

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Key Word Index—*Dryopteris villarii*; *D. arguta*; *Currania robertiana*; Pteridaceae; ferns, leaf exudates; acylphloroglucinols; ultrastructure of glandular trichomes.

Abstract—The sparse resinous leaf exudates of *Dryopteris villarii* and *D. arguta* contain acylphloroglucinols that have previously only been found in the rhizomes of *Dryopteris* species. The phloroglucinol derivatives were characterized as representatives of the para-aspidin, desaspidin and albaspidin series by 2-D NMR spectroscopy and atmospheric pressure chemical ionization mass spectrometry (APCI-MS-MS). Mass fragmentation proved to be of diagnostic value to identify the acyl side chains and also allowed distinction between desaspidins and isomeric albaspidin analogues. The frond exudate of *Currania robertiana* was devoid of acylphloroglucinols, but was found to contain flavonoid aglycones. The acylphloroglucinols and flavonoid aglycones are presumably produced by external glands on the leaves, the presence and ultrastructure of which was examined by scanning electron microscopy.

INTRODUCTION

Extensive studies on the chemical composition of frond exudates from Gymnogrammoid ferns (genera *Cheilanthes*, *Notholaena*, *Pityrogramma* and some other Pteridaceae) have revealed the presence of a rich array of flavonoid aglycones [1–3], diterpenoids and triterpenoids [4–6]. All fern species previously examined for exudate flavonoids or other phenolics [7] originate from both Americas, from Asia, and from Australia (*Platyzoma* [8]). This is the first study of the foliar exudates of two European Alpine ferns, *Currania robertiana* (Hoffm.) Wherry [Syn.: *Gymnocarpium robertianum* (Hoffm.) Newm.; *Dryopteris robertiana* (Hoffm.) C. Chr.] and *Dryopteris villarii* (Bell.) Woynar.

Preliminary TLC analysis of the frond exudates of *D. villarii* and *D. arguta* (Kaulf.) Watt. from California indicated the presence of non-flavonoid phenolics which were subsequently isolated and identified as acylphloroglucinols by spectroscopic techniques. Although acylphloroglucinols are well-known as internal gland products from rhizomes of many ferns, in particular *Dryopteris* [9, 10], their presence in leaf exudates produced by external glands is reported here for the first time.

RESULTS AND DISCUSSION

Identification of leaf exudate constituents

In the following, Penttilä and Sundman's system for assigning names to acylphloroglucinols [11–13] and numbering of atoms as in Ref. [14] is used. The frond exudate of *D. arguta* yielded para-aspidin AA (**1-AA**) and four acylphloroglucinols of the desaspidin series (**2-AP**, **2-PP**, **2-AB**, and **2-PB**) by column chromatography on polyamide and semi-preparative HPLC on RP-18. In addition, six acylphloroglucinols were isolated from the frond exudate of *D. villarii* which were identified as the albaspidin homologues **3-AP**, **3-PP**, **3-AB**, **3-PB**, **3-BB** and aspidinol (**4**). Structure assignment was achieved by a combination of atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and 2-D NMR spectroscopy. By co-TLC with markers, the frond exudate of *C. robertiana* was found to contain the flavones, apigenin, apigenin-7-methylether, apigenin-4′-methylether, apigenin-7,4′-dimethylether and the flavonols, kaempferol, kaempferol-3-methylether, kaempferol-4′-methylether, kaempferol-3,4′-dimethylether,

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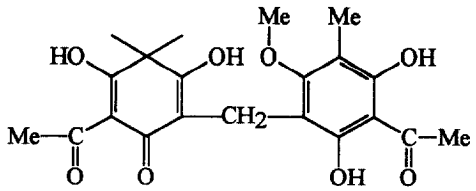
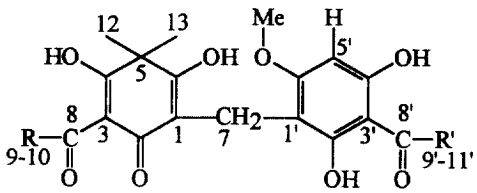
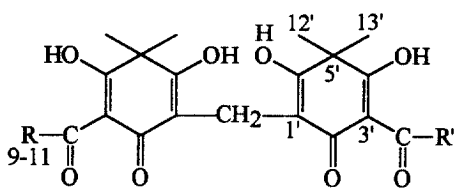


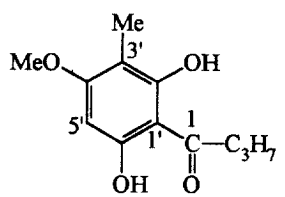
Para-aspidin
1-AA



Desaspidins
2-AA R=R'=Me
2-AP R=Me, R'=Et
2-AB R=Me, R'=Pr
2-PB R=Et, R'=Pr



Albaspidins
3-AA R=R'=Me
3-AP R=Me, R'=Et
3-PP R=R'=Et
3-AB R=Me, R'=Pr
3-PB R=Et, R'=Pr
3-BB R=R'=Pr



4 Aspidinol B

kaempferol-3,7,4′-trimethylether and herbacetin-3,8,4′-trimethylether. The latter flavonol is a rather rare compound which, to our knowledge, was previously found only five times from natural sources, including a fern *Pityrogramma triangularis* var. *triangularis* (now *Pentagramma*) [15]. The foliar exudates of *D. villarii* and *D. arguta* were found to be devoid of flavonoids.

Compound **2-AB** (M_r 418, $C_{22}H_{26}O_8$) was obtained as the major constituent of the frond exudate of *D. arguta*. Its ^{1}H NMR spectrum showed characteristic signals for an acylphloroglunicol. Four major low-field resonances attributable to (hydrogen-bonded) hydroxyls (δ_H 9.98, 11.31, 16.39 and 18.40), accompanied by some four minor signals due to keto-enol tautomerism. The methylene bridge linking the two ring systems appeared as a broad singlet at δ_H3.53 which interacted with C-1 and C-1′ of both rings in the HMBC spectrum. The H-5′ singlet at δ_H6.05 was assigned to an aromatic proton, indicating that one ring system was aromatic in nature. The other ring was identified as a filicinic acid unit with a *gem*-dimethyl group resonating at δ_H1.55. This signal coupled with the ^{13}C resonance at δ_C24.8 in the HMQC spectrum of **2-AB**. The substitution pattern of the aromatic (phloroglucinol) ring was determined to be 3′-acyl, 5′-H, 6′-OMe. The aromatic proton signal showed interactions with all ring carbons except C-2′ bearing a free hydroxyl with δ_H 16.39, which excludes the possibility of **2-AB** being an iso-desaspidin homologue. Furthermore, a methoxy group at C-6′ followed from the observation that the free hydroxyl *ortho* to C-5′ interacted with C-3′, C-5′ and with the carbon to which the hydroxyl is attached, C-4′. The ^{1}H NMR spectrum further demonstrated the presence of an acetyl and a butyryl side chain. Table 1 lists all ^{1}H and ^{13}C shifts for **2-AB** assigned on the basis of ^{1}H–^{13}C couplings observed in the HMQC and HMBC spectra as well as by comparison with NMR data reported for desaspidin BB [14]. The acetyl function was located on the filicinic acid unit by mass spectrometry (see below). Compound **2-AB** was thus identified as desaspidin AB.

The accompanying acylphloroglunicols from *D. arguta* were identified by ^{1}H NMR and mass spectrometry only. Compounds **2-AA**, **2-AP** and **2-PB** showed ^{1}H NMR spectra similar to that of **2-AB**, except for the acyl side chain signals, and were therefore identified as desaspidin homologues. Compound **1-AA** (M_r 390, $C_{20}H_{22}O_8$) lacked the aromatic H-5′ signal but showed a C-methyl at δ_H 2.14 instead. The ^{1}H NMR spectrum of **1-AA** further showed two acetyl methyls. The structure of **1-AA** was assigned as para-aspidin AA and confirmed by mass spectrometry.

The M_r of compound **3-BB** (M_r 460, $C_{25}H_{32}O_8$) suggested that **3-BB** was identical with or closely related

Table 1. ^{1}H and ^{13}C NMR data for phloroglucides 2-AB, 3-PB and 3-BB

Atom No.	**2-AB** δ_H	**2-AB** δ_C	**3-PB** δ_H	**3-PB** δ_C	**3-BB** δ_H	**3-BB** δ_C
1		108.65		110.87		110.88
2		187.63		187.76		187.77
3		111.39		108.21		108.21
4		199.04		199.28		199.28
5		44.48		44.52		44.52
6		172.09		173.41		173.42
7	3.53 *br s*	16.81	3.31 *s*	18.18	3.31 *s*	18.23
8		203.71		206.52		206.53
9	2.73 *s*	29.54	3.16 *m*	43.13	3.15 *m*	43.13
10			1.17 *t* (7.2)†	18.23	1.69 *m*	18.23
11					1.01 *t* (7.4)†	14.17
12	1.55 *s*	24.77	1.551 *s**	25.51*	1.54*	25.50*
13	1.55 *s*	24.77	1.48 *s**	24.38*	1.48 *s**	24.38*
1′		104.79		110.85		110.88
2′		163.41		187.80		187.77
3′		106.59		108.14		108.21
4′		164.20		198.67		199.28
5′	6.05 *s*	93.12		44.40		44.52
6′		162.37		173.41		173.42
8′		206.62		207.44		206.53
9′	2.99 *m*	45.91	3.23 *m*	35.08	3.15 *m*	43.13
10′	1.70 *m*	18.37	1.69 *m*	8.59	1.69 *m*	18.23
11′	0.99 *t* (7.4)†	14.25	1.01 *t* (7.3)†	14.17	1.01 *t* (7.4)†	14.17
12′			1.545 *s**	25.48*	1.54 *s**	25.50*
13′			1.47 *s**	24.38	1.48 *s**	24.38*
4-OH	18.40 *s*		18.64 *s*		18.64 *s*	
6-OH	9.98 *s*		12.35 *s*		12.35 *s*	
2′-OH	16.39 *s*					
4′-OH	11.31 *s*		18.57 *s*		18.64 *s*	
6′-OH			12.35 *s*		12.35 *s*	
6′-OMe	3.85 *s*	55.82				

* Assignments interchangeable.
† (*J* in Hz).

to the known fern phloroglucinol, aspidin BB. However, this possibility was immediately ruled out because of the absence of *O*-methyl resonances in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, there were only 12 carbon signals indicating a symmetrical molecule consisting of two butyrylfilicinic acids linked through a methylene bridge. Unambiguous assignment of the ^{1}H and ^{13}C resonances followed from ^{1}H–^{13}C HMQC and HMBC spectroscopy (Table 1). The structure of **3-BB** being identical with albaspidin BB was confirmed by comparison of our NMR data with those reported for a number of acylphloroglucinols [14]. In the HMBC spectrum, the *gem*-dimethyl groups did not interact with C=O carbon of the filicinic acid rings. This indicates that the preferred solution confirmation is the tautomer with the *gem*-dimethyl *para* to the keto function. From the HMQC spectrum it became clear why only 12 signals were observed and not the expected 13 signals: it appeared that carbons 7, 10 and 10′ all resonated at δ_C 18.23, since there were two cross peaks for this signal, one with H-7 at δ_H 3.31 and the other with the multiplet at δH 1.69 assigned to H-10/10′ by HMBC correlations (Table 1).

Compound **3-PB** (M_r 446. $C_{24}H_{30}O_8$) was identified as albaspidin PB in a similar fashion. Due to the near-symmetry of the molecule, double signals were observed for the ring carbons of both filicinic acid units. The carbons resonating at the same frequencies as the ring carbons in albaspidin BB were identified as those belonging to the butyrylfilicinic acid unit. This piece of information could not be inferred from HBMC correlations because there were no interactions between nuclei across the acyl C=Os. Assignment of the butyryl and propionyl signals readily followed from the HMBC spectrum. The acyl carbon at δ_C 206.5 showed cross peaks with two methylene proton signals (δ_H 3.16 and 1.69) and the terminal methyl protons at δ_H 1.01, which were therefore attributed to the butyryl function. Likewise, within the propionyl side chain, acyl C-8′ interacted with the methylene protons at δ_H 3.23 (H-9′) and the methyl protons at δ_H 1.17 (H-10′). Full assignment of signals is given in Table 1.

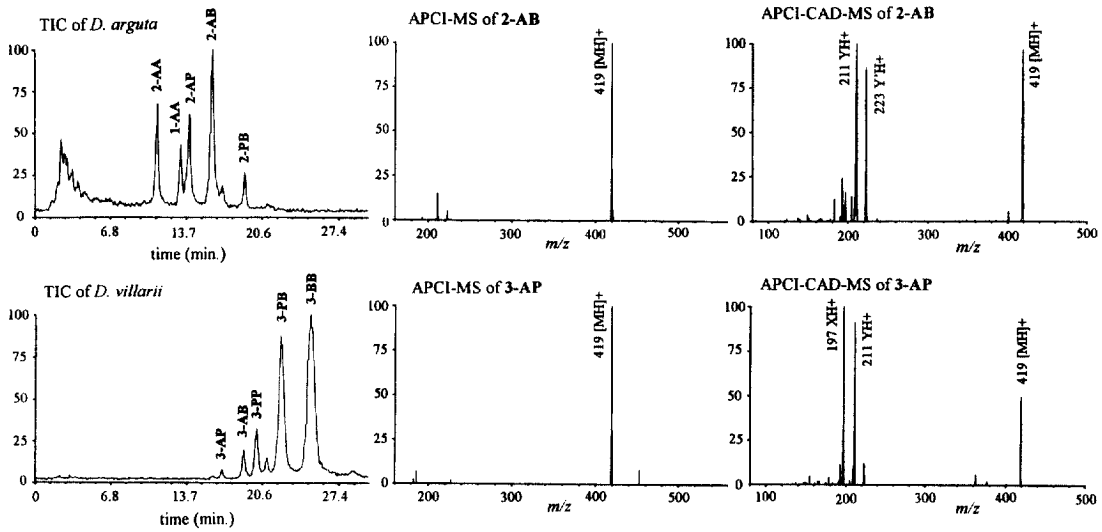


Fig. 1. HPLC-APCI-MS analysis of crude phloroglucinol mixtures of *D. arguta* (upper panels) and *D. villarii* (lower panels). Total ion current (TIC) chromatograms were recorded in the positive ion mode from *m/z* 160 to *m/z* 560 (left). The acylphloroglucinols yielded predominantly *pseudo*-molecular $[MH]^+$ ions (middle) which were collisionally activated to give X and Y cleavage ions in the MS-MS mode (CAD spectra, right).

The accompanying minor acylphloroglucinols, **3-AP**, **3-AB**, and **3-PP**, were obtained from the frond exudate of *D. villarii* in very small amounts. Their structures were tentatively assigned as the albaspidin homologues AP, PP and AB by mass spectrometry.

Compound **4** (M_r 224, $C_{12}H_{16}O_4$) was identified as aspidinol B by ^1H, ^{13}C, ^1H–^{13}C HMQC and HMBC spectroscopy. The butyryl function was located *para* to the methoxy substituent, because the aromatic proton (H-5′, at δ_H 5.96) correlated with all ring carbons but C-2′. This indicates that the aromatic proton is *para* to C-2′ and *meta* to C-1′ carrying the butyryl side chain.

Mass spectrometry

The composition of the crude phloroglucinol mixtures of *D. arguta* and *D. villarii* was examined by HPLC-APCI-MS in the single and tandem MS modes (Fig. 1). The acylphloroglucinols yielded intense protonated molecular $[MH]^+$ ions without significant fragmentation in the single MS mode. Fragmentation of the $[MH]^+$ ions was induced by collisions with Ar gas molecules in the tandem-MS mode. Basically, two fragmentation pathways were discerned, (1) cleavage of the methylene bridge on either side of C-7, and (2) loss of the acyl side chain [16]. The first route yielded the most intense fragment ions which proved to be of great diagnostic value in the identification of both acyl residues (Table 2, Fig. 2). In the albaspidins, the charge is preferentially retained on the filicinic acid fragments, XH^+ and YH^+, after cleavage of the methylene bridge. By contrast, para-aspidin-AA and the desaspidins yielded predominantly $Y'H^+$ and YH^+ ions as inferred from homologues whose XH^+ and YH^+ fragments differ in mass. Hence it seems reasonable to assume that the contribution of the YH^+ ions to the ion current is significantly larger than the contribution of XH^+ ions for all desaspidins. This indicates that the charge is retained on the phloroglucinol unit rather than on the filicinic acid unit (cf ion intensities in Table 2). Clearly, charge retention is favoured on the least acidic oxygen substituents, that is, on the phloroglucinol hydroxyl because the filicinic acid oxygen substituents participate in tautomeric proton-exchange processes. As can be seen from Table 2, differences in the ion intensities of the $Y'H^+$ ions allow distinction between desaspidins and their isomeric albaspidins. Consider, for instance, the two isomeric acylphloroglucinols, **2-AB** and **3-AP**, whose X cleavage products are of equal mass (and Y products of equal mass). They differ significantly in the intensities of the $Y'H^+$ ions: 86% observed for **2-AB** and only 12% for **3-AP** (Fig. 1, Table 2).

In the second pathway, fragment ions are formed by loss of an acyl neutral, O=C=CHR, from the $[MH]^+$ ion which are of additional diagnostic value in the albaspidins. Loss of the acyl substituent from both XH^+ and YH^+ yields protonated filicinic acid with *m/z* 155. Although the desaspidins contain an acylfilicinic acid unit, the *m/z* 155 ion was only observed in spectra of the albaspidins ([X–O=C-=CHR, usually > 10% of the base peak), and thus provides another diagnostic ion to distinguish between desaspidins and albaspidins. Additional fragment ions may arise as a result of loss of water from $[MH]^+$, XH^+ and YH^+ ions.

Acylphloroglucinols have previously been analysed by other 'soft' ionization techniques, i.e. CI-MS, FI-MS, FD-MS and FAB-mass (cf [17] and refs cited

Table 2. APCI-CAD* mass spectral data for phloroglucides **1–3** [*m/z* (rel. int.) †]

Compound	M_r	XH^+	$Y'H^+$	$X'H^-$	YH^+
Para-aspidin					
1-AA	404	197 (100)	**209** (98)	209 (98)	**197** (100)
Desaspidins					
2-AA	390	197 (12)	**195** (100)	209 (58)	**183** (93)
2-AP	404	197 (100)	**209** (88)	209 (88)	**197** (100)
2-AB	418	197 (16)	**223** (86)	209 (32)	**211** (100)
2-PB	432	211 (100)	**223** (88)	223 (88)	**211** (100)
Albaspidins					
3-AA	404	**197** (100)	209 (68)	209 (68)	**197** (100)
3-AP	418	**197** (100)	223 (12)	209 (9)	**211** (91)
3-AB	432	**197** (100)	237 (23)	209 (25)	**225** (97)
3-PP	432	**211** (100)	223 (36)	223 (36)	**211** (100)
3-PB	446	**211** (99)	237 (235)	223 (25)	**225** (100)
3-BB	460	**225** (100)	237 (56)	237 (56)	**225** (100)

* Atmospheric pressure chemical ionization-collision activated decomposition. Compare Fig. 2 for structures of fragment ions. Minor fragments are listed in the Experimental section.

† Principal ions are printed in bold type face. See also text for further explanation.

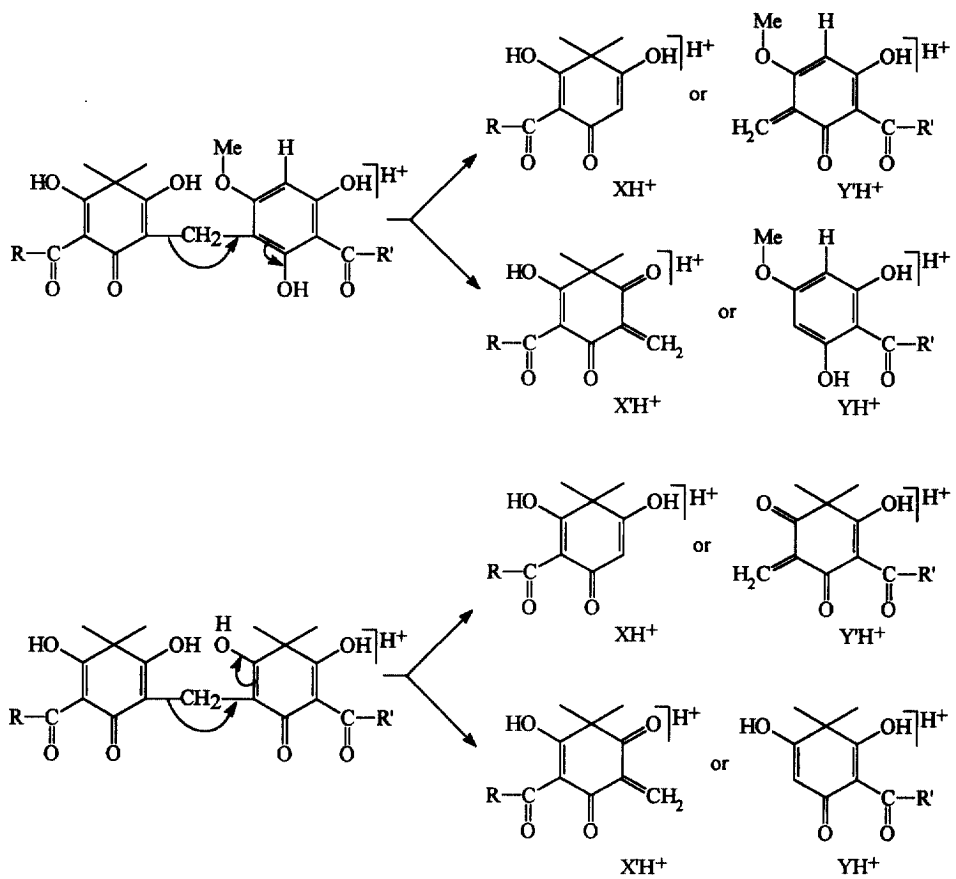


Fig. 2. APCI-CAD mass fragmentation of desaspidins (top) and albaspidins (bottom). For intensities of X and Y cleavage ions, see Table 2.

therein). By FAB mass spectrometry, Widén *et al.* [17] were able to obtain $[M]^+$s of albaspidins up to the hexamer. Dimeric albaspidins yielded similar spectra with APCI-MS in our study. Without further examples of APCI-MS analysis of olgomeric phloroglucinols it is difficult to estimate the upper limit of

the molecular range up to which molecular information can be obtained by APCI-MS. Clearly, APCI has the advantage over FAB mass spectrometry in that the lower mass region is not obscured by matrix ions and that it is well-suited for coupling with analytical HPLC. Although vaporization of the HPLC effluent involves heat, artifacts due to Rottlerone rearrangement, often seen in EI mass spectra of asymmetric oligomeric acylphloroglucinols [18], were not observed in our APCI mass spectra of asymmetric albaspidins. For instance, APCI of **3-PB** [MH^+ at *m/z* 447] did not give rise to expectable Rottlerone artifacts with *m/z* 433 and *m/z* 461. Hypothetically, Rottlerone rearrangement of an albaspidin X—CH_2—Y may give rise to two symmetric albaspidins X—CH_2—X and Y—CH_2—Y by recombination of a neutral fragment X with $X'H^+$ or Y with $Y'H^+$ in the ion source (cf Fig. 2).

Localization and distribution of acylphloroglucinols in Dryopteris

Light microscopic examinations of the three fern species revealed the presence of glandular trichomes on their leaf surfaces. The ultrastructure of the leaf glands of *D. villarii* and *C. robertiana* was investigated by scanning electron microscopy of fresh material (Fig. 3). The glandular trichomes of both species were similar. They appear to be unicellular trichomes with round to orbicular heads (Fig. 3(c)), thus corresponding with the glandular trichomes depicted earlier for *D. sparsa* [19] or reported for *Arachnoides* species [20]. According to these authors, the exudate is deposited on the distal end of these trichomes, possibly between cell wall and cuticle [21]. In view of the earlier discussed correlation between flavonoid aglycones and the presence of secretory structures [22], the leaf surface phloroglucinols of both *Dryopteris* ferns are presumably produced by their glandular trichomes. This hypothesis also parallels earlier observations that *Dryopteris* phloroglucinols are excreted along with other resinous materials by internal glands present in the rhizomes and petiolar bases [23]. Drawings as well as electron microscopic pictures showing the internal glandular hairs of *D. assimilis* Walker (= *D. expansa* (Presl) Fraser-Jenkins and Jermy) were published in 1979 [21]. In that species, glandular hairs of 60–90 μm length were observed in large intercellulars in the rhizomes. It was demonstrated that the phloroglucinols were biosynthesized in the glandular cytoplast and subsequently accumulated in a subcuticular excretion layer. Some years earlier, a drawing of the external secreting hairs of *D. fragrans* (L.) Schott was published [24]. These glandular hairs occur on the epidermis of the rhizomes and petiolar bases. They were said to contain much secreted material between the cuticle and the outer layer of the cell. Internal secreting hairs were not round in this species. Similar drawings exist for *D. campyloptera* (Kuntze) Clarkson, *D. expansa*, and *D. intermedia* (Muehl) A. Gray [25].

Comparison of the leaf surface phloroglucinols identified in this study with rhizome phloroglucinols reported for *D. villarii* and *D. arguta* [26] indicates that the leaves differ from the rhizomes with regard to phloroglucinol composition. Widén *et al.* [26] detected flavaspidic acids (BB and AB), *para*-aspidin **BB**, and filixic acids (BBB and ABB) in the rhizomes of both *D. villarii* and *D. arguta*. In *D. villarii* rhizomes, these acylphloroglucinols were found along with small amounts of desaspidin BB and albaspidin **BB**. The qualitative compositions of *D. villarii* and *D. arguta* rhizomes from different sources proved to be similar [26]. At this point it is unknown to what extent the qualitative differences are associated with organ to organ variability. Comparative analyses of leaves and rhizomes from individual plants will shed more light on this question. On the other hand, the absence of acylphloroglucinols on the leaf surface of *C. robertiana* is in agreement with an earlier report of absence of acylphloroglucinols in the rhizomes of this fern [9].

At this point we can only speculate about the ecophysiological function of leaf surface phloroglucinols of *Dryopteris*. It is conceivable that the distribution of leaf surface acylphloroglucinols reaches beyond these two species of *Dryopteris*. As with externally accumulated flavonoid aglycones, the aspect of localization of phloroglucinols in the plant certainly deserves attention when biological effects are of interest. For example, literature reports on the neurotoxic effects of acylphloroglucinols in cattle (drowsiness and blindness) eating the rhizomes and leaves of male fern (*D. filix-mas*) are exclusively focused on the rhizomes [27].

EXPERIMENTAL

Plant material

Dryopteris villarii was collected by R. Mues (Saarbrücken) in August 1995 and by E. Wollenweber (Darmstadt) in October 1996 at the Schynige Platte in the Berner Oberland, Switzerland (alt. ca 1960 m). *Dryopteris arguta* was collected by J. N. Roitman on 14 March, 1994, in oak woodland near the El Carise Oaks Campground on hwy 74, 23 miles east of Interstate 5 (San Juan Capistrano)(elev. ca 800 m), Riverside Co., CA. *Currania robertiana* was collected by R. Mues in July 1995 near Ilanz in Graubünden, Switzerland and by E. Wollenweber in the Botanischer Garten der TH Darmstadt. Fronds were carefully clipped in the field and air-dried in paper bags. Vouchers are kept at the Universities of Saarbrücken and Darmstadt.

NMR

^1H (600 MHz) and ^{13}C (150.9 MHz); $CDCl_3$ at room temp. Albaspidins **2-PB** and **2-BB** were run at 283 K

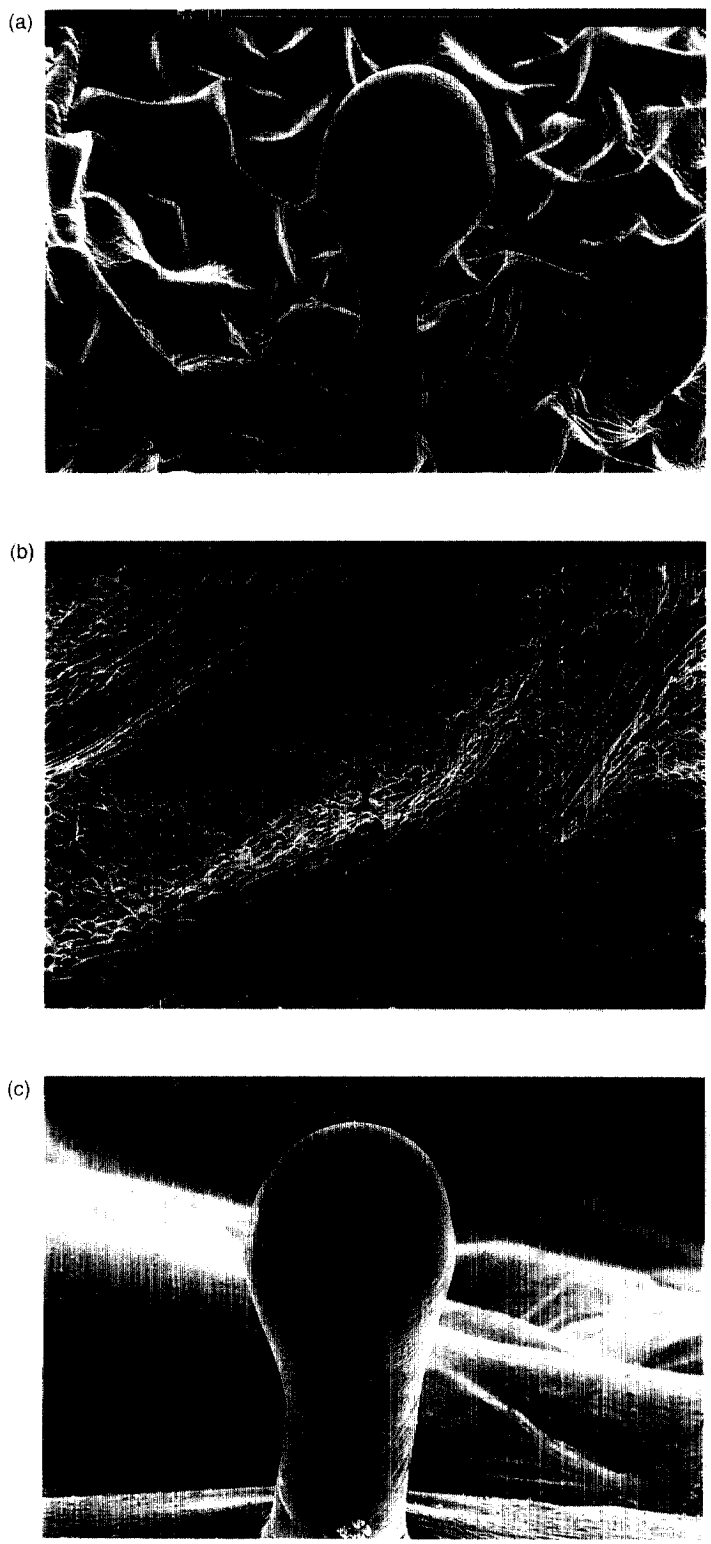


Fig. 3. SEM views of glandular trichomes on the leaf surface of (a) *D. villarii* (epidermal walls shrank on drying); (b) *C. robertiana* (lower leaf surface, survey); (c) *C. robertiana* (detail). For magnification see scale bar on the photographs.

to reduce keto-enol tautomerism. TMS and $CHCl_3$ resonances (δ_H 7.26 ppm, δ_C 73.23 ppm) were used as int. chemical shift references. ^1H–^{13}C HMQC and HMBC experiments were performed using standard pulse sequences. Spectral widths of 20 and 220 ppm were used in the H and C dimensions, respectively.

Mass spectrometry

APCI-MS: PE Sciex API III plus triple quadrupole instrument. Samples were introduced by loop injection or by HPLC via a heated nebulizer interface kept at 400° which heats the column effluent to ca 120°. Ionization of the analyte vapour mixt. was initiated by a corona discharge needle at ca 6 kV and a discharge current of ca 3 μA. The orifice plate voltage was set at 55–65 V in the positive ion mode. MS-MS experiments were performed with Ar–N_2 (9:1) as collision gas at a thickness of ca 1.9×10^{14} atoms^{-2}. The collision energy was 15 V. Other operating conditions were standard.

Isolation and chromatographic procedures

Field-collected air-dried materials as well as fresh leaves from the Botanical Garden were briefly rinsed with Me_2CO to dissolve the exudates. After evapn of the solvent, the residues of *D. villarii* and *D. arguta* were redissolved in a small amount of boiling MeOH, cooled to −18° and centrifuged to eliminate the major part of the lipophilic constituents. The supernatant was chromatographed on polyamide SC-6, eluted with toluene and increasing amounts of MeCOEt and MeOH. In *C. robertiana* the residue was directly subjected to CC on acetylated polyamide. Fractions were monitored by TLC on polyamide (DC-11, Macherey-Nagel) with solvents A [petrol (bp100–140°)–toluene–MeCOEt–MeOH 12:6:1:1], B [toluene–petrol (bp100–140°)–methyl ethyl ketone–MeOH 12:6:2:1] and C (toluene–MeCOEt–MeOH 12:5:3) and on silica with solvents D (toluene–MeCOEt 9:1) and E (toluene–dioxane–HOAc 18:5:1). Chromatograms were viewed under UV (366 nm) before and after spraying with Naturstoffreagenz A. Identification of flavonoids was achieved by co-TLC with authentic markers available in E.W's lab.

HPLC

Analyt. RP-HPLC: 5 μm LiChrospher RP-18 column (250 × 4 mm) at 1 ml min^{-1} with a linear solvent gradient from 80% to 95% MeCN in 1% aq. HCOOH over 15 min followed by a further 15 min at 95% MeCN. Semi-prep RP-HPLC: 10μm Econosil RP-18 column (250 × 10 mm) at 5 ml min^{-1}. *D. villarii* phloroglucinols were separated isocratically with 90% MeCN in 1% aq. HCOOH. For semi-prep isolation of *D. arguta* phloroglucinols, a linear solvent gradient from 80% to 95% MeCN in 1% aq. HCOOH over 15 min was used, followed by a further 15 min at 95% MeCN. The UV trace was recorded at 280 nm. Peak frs were collected manually and evapd on a rotavapor and by lyophilization. Milligram or sub-mg amounts were obtained as white to cream-coloured amorphous powders.

Para-aspidin-AA (**1-AA**). ^{1}H NMR: δ 1.55 (6H, *s*, > C(Me)$_2$), 2.14 (3H, *s*, arom. Me), 2.71 and 2.74 (each 3H, *s*, 2 × acetyl Me), 3.57 (2H, *br*, *s*, H-7), 3.74 (3H, *s*, OMe), 10.00, 11.50, 15.58 and 18.41 (*s*, 4 × OH); APCI-MS, *m/z* (rel. int.): 405 [MH]$^+$(100). APCI-CAD-MS: Table 2 and *m/z* 387 [MH − H_2O]$^+$(10), 179 [YH–H_2O]$^+$ (22).

Desaspidin (**2-AA**). ^{1}H NMR: δ 1.54 (6H, *s*, > C(Me)$_2$), 2.63 and 2.73 (each 3H, *s*, 2 × acetyl Me), 3.54 (2H, *br s*, H-7), 3.85 (3H, *s*, OMe), 6.05 (1H, *s*, H-5′), 9.92, 11.35, 16.23 and 18.41 (*s*, 4 × OH); APCI-MS, *m/z* (rel. int.): 391 [MH]$^+$(100). APCI-CAD-MS: Table 2 and *m/z* 373 (MH–H_2O] (6), 165 [YH–H_2O]$^+$ (22).

Desaspidin AP (**2AP**). ^{1}H NMR: δ 1.17 (3H, *t*, *J* = 7.2 Hz, H-10′), 1.56 (6H, *s*, > C(Me)$_2$), 2.73 (3H, *s*, acetyl Me), 3.05 (2H, *m*, H-9′), 3.53 (2H, *br s*, H-7), 3.85 (3H, *s*, OMe), 6.06 (1H, *s*, H-5′), 9.97, 11.31, 16.34 and 18.41 (*s*, 4 × OH). APCI-MS, *m/z* (rel. int.): 405 [MH]$^+$ (100); APCI-CAD-MS: Table 2 and *m/z* 387 [MH–H_2O]$^+$ (4), 179 [YH–H_2O]$^+$ (21).

Deaspidin AB (**2-AB**). ^{1}H and ^{13}C NMR: Table 1; APCI-MS, *m/z* (rel. int.): 419 [MH]$^+$ (100); APCI-CAD-MS: Table 2 and *m/z* 401 [MH–H_2O] (5), 193 [YH–H_2O]$^+$ (24).

Desaspidin PB (**2-PB**). ^{1}H NMR: δ 0.99 (3H, *t*, *J* = 7.4 Hz, H-11′), 1.17 (3H, *t*, *J* = 7.2 Hz, H-10), 1.54 (6H, *s*, > C(Me)$_2$), 1.70 (2H, *m*, H-10′), 2.93–3.07 (4H, *m*, H-9 and H-9′), 3.53 (2H, *br s*, H-7), 3.85 (3H, *s*, OMe), 6.05 (1H, *s*, H-5′), 9.94, 11.33, 16.38 and 18.47 (*s*, 4 × OH); APCI-MS, *m/z* (rel. int.): 433 [MH]$^+$ (100). APCI-CAD-MS: Table 2 and *m/z* 415 [MH–H_2O]$^+$ (4), 363 [MH − O=C=CH—C_2H_5]$^+$ (2), 193 [YH–H_2O]$^+$ (18).

Albaspidin AA (**3-AA**). APCI-MS, *m/z* (rel. int.); 405 [MH]$^+$ (100); APCI-CAD-MS: Table 2 and *m/z* 363 [MH − O=C=CH_2]$^+$ (6), 179 [XH–H_2O]$^+$ (43), 155 [filicinic acid + H]$^+$ (27). This product was not isolated but was kindly provided by Prof. C. J. Widén for comparison purposes.

Albaspidin AP (**3-AP**). APCI-MS, *m/z* (rel. int.): 419 [MH]$^+$ (100). APCI-CAD-MS: Table 2 and *m/z* 377 [MH–O=C=CH_2]$^+$ (2), 363 [MH–O=C=CH—CH_3]$^+$ (6), 179 [XH–H_2O]$^+$ (4), 193 [YH–H_2O]$^+$ (11), 155 [filicinic acid + H]$^+$ (4).

Albaspidin AB (**3-AB**). APCI-MS, *m/z*, (rel. int.): 433 [MH]$^+$ (100). APCI-CAD-MS: Table 2 and *m/z* 391 [MH–O=C=CH_2]$^+$ (4), 363 [MH–O=C=CH=C_2H_5]$^+$ (22), 207 [YH–H_2O]$^+$ (45), 179 [XH–H_2O]$^+$ (14), 155 [filicinic acid + H]$^+$ (14).

Albaspidin PP (**3-PP**). APCI-MS, mz (rel. int.): 433 [MH]$^+$ (100). APCI-CAD-MS: Table 2 and *m/z* 377 [MH–O=C=CH—CH_3]$^+$ (16), 193 [XH–H_2O]$^+$ (42), 155 [filicinic acid + H]$^+$ (14).

Albaspidin PB (**3-PB**). ^{1}H and ^{13}C NMR: Table 1; APCI-MS, *m/z* (rel. int): 447 [MH)$^+$ (100). APCI-CAD-MS: Table 2 and *m/z* 391 [MH–O=C=CH—CH_3]$^+$ (16), 377 [MH–O=C=CH—C_2H_5]$^+$ (28), 207 [YH–H_2O]$^+$ (26), 193 [XH–H_2O]$^+$ (26) [filicinic acid + H]$^+$ (14).

Albaspidin PB (**3-PB**). ^{1}H and ^{13}C NMR: Table 1; APCI-MS, *m/z* (rel. int): 461 [MH]$^+$ (100). APCI-

CAD-MS: Table 2 and *m/z* 391 $[MH-O{=}C{=}CH{-}C_2H_5]^+$ (63), 207 $[XH-H_2O]^+$ (55), 155 [filicinic acid + H]$^+$ (19). **3-BB** was identical with authentic albaspidin BB by HPLC and APCI-MS-MS.

Aspidinol B (**4**). (2′,6′-Dihydroxy-4′-methoxy-3′-methyl-l′-butyrophenone). ^{1}H NMR: δ 1.00 (3H, *t*, J = 7.2 Hz, H-4), 1.73 (2H, *m*, H-3), 2.02 (3H, *s*, Me-3′), 3.08 (2H, *t*, J = 7.2 Hz, H-2), 3.81 (3H, *s*, OMe-4′), 5.96 (1H, *s*, H-5′), 9.69 and 10.24 (2′ and 6′-OH); ^{13}C NMR: δ 7.2 (Me-3′), 14.1 (C-4), 18.5 (C-3), 46.3 (C-2), 55.9 (OMe-4′), 104.3 (C-3′), 92.1 (C-5′), 150.5 (C-1′), 160.3 (C-2′), 161.4 (C-6′) 163.6 (C-4′), 204.6 (C-1). APCI-MS, *m/z* (rel. int.), 225 [MH]$^+$ (100); APCI-CAD-MS, *m/z*: 225 [MH]$^+$ (100), 207 $[MH-H_2O]^+$ (79), 183 (45), 179 $[207-CO]^+$ (70), 151 $[207-2CO]^+$ (70), 113 (36). Compound **4** was identical with authentic aspidinol B by HPLC and APCI-MS-MS.

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