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Phloroglucinol derivatives in *Dryopteris* sect. *Fibrillosae* and related taxa (Pteridophyta, Dryopteridaceae)

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The phloroglucinol compositions of 18 species (including subspecies) belonging to *Dryopteris* Adanson sect. *Fibrillosae* Ching have been investigated on a world-wide basis, and the taxonomic implications discussed. The main emphasis is on *D. affinis* (Lowe) Fraser-Jenkins, its subspecies and varieties, as well as on *D. wallichiana* (Sprengel) N. Hylander and its relatives. The phloroglucinols of the ferns of sect. *Fibrillosae* proved to be remarkably constant in most taxa: large amounts of flavaspidic acids (*5*) and slightly varying amounts of filixic acids (*19*) were found in virtually all taxa. Traces or small amounts of norflavaspidic acids (*4*), albaspidins (*10*), oligoflavaspidic acids (*23*, *26*) and tetraalbaspidins (*25*) occur as well. Only *D. acutodentata* Ching and *D. affinis* subsp. *borreri* (Newman) Fraser-Jenkins, both partly derived by crossing with species outside the section, differ clearly from the other taxa in containing para-aspidins (*7*) and trispara-aspidin (*20*), while *D. fusco-atra* (Hillebrand) W. Robinson shows a different phloroglucinol pattern altogether. The high levels of similarity between taxa may reflect their common origin from a few diploid apomictic taxa by hybridization with sexual diploids. The different subspecies and varieties of *D. affinis* showed very similar phloroglucinol spectra except for subsp. *borreri*. The triploid apogamous subspecies *cambrensis* Fraser-Jenkins, *pseudo-disjuncta* (Fraser-Jenkins) Fraser-Jenkins and *persica* Fraser-Jenkins may have evolved from the diploid apomictic subsp. *affinis* and *D. oreades* Fomin of sect. *Dryopteris*, whereas the triploid apogamous subsp. *borreri* may have its origin from subsp. *affinis* and *D. caucasica* (A. Braun) Fraser-Jenkins & Corley of sect. *Dryopteris*. The precursor of apomictic diploid *D. wallichiana* subsp. *wallichiana* is discussed. *Dryopteris crassirhizoma* Nakai, a diploid sexual species from Japan in sect. *Fibrillosae*, is suitable from a chemical point of view, but not from its morphology. *Dryopteris conjugata* Ching in sect. *Hirtipedes* Fraser-Jenkins initially appeared to be suitable both in morphology and chemistry, provided that norflavaspidic acid (*4*) was biotransformed to flavaspidic acid (*5*) in *D. wallichiana* subsp. *wallichiana* as suggested in the present work. However, cytological investigation of one accession of this species has shown it to be diploid but apomictic, and therefore inappropriate as a sexual diploid ancestor of the diploid apomictic *D. wallichiana* subsp. *wallichiana*. Five new taxa are described: *Dryopteris wallichiana* subsp. *reichsteinii* Fraser-Jenkins, *D. affinis* subsp. *affinis* var. *jessenii* Fraser-Jenkins, *D. affinis* subsp. *affinis* var. *kerryensis* Fraser-Jenkins, *D. affinis* subsp. *cambrensis* var. *insubrica* Fraser-Jenkins and *D. affinis* subsp. *pontica* Fraser-Jenkins.

Key words: *Dryopteris* sect. *Fibrillosae*, phloroglucinol derivatives, Pteridophyta, taxonomy

INTRODUCTION

Nearly all species of *Dryopteris* Adanson and a few closely related genera of the Dryopteridaceae contain in their rhizomes and stipe-bases phloroglucinol derivatives or (acyl)phloroglucinols, which in most cases are typical for each species (see reviews by Berti & Bottari 1968, Penttilä & Sundman 1970, von Euw *et al.* 1980, Soeder 1985, Widén *et al.* 1983, 1991, 1992, 1993, Hegnauer 1962–1986, Murakami & Tanaka 1988, Gibby *et al.* 1992, Fraser-Jenkins & Widén 1993).

Considerable infraspecific variation in the phloroglucinol spectrum has only been observed in a few species e.g. *D. expansa* (C. Presl.) Fraser-Jenkins & Jermy (= *D. assimilis* S. Walker) and *D. pallida* (Bory) C. Chr. ex Maire & Petitm., either for plants from different parts of the world or sometimes occurring even within the same populations (von Euw *et al.* 1980, Widén *et al.* 1991 and references therein).

The first attempts to divide the genus *Dryopteris* into sections were made by Itô (1935, 1936, 1939) emended by Serizawa (1976), or into different groups by Ching (1936–1938, 1965, 1978). These works were based on material from Japan or from China and the Sikkim Himalaya respectively. A more elaborate classification on a world-wide basis was recently proposed by one of the present authors (Fraser-Jenkins 1986), who listed c. 225 species and 89 hybrids as well as several subspecies. He divided the genus *Dryopteris* into four subgenera and 16 sections corresponding partly to Itô's sections and Ching's groups.

The phloroglucinol derivatives of 17 taxa of sections *Dryopteris*, *Remotae* Fraser-Jenkins, *Pallidae* Fraser-Jenkins and *Marginatae* Fraser-Jenkins, all belonging to subgenus *Dryopteris*, were recently studied by Widén *et al.* (1991). In continuation of this and other recent work (Widén *et al.* 1992, 1993, Fraser-Jenkins & Widén 1993, Gibby *et al.* 1992) we have now investigated the phloroglucinols of 16 out of 18 species in subgenus *Dryopteris*, sect. *Fibrillosae* Ching (Fraser-Jenkins 1986).

The taxonomy and distribution of all these species are presented briefly below (see also detailed discussions by Fraser-Jenkins 1980, 1986, 1988, 1989, 1994). *Dryopteris hawaiiensis* (Hillebrand) W. Robinson, which was misidentified and therefore included in sect. *Fibrillosae* by Fraser-Jenkins (1986), has been transferred by him (1994) to sect. *Remotae*, which explains the anomalous chemical results of Patama and Widén (1991). Consequently it is excluded from the present paper. We have also investigated *D. zayuensis* Ching & S. K. Wu, which was not listed by Fraser-Jenkins (1986).

Of the *Dryopteris* species discussed here only *D. affinis* (Lowe) Fraser-Jenkins, *D. crassirhizoma* Nakai, *D. fusco-atra* (Hillebrand) W. Robinson, *D. polylepis* C. Chr. and *D. wallichiana* (Sprengel) N. Hylander have been investigated previously (Hisada & Noro 1961, Hisada 1966, Fikenscher & Hegnauer 1963, Hisada *et al.* 1971, 1972, Tryon *et al.* 1973, Noro *et al.* 1973, Widén *et al.* 1975ab, Tanker & Çoşkun 1978, Patama & Widén 1991).

The taxonomy (including synonymy), distribution and cytology of each of the taxa investigated are discussed below.

Synopsis of *Dryopteris* sect. *Fibrillosae*

Dryopteris sect. *Fibrillosae* consists mainly of apomictic ferns, either diploids related to *D. wallichiana* subsp. *wallichiana,* or higher polyploids, probably derived from the apomictic diploid species *D. wallichiana* subsp. *wallichiana* or *D. affinis* subsp. *affinis* or their relatives by hybridization with a sexual diploid species to form a new apomictic species or subspecies (Fraser-Jenkins 1980, 1986, 1988, 1989, 1994). Morphologically this section resembles sect. *Dryopteris* and perhaps even more closely sect. *Hirtipedes*, both belonging to subgenus *Dryopteris* (Fraser-Jenkins 1986). The salient features of its species are that the pinnae are divided into more-or-less rectangular, parallel-sided lobes or pinnules, either unlobed or becoming shallowly lobed in the lower lamina, the stipe and rachis usually densely scaly and fibrillose with a strong tendency towards narrow scales, and the lamina is usually lanceolate and not widely deltate. Being apomictic, several species are markedly variable and have been divided into subspecies and varieties, e.g. *D. affinis* and *D. wallichiana* (see below). This, as well as the existence of numerous synonymic names, often leads to serious problems in the correct interpretation of taxa. Also problematical is that the sexual diploid species involved in the triploid taxa may be members of another section (sect. *Dryopteris*, sect. *Hirtipedes* or sect. *Pallidae*), causing further problems in classification to section of the derived triploids.

The Dryopteris wallichiana complex and related taxa

Dryopteris wallichiana subsp. *wallichiana* (= *D. parallelogramma* (Kuntze) Alston) is a diploid apomictic species with a world-wide tropical and subtropical distribution. It exhibits considerable morphological variation even in the Himalaya and SW China, the centre of its distribution. In the Darjeeling area and in E Nepal and SW China there is a morphological variant viz. the "Darjeeling variety" of Fraser-Jenkins (1989) with slightly larger pinnules with impressed veins and darker scales, while in SW China another variant occurs with very black scales. In Hawai'i and tropical Central and South America many, but not all populations have brown rather than blackish scales, though both colours occur in the Himalaya, darker scales being predominant. Three very closely related taxa described originally as species, or in one case as a subspecies belonging to *D. affinis* have now been reduced to subspecies of *D. wallichiana*, and differ from it only in cytotype (all of them being triploid) and in minor morphological characteristics. These are:

1. subsp. *coriacea* (Fraser-Jenkins) Fraser-Jenkins, from the Caucasus, Anatolian Turkey and Iran,
2. subsp. *madrasensis* (Fraser-Jenkins) Fraser-Jenkins, from S India (with subsp. *wallichiana* as a rarity there), Sri Lanka and parts of SE Asia (Fraser-Jenkins 1980, 1988, 1994)
3. subsp. *reichsteinii* Fraser-Jenkins, *subsp. nov.* [*Subspecies similis ad subsp. wallichianam, sed pinnulis latioris et latiore conjunctis, pinnis angustiore acutis ad apices earum et cytotypo triploideo differt.* Holotype: Zimbabwe. Inyanga Mountains, source of Pungwe River, under *Widdringtonia*, c. 2 100 m, *Schneller 237*, 3.III.1979 triploid; BM.]

The last subspecies may be related to the taxon described as *D. paleacea* (Lagasca ex. Sw.) C. Chr. var. *madagascariensis* C. Chr. from northern Madagascar, but that has never been examined in detail nor has the cytology or chemistry been studied, and its frond morphology is perhaps more similar to *D. wallichiana* subsp. *wallichiana*.

In the W Himalaya *Dryopteris wallichiana* is replaced by the diploid sexual species, *D. redactopinnata* S. K. Basu & Panigr. in Pakistan, Kashmir, Uttarkashi, W and central Nepal, SW China, Taiwan and SE Tibet which is intermediate in morphology between *D. wallichiana* and *D. xanthomelas* (Christ) C. Chr. At higher altitudes in the Himalaya *D. wallichiana* is replaced by the diploid apomict *D. xanthomelas* (= *D. pulcherrima* Ching) growing in Pakistan, India, Nepal, Bhutan, SE Tibet and W China (Fraser-Jenkins 1989, 1992). In the central and E Himalaya another rather rare triploid apomict, *D. neorosthornii* Ching, occurs. It is known from India, Nepal, SE Tibet and China and is close to *D. xanthomelas* but is larger with rounded segments and more scattered, wider rachis scales. Another species from the same region is the poorly known taxon *D. zayuensis* Ching & S. K. Wu. It is intermediate in morphology between *D. xanthomelas* and *D. neorosthornii* and is therefore difficult to identify with certainty though it has wider and more numerous dark scales than either and is bigger than *D. xanthomelas*, but narrower and more compact than *D. neorosthornii* with smaller segments. A lobed form of this, described as *D. incisolobata* Ching and S. K. Wu, has also been found by Fraser-Jenkins in Nepal and Tibet, but is not accepted here as a species.

Dryopteris yigongensis Ching is a triploid apomict growing in India, SE Tibet and China. It has a wider lamina-base than in *D. xanthomelas*, and is slightly variable in morphology and may consist of two species preliminarily designated here as "Hattu 1" and "Hattu 2". True *D. yigongensis* ("Hattu 1") is closer to *D. xanthomelas* and the putative new species ("Hattu 2") is slightly more intermediate towards *D. juxtaposita* Christ. True *D. yigongensis*, or perhaps both, may have been derived from *D. xanthomelas* and a member of another section at some stage in the past (Fraser-Jenkins 1989).

Dryopteris lepidopoda Hayata is a diploid apomict with a broad distribution in India, Nepal, Bhutan, SE Tibet, China and Taiwan. This species is usually distinguishable by its wider lamina, more acute teeth, longer stipe and narrower dark scales from the related species, *D. wallichiana*. However, *D. lepidopoda* is somewhat variable and some specimens imitate *D. wallichiana* in having browner scales (CRFJ 8832, 8835, 8840). A further unnamed taxon from the central Himalaya that appears intermediate between the two has not been investigated here.

Dryopteris khullarii Fraser-Jenkins is a triploid apomict known so far only from the W Himalaya in India. It is intermediate in morphology between

Fig. 1. *Dryopteris affinis* (Lowe) Fraser-Jenkins subsp. *affinis* var. *jessenii* Fraser-Jenkins (holotype, BM).

D. wallichiana and *D. nigropaleacea* (Fraser-Jenkins) Fraser-Jenkins (sect. *Pallidae*) and appears likely to have been derived by hybridization between these two species (Gibby 1985, Fraser-Jenkins 1989).

Dryopteris acuto-dentata Ching is a triploid apomict growing in India, Nepal, S and SE Tibet, and China (Gibby 1985, Fraser-Jenkins 1989). It is intermediate in morphology between *D. xanthomelas* and a species such as *D. alpestris* Tag. or *D. komarovii* Koss. (= *D. barbigera* (T. Moore ex Hook.) Kuntze subsp. *komarovii* (Koss.) Fraser-Jenkins) (sect. *Dryopteris*).

In S India and possibly also Sri Lanka there is a rare, tetraploid, probably apomictic species, *Dryopteris sledgei* Fraser-Jenkins.

In New Guinea *Dryopteris wallichiana* is mainly replaced by a distinct triploid species, *D. parrisiae* Fraser-Jenkins. However *D. wallichiana* also occurs in Java and Borneo as well as (possibly) Madagascar and (rare) in New Guinea, Hawai'i, Central and South America, the South Atlantic Islands and the Caribbean (Fraser-Jenkins 1980, 1989, Gibby 1985).

In Mexico there is an additional triploid apomict, *Dryopteris pseudo-filix-mas* (Fée) Rothm. (= *D. chrysocarpa* (Fée) Rothm.) which is morphologically similar to *D. affinis* subsp. *borreri* (Newman) Fraser-Jenkins.

In Hawai'i two more species of the complex have been found in addition to *Dryopteris wallichiana*. One of these, *D. fusco-atra* consists of two morphological variants, viz. var. *fusco-atra* and var. *lamoureuxii* Fraser-Jenkins (see Fraser-Jenkins 1994). It is intermediate between *D. wallichiana* and the diploid sexual *D. hirtipes* (Bl.) Kuntze (sect. *Hirtipedes*), and may be a triploid apomict derived by hybridisation of these two species. The other one, *D. subbipinnata* W. H. Wagner (= *D. palikuensis* Herat ex Fraser-Jenkins) may also be a triploid apomict, probably derived by hybridisation from *D. wallichiana* and some other tripinnate diploid sexual species in Hawai'i.

In E Asia two additional less closely related sexual diploids are found in the *Dryopteris wallichiana* complex. These are *D. polylepis* C. Chr., which is found in Japan and China, and *D. crassirhizoma* Nakai distributed in Japan, Sakhalin, southern Kuriles, Korea and N and central China (Hirabayashi 1974).

The Dryopteris affinis complex

Dryopteris wallichiana and its relatives are lacking from Europe, but are replaced by the closely related complex species *D. affinis*. In fact it seems likely that the same genome which has given rise to *D. wallichiana* may also be present in the *D. affinis* complex (Fraser-Jenkins 1980). However the present apomictic diploids could not have been the direct ancestors, as a sexual diploid form would be needed. This has not been discovered so far.

Dryopteris affinis s. lat. in Europe is divided into four subspecies, viz. subsp. *affinis*, a diploid apomict, subsp. *borreri*, a triploid apomict, subsp. *cambrensis* Fraser-Jenkins (= *D. cambrensis* (Fraser-Jenkins) Beitel & Buck), a triploid apomict, and subsp. *pseudo-disjuncta* (Fraser-Jenkins) Fraser-Jenkins (= var. *setosa* (Christ) Fraser Jenkins), also a triploid apomict. Because of discontinuous morphological variability, subsp. *affinis* is subdivided into five varieties:

1. var. *affinis*.
2. var. *jessenii* Fraser-Jenkins, *var. nov.* [*Var. affinis paleis stipitis nigris, apicibus pinnularum valde rectangularibus, dentibus longioribus differt.* Holotype: Romania. Oltenia, slope beside road, 15 miles up the Cerna valley from Baile Herculane, north of Turnu Severin, *Fraser-Jenkins 3503*, 27.VII.1971 (BM; Fig. 1). Isotypes: ditto (G, H)]. Occurs in the Transylvanian Alps in south central Romania and in one locality near Novi Pazar in Serbia (formerly Jugoslavia).
3. var. *kerryensis* Fraser-Jenkins, *var. nov.* [*Var. affinis lamina minoris, pinnulis confertis, apicibus rectangularioribus, saepe devexis vel obtusis, dentibus prominentibus differt.* Holotype: Ireland. Co. Kerry, road bank between Tahille and Rossdohan, on side road off Kenmare to Sneem and Cahersiveem road, W of Kenmare on way to Rossdohan, *Fraser-Jenkins 15174*, 6.VI.1988 (BM; Fig. 2). Isotype: ditto (G)].
4. var. *paleaceo-lobata* (Moore) Fraser-Jenkins.
5. var. *splendens* (Ehrler ex Becherer) Fraser-Jenkins (= var. *punctata* Oberholzer & Tavel ex Fraser-Jenkins).

For the same reason *Dryopteris affinis* subsp. *cambrensis* is subdivided into two varieties, var. *insubrica* Oberholzer & Tavel ex Fraser-Jenkins, *var. nov.* [*Var. paleaceo-crispa paleis stipitis nitidioris russo-succineis, pinnulis acute-lobatis ad margines, dentibus longioribus acutioribusque ad apices pinnularum, indusiis et axibus laminae glandulosioris differt.* Holotype: Switzerland. Tessin, Lugano, an Fuss einer Mauer an der Strasse von Breganzona nach Muzzano, Südlage, 430 m, *Tavel s. n.*, 14.X.1916 (BERN)]. It occurs in Central Europe from Belgium and SE France throughout the Alps to Italy, S Germany, Austria, former Jugoslavia, W Hungary, Bulgaria, Czech Republic, Slovakia and S Poland. The other variety is var. *paleaceo-crispa* (Moore) Fraser-Jenkins, from Britain, Norway, N France, Germany and N Turkey.

In SW Asia two additional subspecies occur, both triploid apomicts, viz. *Dryopteris affinis* subsp. *persica* Fraser-Jenkins, and subsp. *pontica* Fraser-Jenkins, *subsp. nov.* [*Subspecies subsp. persica similis, sed paleis stipitis fusco-lineatis, pinnulis latioris ad bases earum, valde lobatis a lobis rectangularis, apicibus obtusis vel angustis truncatis differt.* Holotype: Turkey. Ordu vilhayet, 0.5 km below Kurtibes, S of Gölköy, 5 km N of top of Harçbeli Pass, Gölköy to Mesudiye, S of Ordu, *Fagus* woods, *Fraser-Jenkins 14065*, 15.XI.1987 (BM; Fig. 3). Isotypes: ditto (G, H). Paratypes: ditto, *14058-14064, 14066-14068* (BM, H, herb. CRFJ)]. Occurs along the Caspian Coast of Iran, in Transcaucasia and in N Anatolian Turkey. The taxonomy of these taxa is only poorly known as yet.

Fig. 2. *Dryopteris affinis* (Lowe) Fraser-Jenkins subsp. *affinis* var. *kerryensis* Fraser-Jenkins (holotype, BM).

It seems possible that the triploid *Dryopteris affinis* subspp. *cambrensis*, *pseudo-disjuncta* and *persica* have evolved from diploid *D. affinis* subsp. *affinis* and *D. oreades* Fomin, a diploid sexual species in sect. *Dryopteris*. The triploid subsp. *borreri* in turn may have evolved from subsp. *affinis* and the diploid sexual species *D. caucasica* (A. Braun) Fraser-Jenkins & Corley, also in sect. *Dryopteris* (Fraser-Jenkins in Prelli & Prelli 1990). The origin of subsp. *pontica* is not clear. (See p. 94).

Fig. 3. *Dryopteris affinis* (Lowe) Fraser-Jenkins subsp. *pontica* Fraser-Jenkins (holotype, BM).

MATERIAL AND METHODS

Plant material

Voucher specimens are preserved in H. For the collector abbreviations, see Table 1, footnote 2.

Dryopteris parrisiae: CPG 3396 (ex hort. Chelsea Physic Garden 19.IX.1993), Parris s. n., 19.XII.1980, Papua New Guinea, Western Highland Province, Tomba Path, Mt. Hagen, c. 2 600 m (1 dry rhizome); triploid, apomict (2n = 123) (Gibby 1985, sub *D. wallichiana*).

Dryopteris xanthomelas: CRFJ 7520-24, 21.VIII.1978, India, Kashmir, Liddar valley (5 dry rhizomes, 130 g). CRFJ 7525-6, 21.III.1978, India, Kashmir, Chatponsal, 3 400 m (2 rhizomes, 42 g). CRFJ 7611, 26.VIII.1978, India, Himachal Pradesh, Simla area, Narkanda, NE side of Mt. Hattu, below top, 3 000 m (1 rhizome, 15 g). CRFJ 7832-3, 7835, 10.IX.1978, India, Satrundi, N of Tissa, NW of Chamba, 3 300 m (3 rhizomes). CRFJ 8371, 25.X.1978, India, Uttar Pradesh, Chamoli, Trijugi Naryan to Mongu, N of Rudraprayag, 3 300 m (1 big rhizome).

Dryopteris zayuensis: CRFJ 7837, 10.IX.1978, same place and date as CRFJ 7832 (under *D. xanthomelas*; 1 rhizome) [previously identified as "*D. pulcherrima*" (Fraser-Jenkins 1989) but the subspecies subsequently accepted and its nomenclature accepted by Fraser-Jenkins (1992a)].

Dryopteris zayuensis (lobed form, corresponding to the synonym *D. incisolobata*): Kiang & Chiao 296, 1.XI.1967, China, Tibet, Gyirong, 3 380 m.

Dryopteris crassirhizoma: Seki s. n., 28. VIII. 1973 (2 rhizomes) and Iwatsuki s. n., 6.VII.1973, Japan (10 rhizomes) (see Widén *et al.* 1975 ab).

Dryopteris neorosthornii: CRFJ 8363, 8364, 8370, 25.X.1978, India, Uttar Pradesh, Chamoli, Trijugi Naryan to Mongu, N of Rudraprayag, 3 300 m (3 rhizomes).

Dryopteris redactopinnata: CRFJ 7590, 7592, 7593, 7641, 7662, 7663, 26.VIII.1978, India, Himachal Pradesh, NE of Simla, SE of Narkanda, Mt. Hattu, below top (6 rhizomes); diploid (2n = 82) (Gibby 1985). IK 6939, 22.VI.1972, India, Himachal Pradesh, E of Manali, Nagar, Chanderchani Pass, 2 800 m (piece of rhizome). CRFJ 7838-40, 10.IX.1978, India, 15 km below Satrundi, Chamba, 2 700 m (3 rhizomes).

Dryopteris yigongensis ("Hattu 1 species"): CRFJ 7584, 7589, 7610, 7617, 7632, 7634, 7636, 7637, 26.VIII.1978, India, Himachal Pradesh, Simla, Narkanda, NE side of Mt. Hattu, 3 000 m, below top (9 small rhizomes, total 100 g). CRFJ 7643, 7645, 7648, same place and date (4 rhizomes, 170 g). CRFJ 7585, same place and date (1 small rhizome, 7 g). CRFJ 7586, 7609, same place and date (2 rhizomes) [CRFJ 7612 from the same locality and date as 7584-7637 represents "Hattu 1" and is triploid, 2n = c. 123 (Gibby 1985)]. ("Hattu 2 species"): CRFJ 7652, 7654-56, 7658, as 7584-7637 above (five rhizomes, 270 g) [CRFJ 7647 and 7657 from the same locality and date as 7652-7658 represent "Hattu 2" and are triploid, 2n = 123 (Gibby 1985)].

Dryopteris acuto-dentata: CRFJ 7796-7806, 10.IX.1978, India, Himachal Pradesh, 2 km below Satrundi, N of Tissa, N of Ravi valley, Chamba, 3 300 m (type locality; eleven rhizomes, 70 g); triploid apomict, 2n = 123 (Gibby 1985).

Dryopteris wallichiana subsp. *wallichiana:* Puri 20.4 (= TR 4405), raised ex spores from plant from India, Central Himalaya, Nainital (29°24′N, 69°28′E); diploid apomict, 2n = 82 (det. M. Gibby in litt. 10.I.1980). Rhizome (80 g) collected and dried 14.–16.III.1986 ex cult. Agarone, Switzerland by TR. CRFJ 8356-59, 25.X.1978, India, Uttar Pradesh, Chamoli, Trijugi Naryan to Mongu, N of Rudraprayag, c. 2 400 m (3 rhizomes). CRFJ 8483, 8485-8487, 15.XI.1978, India, West Bengal, W of Darjeeling, N of Tonglo, Khalpokri to Gairibas, 2 900 m (4 rhizomes, 1 180 g). TR 2419 (= CRFJ 10926), raised ex spores by Reginald Kaye, Silverdale (Great Britain), obtained from a commercial dealer, Ghose & Co., Darjeeling, N India, with the indication that they were collected in Kashmir. [The species is not known from Kashmir, and the source is probably near Darjeeling where similar plants can be found (see Fraser-Jenkins 1989).

The identification of the plant was confirmed by the late R. C. Ching in PE]. Rhizome (180 g) ex cult. Agarone and dried 14.–16.III.1986 by TR. TR 4166 raised ex spores from plant from Japan, rhizome collected by Dr K. Iwatsuki, X.1976 on Mt. Sakurajima (Kagoshima, Kyushu). Rhizome (48 g) collected ex cult. Agarone and dried 14.–16.III.1986; diploid apomict, det. J. J. Schneller (in Fraser-Jenkins 1989). CRFJ 13108, 13.IV.1987, Mexico, Chiapas, 9 km SE of El Porvenir, c. 2 300 m (1 large rhizome). CRFJ 135252, 1.V.1987, Mexico, Oaxaca, Ixtlan, Llano Verde (1 rhizome). CRFJ 14773, 14775-6, 14777, 14782-3, 25.II.1988, Hawai'i, East Maui, W side of Mt. Haleakala S of Pukalani, Waipoli Road, above Kula, c. 1 200–1 500 m (7 rhizomes). CRFJ 14710-1, 14.II.1988, Jamaica, Surrey, NE of Hagley Grap and Mavis Bank on path to Blue Mountain Peak, 2 500 m (2 rhizomes). CRFJ 13004-5, same place, 3.IV.1987 (2 rhizomes). "Darjeeling variety": CRFJ 8536, 16.XI.1978, India, West Bengal, W of Darjeeling, between Gairibas and Tonglo, 2 700 m (1 rhizome, 235 g). CRFJ 8538, same place and date (1 rhizome, 250 g).

Dryopteris wallichiana subsp. *coriacea*: PA 33122, 11.VII.1977, Georgia, Adzharian ASSR, Mt. Mtirala, 600 m (piece of rhizome; the Finnish Dendrological Society, summer excursion to Caucasus). CRFJ 5931-3 (= TR 4532, 4294, 4292), 30.VI.1977, Iran, Gilan, Assalem, 5.5 km up Nav valley, 110 m (3 rhizomes).

Dryopteris wallichiana subsp. *reichsteinii*: TR 4089, raised ex spores from plants from Zimbabwe, Inyanga, source of Pungwe river under *Widdringtonia*, c. 2 100 m, S exposed, 3.III.1979, Schneller 237; triploid, det. J. J. Schneller. Rhizome (100 g) collected ex cult. Agarone and dried 14.–16.III.1986 by TR.

Dryopteris wallichiana subsp. *madrasensis*: CRFJ 9204, 9206, 9207, 20.XII.1978, India, Tamil Nadu, Palni Hills, SE of Kodaikanal, c. 1 200 m (three rhizomes, 850 g). CRFJ 9359, 26.XII.1978, 4 km above Ootacamund on W side of Dodabetta Mt., Nilgiri Hills, 2 450 m. CRFJ 9362, 26.XII.1978, E side of Ootacamund, Nilgiri Hills, 2 600 m (1 rhizome, 70 g); triploid apomict, 2n = 123 (Gibby 1985).

Dryopteris affinis subsp. *affinis* var. *affinis*: CJW 78/6, 3.I.1978, Madeira, Santo di Serra, 600 m (1 rhizome). TR 2616 cult. in Agarone, Madeira, Santo di Serra, 800 m, 17.IV.1969, C. J. de Joncheere, J. D. Lovis, W. Pickering, H. L. & T. Reichstein s. n. (1 rhizome, 62 g); diploid with 82 bivalents in cell from 8-celled sporangium, det. G. Vida. TR 3548, Azores, Sao Miguel, along road between Furnas and Salga, c. 650 m, 2.V.1973 (2n = c. 82, det. G. Vida in litt. 20.X.1973). 1 rhizome, 32 g ex cult. Agarone, TR & S & S s. n., 17.VIII.1948, Portugal, Serra do Geres, albegaria, margems do rio Homen (piece of rhizome from herbarium specimen), Dyce & Parker 11824, 30.VII.1968, England (see Widén *et al.* 1971; piece of rhizome from herbarium specimen). [Fraser-Jenkins has found that 11824 is a mixed gathering and is either subsp. *affinis* or subsp. *borreri* in different herbaria; the actual specimen investigated in H is subsp. *affinis*, though other specimens in H are subsp. *borreri*]. CRFJ 12700, 12702-3, 15.IX.1986, Scotland, Dunbartonshire, N of Dumbarton, Loch Lomond, roadside wood c. 1 mile N of Tarbert (3 rhizomes). TR 293, 16.XI1986, Germany (see Widén *et al.* 1971; 1 rhizome, 26 g). CRFJ 11729, 11733, 21.X.1985, Yugoslavia, Hrvatska, c. 500 m up side turn above Macelj Gora, on Trakosan road, N side of mountains S of Ptuj, off Zagreb road, N of Krapina, c. 65 km N of Zagreb (2 rhizomes).

— var. *paleaceo-lobata*: CRFJ 12784, 19.IX.1986, Wales, Glamorgan, N of Treorchy, road to Nant-y-moel, c. half a mile below NE side of the Bwlch pass (1 rhizome).

— var. *splendens*: CRFJ 11590, 13.X.1985, Switzerland, Kt. Nidwalden (Unterwalden), Haltiwald, S of Luzern, N of Hergeswil (1 rhizome). TR 740, 3.X.1962, Kt. Zürich, N side of the road to Hoher Rohn above Hütten, 950 m, E. Oberholzer s. n.; diploid, apomict (c. 82 bivalents), det. G. Vida in litt. 1967, ex cult. Basel (1 rhizome, 40 g), coll. 16.IX.1986. CRFJ 11647, 16.X.1985, Kt. Luzern, S of Luzern on Horw Road, W side of Bireggwald, 480 m. CRFJ 11751, 21.X.1985, Yugoslavia, Hrvatska, same place and date as CRFJ 11729, 11733 (cited under var. *affinis*).

— var. *jessenii*: CRFJ 14440, 11.XI.1987, Romania, Sibui Judeţul, Fagaraş Mts, 6 miles from Cirţişoara turn (1 rhizome). CRFJ 14422-3, 11.XI.1987, ditto (3 rhizomes).

Dryopteris affinis subsp. *persica*: CRFJ 5922, 13.VI.1977, Iran, Gilan 5.5 km up Rudkanceh-e-Nav valley, above Assalem, S of Siadun (1 rhizome, ex cult. Bridgend).

Dryopteris affinis subsp. *pontica*: CRFJ 14058, 14060-1, 14064, 15.X.1987, Turkey, Anatolia, Kurtibeş, Gölköy, S of Ordu (4 rhizomes). CRFJ 5948 (= TR 4264A), dry weight 358 g, ex cult. Basel (1 rhizome) coll. 21.X.1985, Iran, Assalem, calc. gorge c. 200 m, 11 km up Rudkanceh-e-Nav valley.

Dryopteris affinis subsp. *cambrensis* var. *paleaceo-crispa*: JAC & Jermy 1873, 12.IX.1962, Scotland, Vice-County 88, Mid Perth, Carbane Castle, Glen Lyon (piece of rhizome). CRFJ 12507-09, 6.IX.1986, Perthshire, NW of Crieff, Glen Ogle, c. 2 miles S of junction with Killin road, along track to N of small loch (3 rhizomes). CRFJ 12561, 12581, 12592, 9.IX.1986, Scotland, (see Fraser-Jenkins & Widén 1993).

— form imitating *D.* x *complexa*: CRFJ 12634, 9.IX.1986, Scotland, Inner Hebrides, NW Skye, Woods in from gate just S of Dunvegan Castle car park (1 rhizome). CRFJ 11702, 19.IX.1985, Austria, Salzburg, c. 1 km W of SE tip of Mond See, W of Unterach, Salzburg to Bad Ischl (1 rhizome).

— var. *insubrica*: CRFJ 11838, 30.X.1985, Switzerland, Kt. Ticino, Monti di Ditto to Monti Motti, above Cugnasco, W of Bellinzona (1 rhizome). CRFJ 11841-42, 11846, 11848, 11849, same place and date (5 rhizomes). TR 2293, Switzerland, Kt. Ticino, 3 km NW of Intragna between Ca1asco and Delri at c. 1 000 m, 1.VII.1967, Seitter s. n. (triploid, 2n = 123, det. G. Vida in litt. 20.IX.1968; 123 bivalents in 8-celled sporangia, n = c. 37^{II} + 45 and 38^{II} + 50^{I}, H. Rasbach in litt. 3.XII.1986; rhizome taken 15.XI.1986, 28 g, ex cult. Basel). TR 2845, Switzerland, Kt. Graubünden, upper Bergell (Val Bregaglia) of the Orlegne alluvion of Cavril at c. 1 600 m, 24.X.1968, Seitter s. n. (coll. living, triploid, 2n = c. 123, det G. Vida in litt. 23.I.1970; rhizome taken 15.XI.1986, 26 g, ex cult. Basel). CRFJ 11741, 21.X.1985, Yugoslavia,

Table 1. Semiquantitative results showing the phloroglucinol composition in different taxa of subgenus *Dryopteris* section *Fibrillosae* and related taxa. Estimated from intensity of spots in TLC and yield in preparative column chromatography. +++ = 20% or more of the crude filicin mixture; ++ = 10–20%; + = 5–10% ; (+) = 1–5%; – = < 1%.

Taxon and ploidy, reproduction[1] Collection number or lit.ref.[2]	Origin[3]	Aspidinol-B (*2*-B)[4]	Norflavaspidic acid-BB (*4*-BB)[5]	Norflavaspidic acid-AB (*4*-AB)	Flavaspidic acid-BB (*5*-BB)[5]	Flavaspidic acid-AB (*5*-AB)	Para-aspidin-BB (*7*-BB)[5]	Para-aspidin-AB (*7*-AB)	Albaspidin-BB (*10*-BB)[6]	Albaspidin-AB (*10*-AB)[7]	Albaspidin-AA (*10*-AA)	Desaspidin-BB (*8*-BB)[5]	Filixic acid-BBB (*19*-BBB)[8]	Filixic acid-ABB (*19*-ABB)[9]	Filixic acid-ABA (*19*-ABA)	Trispara-aspidin-BBB (*20*-BBB)	Trisdesaspidin-BBB (*21*-BBB)	Trisflavaspidic acid-BBB (*23*-BBB)	Trisflavaspidic acid-ABB (*23*-ABB)	Tetraalbaspidin-ABBA (*25*-ABBA)	Tetraalbaspidin-BBBB (*25*-BBBB)	Tetraflavaspidic acid-BBBB (*26*-BBBB)
Sect. Fibrillosae																						
D. parrisiae 3x, a	CPG3396	New Guinea	–	–	–	+	+++	–	–	–	–	–	–	+	++	++	–	–	(+)	+	+	–	–
D. xanthomelas	CRFJ7520-24	Kashmir	–	–	–	+++	+++	–	–	–	–	–	–	(+)	(+)	(+)	–	–	+	+	+	–	+
2x, a	CRFJ7525-26	Kashmir	–	–	–	+++	+++	–	–	–	–	–	–	(+)	(+)	(+)	–	–	+	+	+	–	+
	CRFJ7611	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	(+)	+	–	–	+	+	+	–	+
	CRFJ7832-33	N India	–	–	–	+++	+++	–	–	–	–	–	–	+	+	+	–	–	+	+	+	–	+
	CRFJ7835	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
	CRFJ8371	N India	–	–	–	+++	+++	–	–	–	–	–	–	–	+	+	–	–	+	+	(+)	–	+
D. zayuensis 2x, a	CRFJ7837	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
"lobed form"	King & Chiao 296	Tibet	–	–	–	++	++	–	–	–	–	–	–	+	++	++	–	–	+	+	–	–	–
D. polylepis 2x, s	Widén *et al.* (1975)	Japan	–	–	–	+++	+++	–	–	(+)	?	?	–	++	++	++	–	–	–	–	++	–	–
	Hisada & Noro (1961), Hisada & Noro (1972)	Japan	(+)/–	–	–	++	++	–	–	+/–	–	–	–	+	–	–	–	–	–	–	+[10]	–	–
D. crassirhizoma 2x, s	Widén *et al.* (1975ab)	Japan	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+++	–	–
	Hisada & Noro (1961), Noro *et al.* (1973)	Japan	(+/–)	–	–		+/–	–	–	–	–	+/–	–	–	–	+	–	–	–	–	++[10]	–	–
D. neorosthornii 3x, a	CRFJ8363-64	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	(+)	–	+
	CRFJ8370	N India	–	–	–	+++	+++	–	–	–	–	–	–	–	+	+	–	–	+	+	(+)	–	+
D. redactopinnata 2x, a	CRFJ7590, 7641, 7662-63	N India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	+	–	+
	CRFJ7592-93	N India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	(+)	–	+
	CRFJ7838-40	N India	–	–	–	++	++	–	–	+	(+)	–	–	–	++	++	–	–	(+)	–	–	–	+
	IK6939	N India	–	–	–	+	+++	–	–	(+)	(+)	–	–	–	–	++	–	–	++	+	–	–	–
D. yigongensis 3x, s	CRFJ7584	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
	CRFJ7585	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
"Hattu 1"	CRFJ7589, 7617, 7632-34, 7636-37	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
	CRFJ7610	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
	CRFJ7643-45	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
	CRFJ7648, 7586, 7609	N India	–	–	–	+++	+++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	+	–	+
"Hattu 2"	CRFJ7652	N India	–	–	–	+++	+	–	–	(+)	(+)	–	–	(+)	(+)	–	–	–	+	(+)	–	–	+
	CRFJ7654-56	N India	–	–	–	+++	+	–	–	(+)	(+)	–	–	(+)	(+)	–	–	–	+	(+)	–	–	+
	CRFJ7658	N India	–	–	–	+++	+	–	–	(+)	(+)	–	–	(+)	(+)	–	–	–	+	(+)	–	–	+
D. acutodentata 3x, a	CRFJ7796-7806	N India	(+)	–	–	++	++	++	+	(+)	–	–	–	(+)	(+)	+	+	–	+	–	+	+	+
D. wallichiana	HSP20.4(=TR-4405)	N India	–	–	–	(+)	+++	–	–	–	(+)	++	–	–	(+)	++	–	–	+	+++	+	–	+
ssp. *wallichiana*[11]	CRFJ8356-59	N India	–	–	–	+	++	–	–	(+)	(+)	(+)	–	(+)	(+)	+	–	–	(+)	(+)	+	+	+
2x, a	CRFJ8483, 8485-87	N India	–	–	–	–	+++	–	–	–	–	+	–	–	(+)	++	–	–	–	(+)	+	–	(+)
	TR2419	N India	–	–	–	+	+++	–	–	–	+	++	–	–	–	+	–	–	–	+++	+	–	+
	TR4166	Japan	–	–	–	–	+++	–	–	–	(+)	++	–	–	–	++	–	–	–	+++	+	–	+
	Hisada & Noro (1961)	Japan	(++)?	–	–	++	–	–	–	+/–	–	–		+	–	–	–	–	–	–	+	–	+
	Tryon *et al.* (1973)[12]	Mexico	–	–	–	+	+++	–	–	–	–	–	–	–	(+)	+	–	–	–	+[12]	–	–	–
	CRFJ13108	Mexico	–	–	–	+	(+)	+++	–	–	–	–	–	–	(+)	–	+	–	–	(+)	++	–	–
	CRFJ13252	Mexico	⁻	–	–	–	+	(+)	+++	–	–	–	–	–	–	(+)	–	+	–	–	(+)	++	–
	CRFJ14773, 14775-76	Hawai´i	–	–	–	(+)	(+)	+++	–	–	–	–	–	–	(+)	–	+	–	–	(+)	++	–	–
	CRFJ14797-14782-83	Hawai´i	⁻	–	–	(+)	(+)	+++	–	–	–	–	–	–	(+)	–	+	–	–	(+)	++	–	–
	CRFJ13004-5, 14710, 14711	Jamaica	-	-	(+)	(+)	+++	–	–	–	–	–	–	(+)	–	+	–	–	(+)	++	–	–	–
"Darjeeling variety"	CRFJ8536	N India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	+	–	+
	CRFJ8538	N India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	+	–	+
ssp. *coriacea*	PA33122	Georgia	–	–	–	(+)	+++	–	–	–	(+)	+	–	–	(+)	+	–	–	–	+	+	–	–
3x, a	CRFJ5931-33	Iran	–	–	–	(+)	+++	–	–	–	+	+	–	–	(+)	+	–	–	–	+	+	–	–
ssp. *reichsteinii*	TR4089	Zimbabwe	–	–	–	++	++	–	–	(+)	(+)	(+)	–	–	+	+	–	–	(+)	++	(+)	(+)	+
ssp. *madrasensis*	CRFJ9204	S India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	(+)	(+)	+
3x, a	CRFJ9206-07	S India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	(+)	(+)	+
	CRFJ9359	S India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	(+)	(+)	+
	CRFJ9362	S India	–	–	–	+++	+++	–	–	–	+	+	–	–	+	+	–	–	+	+	(+)	(+)	+

(Continues)

Table 1 continued.

			Aspidinol-B (*2-BB*) [4]	Norflavaspidic acid-BB (*4-BB*) [5]	Norflavaspidic acid-AB (*4-AB*)	Flavaspidic acid-BB (*5-BB*) [5]	Flavaspidic acid-AB (*5-AB*)	Para-aspidin-BB (*7-BB*) [5]	Para-aspidin-AB (*7-AB*)	Albaspidin-BB (*10-BB*) [6]	Albaspidin-AB (*10-AB*) [7]	Albaspidin-AA (*10-AA*)	Desaspidin-BB (*8-BB*) [5]	Filixic acid-BBB (*19-BBB*) [8]	Filixic acid-ABB (*19-ABB*) [9]	Filixic acid-ABA (*19-ABA*)	Trispara-aspidin-BBB (*20-BBB*)	Trisdesaspidin-BBB (*21-BBB*)	Trisflavaspidic acid-BBB (*23-BBB*)	Trisflavaspidic acid-ABB (*23-ABB*)	Tetraalbaspidin-ABBA (*25-ABBA*)	Tetraalbaspidin-BBBB (*25-BBBB*)	Tetraflavaspidic acid-BBBB (*26-BBBB*)
D. affinis [13]	CJW78/6	Madeira	–	–	–	++	+	–	–	(+)	(+)	(+)	–	(+)	(+)	(+)	–	–	+	+	(+)	(+)	+
ssp. *affinis*	TR2616	Madeira	–	(+)	(+)	++	++	–	–	–	–	–	–	+	+	+	–	–	++	++	–	–	–
var. *affinis*	TR3548	Azores	–	(+)	(+)	+	++	–	–	–	–	–	–	(+)	(+)	+	–	–	+	+	–	–	–
2x, a	S & S	Lu	–	–	–	++	++	–	–	(+)	(+)	(+)	–	(+)	(+)	(+)	–	–	+	+	–	+	+
	CRFJ10813,																						
	Fr.-Jenk. & Wid. (1993)	Hs	–	–	–	+	+	–	–	(+)	(+)	(+)	–	(+)	(+)	(+)	–	–	+	+	(+)	(+)	–
	JAC *et al.* 11824	England	–	–	–	++	+	–	–	(+)	(+)	(+)	–	(+)	(+)	(+)	–	–	(+)	+	+	+	(+)
	CRFJ11694-95,																						
	Fr.-Jenk. & Wid. (1993)	Austria	–	–	–	++	+++	–	–	(+)	+	(+)	–	+	+	+	–	–	+	++	(+)	(+)	–
	CRFJ12700,																						
	12702-03	Scotland	–	–	–	+	++	–	–	–	–	–	–	(+)	(+)	(+)	–	–	(+)	+	–	–	–
	TR293 [14]	Ge	–	–	–	++	++	–	–	–	–	–	–	(+)	(+)	(+)	–	–	+	+	–	–	–
	CRFJ11729	Ju	–	+	+	++	++	–	–	(+)	(+)	(+)	–	+	+	+	–	–	+	+	–	–	–
	CRFJ11733	Ju	–	(+)	(+)	++	++	–	–	(+)	(+)	(+)	–	(+)	+	(+)	–	–	+	+	–	–	–
	Fik. & Hegn. (1963)	Hs	–	–	–	++	++	–	–	+/–	–	–	–	+	–	–	–	–	–	–	–	–	–
var. *paleaceo-lobata* 2x, a	CRFJ12784	Wales	–	–	–	++	++	–	–	–	–	–	–	(+)	(+)	(+)	–	–	+	+	–	–	–
var. *splendens*	CRFJ11590	He	–	–	–	++	++	–	–	(+)	(+)	(+)	–	(+)	+	(+)	–	–	+	+	(+)	(+)	–
2x, a	TR740	He	–	+	+	++	++	–	–	–	–	–	–	+	+	(+)	–	–	+	+	–	–	–
	CRFJ11647	He	–	–	–	++	++	–	–	(+)	(+)	(+)	–	(+)	+	(+)	–	–	+	+	(+)	(+)	–
	CRFJ11751	Ju	–	–	–	+++	+++	–	–	+	+	–	–	+	+	+	–	–	+	+	(+)	(+)	–
var. *jessenii*	CRFJ14422-23	Romania	–	+	+	++	+++	–	–	–	–	–	–	+	+	+	–	–	(+)	+	+	–	–
2x, a	CRFJ14440	Romania	–	+	+	++	+++	–	–	–	–	–	–	+	+	+	–	–	(+)	+	+	–	–
ssp. *persica* 3x, a	CRFJ5922	Iran	–	–	–	++	++	–	–	–	–	–	–	+	++	+	–	–	+	(+)	+	–	–
ssp. *pontica*	CRFJ14058, 14060-61,																						
3x, a	14064	An	–	+	+	+++	+++	–	–	–	–	–	–	+	+	+	–	–	+	+	+	–	–
	CRFJ5948																						
	(=TR4264A)	An	–	–	–	++	++	+	+	(+)	(+)	(+)		(+)	(+)	(+)	–	–	+	+	(+)	(+)	(+)
ssp. *cambrensis*																							
var. *paleaceo-crispa*	JAC1873	Scotland	–	–	–	++	+	–	–	–	(+)	(+)	–	(+)	(+)	(+)	–	–	+	+	(+)	–	+
3x, a	CRFJ12507-09	Scotland	–	(+)	(+)	++	++	–	–	–	–	–	–	(+)	+	++	–	–	+	+	–	–	–
	CRFJ12561	Scotland	–	(+)	(+)	++	++	–	–	–	–	–	–	(+)	+	++	–	–	+	+	–	–	–
	CRFJ12581	Scotland	–	(+)	(+)	++	++	–	–	–	–	–	–	(+)	+	++	–	–	+	+	–	–	–
	CRFJ12592	Scotland	–	+	+	++	(+)	–	–	–	–	–	–	(+)	+	++	–	–	+	(+)	–	–	–
"form similar to	CRFJ12634	Scotland	–	+	+	++	+	–	–	–	–	–	–	(+)	+	++	–	–	+	(+)	–	–	–
D. x complexa"	CRFJ11702 [15]	Austria	–	–	–	++	+	–	–	(+)	(+)	(+)	–	++	+	+	–	–	+	+	–	–	–
3x?, a																							
var. *insubrica*	CRFJ11838	He	–	–	–	++	++	–	–	+	+	–	–	+	+	+	–	–	+	+	–	–	–
3x, a	CRFJ11841-42	He	–	–	–	++	+++	–	–	(+)	(+)	–	–	(+)	+	+	–	–	+	++	(+)	(+)	–
	CRFJ11846,																						
	11848-49	He	–	–	–	++	+++	–	–	(+)	(+)	(+)	–	(+)	+	+	–	–	+	++	(+)	–	–
	TR2293	He	–	+	+	++	++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	–	–	–
	TR2845	He	–	+	+	++	++	–	–	–	–	–	–	(+)	+	+	–	–	+	+	–	–	–
	CRFJ11741	Ju	–	–	–	++	++	–	–	(+)	(+)	–	–	+	+	+	–	–	(+)	(+)	–	–	–
ssp. *pseudo-disjuncta*	CRFJ11606	He	–	–	–	++	++	–	–	(+)	(+)	(+)	–	+	+	(+)	–	–	+	+	–	–	–
	Widén *et al.* (1971) [16]	He	–	–	–	++	++	–	–	(+)	(+)	(+)	–	+	+	(+)	–	–	–	–	–	–	–
3x, a	Fik. & Hegn. (1963) [16]	He	–	–	–	++	++	–	–	+/–	–	–	–	+	–	–	–	–	–	–	–	–	–
ssp. *borreri*	CRFJ11618,																						
3x, a	Fr.Jenk. & Wid. (1993)	He	(+)	–	–	++	+	++	+	(+)	(+)	(+)	–	(+)	(+)	(+)	(+)	–	+	(+)	–	–	–
	Widén *et al.* (1971) [17]	He	+	–	–	++	+	++	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–
	CRFJ11747	Ju	(+)	–	–	++	+	++	+	(+)	(+)	(+)	–	+	+	+	(+)	–	+	(+)	–	–	–
	CRFJ11714-15	Austria	(+)	–	–	++	+	++	+	(+)	(+)	(+)	–	(+)	+	+	(+)	–	+	(+)	–	–	–
	CRFJ4089 (=TR3685)	Turkey	(+)	–	–	++	(+)	+++	+	–	–	–	–	+	+	+	(+)	–	+	(+)	–	–	–
	JAC *et al.* 1253	Wales	(+)	–	–	++	+	+++	++	(+)	(+)	(+)	–	(+)	(+)	(+)	+	–	+	(+)	–	–	–
	JAC *et al.* 11782 [17]	England	+	–	–	++	+	+++	++	(+)	(+)	(+)	–	(+)	(+)	(+)	(+)	–	+	(+)	–	–	–
	JAC *et al.* 11823	England	(+)	–	–	++	++	++	–	(+)	(+)	–	–	++	+	(+)	–	–	++	+	–	–	–
	CRFJ10812,																						
	Fr.Jenk. & Wid. (1993)	Hs	(+)	–	–	++	+	+++	+	(+)	(+)	(+)	–	(+)	(+)	(+)	–	–	+	(+)	–	–	–
	Fik. & Hegn. (1963) [18]	Belgium	++	–	–	++	+	+/–	–	+/–	–	–	–	+	–	–	–	–	–	–	–	–	–
D. fusco-atra [19]																							
var. *fusco-atra*	CRFJ14901-03	Hawai´i	–	+	++	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	(+)	–	–
3x?, a	CRFJ14905,																						
	14906, 14908	Hawai´i	–	+	++	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	(+)	–	–
	CRFJ14765	Hawai´i	–	+	++	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	(+)	–	–
var. *lamoureuxii*	CRFJ14911	Hawai´i		+	++	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	(+)	–	–

(Continues)

Table 1 continued.

			Aspidinol-B (*2*-B) [4]	Norflavaspidic acid-BB (*4*-BB) [5]	Norflavaspidic acid-AB (*4*-AB)	Flavaspidic acid-BB (*5*-BB) [5]	Flavaspidic acid-AB (*5*-AB)	Para-aspidin-BB (*7*-BB) [5]	Para-aspidin-AB (*7*-AB)	Albaspidin-BB (*10*-BB) [6]	Albaspidin-AB (*10*-AB) [7]	Albaspidin-AA (*10*-AA)	Desaspidin-BB (*8*-BB) [5]	Filixic acid-BBB (*19*-BBB) [8]	Filixic acid-ABB (*19*-ABB) [9]	Filixic acid-ABA (*19*-ABA)	Trispara-aspidin-BBB (*20*-BBB)	Trisdesaspidin-BBB (*21*-BBB)	Trisflavaspidic acid-BBB (*23*-BBB)	Trisflavaspidic acid-ABB (*23*-ABB)	Tetraalbaspidin-ABBA (*25*-ABBA)	Tetraalbaspidin-BBBB (*25*-BBBB)	Tetraflavaspidic acid-BBBB (*26*-BBBB)
D. lepidopoda [20]	HSP20.16																						
2x, a	(=TR4406)	N India	–	–	–	+	++	–	–	(+)	(+)	(+)	–	–	(+)	(+)	–	–	(+)	++	(+)	(+)	+
	CRFJ8826, 8829-30	N India	–	–	–	+	++	–	–	(+)	+	+	–	(+)	(+)	(+)	–	–	–	(+)	–	–	–
	CRFJ8837-39	N India	–	–	–	+	++	–	–	(+)	+	+	–	(+)	(+)	(+)	–	–	–	(+)	–	–	–
	CRFJ8841-42	N India	–	–	–	+	++	–	–	(+)	+	+	–	(+)	(+)	(+)	–	–	–	(+)	–	–	–
	CRFJ8832	N India	–	–	–	++	++	–	–	–	(+)	+	–	(+)	(+)	(+)	–	–	–	(+)	(+)	–	(+)
	CRFJ8835-36	N India	–	–	–	++	++	–	–	(+)	+	–	–	(+)	(+)	(+)	–	–	–	(+)	(+)	–	(+)
	CRFJ8840	N India	–	–	–	+	++	–	–	–	(+)	(+)	–	(+)	(+)	(+)	–	–	–	(+)	–	–	(+)
	TR4082	Taiwan	–	–	–	++	++	–	–	(+)	(+)	(+)	–	(+)	(+)	–	–	–	+	+	–	(+)	(+)
D. sledgei [21]	CRFJ9349-58	S India	–	+	+	(+)	+	–	–	–	(+)	(+)	–	+	++	++	–	–	–	–	–	++	–
4x, ?a.																							
D. pseudo-filix-	CRFJ13155, 13157	Mexico	–	–	–	+++	++	–	–	–	–	–	–	++	++	+	–	–	–	+	+	–	–
mas	CRFJ13428a,b,c	Mexico	–	–	–	+++	++	–	–	–	–	–	–	++	++	+	–	–	–	+	+	–	–
3x, a	CFRJ13457-61	Mexico	–	–	–	+++	++	–	–	–	–	–	–	++	++	+	–	–	–	+	+	–	–
D. khullarii		N India	–	(+)	(+)	+	++	–	–	(+)	(+)	(+)	–	(+)	+	+	–	–	–	(+)	+	–	–
3x, a	CRFJ8236-37, 8267	N India	–	(+)	(+)	+	++	–	–	(+)	(+)	(+)	–	(+)	+	+	–	–	–	(+)	+	–	–
	CRFJ8350-54	N India	–	(+)	(+)	+	++	–	–	(+)	(+)	(+)	–	(+)	+	+	–	–	–	(+)	+	–	–
D. subbipinnata	CRFJ14795,																						
3x, a	15068	Hawai´i	–	–	–	++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sect. Dryopteris																							
D. oreades [22]	ACJ8515	Scotland	–	–	–	++	+	–	–	–	–	–	–	++	+	(+)	–	–	+	(+)	–	–	–
2x, s	CRFJ13837-45	Wales	–	+	+	++	+	–	–	–	–	–	–	+	(+)	(+)	–	–	+	(+)	–	–	–
	Fr.-Jenk. & Wid. (1993)	Corsica	–	+	+	++	+	–	–	–	–	–	–	+	(+)	(+)	–	–	+	(+)	–	–	–
	Fr.-Jenk. & Wid. (1993)	An	–	+	+	++	+	–	–	–	–	–	–	+	(+)	(+)	–	–	+	(+)	–	–	–
	v. Euw *et al.* (1980)	Italy	–	–	–	++	+	–	–	–	–	–	–	+	(+)	(+)	–	–	+	–	–	–	+
	Widén *et al.* (1971)	Scotland	–	–	–	++	+	–	–	–	–	–	–	–	(+)	(+)	–	–	+	–	–	–	–
D. caucasica	Widén *et al.* (1973)	Turkey	+	–	–	+	–	+++	–	–	–	–	++	+++	–	–	–	+	–	–	–	–	–
2x, s																							
D. barbigera	Widén *et al.* (1991)	N India	+	–	–	+++/++	+/–	+++/+	–	(+)	(+)	–	–	++/+	+	(+)	+/(+)	–	+/–	–	–	–	–
2x, s																							
D. filix-mas	v. Euw *et al.* (1980)	He	–	–	–	+++	+	+	–	–	–	–	+	++	+	(+)	–/+	–	–/+	(+)	–	–	(+)
4x, s	v. Euw *et al.* (1980)	Canada	–	–	–	+++	+	+/+++	–	–	–	–	+/++	+/++	+	(+)	–/+	(+)	–/+	(+)	–	–	(+)
	v. Euw *et al.* (1980)	USA	–	–	–	+++	+	+	–	–	–	–	++	++	+	(+)	+	(+)	(+)	(+)	–	–	(+)
Sect. Hirtipedes																							
D. conjugata [21]	CRFJ15779-15785	Nepal	–	+++	+++	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	+	–	–
2x, a																							
D. hirtipes	CRFJ9164-67,																						
ssp. *atrata* [21]	9170-72, 9173-74	S India	–	++	+++	–	–	–	–	+	++	+	–	+	++	+	–	–	–	–	–	–	–
4x, s?	CRFJ9131-33	S India	–	++	++	–	–	–	–	+	++	++	–	(+)	++	++	–	–	–	–	–	–	–
ssp. *hirtipes* [21]	CRFJF.n.161-171,																						
2x, s	15.9.93	Sri Lanka	–	+++	++	–	–	–	–	–	–	–	–	+	+	(+)	–	–	–	–	–	–	–
Sect.Pallidae																							
D. nigropaleacea [23]	Widén *et al.* (1991)	Pakistan	–	–	–	+++/+++++/+		–	–	+/–	(+)/–	(+)/–	–	+	+	–/++	–	–	(+)/+	–	(+)/–	–(+)/	
2x, s	Widén *et al.* (1991)	N India	–	–	–	+++	++	–	–	+	(+)	(+)	–	+	+	–	–	–	(+)	–	(+)	(+)	(+)

[1] 2x = diploid, 3x = triploid, 4x = tetraploid, a = apomictic, s = sexual.
[2] CPG = Chelsea Physic Garden , CRFJ = C.R. Fraser-Jenkins, TR = Tadeus Reichstein, JAC =J.A. Crabbe, CJW = C.J. Widén, S&S = F. Sousa & A. Santos, HSP = H.S. Puri, IK = I. Kukkonen, PA = Pentti Alanko, WG = W. Gätzi.
[3] Geo = Georgia (Caucasus), Lu = Lusitania (Portugal), Hs = Hispania (Spain), Ge = Germania (Germany), He = Helvetia (Switzerland), Ju = Yugoslavia, An = Anatolian Turkey, Turkey = European Turkey.
[4] Aspidinol-B (*2*-B) is mostly an artefact.
[5] This is often a mixture of BB and PB homologues which do not separate well in TLC.
[6] This spot, which has formerly often been designated as "albaspidin-1" may be provoked by a mixture of the homologues *10*-BB, -PB and -PP.
[7] This spot is often designated as "albaspidin-2" and may be provoked by a mixture of homologues *10*-BA and -PA.
[8] This spot is often designated as "filixic acid 1" and may be produced by a mixture of homologues *19*-BBB, -PBB and -PBP.
[9] This spot is called "filixic acid 2" and may be provoked by a mixture of homologues *19*-ABB and -ABP.
[10] First identified as an unknown substance F (Hisada & Noro 1961), that later proved to be dryocrassin (*25*-ABBA) (Noro *et al.* 1973).
[11] *D. wallichiana* subsp. *wallichiana* from N India also contains Pa-1 = tricosanol, a non-phloroglucinolic compound, as well as trace amounts of the unknown phenolic compounds Le-2 and Le-3. In subsp. *wallichiana* from Mexico, Hawaii and Jamaica considerable amounts of two unknown compounds Wa-1 and Wa-2 occur (see RESULTS).

Hrvatska, same place and date as CRFJ 11729, 11733 (cited under var. *affinis*).

Dryopteris affinis subsp. *pseudo-disjuncta*: CRFJ 11606, 13.X.1985, Switzerland, Kt. Nidwalden (Unterwalden), Haltiwald, S of Luzern, N of Hergeswil (1 rhizome).

Dryopteris affinis subsp. *borreri*: CRFJ 11618, 13.X.1985, Switzerland (see Fraser-Jenkins & Widén 1993). CRFJ 11747, 21.X.1985, Yugoslavia, Hrvatska, same place and date as CRFJ 11729 (cited under var. *affinis*). CRFJ 11714-5, 19.IX.1985, Austria, Salzburg, c. 1 km W of SE tip of Mond See (2 rhizomes). CRFJ 4089 (= TR 3685), 30.VIII.1973, Turkey, Trabzon, Ziğana Pass, 1 500 m, coniferous wood (triploid, det. G. Vida in litt. 8.V.1975; 1 rhizome, 25 g, ex cult. from spores at Basel). JAC & Jermy 1253, 24.IX.1961, Wales, vice-county 49, Caernarvon, Prenteg, 4 km S of Beddgelert, towards top of ravine beside drive to Aberdunant Hall (piece of rhizome from herbarium specimen), Dyce & Parker 11823, England, vice-county 5, Somerset, Over Stowey parish, Round Hill, 3 km SW of Nether Stowey, 180 m (piece of rhizome from herbarium specimen). JAC & Jermy 11782, England (see Widén *et al.* 1971; piece of rhizome from herbarium specimen). CRFJ 10812, Spain (see Fraser-Jenkins & Widén 1993).

Dryopteris fusco-atra var. *fusco-atra*: CRFJ 14765, 25.II.1988, Hawai'i, East Maui, W side of Mt. Haleakala, S of Pukalani, Waipoli Road above Kula ("lower locality"), c. 3 500–4 000 ft (1 rhizome). CRFJ 14901-3, 1.III.1988, N slope of Mt. Haleakala, E of Pukalani, c. 1.5 miles above bottom gate into Haleakala Ranch on road to Waikamoi Gulley and Flume, above Olinda, Makawao forest, 1 250 m (3 rhizomes). CRFJ 14905-6, 14908, 3.VIII.1988, as CRFJ 14901 (3 rhizomes).

— var. *lamoureuxii*: CRFJ 14911, 1.III.1988, same place and date as CRFJ 14901 (cited under var. *fusco-atra*; 1 rhizome).

Dryopteris lepidopoda: Puri 20.16 in litt., 1.XII.1977 (= TR 4406), India, Sikkim, Kewsey, 3 000 m, 3 km E of Lachung, near the Tibet border (diploid, apomict, det. M. Gibby in litt. 10.I.1980; rhizome, 66 g, dried 14.–16.III.1986 ex cult. from spores in Agarone by TR). CRFJ 8826, 8829, 8830, 8837-9, 8841-2, 24.XI.1978, India, Meghalaya, 10 km above Shillong road to Shillong Peak, Khasi Hills, 1 800 m (8 rhizomes). CRFJ 8832, same place and date as CRFJ 8826 (1 rhizome, 80 g). CRFJ 8835-6, same plca and date as CRFJ 8826 (2 rhizomes, 200 g). CRFJ 8840, same place and date as CRFJ 8826 (1 rhizome, 70 g). TR 4082 (= CRFJ 10929), Taiwan, Mt. Ari-san, 2 300 m, Kagi pref., Shiraiwa 5437, 31.VII.1976 (diploid, 2n = 82, Gibby (1985) sub *D. wallichiana*; rhizome, 48 g, ex cult. from spores in Agarone, dried 14.–16.III.1986).

Dryopteris sledgei: CRFJ 9349-9358, 26.XII.1978, India, Tamil Nadu, Nilgiri Hills, 4 km above Ootacamund, Dodabetta Mt, 2 450 m (10 rhizomes, 430 g; tetraploid, 2n = 164, Gibby 1985).

Dryopteris pseudo-filix-mas: CRFJ 13155-13157, 20.IV.1987, Mexico, Chiapas, San Cristobal, Banabil (2 rhizomes). CRFJ 13428a, b, c, 18.V.1987, Oaxaca, Cerro de San Felipe (3 rhizomes). CRFJ 13457-l3461, 22.V.1987, Mexico state, E of Ciudad Mexico, E side of volcan Popocatepetl (3 rhizomes).

Dryopteris khullarii: CRFJ 8236-7, 8267, 24.X.1978, India (W Himalaya), Uttar Pradesh, Chamoli, Trijugi Naryan to Mongu, N of Rudraprayag, c. 1 900 m (3 rhizomes, 260 g). CRFJ 8350-8354, 25.X.1978, Trijugi Naryan to Mongu, N of Rudraprayag, 2 400 m (5 big rhizomes, 1 020 g; triploid apomict, 2n = 123, Gibby 1985).

Dryopteris subbipinnata: CRFJ 14795, 15068, 3.III.1988, Hawai'i, E Maui, W side of Mt. Haleakala, gulley above treeline just above road up to Haleakala, 2 300 m (2 rhizomes).

Dryopteris oreades: ACJ 8515, Scotland, Isle of Mull, Sron nam Boc, Broless, 150 m (1 rhizome). CRFJ 13837-13845, 13.IX.1987, Wales, Glamorgan, Craig-y-Llyn (7 rhizomes).

Dryopteris conjugata Ching: CRFJ 15779-15785, 16.XI.1989, Nepal, N of Kathmandu, Sheopuri, Bagdwar (7 rhizomes). CRFJ 15923 (with N. Punetha and B. S. Kholia) 14.I.1990, India, Uttar Pradesh, Pithoragarh, Satgarh, Dhaj mountain, ex hort. Chelsea Physic Garden, London, 1993 (2n = 82, apomict, det. M. Gibby).

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[12] In Tryon *et al.* 1973 the material was listed as "*D. parallellogramma*". Trisflavaspidic acid ABA (*19*-ABA) was identified later in TLC with improved methods.

[13] The lack of or occurrence of minute amounts of norflavaspidic acids (*4*) in *D. affinis s.lat.* and other related taxa is hardly of any taxonomic importance, because this taxon in general contains only small amounts of phloroglucinols (crude filicin). We consider that other collections as well listed in the table may well contain trace amounts of norflavaspidic acids (*4*-BB and *4*-AB) if re-investigated with our improved methods.

[14] See also previous results on TR-293 sub "*D. borreri* var. *disjuncta*" (Widén *et al.* 1971).

[15] Identity uncertain, possibly a hybrid.

[16] Listed in Widén *et al.* (1971) and Fikenscher & Hegnauer)1963) as "*D. borreri* var. *pseudo-disjuncta*".

[17] This collection has been re-investigated with improved methods, *cf.* also previous results in Widén *et al.* (1971) sub "*D. borreri* triploide Sippe".

[18] Listed as *D.* x *tavelii* No. 1961-862, 1961-867, 1961-885 in Fikenscher & Hegnauer (1963), see also Discussion.

[19] *D. fusco-atra* also contains considerable amounts of three unknown compounds Fu-1, Fu-2 and Fu-3 (see Results).

[20] In addition to the compounds given in the table, *D. lepidopoda* contains minute amounts of the unknown compounds Le-1, Le-2 and Le-3 (see Results).

[21] In addition to the compounds listed in the table, *D. sledgei* contains the unknown compounds Sl-1 and Sl-2, which are probably identical with stenolepin 4 and stenolepin 5 (see Results). *D. conjugata* and *D. hirtipes* subsp. *atrata* and subsp. *hirtipes* also contain large percentages of these compounds. F.n. = Field number.

[22] Listed as "*D. abbreviata*" in Widén *et al.* (1971).

[23] In addition to compounds listed in the table, *D. nigropaleacea* contains trace amounts of penta-albaspidin-BBBBB (*37*-BBBBB), hexa-albaspidin-BBBBBB (*38*-BBBBBB), hexaflavaspidic acid-BBBBBB (*39*-BBBBBB) as well as the unknown compounds Ju-2 = Le-2 and Ju-3 = Le-3. Sometimes stenolepin-B (*28*-B) is also present.

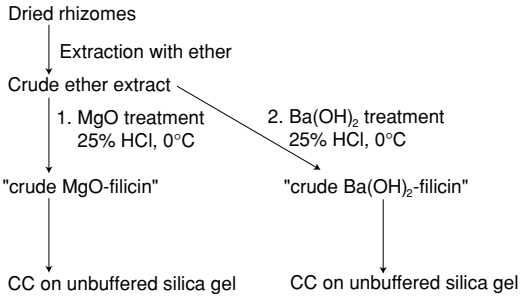


Fig. 4. Extraction of rhizomes and separation of the crude ether extract by the old standard method (Ackerman & Mühlemann 1947, von Euw *et al.* 1980). CC = column chromatography.

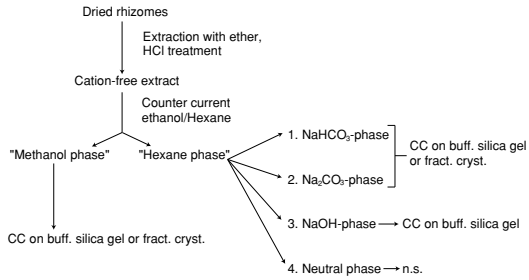


Fig. 5. Extraction of rhizomes and separation of the cation-free ether extract by the "improved method" (von Euw *et al.* 1980, 1985, Patama & Widén 1991); n.s. = not studied. The "hexane phase" can be separated as such by column chromatography or further divided into four fractions of different acidity.

Dryopteris hirtipes (Blume) Kuntze subsp. *atrata* Kuntze (Fraser-Jenkins): CRFJ 9164-9167, 9169-74, 19.XII.1978, India, Tamil Nadu, 19.5 km N of Kodaikanal on Palni road, 1 650 m (8 rhizomes, 480 g; CRFJ 9169 tetraploid, see Gibby 1985). CRFJ 9231-33, 21.XII.1978, Tamil Nadu, Palni Hills, 4.5 miles NE of Kodaikanal on Perumalmalai road (3 rhizomes, 300 g).

Dryopteris hirtipes subsp. *hirtipes*: CRFJ (field numbers 161-171), 15.IX.1993, Sri Lanka, Rattota to Laggala, Matale (11 rhizomes of which 6 investigated by TLC).

Extraction procedure and analysis of rhizomes

For the preparation of crude extractives we previously used the "standard method" (Fig. 4). In this method the use of ice-cooled (0°C) reagents, quick performance and addition of Na_2SO_3 as antioxidant is essential in order to minimize the formation of artefacts (see Ackermann & Mühlemann 1947). The bulk of "phenolics" is usually obtained in the "MgO-filicin", but some less acidic phenolics like aspidinol (*2*, mostly an artefact) are concentrated in the $Ba(OH)_2$-filicin. The crude filicins were then separated on unbuffered silica gel giving losses and deterioration of many sensitive compounds (von Euw *et al.* 1980, 1985).

In later separations we used an improved method avoiding the detrimental steps discussed above (von Euw *et al.* 1980, 1985). In this method the "hexane phase" still containing non-phenolic extractives could be separated by column chromatography on polyamide, cellulose powder or perhaps most successfully on buffered silica gel. However we also used the modified method of Patama and Widén (1991) in which the phloroglucinols could be separated in four fractions of different acidity (Fig. 5). Although some decomposition may occur, all extractives can be excluded and further separation of the "hexane phase" is possible (Patama & Widén 1991). Whether a certain phloroglucinol occurs naturally or not can be decided by direct chromatography of crude ether extracts never exposed to alkali or heat on TLC (von Euw *et al.* 1980, 1985).

In Experimental we give three examples of using the "improved method" in the isolating procedure.

For final identification of individual phloroglucinols we had a complete set of pure reference substances. For structures and numbering see table 4 in Widén *et al.* (1991) and Widén *et al.* (1993). The structures of the phloroglucinol derivatives discussed in the present work are given in Fig. 6.

TLC of naturally occurring phloroglucinols

System I: Silica gel G plates buffered at pH 6, solvent hexane-chloroform (50:50), plates developed 3 × 15 cm (Widén *et al.* 1970, 1976).

System II: Silica gel G plates buffered at pH 6, solvent hexane-chloroform-abs.ethanol (47.5:47.5:10.0), plates developed 1 × 15 cm (Widén *et al.* 1970, 1976).

System III: Silica gel H plates buffered at pH 6, solvent methanol-isopropylether-cyclohexane (10:35:55), plates developed 1 × 15 cm (von Euw *et al.* 1980, 1985).

System IV: Silica gel H plates buffered at pH 4, solvent hexane-chloroform-abs.ethanol-glacial acetic acid (45:35:16:4), plates developed 1 × 15 cm (von Euw *et al.* 1980, 1985).

After development the plates were first viewed in UV-light (254 nm, 336 nm) and then stained with fast blue salt B. In Fig. 7 we give an example of the separation of the polar flavaspidic acids by TLC, system IV.

The total amount of homologues in representative samples of most taxa were estimated after mild reductive cleavage of a small part of crude "MgO-filicin" (10 mg) and subsequent analysis of the mixture formed of monocyclic compounds in paper chromatography (PC) and thin-layer chromatography (TLC), for details see Widén *et al.* (1992). As the variation of the acyl side-chain mainly concerns the geminally substituted filicinic acid rings it is, for example, possible to study the distribution of the homologous flavaspidic acids BB and PB (5-

BB, -PB) or para-aspidins BB and PB (*7*-BB, -PB) although they do not separate in TLC.

RESULTS

The analytical results from the taxa studied are summarized in Table 1. Compounds not present in *Dryopteris* sect. *Fibrillosae* or the other taxa of sections *Dryopteris* and *Hirtipedes* studied here are omitted from the Table (see von Euw *et al*. 1980, 1985, Widén *et al*. 1992, 1993). Compounds of unknown structure are mentioned only in the footnotes and are discussed in more detail below. For comparison, previous results for the same taxa are included in Table 1. Amounts of crude or cation-free ether extracts and crude filicins obtained for each sample are given in Table 2, the approximate total amounts of homologues in Table 3 and of pure crystalline compounds in Table 4 (see Experimental).

Compounds of unknown structure

In addition to the crystalline compounds of known structure (see von Euw *et al*. 1980, 1985), some unknown compounds were also isolated. These are discussed briefly below.

Wa-1, Wa-2. The *Dryopteris wallichiana* samples from Hawai'i (CRFJ all numbers) contained two unknown compounds designated Wa-1 and Wa-2. These were obtained from the "hexane phase" by further treatment with $NaHCO_3$ and Na_2CO_3 solutions according to Patama and Widén (1991) (see Experimental). In El/MS the peak at highest mass was found at m/z 612 (weak) and was probably filixic acid ABA (*19*-ABA). Two other putative M^+ peaks occurred at m/z 538 (weak) and m/z 510 (weak). These are located just in between a two-ring and a three-ring compound. Characteristic peaks at m/z 432 (weak), 418 (weak), and 404 are most likely due to the albaspidins homologues PP, AP and AA (*10*-PP, AP, AA) respectively. We consider that both the filixic acid (*19*-ABA) and the albaspidins (*10*-PP, AP, AA) are artefacts that are formed in the ionization chamber by the rottlerone change and analogous reactions (cf. Lounasmaa *et al*. 1971, Richter *et al*. 1987, Widén *et al*. 1994). Significant signals were also found at m/z 208, 196, 195, 165 and 153.

According to ^1H NMR and investigation of the monocyclic phloroglucinols formed by reductive alkaline cleavage Wa-1 and Wa-2 contain an acetylfilicinic moiety, which is connected by a methylene group to an aromatic ring with a methyl group.

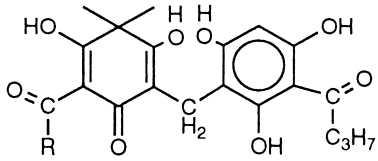
In addition to acetyl, also propionyl and butyryl side chains were observed. For chromatographic properties of Wa-1 and Wa-2, see Experimental.

Pa-1. This proved to be a non-phloroglucinolic compound consisting of the aliphatic saturated alcohol l-tricosanol ($C_{23}H_{27}O_{11}$), see Table 4, footnote. It was isolated from *Dryopteris wallichiana* subsp. *wallichiana* from N India.

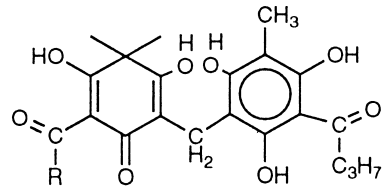
Fu-1, Fu-2. *Dryopteris fusco-atra* from Hawai'i (CRFJ all numbers) contained two unknowns Fu-1 and Fu-2 with different chromatographic properties when compared with Wa-1 and Wa-2 (Patama & Widén 1991). However, apart from the non-occurrence of the putative molecular peak at m/z 538, the same structural features were observed in mass and NMR spectra of Fu-1 and Fu-2 as in Wa-1, Wa-2 of *D. wallichiana*. Accordingly these substances must be closely related though not identical.

Fu-3. In addition to Fu-1 and Fu-2 *Dryopteris fusco-atra* contained a third unknown Fu-3. According to mass and NMR spectroscopy Fu-3 may consist of a four-ring phloroglucinol (M^+ at m/z 794) consisting only of aromatic rings connected by methylene bridges and having acetyl and butyryl side chains (Patama & Widén 1991).

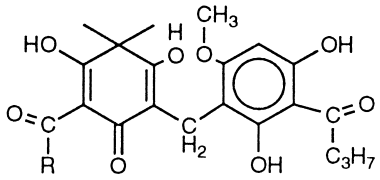
Le-2, Le-3. From *Dryopteris lepidopoda* (CRFJ 8826, 8829-30, 8837-40, 8841-42) two unknown amorphous substances with putative three-ring structures [M^+ at m/z 652 (weak), 648 (weak) and 620] were isolated and a third unknown Le-1 was detected by TLC. Le-1, Le-2 and Le-3 proved to be identical with Ju-1, Ju-2 and Ju-3 from *D. juxtaposita* and Ni-1, Ni-2, Ni-3 from *D. nigropaleacea* and are most likely to be closely related to pentherin I (brown unknown) from *D. fadenii* Pichi-Serm. (earlier identified as *D. pentheri* (Klasser) C. Chr.) and *D. ardechensis* Fraser-Jenkins (see detailed discussions in Widén *et al*. 1973, 1991 and Fraser-Jenkins & Widén 1993). However, re-investigation of the high resolution mass data revealed that the exact mass of the peak at m/z 648 is more likely 648.3816 corresponding to $C_{36}H_{56}O_{10}$, and that of the peak m/z 620 is 620.3587 corresponding to $C_{37}H_{52}O_{10}$ as given in Widén *et al*. (1991). After reductive alkaline cleav-



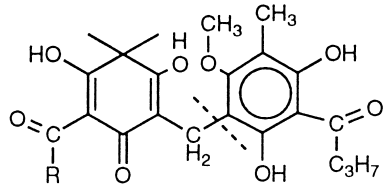
4-BB R=C_3H_7
Norflavaspidic acid-BB
4-AB R=CH_3
Norflavaspidic acid-AB



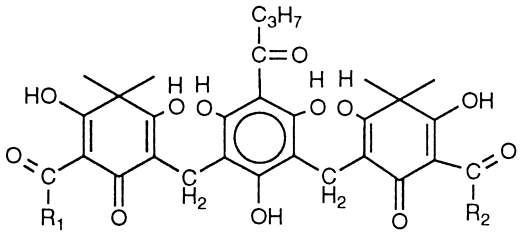
5-BB R=C_3H_7
Flavaspidic acid-BB
5-AB R= CH_3
Flavaspidic acid-AB



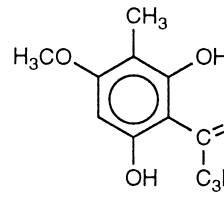
8-BB R=C_3H_7
Desaspidin-BB
8-AB R=CH_3
Desaspidin-AB



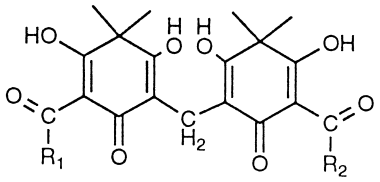
7-BB R=C_3H_7
Para-aspidin-BB
7-AB R=CH_3
Para-aspidin-AB



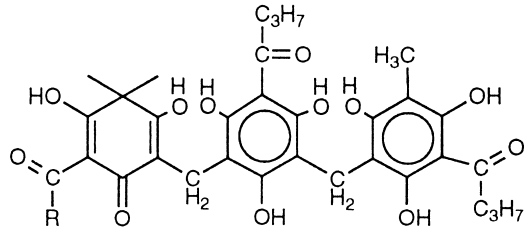
19-BBB R_1=R_2=C_3H_7
Filixic acid-BBB
19-ABB R_1=CH_3; R_2=C_3H_7
Filixic acid-ABB
19-ABA R_1=R_2=CH_3
Filixic acid-ABA



2-B
Aspidinol-B

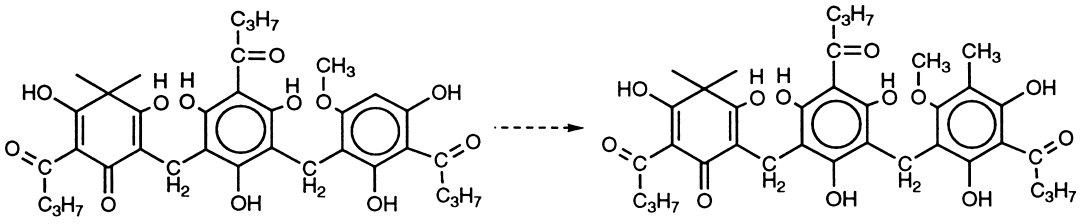


10-BB R_1 = R_2 = C_3H_7
Albaspidin BB
10-AB R_1=CH_3; R_2=C_3H_7
Albaspidin AB
10-AA R_1= R_2=CH_3
Albaspidin AA



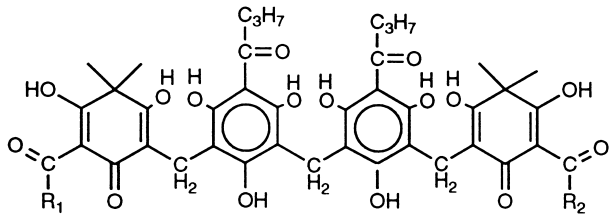
23-BBB R=C_3H_7
Trisflavaspidic acid-BBB
23-ABB R_1=CH_3
Trisflavaspidic acid-ABB

Fig. 6. Chemical structures of the phloroglucinol derivatives investigated.

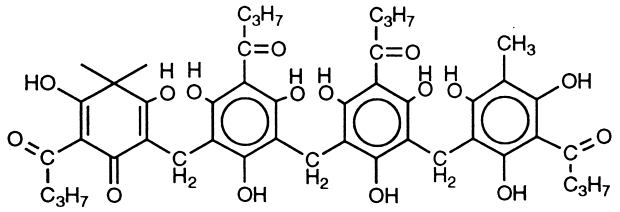


21-BBB
Trisdesaspidin-BBB

20-BBB
Trispara-aspidin-BBB



25-BBBB $R_1=R_2=C_3H_7$
Tetraalbaspidin-BBBB=Methylene-bis-norflavaspidic acid-BBBB
25-ABBA $R_1=R_2=CH_3$
Tetraalbaspidin-ABBA=Methylene-bis-norflavaspidic acid-ABBA
="Dryocrassin"



26-BBBB
Tetraflavaspidic acid-BBBB

Fig. 6 continued.

age of Le-2 and Le-3 butyryl-filicinic acid *(36)* and its acetyl homologue (*36*-C_2H_4)were found in paper chromatography (PC), and butyryl-phloroglucinol (*27*) and butyryl-methylphloroglucinol (*31*) in thin-layer chromatography (TLC), for structural formulae see Widén *et al.* (1991: 90). Accordingly the peak at m/z 648 may be attributed to the B homologue and that at m/z 620 to the A homologue. That means that the unknown part of the molecule may consist of $C_{12}H_{27}O_2$ (203) and not $C_{12}H_{11}O_3$ as postulated by Widén *et al.* (1973) or $C_{16}H_{25}$ as later considered (Fraser-Jenkins & Widén 1993).

Also in *Dryopteris wallichiana* from N India (CRFJ 8483, 8485-87) at least Le-2 and Le-3 were present in minute amounts (TLC, dark brown coloration with fast blue salt B).

Sl-1, Sl-2. From *Dryopteris sledgei* (CRFJ 9349-58) two unknown amorphous compounds Sl-1 and Sl-2 were isolated in amorphous form from the "hexane phase". In negative fast atom bombardment

mass spectrum (FAB/MS) putative molecular peaks were observed at m/z 633 (MW^+ 634), 619 (MW^+ 620) and 605 (MW^+ 606). Except for MW^+ 634 the other putative mass peaks were exactly the same as those in stenolepin 4 + 5 from *D. stenolepis* (Baker) C. Chr. from India (cf. Widén *et al.* 1991). After reductive alkaline cleavage only acetylfilicinic acid as well as some butyryl and propionylfilicinic acid were found; i.e. exactly the same decomposition products as those of stenolepin 4 + 5. According to TLC and coloration with fast blue salt B (dark-brown) these substances are indeed identical apart from the absence of the homologue at m/z 634.

From the chromatographic behaviour, mass spectra and products obtained by reductive alkaline cleavage we consider that the stenolepins 4 + 5 and Sl-1, Sl-2 may simply be lower homologues of Le-1, Le-2, Le-3 (Ju-1, Ju-2 Ju-3) and one of them (perhaps Le 1= Ju-1) may also be identical with pentherin I. Interestingly these substances always show six units lower molecular weights as compared with the corresponding filixic acids (cf. Widén *et al.* 1992). These facts, as well as the similar chromatographic behaviour of the "unknowns" in TLC as compared with the corresponding filix acids (*19*), point to a close structural relationship of all these substances (cf. Widén *et al.* 1973, Widén *et al.* 1991). Unfortunately we have been unable to get any of these easily decomposing unknown substances in pure crystalline form to determine their complete structure.

DISCUSSION

The main purpose of the present work was to establish how chemical composition reflects taxonomic relationships in the 18 taxa of *Dryopteris* sect. *Fibrillosae* investigated. As seen from Table 1 their phloroglucinol composition is indeed remarkably similar, with only a few exceptions (see below). Most taxa contain considerable amounts of flavaspidic acids (*5*) combined with varying amounts of filixic acids = trisalbaspidins (*19*). Traces or small amounts of norflavaspidic acids (*4*), albaspidins (*10*), oligoflavaspidic acids (*23, 26*) and tetraalbaspidins = methylene-bis-norflavaspidic acids (*25*) also occur frequently. This is easily understandable from a biosynthetic point of view (cf. Geissman 1967, Penttilä 1967) as all these substances contain the same building units connected by one to three methylene bridges. The differences in chemical composition are in fact so small that it is difficult to find any clear differences between most of the taxa investigated. This is in agreement with the morphological similarity of the different taxa of sect. *Fibrillosae*. However similar phloroglucinol compositions have also been found in the *D. filix-mas* and *D. villarii* complexes, sect. *Dryopteris*, of which certain taxa are included in Table 1 for comparison, as well as in some taxa of sect. *Pallidae* (Widén *et al.* 1991, Fraser-Jenkins & Widén 1993 and references therein). In the following we discuss in more detail the phloroglucinol compositions of the taxa investigated.

Dryopteris wallichiana and related taxa

Diploid taxa

There are six diploids, viz. *Dryopteris wallichiana* subsp. *wallichiana*, *D. redactopinnata*, *D. xanthomelas*, *D. lepidopoda*, *D. polylepis* and *D. crassirhizoma*. In addition the poorly known species *D. zayuensis* may be diploid and is also discussed below.

All these taxa show very similar phloroglucinol compositions as outlined above. The existence of minute amounts of norflavaspidic acid (*4*) in *Dryopteris wallichiana* subsp. *wallichiana* from the New World is hardly of any taxonomic importance, because the material from the Old World may contain *4* in trace amounts if re-investigated with our improved methods (von Euw *et al.* 1980, 1985). The chemical similarity could be interpreted as a useful new piece of evidence to support Fraser-Jenkins' contention in contrast to Alston (1957) and others that the New World *D. parallelogramma* represents the same species (and subspecies) as the Asiatic *D. wallichiana*. However the similarity in chemistry of many of the diploid species in this group is unable to clarify the situation. The same is true of *D. affinis* and its subspecies and varieties as well as of other related taxa. An extensive collection of the diploid apogamous, morphologically variable species *D. wallichiana* ssp. *wallichiana* (Gibby 1985, Fraser-Jenkins 1989) from N India, Japan, Mexico, Hawai'i and Jamaica has been investigated by us. It exhibits a slightly varying phloroglucinol composition, but flavaspidic acid AB (*5*-AB) seems to be the main compound in most cases. The other phenolics are present only in minute amounts and propionyl homologues were totally lacking (Chemotype I).

CRFJ 8536 and 8538, the "Darjeeling variety" of *Dryopteris wallichiana* subsp. *wallichiana* showed a slightly different phloroglucinol spectrum in containing great percentages of both flavaspidic acids AB and BB (*5*-AB, -BB); they may belong to a different chemotype (Chemotype II). It appears that there might be good chemical evidence for formally separating this taxon from subsp. *wallichiana*. Unfortunately there appear to be intermediates existing in Nepal, Sikkim etc. between these two taxa. Further study may allow a more informed decision on the taxonomic significance of variation in this taxon.

Several of the investigated numbers also contained albaspidin homologues (*10*) in slightly varying amounts. Albaspidin (*12*) may be an artefact that is easily formed from other phloroglucinols (here probably flavaspidic acid (*5*) or filixic acid (*19*)) by the so-called rottlerone change (see Euw *et al*. 1980, 1985). However, as all rhizomes were similarly stored and treated this seems unlikely. In any case, HSP 20.4 (= TR 4405) and TR 2419 from N India, as well as TR 4166 from Japan, contain considerable percentages of albaspidin AA (*10*-AA) and may represent a third chemotype (Chemotype III) also containing much trisflavaspidic acid ABB (*23*-ABB). They do not appear to differ morphologically from normal subsp. *wallichiana*.

The possible existence of aspidinol (*2*) ((++) in Table 1) in Japanese *Dryopteris wallichiana* subsp. *wallichiana* (reported by Hisada & Noro 1961) cannot be correct as this species is totally lacking paraaspidin (*7*) which is easily converted to aspidinol (*2*) on decomposition (Fig. 6, cf. Penttilä & Sundman 1966, von Euw *et al*. 1980). Moreover aspidinol (*2*) is very probably not a naturally occurring phloroglucinol. It may be noted that in 1961 only butyryl-homologues of the phloroglucinols were known. Therefore the results of Hisada and Noro (1961) are not comparable with ours in every single detail.

The unknown substances Wa-1 and Wa-2 were found only in the Hawai'ian material of *Dryopteris wallichiana*, whereas the unknown substances Le-2 and Le-3 occur only in the N Indian taxon (see Results). However, the taxonomic value of these observations is questionable. They appear not to be related to morphological variation, but may illustrate geographical variation.

We had a large collection of *Dryopteris lepidopoda* from N India and one sample from Taiwan. It exhibits a phloroglucinol spectrum close to *D. wallichiana* and especially to the "Darjeeling variety" (CRFJ 8356, 8358). Flavaspidic acid AB (*5*-AB) is the main compound, but CRFJ 8832 and 8835-36 from N India and TR 4082 from Taiwan contain percentages of the flavaspidic acids BB and AB (*5*-BB, *5*-AB) about equal to the "Darjeeling variety". These specimens also approach *D. wallichiana* in morphology. The existence of minute amounts of albaspidin homologues (*5*-BB, -AB, -AA) in all samples investigated is also noteworthy. As in the case of *D. wallichiana* propionyl homologues were totally lacking.

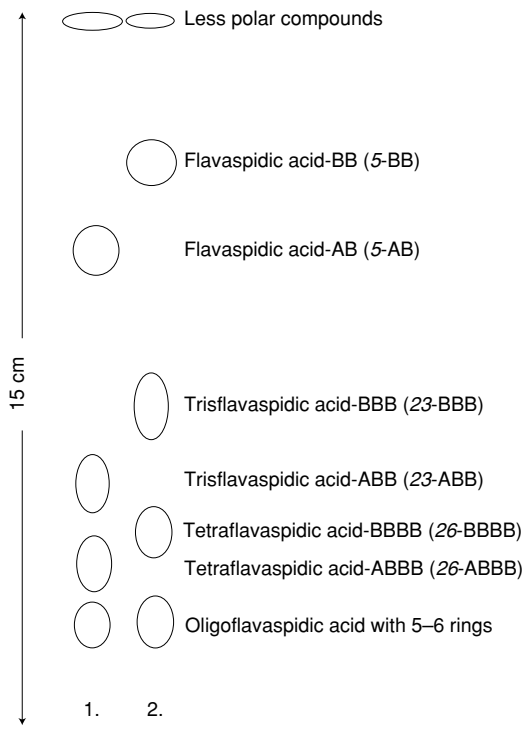


Fig. 7. Chromatography of crude filicin of (1) *Dryopteris wallichiana* (Sprengel) N. Hylander (CRFJ 8483, 8485-87 containing only acetyl homologues) and (2) *D. aitoniana* Pichi-Sermolli (CJW s. n. containing virtually only butyryl homologues) on silica gel H buffered at pH 4.0. Solvent: *n*-hexane-chloroform-absolut ethanol-glacial acetic acid 45:35:16:4.

Turning to the group of dark-scaled, high altitude members of the section, the phloroglucinol composition of *Dryopteris xanthomelas* from N India is very similar to that of *D. polylepis* from Japan. On morphological grounds it is quite acceptable to consider these as being related and in the same subgroup as *D. polylepis* has dark scales similar to those of *D. xanthomelas* (and also the uninvestigated

Table 2. Amounts of crude extractives in the *Dryopteris* material and related taxa investigated. For abbreviations and other data see Table 1.

Taxon	Collection number or lit.ref.	Origin	Dry rhizome in g	Ether extract in g (in %)		Crude filicin in g (in %)	
				crude	cation free	Mg	$Ba(OH)_2$
D. parrisiae	CPG3396	New Guinea	0.41	0.02 (4.8)	n.s.	n.s.	n.s.
D. xanthomelas	CRFJ7520-24	Kashmir	104.9	2.62 (2.50)	n.s.	1.01 (0.96)	0.15 (0.14)
	CRFJ7525-26	Kashmir	36.1	2.02 (5.60)	n.s.	0.68 (1.88)	0.32 (0.89)
	CRFJ7611	Kashmir	6.5	0.37 (5.69)	n.s.	n.s.	n.s.
	CRFJ7832-33, CRFJ7835, 7837[1)]	N India	265.3	8.89 (3.35)	n.s.	2.89 (1.09)	0.12 (0.05)
	CRFJ8370-71[2)]	N India	198.8	10.31 (5.19)	n.s.	4.59 (2.31)	0.45 (0.23)
D. zayuensis "lobed form"	Kiang & Chiao 296	Tibet	2.3	0.12 (5.2)	n.s.	n.s.	n.s.
D. polylepis	Widén *et al.* (1975)	Japan	120.6	18.2 (15.1)	n.s.	3.07 (2.6)	3.58 (2.97)
D. crassirhizoma	Widén *et al.* (1975)	Japan	275.0	21.1 (7.7)	n.s.	5.22 (1.9)	6.62 (2.4)
D. neorosthornii	CRFJ8363-64 CRFJ8370[2)]	N India	238.6	6.69 (2.80)	n.s.	2.41 (1.01)	0.25 (0.10)
D. redactopinnata	CRFJ7838-40	N India	16.9	0.71 (4.20)	n.s.	n.s.	n.s.
	CRFJ7590,7641, 7662-63,7592-93	N India	426.2	1.23 (0.29)	n.s.	0.46 (0.11)	0.07 (0.02)
	IK6939	N India	6.2	0.32 (5.16)	n.s.	n.s	n.s.
D. yigongensis	CRFJ7584,7589,7617, 763234,7636-69,7610	N India	89.2	6.08 (6.82)	n.s.	2.28 (2.56)	0.19 (0.21)
"Hattu 1"	CRFJ7585	N India	4.8	0.26 (5.42)	n.s.	n.s.	n.s.
	CRFJ7643-45,7648	N India	154.6	10.48 (6.78)	n.s.	1.94 (1.25)	0.65 (0.42)
	CRFJ7586,7609,7645-46	N India	226.8	14.86 (6.55)	n.s.	2.76 (1.22)	2.37 (1.04)
"Hattu 2"	CRFJ7652,7654-56,7658	N India	254.9	14.06 (5.52)	n.s.	2.11 (0.83)	1.37 (0.54)
D. acutodentata	CRFJ7796-7806	N India	57.6	3.73 (6.48)	n.s.	1.58 (2.74)	0.07 (0.12)
D. wallichiana	HSP20.4 (= TR4405)	N India	22.0	n.s.	0.70 (3.2)	n.s.	n.s.
ssp. *wallichiana*	CRFJ8356-59	N India	489.1[3)]	21.22 (4.34)	n.s.	n.s.	n.s.
	CRFJ8483,8485-87	N India	912.1[3)]	36.8 (4.03)	36.33 (3.98)	n.s.	n.s.
	CRFJ8483,8485-87	N India	24.9	1.0 (4.03)	n.s.	0.49 (1.97)	0.09 (0.36)
	TR2419 (= CRFJ10926)	N India	20.6	n.s.	0.71 (3.45)	n.s.	n.s.
	TR4166	Japan	20.6	n.s.	0.64 (3.11)	n.s.	n.s.
	Tryon *et al.* (1973)	Mexico	172.0	10.5 (6.1)	n.s.	1.88 (1.10)	n.s.
	CRFJ13106,13108	Mexico	9.4	0.45 (4.8)	n.s.	n.s.	n.s.
	CRFJ13252	Mexico	7.6	0.22 (2.9)	n.s.	n.s.	n.s.
	CRFJ14773,14775-76	Hawai´i	21.3	1.42 (6.67)	n.s.	n.s.	n.s.
	CRFJ14777,14782-83	Hawai´i	20.1	1.00 (4.98)	n.s.	n.s.	n.s.
	CRFJ all numbers	Hawai´i	539.1[3)]	n.s.	24.4 (4.5)	n.s.	n.s.
	CRFJ13004-5,14710	Jamaica	28.6	1.39 (4.86)	n.s.	n.s.	n.s.
"*Darjeeling variety*"	CRFJ8536	N India	217.5	1.31 (0.60)	n.s.	0.90 (0.41)	0.02 (0.01)
	CRFJ-8538	N India	241.1	7.08 (2.94)	n.s.	3.38 (1.40)	0.09 (0.04)
ssp. *coriacea*	PA33122	Geo	1.1	0.08 (7.27)	n.s.	n.s.	n.s.
	CRFJ5931-33	Iran	186.2	4.75 (2.55)	n.s.	n.s.	n.s.
ssp. *reichsteinii*	TR4086	Zimbabwe	25.6	1.17 (4.6)	n.s.	n.s.	n.s.
ssp. *madrasensis*	CRFJ9204,9206-7	S India[3)]	558.3	8.86 (1.59)	n.s.	3.14 (0.56)	0.18 (0.03)
	CRFJ9359	S India	83.4	2.48 (2.97)	n.s.	0.95 (1.14)	0.07 (0.08)
	CRFJ9362	S India	61.8	2.71 (4.38)	n.s.	1.04 (1.68)	0.06 (0.10)
D. affinis ssp.	CJW78/6	Madeira	2.9	0.09 (3.1)	0.08 (2.76)	n.s.	n.s.
affinis var.	TR2616	Madeira	25.2	n.s.	0.69 (2.74)	n.s.	n.s.
affinis	TR3548	Azores	15.1	n.s.	0.59 (3.91)	n.s.	n.s.
	S & S	Lu	4.3	0.26 (6.1)	0.17 (4.0)	n.s.	n.s.
	CRFJ10813	Hs	36.1	2.25 (6.23)	2.09 (5.8)	n.s.	n.s.
	JAC e*t al.*11824	England	3.6	0.11 (3.06)	0.10 (2.78)	n.s.	n.s.
	CRFJ11694-95	Austria	5.5	n.s.	0.35 (6.36)	n.s.	n.s.
	CRFJ12700,12702-03	Scotland	24.3	n.s.	1.11 (4.57)	n.s.	n.s.
	TR293	Ge	15.2	n.s.	0.39 (2.57)	n.s.	n.s.
	CRFJ11729	Ju	2.2	n.s.	0.12 (5.5)	n.s.	n.s.
	CRFJ11733	Ju	2.1	n.s.	0.13 (6.2)	n.s.	n.s.

(Continues)

Table 2 continued.

Taxon Collection number or lit.ref.		Origin	Dry rhizome in g crude	Ether extract in g (in %) cation free	Crude filicin in g (in %) Mg	$Ba(OH)_2$
var. *paleaceo-lobata*	CRFJ12784	Wales	30.7 n.s.	1.40 (4.56)	n.s.	n.s.
var. *splendens*	CRFJ11590	He	2.5 n.s.	0.16 (6.4)	n.s.	n.s.
	TR740	He	19.8 n.s.	0.69 (3.48)	n.s.	n.s.
	CRFJ11647	He	2.0 n.s.	0.10 (5.0)	n.s.	n.s.
	CRFJ11751	Ju	2.2 n.s.	0.13 (5.9)	n.s.	n.s.
var. *jessenii*	CRFJ14422-23,14440	Romania	36.2 2.26 (6.24)	n.s.	n.s.	n.s.
ssp. *persica*	CRFJ5922	Iran	3.0 0.14 (4.67)	n.s.	n.s.	n.s.
ssp. *pontica*	CRFJ5948 (= TR4264A)	Iran	20.4 1.01 (4.95)	0.83 (4.07)	n.s.	n.s.
	CRFJ14058,14060-61, 14064	An	31.6 1.70 (5.38)	n.s.	n.s.	n.s.
ssp. *cambrensis*	JAC1873	Scotland	4.6 0.18 (3.9)	0.15 (3.26)	n.s.	n.s.
var. *paleaceo-crispa*	CRFJ12507-09	Scotland	45.6 n.s.	1.64 (3.57)	n.s.	n.s.
	CRFJ12561	Scotland	8.9 n.s.	0.30 (3.37)	n.s.	n.s.
	CRFJ12581	Scotland	12.9 n.s.	0.69 (5.35)	n.s.	n.s.
	CRFJ12592	Scotland	10.0 n.s.	0.29 (2.9)	n.s.	n.s.
"form similar to *D. x complexa*"	CRFJ12634	Scotland	9.4 n.s.	0.42 (4.47)	n.s.	n.s.
	CRFJ11702	Austria	1.7 n.s.	0.13 (7.7)	n.s.	n.s.
ssp. *pseudo-disjuncta*	CRFJ11606	He	2.0 n.s.	0.11 (5.5)	n.s.	n.s.
	Widén *et al.* (1971)	He	11.8 0.62 (5.3)	n.s.	0.22 (1.86)	n.s.
var. *insubrica*	CRFJ11838	He	2.6 n.s.	0.19 (7.3)	n.s.	n.s.
	CRFJ11841-42	He	6.4 n.s.	0.53 (8.28)	n.s.	n.s.
	CRFJ11846,11648-49	He	9.7 n.s.	0.87 (8.97)	n.s.	n.s.
	TR2293	He	20.2 n.s.	0.71 (3.51)	n.s.	n.s.
	TR2845	He	16.2 n.s.	0.77 (4.75)	n.s.	n.s.
	CRFJ11741	Ju	3.1 n.s.	0.20 (6.5)	n.s.	n.s.
ssp. *borreri*	CRFJ11618	He	2.0 n.s.	0.14 (7.0)	n.s.	n.s.
	Widén *et al.* (1971)	He	25.0 2.77 (11.1)	n.s.	0.75 (3.02)	n.s.
	CRFJ11747	Ju	2.9 n.s.	0.19 (6.6)	n.s.	n.s.
	CRFJ11714-15	Austria	5.7 n.s.	0.39 (6.84)	n.s.	n.s.
	CRFJ4089 (= TR3685)	An	12.7 n.s.	0.96 (7.56)	n.s.	n.s.
	JAC *et al.*1253	Wales	4.6 0.33 (7.2)	0.22 (4.78)	n.s.	n.s.
	JAC *et al.*11782	England	2.9 0.15 (5.2)	0.11 (3.8)	n.s.	n.s.
	JAC *et al.*11823	England	2.6 0.16 (6.2)	0.13 (5.0)	n.s.	n.s.
	CRFJ10812	Hs	31.5 2.70 (8.57)	2.33 (7.4)	n.s.	n.s.
D. fusco-atra	CRFJ14765	Hawai´i	10.0 0.73 (7.3)	n.s.	n.s.	n.s.
var. *fusco-atra*	CRFJ14901	Hawai´i	10.3 0.68 (6.6)	n.s.	n.s.	n.s.
	CRFJ14902	Hawai´i	9.9 0.92 (9.3)	n.s.	n.s.	n.s.
	CRFJ14903	Hawai´i	10.9 1.09 (10.0)	n.s.	n.s.	n.s.
	CRFJ14905	Hawai´i	11.5 1.06 (9.2)	n.s.	n.s.	n.s.
	CRFJ14906	Hawai´i	13.5 1.10 (8.2)	n.s.	n.s.	n.s.
var. *lamoureuxii*	CRFJ14911	Hawai´i	13.0 0.31 (2.4)	n.s.	n.s.	n.s.
	CRFJ all numbers[3)]	Hawai´i	464.1 n.s.	25.76 (5.6)	n.s.	n.s.
D. lepidopoda	HSP20.16 (= TR4406)	N India	19.7 n.s.	0.85 (4.3)	n.s.	n.s.
	CRFJ8826,8829-30, 8837-39,8841-42	N India	236.6[3)] 5.49 (2.32)	5.10 (2.16)	n.s.	n.s.
	CRFJ8826,8829-30, 8837-39,8841-42	N India	50.5 1.17 (2.32)	n.s. 0.11	(0.22) 0.08	(0.16)
	CRFJ8832	N India	20.8 0.83 (3.99)	n.s.	n.s.	n.s.
	CRFJ8835-36	N India	38.4 0.95 (2.47)	n.s.	n.s.	n.s.
	CRFJ8840	N India	16.7 1.02 (6.11)	n.s.	n.s.	n.s.
	TR4082 (= CRFJ10929)	Taiwan	20.1 n.s.	0.84 (4.2)	n.s.	n.s.
D. sledgei	CRFJ9349-58	S India	154.6[3)] 6.28 (4.06)	4.93 (3.19)	n.s.	n.s.
	CRFJ9349-58	S India	34.4 1.40 (4.06)	n.s.	0.33 (0.96)	0.08 (0.23)
D. pseudo-filix-mas	CRFJ13155,13157	Mexico	20.9 0.65 (3.11)	n.s.	n.s.	n.s.
	CRFJ13428	Mexico	27.8 1.34 (4.82)	n.s.	n.s.	n.s.
	CRFJ13457-61	Mexico	8.9 0.36 (4.0)	n.s.	n.s.	n.s.

(Continues)

Table 2 continued.

Taxon and ploidy, reproduction	Collection number or lit.ref.	Origin	Dry rhizome in g	Ether extract in g (in %) crude	cation free	Crude filicin in g (in %) Mg	$Ba(OH)_2$
D. khullarii	CRFJ8236-37,8267	N India	13.2	0.33 (2.50)	n.s. 0.05	(0.38) 0.01	(0.08)
	CRFJ8350-54	N India	494.6[3]	5.89 (1.19)	5.00 (1.01)	n.s.	n.s.
D. subbipinnata	CRFJ14795	Hawai´i	1.65	0.02 (1.21)	n.s.	n.s.	n.s.
	CRFJ15068	Hawai´i	1.57	0.01 (0.64)	n.s.	n.s.	n.s.
D. conjugata	CRFJ15779-15785	Nepal	5.4	0.22 (4.1)	n.s.	n.s.	n.s.
D. hirtipes ssp. *atrata*	CRFJ9164-67,9170-72, 9173-74	S India	411.0	6.98 (1.7)	n.s. 1.65	(0.40) 0.07	(0.02)
	CRFJ9131-33	S India	257.0	9.33 (3.6)	n.s.	3.37 (1.31)	0.09 (0.04)
ssp. *hirtipes*	CRFJF.n. 161-171, 15.9.93	Sri Lanka	11.7	0.74 (6.3)	n.s.	n.s.	n.s.

[1]CRFJ7837 = *Dryopteris zayuensis*, of similar phloroglucinol composition to that of *D. xanthomelas*, was extracted together with CRFJ7832,7833,7835 = *D. xanthomelas* and used for preparative isolation.
[2]CRFJ8371, *Dryopteris xanthomelas*, first identified as *D. neorosthornii* (of similar phloroglucinol composition to that of *D. xanthomelas*), was extracted together with CRFJ8370 = *D. neorosthornii* and used for preparative isolation.
[3]This material was used for preparative isolation.

D. rosthornii (Diels) C. Chr. They contain about equal percentages of flavaspidic acids AB, PB and BB (*5*-AB, -PB, -BB) as the main compounds. *Dryopteris redactopinnata* differs from the above taxa in containing virtually only the homologues PB and BB of flavaspidic acid (*5*-PB, -BB). IK 6939 is also slightly different from other specimens of *D. redactopinnata* in containing much less flavaspidic acid PB (*5*-PB) than the AB homologue (*5*-AB). *Dryopteris redactopinnata* is morphologically intermediate between *D. wallichiana* and *D. xanthomelas* in frond and segment-size and in its scale-colour, which is yellowish-grey, but the wide, glossy stipe-base scales place it more closely to *D. xanthomelas* than to *D. wallichiana*, even though its flavaspidic acid constituents are more similar to those of *D. wallichiana*. Very similar phloroglucinol spectra were also found in *D. zayuensis* from N India including the lobed form of it described as *D. incisolobata* from Tibet. In the latter only minute amounts of flavaspidic acid AB (*5*-AB) were observed. Morphologically *D. zayuensis* is close to *D. redactopinnata* but with completely black scales, similar to but denser and wider than in *D. xanthomelas*, from which it also differs in its large frond, more similar to that of *D. redactopinnata*. Its chemistry appears close to *D. redactopinnata*.

The phenolics of Japanese *Dryopteris crassirhizoma* were rather different from the other species of this group in containing large amounts of tetraalbaspidin-ABBA (= dryocrassin) (*25*-ABBA) and only minute amounts of filixic acid ABA (*19*-ABA) and flavaspidic acid AB (*5*-AB). Concerning the possible occurrence of aspidinol (*2*) and albaspidin (*10*) (reported by Hisada & Noro 1961), see discussions under *D. wallichiana*. This species is somewhat isolated morphologically.

Triploid taxa

These consist of the following eleven taxa, viz. *Dryopteris wallichiana* subsp. *coriacea*, subsp. *madrasensis* and subsp. *reichsteinii, D. yigongensis*, *D. acutodentata*, *D. neorosthornii*, *D. khullarii*, *D. parrisiae, D. pseudo-filix-mas* and probably *D. fusco-atra* and *D. subbipinnata.*

Dryopteris wallichiana subsp. *coriacea* exhibited a phloroglucinol spectrum very similar to that of *D. wallichiana* subsp. *wallichiana* thus giving only little indication of its origin (the other ancestor remains unknown). Its chemistry, with the almost exclusive existence of flavaspidic acid AB (*5-AB*), supports its transfer from being a subspecies of *D. affinis* to a subspecies of *D. wallichiana* which was done on morphological grounds (Fraser-Jenkins 1988, 1994).

Dryopteris wallichiana subsp. *madrasensis* from S India is evidently closely related to *D. wallichiana* subsp. *wallichiana* and perhaps derived from it by hybridisation (Fraser-Jenkins 1989). This was confirmed by our chromatographic investigations, the phloroglucinol compositions being almost identical. Flavaspidic acids BB and AB (*5*-BB, *5*-AB) are the

Table 3. The semiquantitative composition of the acylfilicinic acids (*36* and homologues, see Widén *et al.* 1991) after reductive cleavage of the crude filicins. B = butyryl, P = propionyl, A = acetyl.

Taxon	Collection no. or lit.ref.	Origin	Acylfilicinic acid in % of total amount		
			B	P	A
D. xanthomelas	CRFJ8370-71[1)]	N India	25	15	60
D. polylepis	Widén *et al.* (1975)	Japan	45	5	50
D. crassirhizoma	Widén *et al.* (1975)	Japan	–	5	95
D. neorosthornii	CRFJ8363-64	N India	40	10	50
D. redactopinnata	CRFJ7830-40	N India	5	45	50
D. yigongensis	CRFJ7584, 7889, 7617,				
"Hattu 1"	7632-34, 7636-37, 7610	N India	45	5	10
"Hattu 2"	CRFJ7652, 7654-56, 7658	N India	80	10	10
D. acuto-dentata	CRFJ7796-7806	N India	10	45	45
D. wallichiana	HSP20.4 (TR4405)	N India	5	–	95
ssp. *wallichiana*	CRFJ8356-59	N India	5	–	95
	CRFJ8343, 8485-87	N India	–	–	100
	Tryon *et al.* (1973)	Mexico	2	–	98
ssp. *coriacea*	CRFJ5931-33	Iran	3	3	94
ssp. *madrasensis*	CRFJ9204, 9206-07,	S India	45	10	45
	CRFJ9362	S India	45	10	45
D. affinis ssp. *affinis*	TR293,				
var. *affinis*	Widén *et al.* (1971)	Ge	10	35	55
ssp. *pseudo-disjuncta*	Widén *et al.* (1971)	He	27	31	42
ssp. *borreri*	Widén *et al.* (1971)	He	20	20	60
D. lepidopoda	HSP20.16 (TR4406)	N India	5	–	95
	CRFJ8826, 8829-30,				
	8837-39, 8841-42	N India	40	–	60
	CRFJ8832	N India	40	–	60
	CRFJ8835-36	N India	50	–	50
	CRFJ8840	N India	30	–	70
D. sledgei	CRFJ9349-58	S India	5	15	80
D. khullarii	CRFJ8236-37, 8267	N India	10	–	90

[1)]CRFJ8371, *Dryopteris xanthomelas*, first identified as *D. neorosthornii*, was extracted together with CRFJ8370 = *D. neorosthornii*, see Table 2, footnote 2.

main phloroglucinols. The occurrence of small amounts of albaspidin AB and AA (*10*-AB, *10*-AA) is also noteworthy.

Dryopteris wallichiana subsp. *reichsteinii* from Zimbabwe (Africa) is also related to *D. wallichiana* subsp. *wallichiana* (Fraser-Jenkins 1989) as is evident from its phloroglucinol spectrum too. Trace amounts of albaspidin homologues (*10*-BB, -AB, -AA) are present as well. However diploid *D. wallichiana* is not known from continental Africa, though its nearest station appears to be Gough Island and perhaps Madagascar.

According to Fraser-Jenkins (1989) *Dryopteris yigongensis* from N India may consist of two species, preliminarily designated "Hattu 1" and "Hattu 2" of slightly different morphology (see also Taxonomy, above). This was subsequently confirmed to some extent by our phloroglucinol investigations. "Hattu 1" (as illustrated by Fraser-Jenkins 1989), real *D. yigongensis*, contained great percentages of both flavaspidic acids AB (*5*-AB) and BB (*5*-BB), whereas "Hattu 2", the possible new species, contained much less flavaspidic acid AB (*5*-AB). Moreover traces of albaspidin BB (*10*-BB) and AB (*10*-AB) were found in "Hattu 2" (concerning the occurrence of albaspidin (*10*) see under *D. wallichiana*). However, the differences in phloroglucinol content are relatively small. As suggested by Fraser-Jenkins (1989), true *D. yigongensis* may well have been derived from *D. xanthomelas* in the past; the chemis-

Table 4. Pure crystalline compounds isolated from the analysed taxa. For abbreviations and additional data see Table 1. In mixed crystallisates the main homologues are underlined.

Taxon	Collection nr. or lit.ref.	Origin crystals	Compounds isolated in (mg)
D. xanthomelas[1]	CRFJ8370-71	N India	Filixic acid-ABA, ABB, PBP, BBB = *19*-ABA, ABB, PBP, BBB (3.7); flavaspidic acid-BB, PB = *5*-BB, PB (73.1).
D. polylepis	Widén *et al.* (1975)	Japan	Albaspidin-BB, PB and PP = *10*-BB, PB, PP (0.5) flavaspidic acid-BB (*5*-BB) (172.4), flavaspidic acid-BB, PB, PP = *5*-BB, PB and PP (172.4), flavaspidic acid-AB = *5*-AB (333).
D. crassirhizoma	Widén *et al.* (1975)	Japan	Dryocrassin = *25*-ABBA (127.6) Filixic acid-ABA = *19*-ABA. [2]
D. neorosthornii	CRFJ8370-71[1]	N India	See sub D. xanthomelas.
D. acuto-dentata	CRFJ7796-7806	N India	Filixic acid-PBB, PBP, ABB, ABP = *19*-PBB, PBP, ABB, ABP (35.7) Filixic acid-PBP, ABB, ABP, ABA = *19*-PBP, ABB, ABP, ABA (33).
D. wallichiana ssp. *wallichiana*	HSP20.4 (TR4405)	N India	Trisflavaspidic acid-ABB = *23*-ABB (19) [3]
	CRFJ8343,8485-87	N India	Albaspidin-AA (*10*-AA) (artefact, 12.4), flavaspidic acid-AB = *5*-AB (636), filixic acid-ABA = *19*-ABA (183.2), dryocrassin = tetra-albaspidin-ABBA = *25*-ABBA (20.1), tetraflavaspidic acid-ABBB *26*-ABBB (53.6, not quite pure), PA-1 = tricosanol. (73).[4]
ssp. *wallichiana*	CRFJ8536-59	N India	Albaspidin-BB, AB = *10*-BB, AB (5.3), filixic acid-ABA = *19*-ABA (10.9), filixic acid-BBB, ABB, ABA = *19*-BBB, ABB, ABA (23.9), flavaspidic acid-BB, AB = *5*-BB, AB (148.8), flavaspidic acid-AB = *5*-AB (700.1), tetraflavaspidic acid-BBBB, ABBB = *26*-BBBB, ABBB (20.1, not quite pure).
	CRFJ14773,14775-76, 14777,14782-83	Hawai´i	Flavaspidic acid-AB = *5*-AB (444.4), "Wa 1 + 2" (164).
ssp. *madrasensis*	CRFJ9204 ; 9206-07	S India	Filixic acid-BBB, PBP/ABB, ABA = *19*-BBB, PBP/ABB, ABA (11.2).
D. affinis ssp. *affinis* var. *affinis*	TR293, Widén *et al.* (1971)	Ge	Filixic acid-BBB, PBB, PBP, ABB, ABP, ABA = *19*-BBB, PBB, PBP, ABB, ABP, ABA (3), flavaspidic acid-BB, PB = *5*-BB, PB (6).
ssp. *pseudo-disjuncta*	W.G. *s.n.*, Widén *et al.* (1971)	He	See sub TR-293 (These two samples of similar phloroglucinol composition were mixed before separation)
ssp. *borreri*	W.G. *s.n.*, Widén *et al.* (1971)		Filixic acid-BBB, PBB, PBP, ABB, ABP, ABA = *19*-BBB, PBB, PBP, ABB, ABP, ABA (16), para-aspidin-BB = *7*-BB (6), flavaspidic acid-BB, PB = *5*-BB, PB (20).
D. fusco-atra	CRFJ14765,14901-03, 14905-06,14911, see Patama & Widén (1991)[5]	Hawai´i	Filixic acid-ABP, ABA = *19*-ABP, ABA (230.1), norflavaspidic acid-PB, AB = *4*-PB, AB (37), Fu-3 (209), Fu-1, 2 (1295, not quite pure).
D. lepidopoda	CRFJ8826,8229-30, 8837-39,8841-42	N India	Albaspidin-BB, AB = *10*-BB, AB (3.7), flavaspidic acid-BB, AB = *10*-BB, AB (49.4), tetraflavaspidic acid-BBBB, ABBB = *26*-BBBB, ABBB (4.0, not quite pure).
D. sledgei	CRFJ9348-58	S India	Filixic acid-PBB, PBP/ABB, ABP, ABA = *19*-PBB, PBP, ABB, ABP ABA (52.3). (32.3), filixic acid-PBP/ABB, ABP, ABA = *19*-PBP, ABB, ABP, ABA (52.3), filixic acid-ABA = *19*-ABA (0.3).
D. khullarii	CRFJ8350-54	N India	Flavaspidic acid-AB = *5*-AB (28.9).

[1] Isolated from a mixed sample of *Dryopteris xanthomelas* and *D. neorosthornii*, see Table 2, footnote 2.
[2] Filixic acid-ABA (*19*-ABA) was identified later with our improved methods. However only mixed crystallisates with dryocrassin (*25*-ABBB) were obtained.
[3] Trisflavaspidic acid-ABA (*23*-ABA, amorphous) was identified from its behaviour in TLC and from products obtained by reductive alkaline cleavage (*cf.* von Euw *et al.* 1980,1985). It was not obtained in crystalline form for final characterisation.
[4] Pa-1, m.p. 65–67°C, proved to be 1-tricosanol, an aliphatic, straight-chain alcohol $C_{23}H_{47}OH$, $M^+ = 340$. It was identified from its mass and ^{13}C NMR spectra. It gave no coloration with fast blue salt B in TLC. It has been reported previuosly from *Dryopteris filix-mas* (Thomas & Taurins 1962).
[5] Only provisional numbers were given in Patama and Widén (1991).

try of these two taxa is almost identical. The other ancestor, however, remains unknown. The putative new species "Hattu 2", though similar, is slightly more reminiscent of *D. juxtaposita* in its morphology. This is in agreement with the chemical analysis too; the phloroglucinol compositions of these two taxa are indeed very similar (Widén *et al.* 1991, table 5), but from their morphology it is quite clear that "Hattu 2" is very close to *D. yigongensis* and thus to the *D. xanthomelas* group. Its other ancestor is unknown but could be a diploid sexual species in the *D. odontoloma* (Beddome) C. Chr. group in sect. *Pallidae*. At present it is still unclear whether "Hattu 2" merely represents morphological and chemical variation within *D. yigongensis*, and so it is not formally recognised here.

Dryopteris neorosthornii and *D. khullarii* from N India exhibit phloroglucinol spectra close to those

of the species discussed above. According to Fraser-Jenkins (1989) *D. khullarii* appears to have been formed by hybridization between *D. wallichiana* and *D. nigropaleacea*. This cannot be directly confirmed by chemistry as these two very dissimilar species have very similar phloroglucinol compositions. On the other hand, their chemistry does not reject this suggestion.

Dryopteris acuto-dentata clearly differs from the other taxa so far investigated in containing huge amounts of para-aspidin BB and AB (*7*-BB, *7*-AB). According to Fraser-Jenkins (1989) *D. acuto-dentata* is intermediate in morphology between *D. xanthomelas* and *D. alpestris* or, most likely, *D. komarovii* in sect. *Dryopteris*. We only had material of *D. barbigera* (closely related to *D. komarovii*) for our chromatographic work. *Dryopteris acuto-dentata* has also been confused previously with *D. serrato-dentata* (Beddome) Hayata. Both *D. barbigera* and *D. serrato-dentata* contain considerable amounts of para-aspidin (*7*) (Widén *et al.* 1991, and in prep.), and are indeed promising candidates for one of the ancestors of *D. acuto-dentata*. However, *D. serrato-dentata* is a tetraploid sexual species and can probably be excluded from the candidates, but *D. barbigera, D. komarovii* and probably also *D. alpestris* are diploid sexual species (Gibby 1985, Fraser-Jenkins 1989). Thus the chemical results do indicate that *D. acuto-dentata* could be derived from a cross of *D. xanthomelas* and the *D. barbigera* group as suggested by its morphology. A recent paper by Lu (1990) describing a new sect., *Caespitosae* Lu for *D. barbigera* and *D. serrato-dentata* and their relatives, based on *D. acuto-dentata* as the type, is somewhat unfortunate as *D. acuto-dentata* is almost certainly of mixed ancestry between two sections, and its morphology shows strong signs of *D. xanthomelas*, its ancestor in sect. *Fibrillosae*, in larger plants.

Dryopteris parrisiae from New Guinea is clearly related to *D. wallichiana* in containing flavaspidic acid AB (*5*-AB) as the main phloroglucinol. It is worth noting that both species are known from New Guinea (Fraser-Jenkins 1989). In morphology *D. parrisiae* is close to *D. wallichiana* but smaller and the pinna-lobes are more widely joined together at their bases, perhaps slightly similar to *D. hirtipes*, though its chemistry is too similar to *D. wallichiana* to suggest any second ancestral diploid.

Dryopteris pseudo-filix-mas resembles *D. filix-mas* in its morphology as well as showing similarities with *D. wallichiana*. It differs from *D. filix-mas* in the lack of para-aspidin (*7*), desaspidin (*8*) and trisdesaspidin (*21*), but it contains a relatively large amount of filixic acid (*19*) and in this respect is closer to it than to *D. wallichiana* (Table 1, see also Widén *et al.* 1971, von Euw *et al.* 1980). Its morphology is markedly similar to *D. affinis* subsp. *borreri*, and it is possible that it has had an analogous origin, derived from North American *D. filix-mas* and diploid sexual *"D. wallichiana"*. Its chemistry is distinct enough to preclude a common origin. Further studies of the species are needed.

Dryopteris fusco-atra from Hawai'i exhibits a very different phloroglucinol spectrum from all the other taxa discussed so far. Norflavaspidic acid AB (*4*-AB) is the main compound and small amounts of norflavaspidic acid BB (*4*-BB), flavaspidic acids BB and AB (*5*-BB, -AB), filixic acids BBB, ABB and ABA (*19*-BBB, -ABB, -ABA) and tetraalbaspidin ABBA (*25*-ABBA) occur as well (Patama & Widén 1991). In addition three unknown compounds Fu-1, Fu-2 and Fu-3 were also present (see Results). *Dryopteris fusco-atra* is probably a triploid apomict (Fraser-Jenkins 1994) that may have evolved from diploid apomict *D. wallichiana* and *D. hirtipes* subsp. *hirtipes*, a sexual diploid. *Dryopteris hirtipes* has been investigated by us from material from S India and Sri Lanka, and the results are included in Table 1. Both subspecies of *D. hirtipes* (see Table 1) are rich in norflavaspidic acid (*4*) and also contain albaspidin (*10*) and filixic acids (*19*) in varying amounts. They also contain considerable amounts of the stenolepins 4 + 5 of unknown structure, but no Fu-1, Fu-2 and Fu-3, compounds that occur in *D. fusco-atra*. Nevertheless we find *D. hirtipes* subsp. *hirtipes* a promising candidate for one ancestor of *D. fusco-atra*, assuming that *D. fusco-atra* has developed new, but related compounds during its probable long isolation in Hawai'i. However, *D. hirtipes* does not occur in Hawai'i, although it is found in Samoa, the Cook Islands and the Marquesas Islands (Fraser-Jenkins 1994). The influence of the other supposed ancestor, *D. wallichiana,* is less clear as both putative ancestors contain albaspidin (*10*) and filixic acid (*19*). However the minute amounts of flavaspidic acids (*10*) present could be due to *D. wallichiana* as *D. hirtipes* is lacking in those compounds. Moreover, as discussed under Results, Fu-1 and Fu-2 appear to be closely related in their chemical structures to Wa-1 and Wa-2 from Hawai'ian *D. wallichiana*. Ac-

cordingly, Wa-1 and Wa-2 may well be the precursors of Fu-1 and Fu-2.

The other Hawai'ian probable triploid, *Dryopteris subbipinnata,* is most interesting. Our sample contained only trace amounts of phloroglucinols of which only the homologous flavaspidic acids BB and AB (*5*-BB, -AB) could be detected in TLC. Such a phloroglucinol has never been reported earlier and is consequently unique. Unfortunately few conclusions can be drawn about its parentage: *D. wallichiana* may be one ancestor as suggested by Fraser-Jenkins (1994). It is possible that *D. subbipinnata* may also contain other phloroglucinols detected in *D. wallichiana*, but their existence could not be proved by TLC as their concentrations in the ether extracts were too low. On morphological grounds the other ancestor must be a species with a wider and more dissect lamina.

Tetraploid taxa

Only one tetraploid species, *Dryopteris sledgei*, probably an apomict, is known and occurs in S India (Gibby 1985, Fraser-Jenkins 1989). It was previously confused with *D. wallichiana*. It differs in the occurrence of only minute amounts of flavaspidic acids (*5*) and instead contains a greater quantity of filixic acids (*19*), as well as tetraalbaspidin BBBB (*22*-BBBB), not detected in *D. wallichiana*. The presence of some norflavaspidic acids (*4*) and albaspidin (*10*) is also noteworthy.

The *Dryopteris affinis* complex

Diploid taxa

Only one diploid apogamous taxon, *Dryopteris affinis* subsp. *affinis*, is known from Europe. We had material from the Macaronesian islands, the Pyrenees, the British Isles and several localities in central Europe. The same phenolics were found as are present in the closely related *D. wallichiana* subsp. *wallichiana*, but the percentages of flavaspidic acids AB, PB and BB (*5*-AB, -PB, -BB) varied slightly. In most cases the percentages of these flavaspidic acids present were about equal to those in *D. wallichiana* subsp. *wallichiana* "Darjeeling variety". Moreover, only traces of filixic acids (*19*) and in most cases albaspidin (*10*) were present. Only one sample (CRFJ 11694-95 from Austria) differed in containing relatively more flavaspidic acid AB (*5*-AB) and trisflavaspidic acid ABB (*23*-ABB). No clear differences were found between var. *affinis*, var. *paleaceo-lobata* and var. *splendens* in phloroglucinol composition (var. *kerryensis* has not been investigated), but var. *jessenii* from Romania was somewhat different in containing more flavaspidic acid AB (*5*-AB) than the BB homologue (*5*-BB) and totally lacking albaspidin homologues (*10*). Concerning the occurrence of norflavaspidic acid (*4*) see discussions below *D. wallichiana*. Of all the varieties of subsp. *affinis*, var. *jessenii* is the most distinct morphologically, but due to the presence of some intermediates and its similarity to subsp. *affinis* it is ranked as a variety.

Of the "*Dryopteris borreri*" material investigated by Fikenscher and Hegnauer (1963) only one specimen from Spain (No. 1961-918) was cytologically investigated and found to be diploid and consequently to be subsp. *affinis*. Another specimen *"D. borreri* subsp. *disjuncta*" from Schwarzwald, Germany (No. 1962-347, provided by TR) was also apparently subsp. *affinis*. Both specimens contained flavaspidic acid BB (*5*-BB) and "Stoff Z", obviously flavaspidic acid AB (*5*-AB)(see Wieffering *et al.* 1965) as their major components as well as small amounts of filixic acid (*19*) and ± albaspidin (*10*). This is in line with our investigations on subsp. *affinis* from the same sources (Table 1). However, the observation of small amounts of para-aspidin (*7*) must be wrong as no aspidinol (*2*) was present (see under *D. wallichiana)*.

The rest of the "*Dryopteris borreri*" material from the British Isles, which was investigated by Fikenscher and Hegnauer (1963), showed a similar phloroglucinol spectrum to that discussed above. However, as no chromosome counts were made and, despite a search in appropriate herbaria by Fraser-Jenkins, no voucher material can be located it is impossible to decide whether it belongs to diploid subsp. *affinis* or triploid subsp. *cambrensis* or both. Moreover, as seen in Table 1, both these taxa show very similar phloroglucinol spectra, so that closer examination cannot be made by chromatographic investigations either. For the same reasons as stated above, no para-aspidin (*7*) was present in that material (cf. also Wieffering *et al.* 1965, Penttilä & Sundman 1966).

Triploid taxa

The triploid *Dryopteris affinis* subsp. *cambrensis* (including var. *paleaceo-crispa* and var. *insubrica)* and subsp. *pseudo-disjuncta* from Europe again exhibited similar phloroglucinol spectra. The samples CRFJ 11841-42, 11846, and 11848-11849 from Switzerland (var. *insubrica*) were somewhat different in containing great percentages of flavaspidic acid AB (*5*-AB). Samples CRFJ 12507-09, 12561, 12581, 12592 (var. *paleaceo-crispa*) and CRFJ 12634 (a form of var. *paleaceo-crispa* imitating *D.* x *complexa*) contained relatively large quantities of filixic acid (*19*), the main homologue being *19*-ABA. CRFJ 11702 also contained relatively large quantities of filixic acid (*19*), with homologue *19*-BBB as the prevailing one. This plant is of uncertain identity and has unusual morphology and rather higher spore abortion than in the other apomicts. It may be a hybrid of *D. filix-mas* and *D. affinis*, which could explain the high level of filixic acid (*19*).

Of the European triploids *Dryopteris affinis* subsp. *borreri* was most interesting. We had a large number of samples from several localities in central Europe, Turkey, the British Isles and Spain. All the samples investigated contained para-aspidin (*7*) with the homologue *7*-BB being the main compound in distinct contrast to all other taxa of the *D. affinis* complex so far investigated. Moreover a trace amount of the corresponding three-ring compound trispara-aspidin BBB (*20*-BBB) was also present. The percentages of flavaspidic acid BB, PB (*5*-BB, PB) were always greater than those of the AB homologue (*5*-AB) and only traces or minute amounts of albaspidin (*10*) and filixic acid (*19*) were detected.

Fikenscher and Hegnauer (1963) have previously investigated plants under the name *Dryopteris* x *tavelii* Roth., which, though thought to be the hybrid between *D. affinis* and *D. filix-mas*, is actually synonymous with *D. affinis* subsp. *borreri* (Fraser-Jenkins 1987). They reported large amounts of aspidinol (*2*) and flavaspidic acid BB (*5*-BB) and small amounts of para-aspidin (*7*) and "Stoff Z" = flavaspidic acid AB (*5*-AB). One specimen (1961-867) from Belgium was lacking para-aspidin (*7*), although it contained much aspidinol (*2*), i.e. the para-aspidin was apparently completely decomposed. These results are in good agreement with ours for subsp. *borreri*. However one "*D.* x *tavelii*" from upper Bavaria, Germany, was totally lacking aspidinol (*2*) and para-aspidin (*7*) and showed a phloroglucinol spectrum resembling that of the other *D. "borreri"* material (see above under diploid taxa). Consequently it cannot belong to *D. affinis* subsp. *borreri*.

Tanker and Çoşkun (1978) have investigated "*Dryopteris borreri"* from Turkey. As no chromosome counts were made nor vouchers examined by us it is again difficult to know to which subspecies the material belongs. However the occurrence of aspidinol (*2*) and para-aspidin (*7*) in Nr. 4a, 4c indicate that these samples may have been *D. affinis* subsp. *borreri*. The occurrence of large amounts of desaspidin (*8*) in all these samples must be due to an error as no desaspidin (*8*) is present in *D. affinis s. lat*., as we have found during the course of the present work (Table 1, see also Fig. 6). It was also erroneously reported by Widén *et al.* (1971) to be present in ACJ 11782, but this has been corrected in the present work.

Dryopteris affinis subsp. *persica* from SW Asia gave similar phloroglucinol spectra to European subsp. *cambrensis* and subsp. *pseudo-disjuncta*. Subspecies *pontica* from Turkey was also similar but CRFJ 5948 (TR 4264A), subsp. *pontica* from Iran differed in containing minute amounts of para-aspidin BB and AB (*7*-BB, -AB) and traces of albaspidin homologues (*10*-BB, -AB, -AA). Further sampling from different sources is needed to study the degree of variation in subsp. *pontica*.

Fraser-Jenkins (1980; and quoted in Prelli & Prelli 1990) suggested that *Dryopteris affinis* subsp. *cambrensis* and subsp. *pseudo-disjuncta* may have evolved from a cross of diploid apogamous *D. affinis* subsp. *affinis* and diploid sexual *D. oreades*. This may indeed be the case as all these species contain mainly flavaspidic acid (*5*) and filixic acid (*19*) as well as traces or small amounts of albaspidin (*10*), trisflavaspidic acid (*23*) and sometimes norflavaspidic acid (*4*) as well as tetraalbaspidin (*25*), (Fig. 8). Subsp. *persica* and perhaps subsp. *pontica* may have a similar parentage as determined from their phloroglucinol compositions but further investigations of the chemically slightly variable subsp. *pontica* are needed. On the other hand subsp. *borreri* containing para-aspidin (*7*) cannot have the same parentage, though subsp. *affinis* again seems to be a promising candidate for one of its ancestors. The other one could well be the sexual diploid *D. caucasica*, which could provide para-aspidin (*7*), desaspidin (*8*)

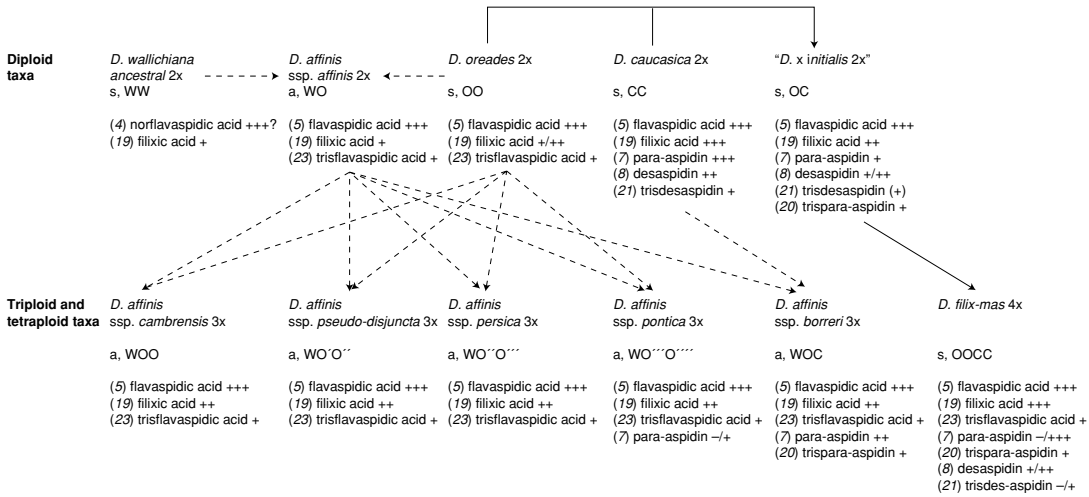


Fig. 8. Genomic relationships and predominant phloroglucinol compositions in the different taxa of *Dryopteris affinis* (Lowe) Fraser-Jenkins and *D. filix-mas* (L.) Schott and their supposed/demonstrated ancestors (cf. Corley 1967, Widén *et al.* 1973, von Euw *et al.* 1980, Prelli & Prelli 1990. "*Dryopteris wallichiana* ancestral 2x" represents a hypothetical taxon that may be extinct today. W, O, C represent genomes in respective diploid taxa and their derived allopolyploid taxa; O'– O'' are somewhat changed *D. oreades* genomes, their existence is only hypothetical. Distribution between homologues (A, P, B) of the different phloroglucinols is not taken into consideration here.

and trisdesaspidin (*21*) (Table 1, Fig. 8). If that is the case we must assume that the biosynthesis of desaspidin (*8*) and trisdesaspidin (*21*) is a recessive character that is suppressed by the biosynthesis of para-aspidin (*7*) and trispara-aspidin (*20*), respectively (cf. Gibby *et al.* 1978). However another even more plausible explanation is that the desaspidin (*8*) and trisdesaspidin (*21*) of *D. caucasica* are biotransformed to para-aspidin (*7*) and trispara-aspidin (*20*), respectively, in subsp. *borreri* (Fig. 6).

The origin of the Dryopteris affinis complex and related taxa

Fraser-Jenkins (1980 etc.), following Manton (1950) and Corley (1967), has suggested that *Dryopteris affinis* subsp. *affinis* may contain two different genomes, W and O. The W genome would have its origin from *"D. wallichiana* ancestral", a diploid sexual taxon which may be extinct today or may possibly still exist. The O genome in turn may be represented by the well known sexual diploid, *D. oreades* in sect. *Dryopteris* (Fig. 8). The diploid apogamous *D. wallichiana* subsp. *wallichiana* of today may contain the same ancestral genomes WW, perhaps in slightly altered form ($W^{I}W^{II}$). A possible alternative that he proposed is that *D. wallichiana* was derived from two distinct genomes (with distinct and complementary or "opposite" morphology), one of which has given rise to the *D. affinis* complex in combination with further genomes. An examination of pairing behaviour in 16-celled sporangia of *D. wallichiana* could be crucial to the understanding of the origin of *D. wallichiana*. Loyal (1960) reported a high degree of bivalent formation in a single cell that he investigated. If this is correct, then the first alternative would be indicated, and a sexual diploid similar to *D. wallichiana* must have existed.

In Fig. 8 the genomic relationships and predominant phloroglucinol compositions in the different taxa of the *Dryopteris affinis* complex and *D. filix-mas* and their supposed/demonstrated ancestors are considered (cf. Manton 1950, Corley 1967, Widén *et al.* 1973, von Euw *et al.* 1980). A good relationship between the phloroglucinol composition and supposed parentage can be observed, though it is also true that the phloroglucinols in sect. *Fibrillosae* are much more similar between species than they are in other sections that have been investigated. Often they are so close that they are of limited use in determining the ancestry of polyploids, or even for the discrimination of species.

As the present day apomictic diploid *Dryopteris wallichiana* subsp. *wallichiana* (or other related

apomicts in sect. *Fibrillosae*) cannot donate the W genome, one of us (CRFJ) has made several efforts to find appropriate sexual diploids from sect. *Fibrillosae* or related sections. No sexual form of *D. wallichiana* has yet come to light from spore studies or cytological investigation. The only promising candidates as parental taxa on the basis of morphology are *D. crassirhizoma* from Japan (sect. *Fibrillosae*) combined with *D. conjugata* from N India and China (sect. *Hirtipedes*) or *D. polylepis* from Japan (sect. *Fibrillosae*).

Dryopteris crassirhizoma contains much tetraalbaspidin ABBA (*25*-ABBA), which is present only as a minor compound in Indian *D. wallichiana* and *D. affinis*. In addition small amounts of flavaspidic acid AB (*5*-AB), the main compound present in *D. wallichiana* and (less distinct) in *D. affinis*, is present only in minute amounts in *D. crassirhizoma* and trisflavaspidic acid (*23*) is totally lacking. Nevertheless *D. crassirhizoma* could be considered as a relative promising candidate for a missing ancestor, but only if another ancestral species was present to provide the missing phloroglucinols. From a morphological point also a second species would have to be involved.

Dryopteris conjugata would be a very promising candidate for the other ancestor, at least on morphological grounds if it were diploid and sexual. However, from the first glance at the chromatograms, the phenolics seem quite unsuitable; it contains considerable amounts of norflavaspidic acids (*4*-AB, -BB), which are only occasionally present in trace amounts in *D. wallichiana* and *D. affinis* and does not contain flavaspidic acids (*5*). Yet *D. conjugata* contains some filixic acid (*19*) and tetraalbaspidin ABBA (*25*-ABBA) which are present in the latter taxa. But the major differences from the expected chromatograms made us suspicious about the possibility of *D. conjugata* as a candidate for the missing ancestor of both *D. affinis* and *D. wallichiana*. However, taking into consideration the close chemical relationship of norflavaspidic acid (*4*) and flavaspidic acid (*5*), it seems quite possible that *5* is formed from *4* by biotransformation (Fig. 6) in both *D. wallichiana* and *D. affinis*. Indeed the trace amounts of norflavaspidic acid (*4*) that have been found in both taxa would support this assumption and suggest that the occurrence of *4* is a primitive character in *Dryopteris*. Unfortunately recent cytological examination of a single accession of *D. conjugata* has revealed that it is diploid, but apogamous, with 82 bivalents at metaphase I of meiosis in 8-celled sporangia, and so this rules out the possibility that it represents a sexual ancestor of *D. wallichiana*. However, it is interesting that unlike most reports for diploid apogamous species of *Dryopteris*, where the chromosomes from 16-celled sporangia are mostly unpaired at meiosis, *D. conjugata* shows a fairly high frequency of bivalents, suggesting that it contains two similar genomes, similar to the report of Loyal (1960) for *D. wallichiana*.

CONCLUSION

Unlike all the other sections in *Dryopteris* that we have investigated, sect. *Fibrillosae* shows a high degree of uniformity of chemical composition. This is the only section where the phloroglucinol chemistry is only marginally useful to separate species and in several cases this is only because they are derived from a member of the section hybridising with a species from another section of *Dryopteris* that has distinct phloroglucinol markers. It is nevertheless possible to recognise various groups, viz. 1) *D. crassirhizoma*, 2) *D. wallichiana*, *D. lepidopoda* and relatives (linked to group 3 via *D. redacto-pinnata*), 3) *D. xanthomelas*, *D. zayuensis* and relatives, and 4) *D. polylepis*. However within these groups some of the species vary so that there are some intermediates (e.g. between *D. lepidopoda* and *D. wallichiana*, or between *D. xanthomelas*, *D. redactopinnata*, *D. neorosthornii* and *D. zayuensis*) and it seems that some of these species (at the diploid level) may not be fully separated as in most other sections. The similarities in chemistry are almost certainly related to the widespread occurrence of apomixis in this section and may reflect a common origin of species at both diploid and polyploid levels from few sexual taxa, possible by multiple origin from the same sexual diploids or from subsequent mutation leading to geographic or ecological variants.

The status of some species where morphological and chemical similarities are so close deserves consideration as to whether they should be recognised at specific level. Two that had been treated as species have now been sunk as subspecies of *Dryopteris wallichiana* (Fraser-Jenkins 1988, 1994 and present paper). But such taxa as *D. zayuensis* and *D. redactopinnata*, for example, could also be

reconsidered in relation to *D. xanthomelas*, though they are maintained here. This view is in contrast to those of Ching (1938 etc.), Ching and Wu (1983) and, to a lesser extent, Lu (1991) who have described every slightly different form as a distinct species, many of which have already been synonymised by Fraser-Jenkins (1986, 1989).

The species we recognise here are normally discontinuous in morphology from adjacent species, even though their chemistry may be close or identical. The similarity in chemistry may reflect a relatively recent separation of the diploid species. Their similarity may be related to their almost universal apomictic mode of reproduction. The diploids may have had similar origins or have been derived from one original diploid apomict through mutation (Schneller & Holderegger 1994).

In the *Dryopteris affinis* complex it is similarly difficult to know whether the almost uniform results for subsp. *cambrensis*, subsp. *pseudodisjuncta* and subsp. *persica* are meaningful but, together with their morphological similarities, they support the hypothesis of an OOW-genome composition of all three subspecies. If the genome composition of all three subspecies does prove to be the same it would be appropriate to treat all three as one subspecies (subsp. *persica* being the earliest name) with the three taxa separated as geographical varieties. As mentioned above the slight variation in chemistry of subsp. *pontica* requires further investigation.

EXPERIMENTAL

Analysis of the cation-free ether extract (36.3 g) of *Dryopteris wallichiana* from N India (CRFJ 8483, 8485-87)

Counter-current distribution

This was performed by manual shaking in 5 separatory funnels as described by von Euw *et al.* (1980, 1985). 36.3 g cation-free ether extract were transferred into the first funnel with methanol/water 95:5 (30 ml) and hexane (400 ml) and shaken (the material is not completely soluble, either in methanol or in hexane alone). After separation the methanol layer containing the more acidic phloroglucinols (the bulk of different flavaspidic acids as well as some albaspidin, filixic acid and dryocrassin was transferred to the second funnel containing hexane (200 ml), then to the third, fourth and fifth (each containing 200 ml hexane). Thereafter new methanol-water (30 ml) was again introduced into the first funnel and, after shaking and separation, transferred to the second and so on. The whole procedure was repeated until 30 methanolic layers had passed the hexane layers containing the less polar phloroglucinols (the bulk of albaspidin, filixic acid and dryocrassin as well as *inter al*. tricosanol and other extractives not investigated here.

After 30 methanolic washings the five remaining hexane layers were dried over Na_2SO_4 and evaporated *in vacuo* giving 11.70 g "hexane phase" (32.2% the cation-free ether extract).

The combined 30 methanolic layers were freed from methanol *in vacuo* and the remaining aqueous solution was extracted three times with the ether. The ether solution was dried over Na_2S0_4 and evaporated *in vacuo*, giving 22.95 g "methanol phase" (63.2% of the cation-free ether extract).

Separation of the "hexane phase"

This was performed by column chromatography of 11.7 g evaporated "hexane phase" on 175 g polyamide. The fractions 1–6 (each 10 ml eluted with hexane) gave 73 mg Pa-I = tricosanol on crystallisation from acetone.

The fractions 7–33 (hexane) gave only mixed crystallisates of Pa-I, albaspidin and filixic acid, that were not separated further. From the fractions 34–102 (hexane) 169 mg pure filixic acid ABA were obtained on crystallisation from acetone. The fractions 103–277 (eluted with hexane, hexane-cyclohexane 3.1 to 1:1, cyclohexane, and cyclohexane-chloroform 1:1) gave only mixed crystallisates of filixic acid and dryocrassin, n.s. In the fractions 278–311 (hexane-cyclohexane 1:1, chloroform) mixtures of filixic acid, dryocrassin, flavaspidic acid and tris- and tetraflavaspidic acid were observed, no crystals were obtained. From the fractions 312–347 (chloroform) 14.7 mg of flavaspidic acid AB were obtained when crystallised from methanol. The fractions 348–450 (chloroform) gave 20.1 mg pure dryocrassin from acetone.

Separation of the "methanol phase"

10.2 g evaporated "methanol phase" were chromatographed on 195 g silica gel buffered at pH 4.0 (see Euw *et al.* 1985). The fractions 31–75 (cyclohexane-chloroform 9:1) gave 12.4 mg albaspidin AA on crystallisation from acetone. Fractions 76–120 (cyclohexane-chloroform 9:1) gave 46.1 mg filixic acid ABA from acetone. The fractions 145–186 (cyclohexane-chloroform 9:1, 3:2) contained filixic acid ABA and dryocrassin; no uniform crystallisates were obtained. Fractions 187–305 (cyclohexane-chloroform 3:2) gave in all 263.7 mg, flavaspidic acid AB, when crystallised from ether or methanol. From the fractions 306–528 (cyclohexane-chloroform 3:2, 1:1, chloroform) 57.9 mg tetraflavaspidic acid ABBB were obtained from ether. The mother liquor contained also some trisflavaspidic ABB that did not crystallise. In the last fractions 529–600 (chloroform, chloroform-methanol 9:1) considerable amounts of tris- and tetraflavaspidic acid were detected; no crystallisates were obtained.

Pa-1, colourless crystals, m.p. 65–70°C (acetone), previously reported n.p. 73.4–73.7°C (Thames & Taurins 1962). According to El/MS (M^+ at m/z 340), ^1H NMR and ^{13}C NMR it consisted of the aliphatic saturated alcohol tricosanol $C_{23}H_{47}OH$. It gave no coloration in TLC after spraying with fast blue salt B.

Albaspidin-AA (*10*-AA), colourless needles, m. p. 168–170°C (acetone). In El/MS one molecular peak (M^+) at m/z 404 (*10*-AA) was recorded. Previously reported m.p. 164–166°C (Patama & Widén 1991).

Filixic acid-ABA (*19*-ABA), yellow plates, m. p. 167–169°C (acetone). In El/MS M^+ at m/z 612 (*19*-ABA) detected. Previously reported m. p. 161–162°C (Tryon *et al.* 1973).

Dryocrassin = tetra-albaspidin-ABBA (*25*-ABBA), yellowish powder, m. p. 182–183°C (acetone, not recrystallised). In El/MS M^+ at 820 (*25*-ABBA) recorded. Previously reported m. p. 210–215°C (recrystallised) (Widén *et al.* 1975).

Tetraflavaspidic acid-ABBB (*26*-ABBB), brownish powder, m. p. 215–225°C from ether. In negative FAB/MS (M-H)-a peak at m/z 833 corresponding to *26*-ABBB (MW 834), and a minor peak at m/z 1041 corresponding to pentaflavaspidic acid-ABBBB (MW 1042) were detected. For chromatographic properties in TLC, see Fig. 7.

Flavaspidic acid-AB (*5*-AB), yellow needles from acetone, m. p. 208–211°C. In El/MS M^+ at m/z 418 was recorded. Previously reported m. p. 209–213°C (Widén *et al.* 1971). For chromatographic properties in TLC, see Fig. 7.

Analysis of the cation-free ether extract (24.43 g) of *Dryopteris wallichiana* from Hawai'i (CRFJ all numbers)

24.43 g cation-free ether extract were divided in a "hexane phase" (residue 14.84 g = 60.7%) and a "methanol phase" (8.56 g = 35.0%) as detailed above.

The "hexane phase" containing the bulk of Wa-1 and Wa-2, dryocrassin, filixic acid and some flavaspidic acids was re-divided into three parts of different acidity: (Patama & Widén 1991): 1) a $NaHC0_3$ fraction (residue 2.43 g = 16%), 2) a Na_2CO_3 fraction (6.49 g = 43.7%), 3) a NaOH fraction (0.70 g = 4.7%) and 4) a residual "neutral phase" (3.43 g = 23.1%). The $NaHCO_3$ and Na_2CO_3 fractions of similar phloroglucinol compositions (containing Wa-1, Wa-2, filixic acid and flavaspidic acid and albaspidin (artefact)) were united and submitted to column chromatography on silica gel (see below). The NaOH fractions contained some flavaspidic acids, as well as albaspidin and acylfilicinic acids as artefacts and was not treated further. The "neutral phase" was devoid of phloroglucinols (no coloration with fast blue salt B) and was discarded.

Separation of the united $NaHCO_3$ and Na_2CO_3 phases

8.43 g of these phases were separated by column chromatography on 250 g silica gel buffered at pH 6.0 (see von Euw *et al.* 1980). The fractions 15–44 (eluted with *iso*propylether-cyclohexane 30:70, followed by methanol-*iso*propylether-cyclohexane 5:35:60) contained albaspidin AA, Wa-1, Wa-2 and some filixic acids; no uniform crystallisates were obtained. The fractions 45–88 (methanol-*iso*propylether-cyclohexane 10:35:55) gave 164.1 mg Wa-1, Wa-2 on crystallisation from hexane (see below). Fractions 89–151 (eluted in turn with methanol-*iso*propylether-cyclohexane 10:35:55 + 1–5% gla-

cial acetic acid, methanol) contained some Wa-2, flavaspidic acid, trisflavaspidic acid; no uniform crystallisates were obtained.

The residue of the "methanol phase" was submitted to direct crystallisation from methanol giving 444.4 mg flavaspidic acid AB, m.p. 202–205°C (MS, TLC).

Wa-1, Wa-2. Yellow powder from hexane, m.p. 151–157°C. For structural features see Results (p. 13). After spraying with fast blue salt B both substances gave in TLC a yellow spot that gradually changed via orange to red. The R_f-values in the four solvent systems tested are listed below:

	I	II	III	IV
Wa-1	0.10	0.53	0.48	0.80
Wa-2	0.07	0.50	0.33	0.77
Fu-1	0.43	0.68	0.20	0.97
Fu-2	0.33	0.60	0.10	0.90
Fu-3	0.57	0.76	0.73	1.00

For comparison the R_f-values of Fu-1, Fu-2 (violet) and Fu-3 (orange) are given also (see Patama & Widén 1991). These "unknown" substances are readily separable from each other in each solvent system tested.

Analysis of the cation-free ether extract (5.10 g)of *Dryopteris lepidopoda* (CRFJ 8826, 8829-30, 8837-39, 8841-42)

5.10 g cation-free ether extract were processed in the same way as in the former case giving 1.30 g (25.5%) "hexane phase" and 2.7 g (52.9%). The less polar "hexane phase" gave on re-division: 1) 0.12 g (9.23%) $NaHCO_3$ fraction, 2) 0.17 g (13.8%) Na_2CO_3 fraction, 3) 0.81 g (62.31%) NaOH fraction and 4) a "neutral fraction", n. s.

The $NaHCO_3$ fraction gave 3.7 mg albaspidin (artefact, Table 4) when submitted to column chromatography on silica gel buffered at pH 6.0. No uniform crystallisates were obtained from the Na_2CO_3 fraction. It contained some Le-1 and albaspidin. In the Na0H fraction additional albaspidin (artefact) and Le-1, Le-2 and Le-3 were detected. It was again separated by column chromatography on silica gel (pH 6.0). The last fractions 220–240 (residue 145.6 mg) eluted with cyclohexane-chloroform 4:1, gave fairly pure Le-2 + 3.

The "methanol phase" was submitted to column chromatography on silica gel buffered at pH 4.0. The fractions 217–243 (cyclohexane-chloroform 4:1) gave 49.4 mg flavaspidic acid BB, AB. The fractions 286–315 (same solvent) gave 4.0 mg slightly impure tetraflavaspidic acid-BBBB, ABBB.

Le-2, Le-3. Brown amorphous resin, in El/MS putative molecular peaks M^+ at m/z 652 (weak, indistinct), 648 (weak), and 620. Other characteristic peaks occurred at m/z 445, 417, 302, 209 and 208. According to MS and TLC Le-2, Le-3 are identical with Ju-2, Ju-3 from *Dryopteris juxtaposita*.

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