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## The phloroglucinols of the *Dryopteris villarii* complex and some related ferns (Pteridophyta, Dryopteridaceae)

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### Abstract

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The 3 species of the European *Dryopteris villarii* complex (*D. villarii*, *D. submontana* and *D. pallida*) including subsp. *balearica* have already been briefly analysed by Widén et al. (1971). We report here on a more detailed study and add results for *D. pallida* subsp. *libanotica*, from Cyprus and Asia Minor subsp. *raddeana* and for the following 12 ± related Asiatic and American taxa: *D. nigropaleacea*, *D. ramosa*, *D. stewartii*, *D. marginata*, *D. barbigera*, *D. blanfordii*, *D. parachrysocoma*, *D. woodsii*, *D. juxta-posita*, *D. odontoloma*, *D. arguta* and *D. fragrans*.

A new classification of the genus *Dryopteris* on world-wide basis was recently published by C. R. Fraser-Jenkins (1986). He divided the genus into 4 subgenera and further into 17 sections. The 18 taxa which we examined are all members of one subgenus (*Dryopteris*) and of only four of its sections (4. *Dryopteris*, 5. *Remotae*, 6. *Pallidae* and 9. *Marginatae*). This may be the reason why the chemical composition of all our 18 taxa is relatively uniform (see Table 5). When comparing chemical composition of “phenolics” (= “Phloroglucinols”) with taxonomic position we sometimes found a good correlation, sometimes not. A good correlation exists between *D. villarii* and *D. filix-mas* both of section 4. *Dryopteris* and both producing very similar phenolics. On the other hand, *D. nigropaleacea* and *D. pallida* both in the same section 6. *Pallidae* are chemically distinct while *D. ramosa* (section 9. *Marginatae*) produces quite similar “phenolics” as *D. pallida*.

We could confirm former results showing that the chemical composition in phenolics is a very conservative character. We found that three subspecies of *D. pallida*, viz. subsp. *pallida*, subsp. *libanotica*, and subsp. *raddeana* produce virtually the same phenolics. They obviously have evolved from the same ancestral stock. Morphology has diversified during very long geographical isolation but chemistry has not and the three taxa also still have homologous genomes.

Chemical results are often also very useful for guessing the origin of allopolyploids which frequently produce exactly the sum of the compounds found in their ancestors.

This was shown to be the case of *D. submontana*, most probably an allotetraploid derived from *D. villarii* and *D. pallida*. It is also in agreement with the assumption that the triploid apomictic *D. stewartii* may have evolved from the two diploid sexual species *D. nigropaleacea* and *D. ramosa*.

*Key words:* *Dryopteris*, phloroglucinols, chemotaxonomy.

## 1. Introduction

Nearly all species of *Dryopteris* (and a few of some closely related genera) contain in their rhizomes and stipe bases some phloroglucinol derivatives ("phloroglucinols") which are  $\pm$  typical for each species (see reviews by Berti & Bottery 1968, Penttilä 1967, Penttilä & Sundman 1970, v. Euw et al. 1980, Widén et al. 1983, and further literature therein). The composition is usually characteristic, remarkably uniform for each species, and little dependent on origin, season of collecting, and age of the specimens. – Only in a few species (e.g. *D. expansa* (Presl) Fraser-Jenkins & Jermy 1977: 938 = *D. assimilis* Walker) a considerable range of variation in the phloroglucinol spectrum was observed for different individual plants from different localities or sometimes even within the same populations (Widén & Sorsa 1969; Widén & Britton 1969, 1971: 247, Widén et al. 1970a; Britton & Widén 1974). In spite of such occasional irregularities the careful chemical analysis can be helpful in solving taxonomical problems in the genus *Dryopteris* even with old herbarium material, provided at least 1 g of dry rhizome is available (e.g. Widén et al. 1976 for *D. remota*). It may also be helpful in estimating relationships between different taxa, particularly in allopolyploid ferns to which belong about 50% of the fern flora of Europe and probably of the world (Dostál & Reichstein in Kramer (ed.) 1984: 13). Some members of the *D. villarii* complex were analysed before (Widén et al. 1971), we give here results of a more detailed study including some related taxa.

## 2. Taxonomy

For a long time the genus *Dryopteris* Adanson (1763) was an assembly of many different groups of ferns. Since Christensen's work (1913, 1920, 1938) and that of Copeland (1947); Holttum (1947, 1949, 1960), Hirabayashi (1974), Pichi Sermolli (1977), Tryon & Tryon (1982), Fraser-Jenkins (1980, 1984) and many other authors it was redefined according to modern standards as reviewed and continued by Fraser-Jenkins (1986), see also Kung (1989a) and Kramer with contributors (1990: 110–112). What remains is still a large but well circumscribed genus with world-wide distribution, and all those of its members which were cytologically examined also have their chromosome numbers uniformly based on  $x=41$  (Löve et al. 1977: 301–321, Gibby 1985, Manickam and Irudayaraj 1988, Wang Ruo-fen 1988). As mentioned above nearly all also produce and store typical acyl-phloroglucinols in particular internal/external glands in their rhizomes. Such phloroglucinols and glands were, however, not only also found in some other closely related genera of the *Dryopteridaceae* (see Widén et al. 1976, 1978, 1981, 1983) but also in a few phanerogams (see v. Euw et al. 1980).

### 2.1 Subgeneric divisions

First successful attempts to divide the genus into sections by Itô (1935, 1936, 1939) emended by Serizawa (1976), or into groups by Ching (1936, 1965, 1978), were based on material from Japan and from China and Sikkim Himalaya, respectively. A more elaborate classification on a world-wide basis was recently worked out by Fraser-Jenkins (1986) who listed (p. 183) c. 225 species and 89 hybrids, and quite many subspecies must be added. He divided the genus into four subgenera (partly corresponding to Itô's sections and Ching's groups), three of them are further divided into 16 sections.

The author pointed out that his sections were less clear-cut groups of species than the subgenera and often have multidirectional relationships. A frequent complication is the existence of allopolyploid species derived from ancestors of two different sections. The allopolyploid had to be assigned to one section or to the other. For a few special cases in which the putative ancestors belong to sections which are so remote from one another that the resultant species cannot be placed in one or the other, e.g. *D. remota* (A. Braun ex Doell) Druce (1908), *D. blanfordii* (see 3.2.6) and *D. corleyi* Fraser-Jenkins (1982) he erected the section 5. *Remotae* while *D. tyrrhena* Fraser-Jenkins et al. (1974) and *D. ardechensis* Fraser-Jenkins (1981) and many other were left in section 4. *Dryopteris*. Some difficulties remain and others turn up because evolution in *Dryopteris* has partly followed a reticulate pattern (as mentioned under allopolyploidy) which in principle cannot be adequately squeezed into a branching pattern. As an illustration we only mention the *D. villarii* complex (Fraser-Jenkins 1977). In the new classification (Fraser-Jenkins 1986) it appears in subgenus *Dryopteris*, but of the three main European species of this complex *D. villarii* is listed in section 4. *Dryopteris* while *D. submontana* and *D. pallida* are placed in section 6. *Pallidae*. In reality *D. villarii* and *D. submontana* are so close in gross morphology (see Rasbach et al. 1982, Fig. 2) that even specialists may in some cases have difficulties to discern them without examination of microcharacters or without counting chromosomes. Chemical data are also better correlated when the three species are treated as members of one complex as originally done by Fraser-Jenkins (1977). Similar difficulties turn up by comparing *D. nigropaleacea* (section 6. *Pallidae*), *D. stewartii* (section 6. *Pallidae*) and *D. ramosa* (section 9. *Marginatae*). These three species also seem to behave like a complex but not as one based on normal allopolyploidy, see 3.2.3. (*D. stewartii*) and discussion in 6.2.3.

In spite of such difficulties we shall use the classification of Fraser-Jenkins (1986) in this study. All 16 taxa which we examined in this study belong to subgenus *Dryopteris* as circumscribed by him. In the list which we give for this material (p. 79–84) the number of the section to which a taxon belongs is given in brackets after its name. Our material includes representatives of sections 3. *Pandae* (*D. chrysocoma*), 4. *Dryopteris* (*D. barbigera*, *D. fragrans* and *D. villarii*), 5. *Remotae* (*D. blanfordii*), 9. *Marginatae* (*D. ramosa*) and 6. *Pallidae* (the other ten species). Additional details on taxonomy and relations are also given in this list (p. 79–84).

### 3. List of plant material which we analysed

Exact collection data are given in the appendix.

#### 3.1 The *Dryopteris villarii* complex

This complex comprises a critical group of ferns which are often difficult to identify. Its European representatives can be placed in three principal taxa, now firmly established



through cytological work by Manton (1950), Panigrahi (1965), Roy (1967), Vida (1969), Fraser-Jenkins and Gibby (1980). However their nomenclatural treatment still varies. Some authors treat them as subspecies of a single collective species (= aggregate species), e.g., Heywood (1964), Fraser-Jenkins & Jermy (1977) and others, while Nardi (1976, 1977, 1979: 445) and Fraser-Jenkins (1977) accept them as three distinct species, which genetically they clearly are. We therefore follow this concept which also results in shorter names but sometimes leads to practical difficulties, as even for the expert, it is not always possible to identify each individual plant correctly, particularly herbarium specimens. For the chemical work we therefore used only material the cytology of which was checked directly or was known beyond doubt for other reasons. Following the nomenclature of Fraser-Jenkins (1977), the three main European taxa are:

3.1.1 *Dryopteris villarii* (Bell.) Woyнар ex Schinz et Thellung (1915), (section 4. *Dryopteris*). This alpine, diploid, sexual taxon ( $n=41^{\text{II}}$ ,  $2n=82$ ) is found on calcareous and dolomitic rocks and screes at (900) 1100–2200 (2500) m alt. from France to Greece, and perhaps still further east. In spite of pronounced variability in gross morphology it is relatively uniform in most characters. Large specimens may, however, be rather difficult to differentiate from *D. submontana* (see Rasbach et al. 1982: 37, Fig. 2). In West-Central Asia it is replaced by *D. mindshelkensis* Pavlov (see Fraser-Jenkins 1986: 192 and footnote).

3.1.2 *Dryopteris submontana* (Fraser-Jenkins & Jermy) Fraser-Jenkins (1977: 311) (section 6. *Pallidae*) = *D. villarii* subsp. *submontana* Fraser-Jenkins & Jermy (1977) a tetraploid, sexual taxon ( $n=82^{\text{II}}$ ,  $2n=164$ ). It is known from calcareous and dolomitic rocks and screes in submontane warmer regions at c. 500–2000 m alt. In Great Britain it is the only representative of the complex, but its main range is from Spain and Algeria to Greece and Turkey. This is a relatively uniform taxon, in morphology  $\pm$  intermediate between *D. villarii* and *D. pallida* and sometimes difficult, occasionally even impossible to differentiate with confidence from them in herbarium specimens. Spore measurements may in favorable cases be helpful (see Table 1). This intermediate morphology and the cytological behaviour of its hybrids with 3.1.1 and 3.1.3 (Manton 1950, Panigrahi 1965, Roy 1967, Vida 1969, Fraser-Jenkins & Gibby 1980) renders it rather likely that *D. submontana* is an allotetraploid, once formed by chromosome doubling from the diploid hybrid *D. pallida*  $\times$  *D. villarii* = *D. vidae* Fraser-Jenkins & Gibby (1980: 306); but rigid proof for its allotetraploid nature is still missing (in spite of the claim made by Fraser-Jenkins & Gibby 1980: 307).

3.1.3 *Dryopteris pallida* (Bory) Fomin (1911 [1913]: 49). (Section 6. *Pallidae*) = *D. villarii* subsp. *pallida* (Bory) Heywood (1964: 44), a rather variable, diploid, sexual taxon ( $n=41^{\text{II}}$ ,  $2n=82$ ). It can be subdivided into the following,  $\pm$  closely related taxa.

3.1.3.a *D. pallida* subsp. *pallida* the most widespread in Europe on calcareous rocks and screes, partly growing under bushes and trees from sea level to 1200 (–1700) m alt. in the central and eastern Mediterranean area from Sardinia and the Italian mainland to Turkey. Contrary to expectation it is hardy in cultivation out of doors at Basel, whereas 3.1.3.b and 3.1.3.c are not.

3.1.3.b *D. pallida* subsp. *balearica* (Litard.) Fraser-Jenkins (1977: 314) = *D. balearica* (Litard.) Nardi (1976: 28–29), endemic to Mallorca and confined to calcareous rocks on the northern part of the island. Closely related to but morphologically slightly distinct from subsp. *pallida*.

3.1.3.c *D. pallida* subsp. *libanotica* (Rosenst.) Nardi (1977: 97); see Fraser-Jenkins (1977: 315), growing in SW-Asia in similar habitats as subsp. *pallida*. In Turkey (perhaps elsewhere) there is introgression with subsp. *pallida* wherever the two closely related taxa grow together. Really pure material is best collected in Cyprus or Lebanon where subsp. *pallida* is not known to occur.

3.1.3.d *D. pallida* subsp. *raddeana* (Fomin) Nardi (1977: 97), see Fraser-Jenkins (1977: 315) = *D. raddeana* (Fomin) Fomin (1911: 50, 1913: 57–58), another diploid, sexual taxon ( $n=41^{\text{II}}$ ,  $2n=82^{\text{I}}$ ) closely related to subsp. *pallida* but geographically separated and morphologically distinct. This is a very vigorous plant, confined to low levels (up to c. 600 m alt.) on the SW coast of the Caspian Sea in the U.S.S.R. and Iran. In cultivation this is the most vigorous of the whole complex and completely hardy out of doors at Basel.

*Note.* We have good reasons to assume that subsp. *raddeana* is able to produce fertile hybrids with subsp. *pallida*, that the two taxa have homologous chromosomes and would intrograde if growing together, i.e. that they are able to exist as distinct taxa only due to geographical and perhaps ecological separation. See the remark on close relation etc. and correction of a former result in the appendix, 8.5. Further proof for this assumption is, however, still desirable.

### 3.2 Related species

From the Asiatic continent and from North America we were able to obtain material for analysis of the following 11 taxa:

3.2.1 *Dryopteris nigropaleacea* (Fraser-Jenkins) Fraser-Jenkins (1986). (Section 6. *Pallidae*) = *D. pallida* subsp. *nigropaleacea* Fraser-Jenkins (1977: 316). This taxon is diploid and sexual ( $n=41^{\text{II}}$ ,  $2n=82^{\text{I}}$ , Gibby 1985 b) and in gross morphology is closely related to *D. pallida*, differing mainly in its dark scales. How closely it is related genetically has so far not been checked. It replaces *D. pallida* further east and grows in Afghanistan, Pakistan, India, Nepal, and perhaps parts of China. It is found mainly on calcareous rocks from 1000–3000 m alt. and is hardy outdoors at Basel. It has often been confused with the triploid *D. odontoloma* (Moore ex Beddome) C. Christensen (1924: 59, see 3.2.9) which grows in Southern India.

3.2.2 *Dryopteris ramosa* (Hope) C. Christensen (1905: 287, original spelling *D. ramosum*) (section 9. *Marginatae*). This is another diploid species ( $n=41^{\text{II}}$ , Loyal in Mehra 1961: 152) growing partly together with *D. nigropaleacea* but clearly distinct in morphology. It is also hardy and can be grown outdoors at Basel. With regard to chemical composition it is rather similar to *D. nigropaleacea* and differs mainly in the presence of para-aspidin (see chapter 6. discussion).

3.2.3 *Dryopteris stewartii* Fraser-Jenkins 1978 (section 6. *Pallidae*). This is a triploid apomictic species ( $n=123^{\text{II}}$ ,  $2n=123$ , Gibby 1985 b: 7) often growing together with *D. nigropaleacea* and *D. ramosa* and morphologically intermediate between these two diploid species. This made correct identification difficult until Fraser-Jenkins discovered that another species was involved. The intermediate morphology and the sympatric distribution made him assume that *D. stewartii* once arose from hybridisation between *D. nigropaleacea* and *D. ramosa*. Results of chemical analysis fit such an assumption well but the cytology shows that is is *not* just an allotetraploid (see discussion). *D. stewartii* is also hardy when grown outdoors at Basel.

3.2.4 *Dryopteris marginata* (Wallich ex C. B. Clarke, see Christ 1905: 39) Christ (1907a: 212) (section 9. *Marginatae*). This is a sexual diploid species (Loyal in Mehra 1961, Mehra & Loyal 1965, Gibby 1985b: 7). Kuo (1985: 31) gave China, Himalaya, India, Ceylon, Taiwan and Malay Peninsula as its distribution. The species has occasionally been confused with *D. nigropaleacea* and *D. odontoloma*. We had material from North India for analysis.

3.2.5 *Dryopteris barbigera* (Moore) O. Kuntze (1891: 812) (Section 4. *Dryopteris*). This is a sexual diploid species (Loyal in Mehra 1961, Mehra & Loyal 1965, Loyal in Mehra & Khullar 1980, Gibby 1985b: 5) from high altitudes (10,000–15,000 feet, see Ching 1938: 432). Mehra & Mittal (1962) related it to the *D. filix-mas* group. A closely related taxon was described as *D. komarowii* Kossinski (1921) from Turkestan and Afghanistan and was accepted as *D. barbigera* subsp. *komarowii* (Koss.) Fraser-Jenkins (1989: 384). We had material of the latter only for cytological work from Pakistan, Hazara 2900 m alt., TR-4547 raised from spores CRFJ 6357 but good material for analysis of subsp. *barbigera* from N. India: CRFJ 7433 (one rhizome = 80 g) from Jammu Kashmir, 2850 m alt., CRFJ 7450 (one rhizome = 60 g) from Baltistan, 3350 m alt., and CRFJ 7715–7720 (6 rhizomes = 350 g) from H. P. 3400 m alt. – We also had material of subsp. *barbigera* for cytological work: TR-4557 raised from spores of CRFJ 6517 from Jammu Kashmir, 3250 m alt., 1 km W. of Menanarg, E. side of Zojila Pass. Cultivation was difficult. A single plant survived the winter 1979–80 and was used for the mentioned fixing in root tips.

3.2.6 *Dryopteris blanfordii* (Hope) C. Christensen (1905: 254) (section 5. *Remotae*). This is a triploid apomictic species (Mehra & Khullar 1980, Gibby 1985b: 5). We had material for analyses from North India and were able to raise progeny (TR-4552) from spores from Kashmir. This was partly hardy outdoors at Basel but succumbed during the cold winter of 1984–85.

3.2.7 *Dryopteris chrysocoma* (Christ) C. Christensen (1905: 257) (section 3. *Pandae*). According to Wang (1985) this is a complex in which he included *D. woodsiiisora* Hayata (= *D. chrysocoma* subsp. *parva* Fraser-Jenkins), *D. tenuissima* Tagawa, *D. alpicola* Ching & Z. R. Wang, *D. fangii* Ching, Fraser-Jenkins & Z. R. Wang and two new species: *D. para-chrysocoma* Ching & Z. R. Wang (a diploid sexual taxon), *D. zinongii* Z. R. Wang & Fraser-Jenkins (autotetraploid, sexual) and *D. x daliensis* Z. R. Wang (triploid, apomictic), the hybrid between the two. According to Kuo (1985: 30) *D. chrysocoma* in the old sense (not including the Japanese plant) is distributed in continental West China, Taiwan, North India, Pakistan, Burma and Luzon (Philippines). *D. chrysocoma* s.l. is very common in the vicinity of Darjeeling (West Bengal) and was proposed by Mehra and Mittal (1961) as a substitute for the European male fern (*D. filix-mas*) for the commercial production of “filicin” (now obsolete).

3.2.7.a *D. para-chrysocoma*. We had material of this taxon CRFJ 7681–88 from North India. The diploid *D. para-chrysocoma* produces much larger spores than do most of the other diploid *Dryopteris* species (see Table 1).

3.2.7.b *D. woodsiiisora*. We had the following material of this species: CRFJ 8754–69, 8771–4, 8777–9 from North India.

3.2.8 *Dryopteris juxtaposita* (Christ 1907b: 138) (section 6. *Pallidae*). This was described from Yunnan (China) but also grows in North India and South India (Nilghiri Hills). The name was long forgotten and the species was sometimes confused with *D. nigropaleacea*

Tab. 1. Exospore length of untreated mature spores embedded in balsam. The figures which are not given in brackets (measured by T.R.) mean that at least 90% of the spores from all available origins of the taxon show this size. The figures in brackets are extreme values. R.V. are the values (range of the means) obtained by R. Viane (1990) with a more precise method

Species	Ploidy level	Origin	Length exospore in $\mu\text{m}$
<i>D. villarii</i>	2x	Italy, France, Switzerland, Germany	(27) 30–36 (39) R.V. 35–39
<i>D. pallida</i> subsp. <i>pallida</i>	2x	Italy, Yugoslavia, Greece, Turkey	(24) 30–36 (39) R.V. 32–36
<i>D. pallida</i> subsp. <i>balearica</i>	2x	Mallorca	(27) 30–36 (39) R.V. 31–37
<i>D. pallida</i> subsp. <i>raddeana</i>	2x	Iran	(27) 30–36 (39) R.V. 33–37
<i>D. submontana</i>	4x	England, Spain, France, Italy, Yugoslavia, Turkey	(27) 34–42 (46) R.V. 37–43
<i>D. nigropaleacea</i>	2x	Iran, N. Pakistan, N. India	(24) 27–33 (34)
<i>D. ramosa</i>	2x	N. Pakistan, N. India	(27) 30–33 (38)
<i>D. stewartii</i>	3x apom.	N. Pakistan, N. India	(34) 42–45 (48)
<i>D. barbiger</i> subsp. <i>barbiger</i>	2x	N. Pakistan, N. India Pakistan	(27) 30–39 (42) (24) 33–39 (42)
<i>D. blanfordii</i>	3x apom.	N. Pakistan, N. India	(36) 39–45 (51)
<i>D. chrysocoma</i> subsp. <i>crysocoma</i>	2x	N. India	(35) 45–48 (54)
<i>D. juxtaoposita</i>	3x apom.	N. India, S. India	(24) 36–42 (52)
<i>D. odontoloma</i>	3x apom.	S. India	(36) 42–48 (51) R.V. (40) 44–50 (51)
<i>D. arguta</i>	2x	Canada, USA	(24) 30–36 (39)
<i>D. fragrans</i>	2x	Canada, USA, Japan, Newfoundland	c. (24) 30–36 (39) (30) 42–45 (48)

and *D. odontoloma*. Although often found at the relatively low altitudes of c. 1300–2000 (3000 m) and also in subtropical regions (South India) it is completely hardy outdoors at Basel. It is a triploid apomict Gibby (1985b: 6 and Fig. 29, 30); found  $n=123^{\text{II}}$  in 8-celled sporangia and  $n=41^{\text{II}}+41^{\text{I}}$  in 16-celled sporangia. She quoted previous reports (under *D. odontoloma*) by Loyal (in Mehra 1961), Mehra & Loyal (1965) and (under *D. paleacea*) by Roy, Sinha & Sakya (1971) which are in reasonable agreement. For analysis we had ample material both from North and South India (see Table 5).

3.2.9 *Dryopteris odontoloma* (Beddome) C. Christensen (1924: 59) (section 6. *Pallidae*). This is a triploid, apomictic species (Gibby 1985b: 6) and is probably confined to South India. Icon. see pl. 114 in Beddome (1863). It has often been confused with *D. nigropaleacea* and with *D. juxtaoposita*. We had material from South India for analysis.

3.2.10 *Dryopteris arguta* (Kaulf.) Watt (1867: 159) (section 6. *Pallidae*) from western North America is another diploid, sexual species ( $n=41^{\text{II}}$ ,  $2n=82$ ) obviously related to the *D. villarii* complex but ecologically distinct, growing mainly in woods and on acidic soil. It is not hardy outdoors at Basel but can well be grown in southern Switzerland (Kt.



Tessin, on acid soil). We had good material from California and Canada for analysis (W. Bennert s.n. = TR-4043).

3.2.11 *Dryopteris fragrans* (L.) Schott (1834: tav. 9) (section 4. *Dryopteris*), a diploid, sexual species (Löve et al. 1977: 310) obviously related to the *D. villarii* complex but with circumboreal distribution. It is somewhat polymorphic, Komarov (1911: 394, Komarov ed. 1934: 38 and Engl. transl. 1968: 30) described *var. lepidopoda* (from northern regions) and *var. remotiuscula* (from the Ussuri forests). We did not succeed in raising adult progeny from spores nor even keep plants obtained from Japan and from Canada alive for more than a few months in cultivation. We had dried material (rhizomes) from Canada (Ontario and Newfoundland), USSR (Lake Baikal region), Mongolia and from Finland.

## 4. Methods

### 4.1 Collection of rhizomes

Rhizomes were collected at the localities given in the appendix. Fibrous roots and leaves were cut off, the rhizomes were washed free of soil and dried (preferably on sieves) with air of 40–45 °C passing from below. One or more pressed fronds of each collection were deposited as vouchers in the Herbaria mentioned in the appendix and, if possible, another in the Botanical Museum of the University of Helsinki (H). The amounts of dry rhizomes used for analysis are given in Table 7 (Appendix).

### 4.2 Collection of spores and measurement of exospore length

For this purpose mature fronds (with sori still black) were pressed in pure paper (flimsies or newspaper never before used for plant pressing). After a few days the dark spore powder was packed in small paper bags, these were put in a labeled plastic envelope and attached to the voucher. A small sample of the pure spores was mounted in balsam and kept as a permanent preparation. This was examined under the microscope with precisely calibrated scale in transmitted light. Only the length of the exospore (visible as a regular  $\pm$  elliptic line, see Fig. 1) was measured. The irregular but often characteristic shape of the surrounding perispore is a very useful character for the identification of individual taxa, but less suitable for estimating the spore size.

Table 1 gives our results for the species we used for chemical investigation. For the *D. villarii* group not only the figures found for the material which we have analysed were included but the given range includes results of quite many specimens from different countries.

### 4.3 Fixings for cytological work

The fixation of immature sporangia or root-tips after pretreatment with 0.1% aqueous colchicin for c. 8 hours at 4–6 °C with glacial acetic acid-ethanol (1:4) was done either in the field or with cultivated plants in Basel. Squashing, staining and analysis was performed by G.V. in Budapest. No documentation on the photographs of cytological results is given here, as the results for most of the species treated here have been or will be published elsewhere. Recent counts of *D. ramosa* and *D. stewartii* were performed by Mary Gibby in London and are published (1985). Cytological examination of *D. fragrans* from the USSR and from Mongolia was done by J. Pintér in Budapest.



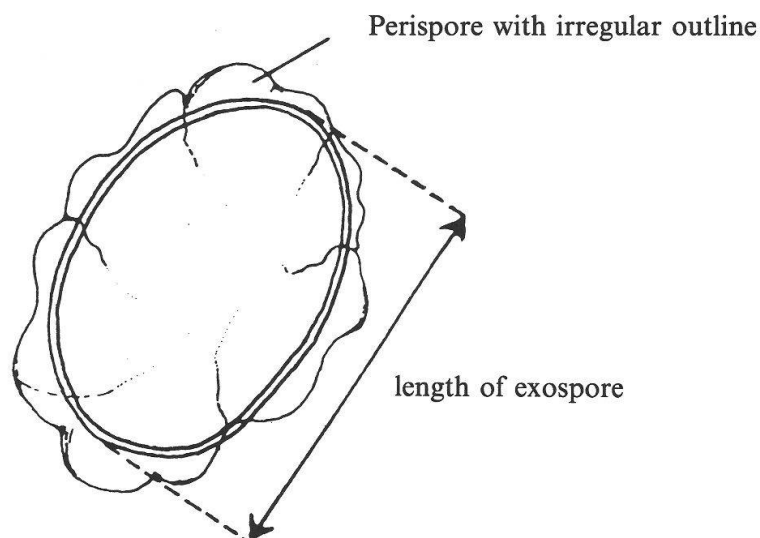


Fig. 1. Spore embedded in balsam as seen under the light microscope

#### 4.4 Chemical analysis

This was mainly carried out using the "standard method" (J. v. Euw et al. 1980: 304). The improved method (avoiding deteriorating steps) as described by v. Euw et al. 1980: 302 and 1985 was unfortunately available only when we worked on part of the Asiatic material, but the main results would not be much different. The main steps in the "standard method" are as follows: 1. Exhaustive extraction of the dried powdered rhizomes (or stipe bases) with peroxidfree ether. The amount of residue after evaporation is given as "Ether extract crude" in Table 6. This mixture can be used directly for thin layer chromatography (TLC). This was performed on silicagel plates buffered at pH 4 or 6 in mainly 3 solvent systems I, II and III with two additional systems IV and V for highly polar compounds (v. Euw et al. 1985: 1253). The silica gel was a commercial product (HF 254 + 366, Typ 66, für Dünnschichtchromatographie, Merck) containing fluorescent additives, allowing non-destructive localisation of all known fern phloroglucinols (Tables 2 and 3) as dark (partly coloured) spots on light background by short wave (centered at 254  $\mu\text{m}$ ) and longwave (centered at 360  $\mu\text{m}$ ) illumination (mercury UV lamps) for short times in a dark box. We also used silica gel GF plates ("Merck") buffered at pH 4 or 6 and the two solvent systems described by Widén et al. 1970: 7–10. After marking the spots were further characterized (this time destructive) by spraying with freshly prepared 0.1% aqueous solution of "fast blue salt Merck" (= tetrazotized di-ortho-anisidin) see Penttilä & Sundman (1961).

Whenever possible the crude ether extract was purified using the MgO procedure (with addition of  $\text{Na}_2\text{SO}_3$  to avoid oxydations) of Ackermann & Mühlemann (1946). In this step the bulk of phenolics (= "MgO-filicins" in Table 6) is separated (as water soluble Mg-salts) from fatty acids and neutral material (remaining in the insoluble filtercake) but in spite of the stabilising effect of the  $\text{Na}_2\text{SO}_3$  some deterioration and losses occur and more than traces of phenolics can remain in the MgO-cake. By treatment of this unreacted part with  $\text{Ba}(\text{OH})_2$  (Widén & Britton 1971, Widén et al. 1976: 1740) a further amount of phenolics (= " $\text{Ba}(\text{OH})_2$ -filicins" in Table 7) can usually be obtained but it normally contains still more artefacts. The "MgO"- and " $\text{Ba}(\text{OH})_2$ -filicins" can

again be examined directly by TLC or first separated by column chromatography. In certain cases heavy losses and decomposition of sensitive compounds can occur when chromatography is performed on columns of unbuffered  $\text{SiO}_2$  or if chloroform is used as a solvent (v. Eeuw et al. 1985). The most reliable identification is always possible when a compound can be isolated in pure state. The TLC examination of the crude ether extract will nearly always permit to test whether a particular compound was already present in the plant or whether it is an artefact. Successful preparative isolation of pure constituents will be mentioned for each species (see Appendix).

For final identification (including identifications of mere spots in TLC) we had a complete set of all known phloroglucinols of ferns to be used as standards, see Tables 3, 4 and 6 (Appendix 8.1). The difficult but unavoidable JUPAC names for each compound are also given there. The chemical structures as presented by v. Eeuw et al. (1980 and 1985) are reviewed in Table 4 with original numbering.

#### 4.1 Remark to Table 4

As mentioned in v. Eeuw et al. 1985: 1256–7 the formulae given are schematized for the compounds containing 2,5-cyclohexadienone rings and do not always represent the true state. Several tautomers (besides dimers or polymers, involving intermolecular H-bonding) are theoretically possible already in simple compounds with only one ring like butyrylfilicinic acid (36). Äyräs et al. (1981a, b, 1982) deduced from  $^{13}\text{C}$ -NMR studies that the tautomer 36b with 2,4-cyclohexadienone structure is exclusively present in ( $\text{D}_6$ )acetone solution. In  $\text{CDCl}_3$  solution, it is still the preferred tautomer, but other forms are present in smaller amounts, perhaps 36a and 36c, the spectra becoming complicated. But according to Äyräs et al. (1981b), the preferred arrangement of albaspidin BB in 10-BB with both rings as 2,5-cyclohexadienones in a particular rotamer stabilized by intramolecular H-bonds. No other tautomer could be observed in ( $\text{D}_6$ )acetone solution, but in  $\text{CDCl}_3$  another unsymmetrical tautomer becomes visible in the spectra as reported by Äyräs et al. (1982). The presence of such a tautomer (and probably small amounts of a third one) is also visible in the  $^1\text{H}$ -NMR spectrum (see v. Eeuw et al. 1985, Fig. 2A and 2B) of a pure sample (freshly sublimed in vacuo to get it completely free of solvent traces). The other compounds with two, three, or more rings may behave in a similar way, but they have not yet been examined in detail. We nevertheless assume that the schematical formulae given for compounds with two or more rings with 2,5-hexadienones represent the closest approximation to the true state among other tautomers, while the actual orientation of the H-bonding is left undetermined.

## 5. Results

These are summarized in the large Table 5 and are discussed briefly in section 6. Amounts of crude (or “cation free”) ether extract and crude “phenolics” obtained for each batch are given in the Appendix 8.3.1 (Table 7), the approximate total amounts of homologues in Table 8 (Appendix 8.3.2) and of pure crystalline compounds in Table 10 (Appendix 8.4.2).

#### 5.1 Remarks to Table 5

The following compounds (and their A- and P-homologues) given in Tables 2 and 6 are omitted from Table 5, as they were not found in the species of ferns treated in this

study: ortho-Desaspidin-BB (9-BB), Phloraspin-BB (11-BB), Phloraspidinol-BB (12-BB), Methylen-bis-desaspidinol-BB (14-BB), Phloropyron-BB (15-BB), Phloraspyron-BB (16-BB), Aemulin-BB (17-BB), Methylen-bis-aspidinol-BB (18-BB), Trisaemulin-BBB (24-BBB). A minus sign (–) means that the corresponding compound is absent (<1% of crude “filicins”). Aspidinol-B (1-B) is sometimes an artefact produced already during long storage of plant material or by the detrimental steps of the “standard procedure”, particularly the treatment with MgO and Ba(OH)<sub>2</sub> and by chromatography on unbuffered SiO<sub>2</sub>. It is mainly formed from para-aspidin. Its original presence can be checked by using the “improved method” (v. Euw et al. 1980, 1985) and examining the “cation free ether extract” directly in TLC.

## 5.2 Compounds of unknown structure

In addition to the crystalline compounds with known structure (1–26, 28, 30, 33–37 in Table 4) three amorphous constituents JU-1, JU-2 and JU-3 were observed in *D. juxtaposita*, partly also in *D. nigropaleacea*. They were characterized by their mobilities in TLC and coloration with fast blue salt B (see Table 3). They were identical with “LE-1, LE-2 and LE-3” found in *A. lepidopoda* (Widén et al. in prep.) and at least closely related to the “Pentherins” (see below, 5.2.1). In the improved isolation method of v. Euw et al. (1980, 1985) these three “unknowns” are concentrated in the hexane phase of the counter

Tab. 2. Correction of provisional names used by Widén et al. (1973) for the four members of the *Dryopteris inaequalis* group from Kenya

Collector and number (progeny)	Ploidy	Misapplied name in Widén et al. (1973)	Correct name Pichi Serm. (1984)
R. B. Faden 71/255 (TR-3274)	4x	“ <i>D. inaequalis</i> ”	<i>D. pentheri</i>
R. B. Faden 71/68 (TR-3224)	2x	“ <i>D. pentheri</i> ”	<i>D. fadenii</i>
R. B. Faden 71/468 (TR-3248)	2x	“ <i>D. schimperana</i> ”	<i>D. spec.</i> not identified
R. B. Faden 71/885 (TR-3305)	4x	<i>D. spec.</i> not identified allo-tetraploid	<i>D. spec.</i> not yet identified tetraploid

Tab. 3. Mobilities (= 100 × R<sub>f</sub> values) and colours after spraying with “fast blue salt B” of the three unknown compounds in three different systems (I–III) on TLC plates buffered at pH 6.0

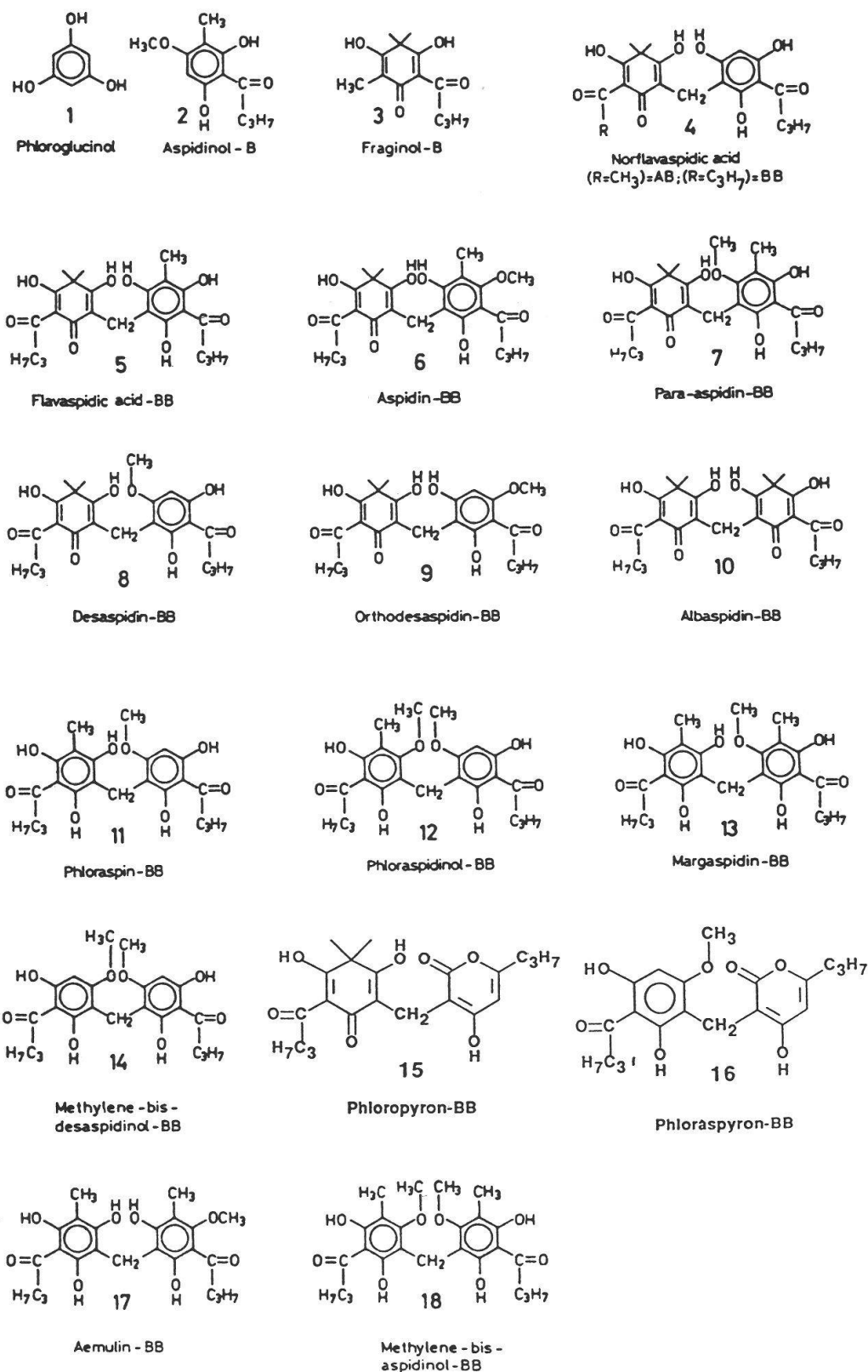
Compound	Colour	System I <sup>1</sup>	System II <sup>2</sup>	System III <sup>3</sup>
JU1 = LE1	brown	3	25	43
JU2 = LE2	brown	3	17	27
JU3 = LE3	brown	3	7	13

<sup>1</sup> Silica gel HF, methanol-isopropylether-cyclohexane 10:35:55 (v. Euw et al. 1985).

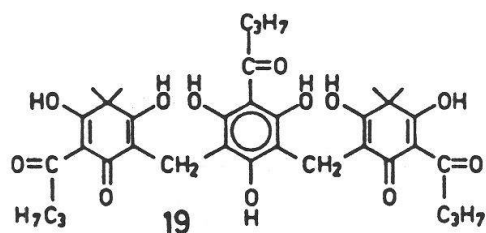
<sup>2</sup> Silica gel GF, chloroform-hexane 50:50 (Widén et al. 1970a).

<sup>3</sup> Silica gel GF, chloroform-hexane ethanol 45:45:10 (Widén et al. 1970a).

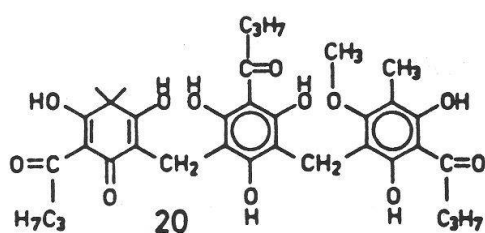
Tab. 4. Structures of the main phloroglucinols isolated from ferns. The unsubstituted Phloroglucinol (1) is not present in ferns. Except for 25 only the B-homologues are given here. In the A-, P-, V-homologues the butyryl side chain is replaced by Acetyl, Propionyl- or Valeryl side chains. For tautomeric forms see remark under 4:1



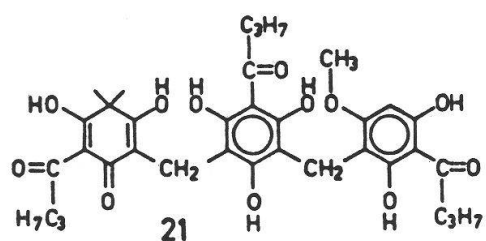
Tab. 4 (continuation)



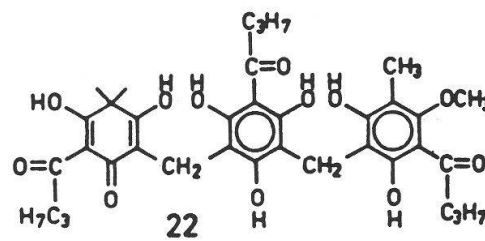
Filixic acid - BBB



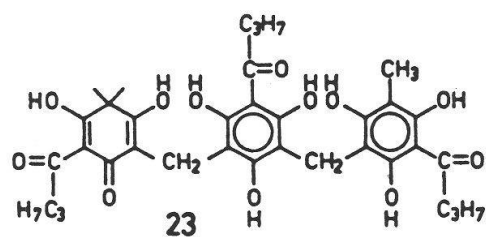
Trispara - aspidin - BBB



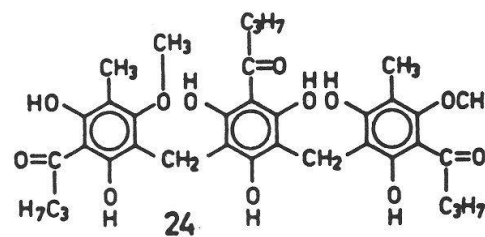
Trisdesaspidin - BBB



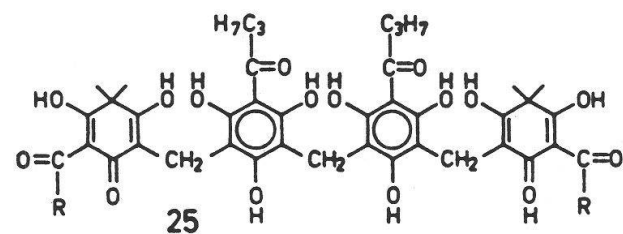
Trisaspidin - BBB



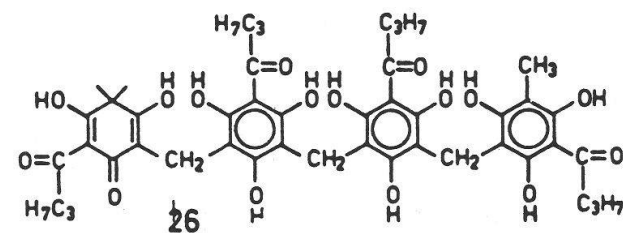
Trisflavaspic acid - BBB



Trisaemulin - BBB



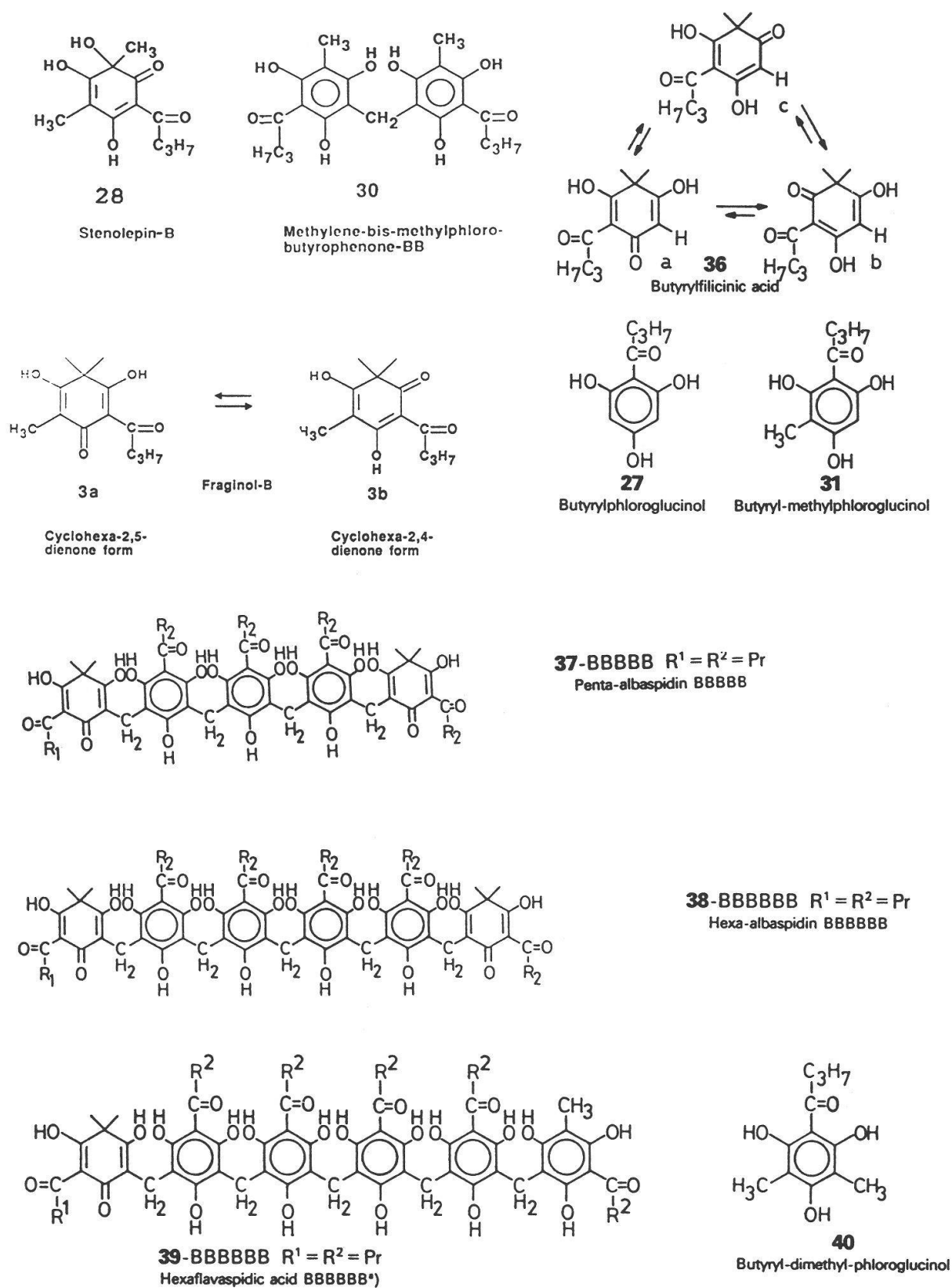
ABBA(R=CH<sub>3</sub>)=Methylene - bis -  
norflavaspic acid - ABBA =  
Dryocrassin  
BBBB(R=C<sub>3</sub>H<sub>7</sub>)=Methylene - bis -  
norflavaspic acid - BBBB



Tetraflavaspic acid - BBBB



Tab. 4 (continuation)



current distribution. From this hexane phase they could be further concentrated in the sodium hydroxyde (=“phenolic”) fraction when their ether solution was shaken with aqueous potassium bicarbonate, sodium carbonate and sodium hydroxyde according to Patama and Widén (1991). After reductive cleavage all three compounds gave butyryl-filicinic acid (36) and its lower homologues (P and A) and weak spots for acyl-phloroglucinols (27, 31 and lower homologues) in TCL and PC. In electron impact (EI) and fast atom bombardment (FAB, see Barber et al. 1981) mass spectra all three “unknowns” gave putative molecular peaks at  $m/z$  648 and 620 (the latter weak in FAB). According to high resolution measurements they correspond to  $C_{39}H_{52}O_8$  (648.3746) and  $C_{34}H_{52}O_{10}$  (620.3587).

5.2.1 The “pentherins” and the four members of *Dryopteris inaequalis* complex in Kenya. When Widén et al. (1973) examined 8 *Dryopteris* species from Kenya the four members of the *Dryopteris inaequalis* group posed particular taxonomic problems. The nomenclatures was controversial. Some authors treated *D. pentheri* (Krasser) C. Christensen (1905: 284) and *D. schimperana* (A. Br.) C. Christensen (1905: 291, given as *D. schimperianum*) as synonyms of *D. inaequalis* (Schlechtendal) O. Kuntze (1891: 813). Cytological and chemical examination (Widén et al. 1973) clearly showed that four distinct species of this group are growing in Kenya to which provisional but partly incorrect names had been attached (see Table 2). Pichi Sermolli (1984) showed that true *D. inaequalis* is restricted to South Africa and that the assumed diploid “*D. pentheri*” analysed by Widén et al. (1973) was a new species: *D. fadeni* Pichi Serm. while the “tetraploid *D. inaequalis*” was true *D. pentheri*. The other two species are still not identified but the cytological and chemical work of Widén et al. (1973) showed that the tetraploid, unnamed species Faden 71/885 is most probably an *allo*-tetraploid derived from chromosome doubling in a hybrid of *D. fadenii*  $\times$  *D. spec.* Faden 71/468.

Pentherin-I (=“brown unknown”) was isolated from *D. fadenii* and from the *allo*-tetraploid *D. spec.* Faden 71/885 while pentherin-II (=“yellow unknown”=compound TR-1498) in Widén et al. (1973: 2147) was found only in *D. fadenii*. True tetraploid *D. pentheri* does not contain any pentherins. Both pentherins are sensitive compounds, deteriorating easily and have perhaps therefore never been isolated in crystalline state.

There was no original pentherin-I left for direct comparison of the three unknown JU-1, JU-2 and JU-3 of this work but there is good reason to assume that pentherin-I is identical with one of them. The mobilities in TLC are similar, the colouration with fast blue salt B the same, reductive cleavage gave identical products and the mass spectra the same putative molecular peak at  $m/z$  648. Pentherin-II gives a yellow spot after spraying with fast blue salt B but otherwise behaves in a similar way, giving the same products after reductive cleavage. Its EI mass spectrum taken under the mildest possible conditions ( $t=80^\circ$  and 75 eV) is given in Fig. 2 (Widén et al. 1973: 2140). The putative molecular peak at  $m/z$  648 is prominent and was assumed to be  $C_{36}H_{40}O_{11}$  (=648) but is obviously the same as found for the 3 unknown JU-1 etc. and for which the empirical formula  $C_{39}H_{52}O_8=648.3746$  was estimated as mentioned by high resolution measurements. Widén et al. (1973) also give a hypothetical partial structure (p. 2136 formula 5) which in principal may be correct in spite of the assumed wrong empirical formula. It can, however, be that this peak at  $m/z$  648 is a fragment or rearrangement product formed from the very sensitive pentherins during registration of the mass spectrum.

Tab. 5. Semiquantitative results for composition<sup>1</sup> of phloroglucinols in the different taxa: + + + = or more than 20% of the crude "filicin" mixture; + + = 10–20%; + = 5–10%; (+) = 1–5%; – = less than 1%. Only the numbers of the compounds could be given in this table, names and chemical structures see Tables 2 and 3

Name of plant, ploidy level <sup>2)</sup> and collectors number <sup>3)</sup>	(Section) and Origin	2-B	3-B	23-B	4-BB	4-AB	5-BB	5-AB	6-BB	6-AB	7-BB	7-AB	8-BB
Footnotes	4)	6)			6)			6)			6)		
<i>D. villarii</i> subsp. <i>villarii</i> (4) 2x	TR 2747 It <sup>14)</sup> TR 4829 CH	+	–	–	–	–	++	(+)	–	–	+++	–	+
		+	–	–	–	–	++	(+)	–	–	+++	–	+
<i>D. pallida</i> subsp. <i>pallida</i> (6) 2x	TR 116 Sd <sup>14)</sup> TR 116 Sd TR 545 It TR 3244 Ju	+	–	–	–	–	++	(+)	–	–	+++	–	+
		+	–	–	–	–	++	(+)	–	–	+++	–	+
		+	–	–	–	–	++	(+)	+++	–	+++	–	–
		+	–	–	–	–	+	–	+++	–	+++	–	–
<i>D. submontana</i> (6) 4x	ACJ s.n. GB <sup>14)</sup> GV s.n. Rm TR 3023 Sp TR 3629 Ga TR 3241 Ju	+	–	–	–	–	++	(+)	–	–	+++	–	(+)
		+	–	–	–	–	++	(+)	+	–	+++	–	+
		+	–	–	–	–	+	(+)	(+)	–	+++	–	(+)
		+	–	–	–	–	+	–	+	–	+++	–	+
		+	–	–	–	–	+	–	–	–	+++	–	(+)
<i>D. pallida</i> subsp. <i>balearica</i> (6) 2x	TR 3253 Ma <sup>14)</sup> TR 3253 Ma1	–	–	–	–	–	++	–	++	–	–	–	+++
		–	–	–	–	–	++	–	(+)	–	–	–	+++
<i>D. pallida</i> subsp. <i>libanotica</i> (6) 2x	TR 1825 Cy TR-3926 = CRFJ 4574 Tk	+	–	–	–	–	+	–	++	(+)	+++	–	(+)
		+	–	–	–	–	+	–	+++	+	+++	–	(+)
<i>D. pallida</i> subsp. <i>raddeana</i> (6) 2x	TR 1545 Ir TR 2742 Ir CRFJ 6034-6;6041-2 Ir PU 19351 Ir	+	–	–	–	–	++	(+)	+	–	+++	–	–
		+	–	–	–	–	+	–	(+)	–	+++	–	–
		+	–	–	–	–	++	–	+	–	+++	–	–
		+	–	–	–	–	+	–	+	+	+++	–	–
<i>D. nigropaleacea</i> (6) 2x	IK 6697 Pak CRFJ 6230-3 Pak CRFJ 7377 Pak CRFJ 8027-41 Pak CRFJ 8235 N.In	–	–	–	–	–	++	++	–	–	–	–	–
		–	–	–	–	–	+++	++	–	–	–	–	–
		–	–	(+)	–	–	+++	++	–	–	–	–	–
		–	–	(+)	–	–	+++	++	–	–	–	–	–
		–	–	(+)	–	–	+++	++	–	–	–	–	–
<i>D. ramosa</i> (9) 2x	CRFJ 6280-3 Pak CRFJ 7373;7379-83 Pak CRFJ 7418-25 N.In CRFJ 7843-51 N.In	+	+	–	–	–	++	+	–	–	++	+	–
		(+)	(+)	–	–	–	++	++	–	–	++	–	–
		(+)	(+)	–	–	–	++	+	–	–	++	–	–
		(+)	(+)	–	–	–	++	+	–	–	+	–	–
<i>D. stewartii</i> (6) 3x (apomictic)	PU 17447 Pak PU 17514 Pak CRFJ 6298 Pak CRFJ 6329 Pak CRFJ 7370-2;7374-6;7378; 7384-8 Pak CRFJ 7426-9 N.In CRFJ 7457 N.In CRFJ 7614;7616;7651 N.In CRFJ 7852-9 N.In CRFJ 7917-21 Pak CRFJ 7938-9 Pak	–	–	–	–	–	++	+	–	–	–	–	–
		(+)	–	–	–	–	++	+	–	–	++	–	–
		–	–	–	–	–	+++	++	–	–	–	–	–
		+	–	–	–	–	+++	++	–	–	++	+	–
		(+)	–	–	–	–	+++	++	–	–	+	–	–
		(+)	–	–	–	–	+++	++	–	–	++	–	–
		–	–	–	–	–	+++	++	–	–	–	–	–
		(+)	–	–	–	–	+++	++	–	–	(+)	–	–
<i>D. marginata</i> (9) 2x	CRFJ 8421-33 N.In CRFJ 8609-12 8614-6;8618-23 N.In CRFJ 8795-8 N.In	–	++	(+)	–	–	+++	–	–	–	–	–	–
		–	++	–	–	–	++	–	–	–	–	–	–
		–	++	–	–	–	++	–	–	–	–	–	–
<i>D. barbigera</i> (4) 2x	CRFJ 7433 N.In CRFJ 7450 N.In CRFJ 7715-20 N.In	+	–	–	–	–	+++	–	–	–	+++	–	–
		+	–	–	–	–	++	+	–	–	+++	–	–
		+	–	–	–	–	+++	+	–	–	+	–	–

Tab. 5 (continuation)

10- BB	10- AB	10- AA	13- BB	30- BB	19- BBB	19- ABB	19- ABA	20- BBB	21- BBB	23- BBB	23- ABB	25- ABBA	25- BBBB	26- BBBB	37- BB BBB	38- BBB BBB	39- BBB BBB	JU1	JU2	JU3
7)	8)				9)	10)												11)	12)	13)
+	-	-	-	-	+++	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(+)	-	-	-	-	+++	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	-	-	-	++	+	-	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	+	(+)	-	++	(+)	+	-	-	-	-	-	-	-	-	-	-
(+)	(+)	-	-	-	-	-	-	++	-	(+)	-	-	-	-	-	-	-	-	-	-
(+)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	++	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	++	-	-	+	-	(+)	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	++	(+)	-	(+)	-	(+)	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	++	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	++	(+)	-	(+)	-	-	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
++	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
(+)	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
++	(+)	-	-	-	-	-	-	++	(+)	(+)	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	++	-	+	-	-	-	-	-	-	-	-	-	-
+	(+)	-	-	-	(+)	-	-	++	-	(+)	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	(+)	-	-	++	-	+	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	-	-	-	++	-	+	-	-	-	-	-	-	-	-	-	-
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(+)	(+)	-	-	-	++	+	(+)	(+)	-	+	-	-	-	-	-	-	-	-	-	-
(+)	(+)	-	-	-	+	+	(+)	+	-	+	-	-	-	+	-	-	-	-	-	-

Tab. 5 (continuation)

<i>D. blanfordii</i>	CRFJ 7401-6	N.In	(+)	-	-	-	-	+++	+++	-	-	+	+	-
(5) 3x	CRFJ 7410-15	N.In	(+)	-	-	-	-	+++	+++	-	-	++	+	-
(apomictic)	CRFJ 7430-1	N.In	(+)	-	-	-	-	+++	+++	-	-	++	+	-
	CRFJ 7441-2	N.In	(+)	-	-	-	-	+++	+++	-	-	++	+	-
<hr/>														
<i>D. chrysocoma</i>	(3)2x and 4x													
<i>D. para-chr.</i>	CRFJ 7681-8	N.In	-	-	-	-	-	+++	+	-	-	-	-	-
<i>D. woodsiiisora</i>														
CRFJ 8754-9;	CRFJ 8771-4	N.In	-	-	-	-	-	+++	++	-	-	-	-	-
	CRFJ 8779	N.In	-	-	-	-	-	+++	++	-	-	-	-	-
<hr/>														
<i>D. juxtaposita</i> (6)														
	CRFJ 7595;7607													
	7615;7621-9;7642;7653	N.In	-	-	-	-	-	++	+	-	-	-	-	-
tripl.	CRFJ 8163-9	N.In	-	-	-	(+)	(+)	+++	++	-	-	-	-	-
apom.	CRFJ 8210-16													
	8218-9;8221-8	N.In	-	-	-	(+)	(+)	++	+	-	-	-	-	-
	CRFJ 8816-9	N.In	-	-	-	(+)	(+)	++	+	-	-	-	-	-
	CRFJ 9215-8	S.In	-	-	-	(+)	(+)	+++	++	-	-	-	-	-
	CRFJ 9222-7	S.In	-	-	-	(+)	(+)	++	+	-	-	-	-	-
	CRFJ 9278;9280-2	S.In	-	-	-	(+)	(+)	++	+	-	-	-	-	-
CRFJ 9365-76;9378-9;9380-3	S.In	-	-	-	(+)	(+)	+++	++	-	-	-	-	-	-
<hr/>														
<i>D. odontoloma</i> (6)														
tripl. apomictic														
	CRFJ 9316;9304-13	S.In	-	-	-	(+)	(+)	+++	++	-	-	-	-	-
	CRFJ 9389-91;9393-9409	S.In	-	-	-	(+)	(+)	+++	++	-	-	-	-	-
<hr/>														
<i>D. arguta</i> (6)	FL 384	Og <sup>15)</sup>	-	-	-	-	-	++	++	-	-	+	-	-
2x L.Bonnell s.n.		BC <sup>15)</sup>	-	-	-	-	-	++	++	-	-	+	-	-
A.Gerber A-1 =	TR 3290	Cal	-	-	-	-	-	++	++	-	-	+	-	-
W.Bennert s.n. =	TR 4043	Cal	-	-	-	-	-	++	++	-	-	+	-	-
<hr/>														
<i>D. fragrans</i>	DMB 1758-61	Ont <sup>16)</sup>	-	-	-	-	-	++	(+)	+++	+	-	-	-
(4) 2x	DMB 1857-9	Ont <sup>16)</sup>	-	-	-	-	-	+	(+)	++	+	+	-	+
P.Kallio s.n.		NF <sup>16,17)</sup>	-	-	-	-	-	++	(+)	+++	+	-	-	-
P.Kallio s.n.		FS <sup>16,17)</sup>	-	+	-	-	-	++	(+)	+++	+	(+)	-	-
J.Pintér s.n.		USSR	-	++	-	-	-	+	(+)	+++	+	-	-	-
J.Pintér s.n.		Mong	-	++	-	-	-	+	(+)	+++	+	+	-	-

<sup>1</sup> Estimated from intensity of spots in TLC and yield in preparative column chromatography.

<sup>2</sup> 2x=diploid, 3x=triploid, 4x=tetraploid, apom.=apomict.

<sup>3</sup> WB=W. Bennert, DMB=D. M. Britton, CRFJ=C. R. Fraser-Jenkins, ACJ=A. Clive Jermy, IK=Kukkonen, FL=Frank A. Lang, PU=P. Uotila, s.n.=sine numero.

<sup>4</sup> BC=British Columbia, Canada; Cal=California, USA; CH=Switzerland; Cy=Cyprus; FS=Finland; Ga=France; GB=Great Britain; N.In=North India; S.In=South India; Ir=Iran; It=Italy; Ju=Yugoslavia; Mal=Mallorca, Balears; Mong.=Mongolia; NF=New Foundland, Canada; Og=Oregon, USA; On=Ontario, Canada; Pak=Pakistan; Rm=Rumania; Sd=Sardinia.

<sup>5</sup> Aspidinol-B (1-B) is sometimes an artefact.

<sup>6</sup> Often a mixture of BB- and BP-homologues which do not well separate on TLC.

<sup>7</sup> The spot which formerly has often been designated as "Albaspidin-1" may be provoked by a mixture of the homologues BB, PB and PP.

<sup>8</sup> The spot is often designated as "Albaspidin-2" and may be provoked by a mixture of the homologues BA and PA.



Tab. 5 (continuation)

-	-	-	-	-	-	(+)	+	-	-	-	+	-	(+)	-	-	-	-	-	-	-
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-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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<sup>9</sup> The corresponding spot in TLC is often designated as "filixic acid-1" and may be provoked by a mixture of homologues BBB, PBB and PBP.

<sup>10</sup> The corresponding spot is often called "filixic acid-2" and may be produced by a mixture of homologues ABB and ABP.

<sup>11</sup> JU-1 = unknown, giving brown spot after spraying, see Table 6, first isolated from *D. lepidopoda* (Widén et al. in prep.) also present in *D. juxtaposita* and *D. nigropaleacea*.

<sup>12</sup> JU-2 = unknown, giving brown spot after spraying, see Table 6, first found in *D. lepidopoda* also present in *D. juxtaposita* and *D. nigropaleacea*.

<sup>13</sup> JU-3 = unknown, giving brown spot after spraying, see Table 6 for morbidity, first also found in *D. lepidopoda*, also present in *D. juxtaposita* and *D. nigropaleacea*.

<sup>14</sup> Reported from Widén et al. (1971).

<sup>15</sup> Reported from Widén & Britton 1971 c.

<sup>16</sup> Reported from Widén & Britton 1971 b.

<sup>17</sup> Reported from v. Schantz & Widén 1967.

## 6. Discussion

In the following chapter we discuss the results for each of the 17 taxa analysed in this study. The main purpose was to establish how far chemical composition reflects taxonomical relations, in particular Fraser-Jenkins' (1986) classification. We have to consider that he divided the genus *Dryopteris* into four subgenera and that all 17 taxa of this study are members of only one, of the subgenus *Dryopteris*. This is one reason why the results of all the 17 taxa are relatively similar (see Table 5). Fraser-Jenkins (1986) divided the subgenus *Dryopteris* into 11 sections. We therefore have to compare chemical results only on the level of sections which he himself considers as less clear-cut groups of species. The material which we analysed (17 taxa) belongs to only 5 of these sections (3. *Pandae*; 4. *Dryopteris*; 5. *Remotae*; 6. *Pallidae* and 9. *Marginatae*). This may be another reason for the relative uniformity of chemical results in our material. None of the 17 taxa of the present study did contain any phloraspidinol (12), and any aemulin (17), all present in *D. aemula* (section 10), and any phloropyron (15) present in most specimens of *D. expansa* (section 11) (reviewed in v. Euw et al. 1980, 1985). Chemical diversity, if any, in our material was restricted to details and was often but not always correlated with taxonomical relationship. Very good correlation was observed in some genetically closely related taxa, particularly in taxa possessing homologous chromosomes like three subspecies of *D. pallida* (see 6.1.4) which showed only morphological diversity and (at least potentially) the phenomenon of introgression. They usually produce exactly the same phloroglucinols. The complex *D. intermedia* – *D. azorica* – *D. maderensis* (= *D. intermedia* subsp. *maderensis*) is a well-known example (Gibby and Walker 1977, Gibby et al. 1977, 1978, Gibby 1979, 1983).

Good correlation with expected values were usually also found in allopolyploid species. They often produce phloroglucinols which correspond exactly to the sum of those found in the putative ancestors. In some cases the formation of a particular compound present in only one ancestor may also be absent in the allopolyploid taxon. Its formation is then suppressed by the other ancestor which is obviously dominant in this respect.

In the majority of taxa which we analysed in this study the true genomic relations are unknown. We found that some members of one section actually produce similar compounds, partly different from those produced in members of other sections. In some cases, however, the opposite is true.

### 6.1 The *D. villarii* complex

The new results for the six European members of this complex agree fairly well with former observations (Widén et al. 1971). We can summarize that in first approximation the six members of this complex produce phloroglucinols similar to those of the *D. filix-mas*, *D. affinis* (former *D. borrieri*), and *D. wallichiana* complexes (Widén et al. in prep.). Some differences exist and these can partly be correlated with Fraser-Jenkins' (1986) classification.

6.1.1 *D. villarii* (section 4. *Dryopteris*) does not produce any aspidin (6) nor any trispara-aspidin (20) but large amounts of filixic acid (19). The same was found for *D. filix-mas* (reviewed in Widén et al. 1971, v. Euw et al. 1980, 1985) which is the type of section 4.

6.1.2 *D. pallida* (section 6. *Pallidae*) is a variable taxon. All four subspecies produce aspidin (6) and three subspecies also tris-para-aspidin (20), absent in subsp. *balearica*. The four subspecies lacked filixic acid (19) or had only traces. Presence or absence of these three compounds (6, 19, 20) can even be used as a chemical marker of differentiating *D. pallida* from *D. villarii* and also *D. pallida* subsp. *balearica* from subsp. *pallida*.

6.1.3 *D. submontana* (section 6. *Pallidae*). This taxon was still without a valid name when Widén et al. (1971) published their results; it was referred to the "tetraploide Sippe" of *D. villarii* in their paper. The chemical results obtained for material of different origin were virtually constant. They are in agreement with the assumption (see chapter 3.1.2) that *D. submontana* may be an allo-tetraploid containing two genomes of *D. villarii* and two of *D. pallida*. As seen in Table 3, in *D. submontana* nearly all of the main phloroglucinols are present which are to be expected from an addition of those present in both putative ancestors. Only trispara-aspidin (20) (present in *D. pallida*) was not found in *D. submontana*, or only in traces. Its formation in *D. submontana* is obviously suppressed by the genomes of *D. villarii* dominant (in this character).

6.1.4 Chemical differences on subspecific level of *D. pallida*. As pointed out before (Widén et al. 1971) subsp. *balearica* differs from subsp. *pallida* by producing more desaspidin (8), no para-aspidin (7), and no trispara-aspidin (17). On the other hand, subsp. *libanotica* and subsp. *raddeana* gave virtually the same chemical results as did subsp. *pallida*. We have good reason to assume that these three subspecies have homologous genomes in spite of the presence of morphological differences. Introgression of subsp. *pallida* and subsp. *libanotica* is obvious wherever both taxa grow sympatrically. Subsp. *raddeana* is geographically separated from subsp. *pallida* and the two subspecies are morphologically distinct. Cytological results in an experimentally produced hybrid with *D. tyrrhena* Fraser-Jenkins et al. (1975) (see Appendix 8.6.) showed that subsp. *raddeana* and subsp. *pallida* also must have homologous genomes. This very close relation is reflected in similar chemical composition. On the other hand subsp. *balearica* is chemically distinct (see Table 5). The following preliminary experiment shows that it may also be genetically distinct. One of us (G.V.) has made the cross of subsp. *balearica* with subsp. *raddeana* (TR-949) and found the spores of this hybrid all bad and aborted (in litt. to T. R. June 28, 1972). Due to his move to the Eötvös Lorand University, Budapest he has not examined the meiosis and the plant was lost. The completely abortive spores, however, indicate that due to difficulties in meiotic chromosome pairing prior to spore formation, an effective reproductive barrier has been built up between subsp. *balearica* and the three other subspecies of *D. pallida*. This could in fact be an argument for the acceptance of specific rank as recommended by Nardi (1976: 28–29).

## 6.2 Related, mainly Asiatic and American species

Among the species with a more eastern distribution which we analysed the following three are probably closest to *D. pallida*: *D. nigropaleacea*, *D. ramosa*, and *D. stewartii*. They may even represent a particular complex.

6.2.1 *D. nigropaleacea* (section 6. *Pallidae*) is a sexual diploid species (Gibby 1985b: 1) which formerly was often confused with the triploid apomictic *D. odontoloma* (Löve et al. 1977: 315, corrected by Gibby 1986b: 6–7) known only from southern India. *D. nigropaleacea* is morphologically distinct from but rather similar to *D. pallida* and was first treated as *D. pallida* subsp. *nigropaleacea* Fraser-Jenkins (1977: 316). However, in its chemical composition *D. nigropaleacea* differs quite considerably from *D. pallida* by

lacking aspidin (6), para-aspidin (7) and trispara-aspidin (20) and by producing filixic acid (19) and sometimes trace amounts of dryocrassin (25-ABBA) and stenolepin (28).

6.2.2 *D. ramosa* (section 9. *Marginatae*) is reported to be diploid ( $n=41^{\text{II}}$ , Loyal in Mehra 1961: 152). Fraser-Jenkins (1978: 271) assumed, on the basis of its very small spores, that this is correct. He also stated that at least two counts ( $n=41^{\text{II}}$ ) of Loyal in Mehra (1961: 152) and Mehra & Loyal (1965: 464–9), published incorrectly under *D. odontoloma* (quoted in Löve et al. 1977: 315), refer to *D. marginata*, not to *D. ramosa*. The latter is morphologically quite distinct from *D. nigropaleacea* (section 6. *Pallidae*) but produces very similar phenolics. The only qualitative difference is the presence in *D. ramosa* of a fair amount of para-aspidin (7) and some trispara-aspidin (20).

6.2.3 *D. stewartii* (section 6. *Pallidae*). This is a triploid, apomictic species (Gibby 1985b: 7), morphologically intermediate between *D. nigropaleacea* and *D. ramosa*. It was first found in Pakistan and N. W. India together with these two species. This caused difficulties in distinguishing the plants in the field among a confusing range of intermediates, until Fraser-Jenkins was able to describe *D. stewartii* as a distinct species. This intermediate morphology, and the sympatric stations, made him assume that *D. stewartii* may have evolved by some hybridisation process from *D. nigropaleacea* and *D. ramosa*. Chemical analysis (see below) would ally with such an assumption. The formation of triploid *D. stewartii* from two diploid ancestors cannot, however, have followed the normal process of chromosome doubling in a diploid hybrid leading to allotetraploids (Manton 1950). We believe that the most probable origin of *D. stewartii* would be through fusion of a normal haploid gamete (Ni) of *D. nigropaleacea* with an unreduced gamete (RmRm) of *D. ramosa* producing a zygote (NiRmRm). We must further assume that, for some unknown reasons this zygote had, the capacity for apomictic reproduction. The occasional formation of unreduced gametes and their participation in hybrid formation is well documented both in natural and experimentally produced fern hybrids. Acquisition of apomictic properties in such triploid hybrids has been postulated as a theoretical possibility (Manton 1950, Lovis 1977: 387–402, Walker 1979, 1985) but was so far observed only with low yield under experimental conditions in triploid *Polystichum* hybrids (Vida and Reichstein 1975). The case of *D. stewartii* may be an ideal subject for checking this hypothesis further, as both putative ancestors are known and easily available.

We have analysed *D. stewartii* from 11 localities. The composition of its phloroglucinols is relatively constant and corresponds fairly well with the expected value when the compounds found in both putative ancestors are added. There is, however, an unexpected detail worth mentioning. Although the amounts of most compounds present in *D. stewartii* were found to be the same for all 11 samples, considerable differences were observed for para-aspidin (7) and trispara-aspidin (20), i.e., exactly for the only two compounds absent from *D. nigropaleacea* and present in *D. ramosa*. Some samples contained amounts of these two compounds corresponding to those found in *D. ramosa*, others contained less, and some none at all, like *D. nigropaleacea*. So far we did observe a similar “splitting” only in one other fern of hybrid origin i.e. in *D. carthusiana* (Vill.) H. P. Fuchs (= *D. spinulosa* Watt), see Widén et al. (1970a), Widén & Britton 1971a.

6.2.4 *D. marginata* (section 9. *Marginatae*) is the type species of section 9. It has often been confused with *D. ramosa* and *D. odontoloma*. In chemical composition it differs from *D. ramosa* (same section) by the presence of methyl-butyryl-filicinic acid (3) and the



absence of the AB-homologue of flavaspidic acid (5-AB), para-aspidin (7), and trispara-aspidin (20).

6.2.5 *D. barbiger* (section 4. *Dryopteris*) is related to the *D. filix-mas* complex but is rather distinct. Its chemical composition is similar to that of *D. filix-mas* but contains small amounts of other compounds not present in *D. filix-mas*.

6.2.6 *D. blanfordii* was placed by Fraser-Jenkins (1986: 190–1) in section 5 where it is next to *D. remota* and *D. corleyi*. In chemical composition our material was, however, closer to *D. filix-mas*, *D. villarii* (section 4), and *D. affinis* (section 2).

6.2.7 The *D. chrysocoma* complex was placed in section 2. *Fibrillosae* Ching (1938) by Fraser-Jenkins (1986: 190–1) together with the *D. affinis* and *D. wallichiana* complexes. The phloroglucinols in our material were indeed similar to those reported for *D. affinis* (Widén et al. 1971 under *D. borrieri*) but in *D. chrysocoma* we found methylene-bis-methylphlorobutyrophenone (30) trisflavaspidic acid (23), and tetraflavaspidic acid (26), so far not reported for *D. affinis*. This may, however, be due to meanwhile improved analytical methods (v. Euw et al. 1980, 1985). We found no difference in chemical composition between *D. parachrysocoma* and *D. woodsiiisora*.

6.2.8 *D. juxtaposita* (section 6. *Pallidae*). We had material from 8 different localities that gave uniform results. The chemical composition differs from that of *D. pallida* by the absence of aspidin (6) (present in large amounts in some specimens of *D. pallida*), para-aspidin (7), small amounts of albaspidin (10), no trispara-aspidin (20) and the presence of filixic acids (19) (usually absent in *D. pallida*), traces of norflavaspidic acid (4), the compounds 25, 26, 30 and the unidentified compounds JU1, JU2 and JU3, see chapter 5.2.

6.2.9 *D. odontoloma* (section 6. *Pallidae*). We had correctly identified material from S. India. It contained virtually the same chemical compounds as *D. juxtaposita*, only the trace compounds 25, JU1, JU2 and JU3 were missing.

6.2.10 *D. arguta* (section 6. *Pallida*) is the north-western North American representative of the *D. villarii* group. It contains similar phloroglucinols as *D. pallida* but less para-aspidin (7), no desaspidin (8), no albaspidin (10), and no tripara-aspidin (20); on the other hand, it contains some filixic acids (19), usually absent from *D. pallida*.

6.2.11 *D. fragrans* (section 4. *Dryopteris*). We had material from North America and from Eurasia. Not all samples contained fraginol (3-B), named after this fern; we found it only in material from Eurasia. The main phloroglucinols found in all five samples are: flavaspidic acid (5), aspidin (6), and traces of albaspidin (10). *D. fragrans* differs from *D. pallida* mainly by the absence or rare occurrence of para-aspidin (7), margaspidin (13), and trispara-aspidin (20).

## 7. General conclusions

The reported observations, with rare exceptions, are in agreement with the following formerly elaborated rules.

### 7.1 Relative stability of phloroglucinol content

The composition of phloroglucinols is usually a highly constant character of a taxon, independent of size, age, or origin of the plant. It remains nearly unchanged after



prolonged storage of the dry rhizomes and can in suitable cases be used for identification of old herbarium material which might otherwise be impossible, e.g. of the *D. remota* type (Widén et al. 1976). It was also of great help in differentiating between the members of the difficult African *D. inaequalis* group (Widén et al. 1973).

In the present study significant variation of a principal component was only observed for aspidin (6) in *D. pallida* subsp. *pallida* from different localities. The same taxon also showed considerable variation for minor compounds: desaspidin (8), albaspidin (10), filixic acid (19), trisdesaspidin (21), and trisflavaspidic acid (27). The other 7 taxa examined in this study were very constant in their chemical composition, independent of their origin. Variation of para-aspidin (7) and trispara-aspidin (20) in *D. stewartii* is a special case and was discussed in chapter 6.2.3. Compounds normally found in trace amounts sometimes also show variation but this may partly be caused by the limits of analytical reliability.

## 7.2 Identical or very similar chemical composition does not prove close taxonomical affinity between different species

A good example is *D. caucasica* (A.Br.) Fraser-Jenkins & Corley which according to Widén et al. (1973) produces virtually the same phloroglucinols as does *D. villarii*. In the present study we found *D. ramosa*, section 9. *Marginatae*, to produce very similar phloroglucinols as does *D. pallida*, section 6. *Pallidae*.

## 7.3 Morphologically closely related taxa may have distinct chemical composition

This was found in our study for *D. pallida* subsp. *pallida* and *D. pallida* subsp. *balearica* (see Table 5). In chemical composition the subsp. *balearica* differs more from subsp. *pallida* than do subsp. *libanotica* and subsp. *raddeana*. It may be recalled that Nardi (1976: 28–29) raised it to specific rank as *D. balearica* (Litard.) Nardi.

## 7.4 Chemical composition is usually a rather conservative character

A well-known example is provided by the following three diploid taxa: *D. intermedia* (Muhlenb. ex Willd.) A. Gray in N. America, *D. azorica* (Christ) Alston in the Azores, and *D. maderensis* Milde ex Alston (= *D. intermedia* subsp. *maderensis* Fraser-Jenkins) on Madeira. By cytogenetic examination they were found to contain homologous genomes (Gibby & Walker 1977, Gibby et al. 1977, Gibby 1979, 1983, 1985a) and to be able to exist as morphologically distinct taxa (Gibby et al. 1977) only owing to prolonged geographical isolation. They would probably intrograde if they were growing sympatrically. We must assume that they developed from an ancestral stock during very long time. Morphological differentiation may have started after the formation of the Atlantic islands. The three taxa are today quite distinct (Gibby et al. 1977) but their chromosomes remained homologous as shown by the pairing behaviour at meiosis of their hybrids (Gibby & Walker 1977, Gibby 1979, 1983, 1985). Remarkably, the composition of phloroglucinols also remained the same (Britton & Widén 1974, Widén et al. 1975, Gibby et al. 1978, see review v. Euw et al. 1980). Quite similar results were reported for the allotetraploids *D. crispifolia* (Azores) and *D. guanchica* (Canary Islands and Iberian peninsula) see Gibby et al. (1977, 1978), Gibby (1979, 1983, 1985). These two plants are morphologically quite distinct but have homologous chromosomes and produce virtually the same phloroglucinols (Widén et al. 1975, Gibby et al. 1978). As mentioned above, a similar correlation could partly be observed for the four subspecies of *D. pallida*. Three

of them, viz. subsp. *pallida*, subsp. *libanotica*, and subsp. *raddeana*, probably have homologous chromosomes and produce virtually the same phloroglucinols; subsp. *balearica* is chemically distinct, but has cytogenetically only tentatively been examined (see 6.1.4).

### 7.5 Similar chemical composition nevertheless often reflects taxonomical relations

As mentioned under 7.4, this is particularly evident in cases of cytogenetical relation. If we compare the chemical composition of the 17 taxa of this study in a more general way with the classification of Fraser-Jenkins (1986) we often find good agreement but sometimes disagreement, see Table 5 and discussion (chapter 6). We must consider the "spectrum" of phenolics which a *Dryopteris* species (or subspecies) produces to be an important taxonomical character, but we must keep in mind that it is only *one* character and therefore not infallible. Some disagreement may also be caused by the fact that the boundaries between sections (Fraser-Jenkins 1986) are not always sharp (e.g. sections 5. *Remotae*, 9. *Marginatae*).

*Note.* We may employ such a correlation for speculating about the possible origin of triploid apomictic *D. remota*. It has been assumed to have originated by a crossing of diploid sexual *D. expansa* with diploid apomictic *D. affinis*. Morphologically this would fit but experiments aimed at producing this hybrid were unsuccessful and the chemical composition does *not* agree (Widén et al. 1970 b). Both morphology and chemistry would fit rather well the assumption of a cross of *D. pallida* or *D. ramosa* with diploid *D. affinis*. We hope that some younger colleague will try to make such hybrids.

## 8. Appendix

Specifies the officially valid JUPAC names (see v. Euw et al. 1985 and Richter et al. 1987) of the most important phloroglucinols (nearly all which are known today). Only the B-homologues with butyryl (= 1-oxobutyl) side chains are given. Many ferns contain in addition (or exclusively) some lower homologues (A-series with acetyl=1-oxoethyl and P-series with propionyl=1-oxopropyl side chains), more rarely also higher homologues (V-series with valeryl=1-oxopentyl side chains) or with ramified side chains. The chemical structures are reviewed in Table 4. The numbering (2-B etc.) of the compounds in the Tables 4, 5 and 6 are the same as used in v. Euw et al. (1980, 1985) and Richter et al. (1987).

### 8.1 Table 6. List of the main phloroglucinols

2-B = Aspidinol B = 1,3-Dihydroxy-5-methoxy-4-methyl-2-(1-oxobutyl)-benzene  
 3-B = Fraginol B = 3,5-Dihydroxy-2,4,4-trimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one (= Methyl-butyryl-filicin acid)  
 4-BB = Norflavaspidic acid = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,4,6-trihydroxy-3-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one  
 5-BB = Flavaspidic acid = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one  
 6-BB = Aspidin BB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,6-dihydroxy-4-methoxy-3-methyl-5-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one  
 7-BB = Para-aspidin BB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,4-dihydroxy-6-methoxy-5-methyl-3-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one  
 8-BB = Desaspidin BB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,4-dihydroxy-6-methoxy-3-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one

- 9-BB = Ortho-desaspidin BB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[2,6-dihydroxy-4-methoxy-3-(1-oxobutyl)-phenyl] methyl-2,5-cyclohexadien-1-one
- 10-BB = Albaspidin BB = 2,2'-Methylene-bis[3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one]
- 11-BB = Phloraspin BB = 1,3,5-Trihydroxy-4-methyl-2-(1-oxobutyl)-6-[2,4-dihydroxy-6-methoxy-3-(1-oxobutyl)-phenyl] methylbenzene
- 12-BB = Phloraspidinol BB = 1,3-Dihydroxy-5-methoxy-4-methyl-2-(1-oxobutyl)-6-[2,4-dihydroxy-6-methoxy-3-(1-oxobutyl)-phenyl]methyl-benzene
- 13-BB = Margaspidin BB = 1,3,5-Trihydroxy-4-methyl-2-(1-oxobutyl)-6-[2,4-dihydroxy-6-methoxy-5-methyl-3-(1-oxobutyl)-phenyl] methyl-benzene
- 14-BB = Methylene-bis-desaspidinol BB = 6,6'-Methylene-bis[1,3-dihydroxy-5-methoxy-2-(1-oxobutyl)-benzene]
- 15-BB = Phloropyron BB = 3[(5-Butanoyl-2,4-dihydroxy-3,3-dimethyl-6-oxocyclohexa-1,4-dienyl)methyl]-4-hydroxy-6-propyl-2*H*-pyran-2-one
- 16-BB = Phloraspyron BB = 3[(3-Butanoyl-2,4-dihydroxy-6-methoxyphenyl)methyl]-4-hydroxy-6-propyl-2*H*-pyran-2-one
- 17-BB = Aemulin BB = 1,3,5-Trihydroxy-4-methyl-2-(1-oxobutyl)-6-[2,6-dihydroxy-4-methoxy-3-methyl-5-(1-oxobutyl)phenyl]methyl-benzene
- 18-BB = Methylene-bis-aspidinol BB = 6,6-Methylene-bis[1,3-dihydroxy-5-methoxy-4-methyl-2-(1-oxobutyl)-benzene]
- 19-BBB = Filixic acid BBB = Trisalbaspidin BBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,4-dihydroxy-3,3-dimethyl-5-(1-oxobutyl)-6-oxo-1,4-cyclohexadienyl]-methylphenyl}-methyl-2,5-cyclohexadien-1-on
- 20-BB = Tris-para-aspidin BBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,4-dihydroxy-6-methoxy-5-methyl-3-(1-oxobutyl)-phenyl]-methyl-phenyl}-methyl-2,5-cyclohexadien-1-one
- 21-BBB = Tris-desaspidin BBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,4-dihydroxy-6-methoxy-3-(1-oxobutyl)phenyl]-methyl-phenyl}-methyl-2,5-cyclohexadien-1-one
- 22-BBB = Trisaspidin BBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,6-dihydroxy-4-methoxy-3-methyl-5-(1-oxobutyl)phenyl]-methyl-phenyl}-methyl-2,5-cyclohexadien-1-one
- 23-BBB = Trisflavaspidic acid BBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{[2,4,6-trihydroxy-3-(1-oxobutyl)-5-[[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]-methyl]phenyl]-methyl}-2,5-cyclohexadien-1-one
- 24-BBB = Trisaemulin BBB = 1,3-Dihydroxy-5-methoxy-2-(1-oxobutyl)-4-methyl-6-{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,6-dihydroxy-4-methoxy-3-methyl-5-(1-oxobutyl)-phenyl]methyl}-benzene
- 25-BBBB = Tetra-albaspidin BBBB = 2,2'-Methylene-bis-{2,4,6-trihydroxy-5-(1-oxobutyl)-1,3-phenylene}-bis-[(methylene)]-[3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one]}. The ABBA homologue was first called "Dryocrassin" = Methylene-bis-norflavaspidic acid
- 26-BBBB = Tetraflavaspidic acid BBBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6{3[3-[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)-phenyl]methly-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl}methyl-2,5-cyclohexadien-1-one
- 28-B = Stenolepin B = 2,3,5-Trihydroxy-2,4-dimethyl-6-(1-oxobutyl)-3,5-cyclohexadien-1-one

30-BB = Methylene-bis-methylphlorobutyrophenone = Methylene-bis[1,3,5-trihydroxy-4-methyl-6-(1-oxobutyl)-benzene]

37-BBBBB = Penta-albaspidin BBBBB = 2-{3-[3-[3-[2,4-Dihydroxy-3,3-dimethyl-5-(1-oxobutyl)-6-oxo-1,4-cyclohexadienyl]]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]-methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)-phenyl}-methyl-3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-cyclohexadien-1-one

38-BBBBBB = Hexa-albaspidin BBBBBB = 2-{3-[3-[3-[3-[2,4-Dihydroxy-3,3-dimethyl-5-(1-oxobutyl)-6-oxo-1,4-cyclohexadienyl]]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl}methyl-3,5-dihydroxy-4,4-dimethyl-2,5-cyclohexadien-1-one

39-BBBBBB = Hexaflavaspidic acid BBBBBB = 3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-{3-[3-[3-[3-[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)-phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)-phenyl}methyl-2,5-hexadien-1-one

40-B = Butyryl-dimethyl-phloroglucinol = 1,3,5-Trihydroxy-2,6-dimethyl-4-(1-oxobutyl)-benzene

## 8.2 Origin of plant material

Abbreviation of collectors' names<sup>3</sup>, and of origins<sup>4</sup> see footnotes for Table 5. When cytological results are given (2x, 3x or 4x) this indicates that chromosomes of at least on plant of the actual population were counted. Former results<sup>14-17</sup> are included for comparison.

8.2.1 *D. villarii*. TR-2747, 15-VII-1969, It.<sup>14</sup> see Widén et al. 1971: 2836. – TR-4829, 20-IX-1978, CH, Kt. Nidwalden, Trübsee, limestone boulders below Joch-Pass, c. 1790 m alt. Several rhizomes. 50 g.

8.2.2 *D. pallida* subsp. *pallida*. TR-116, 1-X-1959, Sd<sup>14</sup>, 15 g dry rhizome.<sup>14</sup> – Second batch from same collection after cult. in Basel, n=41<sup>II</sup> (det. G.V. 17-VI-1969) several rhizomes. 20.4 g. – TR-545, 9-X-1961, It. Lazio, between Itri and Fondi at c. 200 m alt., dry limestone wall with *Cyclamen neapolitanum*, 55.3 g dry weight. – TR-3244, 15-VIII-1971, Ju, Montenegro, sunny limestone rocks near the road at c. 600 m alt. between Kotor and Cetinje. Exospore (27) 32–33 (35) µm long living progeny raised. 1.5 g dried rhizome for analysis.

8.2.3 *D. submontana* G.V.s.n., 16-VIII-1968, Rm<sup>14</sup>, 2n=c. 164 (G. Vida 1960), piece of living plant and spores received in Basel (Nov. 1969), progeny was raised (TR-2862). A dried rhizome (3 g) was used for analysis. – TR-3023 raised in Basel from spores from Spain, Sierra Nevada, N.W. side of the Dornajo (Granada), in shady limestone at 2050 m. leg. H. Metlesics 26-VII-1969, 2n=c 164 (det. G.V. 29-III-1972), 5.3 g dry rhizomes for analysis. – TR-3241, 13-VII-1971, Ju, Valebit, above the Mali Alan Pass, road from Obrovac in Northern direction (to Zagreb), 1200 m alt., on shady limestone rock, E. & M. Meyer, V. Ravnik, H. Kunz, H. Melzer & T. R. Raised from spores in Basel 2n=c 164 (det. G.V.). One rhizome dried 3.8 g for analysis. – TR-3629, 30-IX-1973, Ga, c. 1 km W. of the village Caussols (N. of Grasse), Alpes-mar. in holes of limestone pavement c. 1140 m, H. Rasbach H.L.R. & T.R., 2n=c. 160 (G.V. 26 Oct. 1973 for TR-3569 from the same population); 16 rhizomes gave 255 g dry material.



8.2.4 *D. pallida* subsp. *balearica*. TR-3253, J. Orell s.n. Mai 1971, 5 dry rhizomes = 73 g (see Widén et al. 1971) Mal.<sup>14</sup> A second batch of 35.7 g dry rhizome was obtained from plants raised from spores.

8.2.5 *D. pallida* subsp. *libanotica*. TR-1825 raised in Basel from spores from Cyprus obtained 10-XII-1966 from A. Sleep (Leeds), coll. Merton, diploid. 3.3 g dry rhizome for analysis. – CRFJ-4574 (not quite pure), 4-VIII-1975, Turkey, Antalya, 700 m alt. S. side of Irmosan Pass, N. of Akseki. Nine rhizomes (TR-3926) dried for analysis = 325 g.

8.2.6 *D. pallida* subsp. *raddeana*. TR-1545, 20-VII-1965 Iran, rocks along water coarse running into the Caspian sea near Astara, close to Soviet border (slightly below sea level alt.) leg. E. Hauser, cult. (outdoors) in Basel and propagated from spores  $n=41^{\text{II}}$  (det. G.V. 17-VI-1969). One large rhizome dried (221 g) for analysis – TR-2742 (=TR-949) from same place cult. in Hauser's garden (Lago di Garda), incorrectly reported as subsp. *pallida* (from Mt. Bondone) in Fraser-Jenkins et al. 1975. One rhizome dried for analysis 30 g. – CRFJ-6034–6 and 6041–2, 2-VII-1977, Iran, calc. side gorge, 11 km up Nav valley, above Assalam, Talesh, Gilan, c. 200 m alt. Obtained living and cult. in Basel (TR-4282–4 and 4289–90).  $n=41^{\text{II}}$  (det. M. Gibby, 18-VII-1978), 5 rhizomes dried, 250 g. – PU-19351, 10-VIII-1972, Iran, Mazandaran, 5 km S. of Chalus, 100 m alt., deciduous forest, crevices in stone wall on roadside. Piece of rhizome dried (3.5 g).

8.2.7 *D. nigropaleacea*. IK-6697, 4-VI-1972, Pakistan, Hazara, 2 km N. of Dunga Gali, Murree. Rhizome 6 g dried. – CRFJ-6230–3, 25-VII-1977, Pakistan, Rawalpindi Prov., 1 km N. of Murree on Muzafarabad road, calc., c. 2000 m alt. 4 dry Rhizomes 45 g (TR-4328–4331). In this batch CRFJ-6231=TR-4329 came in by mistake, it was *D. stewartii*. This does not falsify results significantly as this species has phenolics similar to those of *D. nigropaleacea*. – CRFJ-7377, 6-VIII-1978, Pakistan, Murree Hills, Hazara, *Cedrus deodara* forest, c. 3 km N. of Changla Gali, 2400 m. 1 dried rhizome 3.7 g. – CRFJ-8027–41, 11-10-1978, Pakistan, Punjab, 2050 m below *Cedrus* and *Pinus*, c. 3 km N.E. of lower Murree, 2050 m alt. 15 dry rhizomes = 270 g. – CRFJ-8235, 24-X-1978, N. India, U.P. c. 1900 m alt. path banks c. 1.5 km below Trijugi Naryan, Chamoli, one dried rhizome, 75 g.

8.2.8 *D. ramosa*. CRFJ-6280–3, 29-II-1977, Pakistan, Hazara, 0.5 km N. of Changla Gali, Murree-Abbottabad 2450 m alt. (TR-4364-7), four dry rhizomes = 29.2 g. – CRFJ-7373, 7379–3, 6-VIII-1978, Pakistan, Hazara, *Cedrus deodara* forest, 7 km N. of Changla Gali 2400 m alt. together with *D. stewartii*, six dry rhizomes = 100 g. – CRFJ-7418–25, 15-VIII-1978, N. India, Jammu Kashmir, *Pinus* forest, 2400 m alt. S. side of Sind valley, 3 km E. of Gund, together with *D. stewartii*, 8 rhizomes = 580 g dry weight. – CRFJ-7843–51, 10-IX-1978, N. India, H.P., Forest, c. 2700 m alt., 15 km below Satrundi, N. of Tissa, N. of Ravi valley, N.W. of Chamba. nine dry rhizomes = 760 g.

8.2.9 *D. stewartii*. PU-17447, 4-VI-1972, Pakistan, Hazara, 2 km N. of Dunga Gali, N. of Murree, piece of rhizome, 3.7 g. – PU-17514, 4-VI-1972. Pakistan, Hazara, 3 km N. of Changla Gali village, Murree, piece of dry rhizome, 0.7 g. – CRFJ-6298, 25-VII-1977, Pakistan, Hazara, 2450 m alt., 0.5 km N. of Changla Gali (TR-4382), together with *D. nigropaleacea* and *D. ramosa*, one rhizome dried, 10 g. – CRFJ-6329, 25-VII-1977, Pakistan, Hazara, 2400 m alt. calc., 3 km North of Changla Gali, Murree, (TR 4400) together with *D. nigropaleacea*, dry rhizome 20 g. – CRFJ-7370–2, 7374–6, 7378, 7384–8, 6-VIII-1978, Pakistan, Hazara, *Cedrus deodara* forest, c. 3 km N. of Changla Gali, 2400 m alt., calc. 12 rhizomes dried, 210 g. – CRFJ-7426–9, 15-VIII-1978, N. India



Jammu Kashmir, *Pinus* forest, 2400 m alt., S. side of Sind valley, 3 km E. of Gund, with *D. ramosa*, four dry rhizomes, 220 g. – CRFJ-7457, 18-VIII-1978, N. India, Baltistan, 3250 m alt. under open boulders in nullah, c. 2 km above Chanigund, W. of Kirgil, one dry rhizome = 18 g. – CRFJ-7614, 7616, 7651, 26-VIII-1978, N. India H.P. 3000 m alt., rocks under *Cedrus deodara*, N.E. side of Mt. Kattu, below top, 3 dry rhizomes, 60 g. – CRFJ-7852–9, 10-IX-1978, N. India H.P., forest, 2700 m, 15 km below Satrundi, N. of Tissa, together with *D. ramosa*, 8 dry rhizomes, 630 g. – CRFJ-7917–21, 1-X-1978, Pakistan, mid Swat valley, 1450 m alt., stream, 2 km N. of Madyan, 5 dry rhizomes, 130 g. – CRFJ-7938–9, 1-VIII-1978, Pakistan, 1700 m alt., among boulders, c. 5 km above Kolalon, mid Swat valley, 2 dry rhizomes, 60 g.

8.2.10 *D. marginata*. CRFJ-8421–33, 12-XI-1978, N. India, W. Bengal, Darjeeling, c. 2200 m alt., banks + woods above Tensing Norgay rd. c. 1 km N. of Aloo Beri, with *D. nigropaleacea* and others, 13 dry rhizomes = 200 g. – CRFJ-8609–12, 8614–6, 8618–23, 19-XI-1978, N. India, West Bengal, 1550 m, Lebong Forest (*Cryptomeria*), N. of and below Lebong, N. of Darjeeling, 12 dry rhizomes = 50 g. – CRFJ-8795–8, 24-XI-1978, N. India, Assam, 1800 m alt. stream gulley, pine forest, below Peak Lodge, 16 km above Shillong on road to the Peak, Khasi Hills, 4 dry rhizomes = 140 g.

8.2.11 *D. barbiger* subsp. *barbiger*. CRFJ-7433, 16-VIII-1978. N. India, Jammu-Kashmir 2850 m alt., stream bank, Thajwas Valley, c. 2 miles S.W. of Sonamarg, Upper Sind Valley, N.E. of Shrinagar, one dry rhizome 80 g. – CRFJ-7450, 17-VIII-1978. N. India, Baltistan, 3350 m alt., rocks, c. 2 km W. of Meenamarg, E. side of Zojila Pass, Oros-Sanamarg, together with *D. ramosa*, one dry rhizome = 60 g. – CRFJ-7715–20, 1-IX-1978, N. India H.P. 3400 m, steep open meadow, 14 km below top of Rohang Pass on S. side, N. of Manali, N. of Kulu, 6 dry rhizomes = 350 g. Diploid.

8.2.12 *D. blanfordii*. CRFJ-7410–15, 15-VIII-1978 N. India, Jammu-Kashmir, 2400 m alt. *Pinus* forest, S. side of Sind valley, 3 km E. of Gund, N.E. of Shrinagar, 6 dry rhizomes = 420 g. – CRFJ-7401–6, 15-VIII-1978, same place, 6 rhizomes = 115 g. – CRFJ-7430, same place, one rhizome = 12 g. – CRFJ-7431, 15-VIII-1978, same place, one rhizome = 23 g. – CRFJ-7841–2, 10-IX-1978, N. India, H.P., forest, 2700 m alt. 15 km below Satrundi, N. of Tissa, two dry rhizomes = 290 g. Tripl. apog.

8.2.13 *D. para-chrysocoma*. CRFJ-7681–8, 1-XI-1978, N. India N.P. 2450 m alt., rocks in ravine, 5 km above Kothi, S. side of Rohtang Pass, 10 km N. of Manali, N. of Kulu. 8 dry rhizomes = 350 g. Cytology not checked.

8.2.14 *D. woodsii* (= *D. chrysocoma* subsp. *parva*). CRFJ-8754–69, 8771–4, 8779, 22-XI-1978, N. India, W. Bengal, Darjeeling, 1150 m alt., 8 km S. of Kurseong, Siliguri, 20 small dry rhizomes = 250 g. Cytology not checked.

8.2.15 *D. juxtaposita*. CRFJ-7595, 7607, 7615, 7621–9, 7642, 7653, 28-VIII-1978, N. India, H.P. 3000 m alt. rocks under *Cedrus deodara*, N.E. side of Mt. Kattu, below top, near Narkanda, N.E. of Simla, 13 dry rhizomes = 190 g, tripl. apog. – CRFJ-8163–9, 21-X-1978, N. India U.P., 1650 m alt., 6 km N. of Mussoorie on Junna Bridge road, N. of Dehra Dun. 7 dry rhizomes, 400 g. – CRFJ-8210–16, 8218–9, 8281–8, 24-10-1978, N. India U.P., ca. 1900 m alt., path banks, c. 1.5 km below Teijugi Naryan, W. from Sonprayag, N. of Rudraprayag, Chamoli, 17 dry rhizomes = 460 g, tripl. apomictic. – CRFJ-8816–9, 24-XI-1978. N. India, Assam, 1800 m alt. stream gulley, *Pine* forest, below Peak Lodge, 10 km above Shillong on road to the Peak, Khasi Hills, Meghalaya, 4 dry rhizomes = 100 g. – CRFJ-9215–8, 20-12-1978, S. India, Tamil Nadu, Western

Ghats, rocks under woods, above Park, 1100 m alt. 1.5 km S.W. Kodaikanal on Berijam road, Palni Hills, four dry rhizomes = 100 g. – CRFJ-9222–7, same place six dry rhizomes, 70 g. – CRFJ-9278, 9280–2, 25-XII-1978, S. India, Tamil Nadu, 2200 m alt., scattered woods by stream, 1 mile along Lovedale road, S. side of Ootacamund, Nilgiri Hills, Western Ghats, four dry rhizomes = 70 g. – CRFJ-9365–76, 9379, 9381–3, 26-12-1978, S. India, Tamil Nadu, road side, stream banks in forest, 2250 m alt., 16 km W. of Kotagiri on Ootacamund road, Nilgiri Hills, Western Ghats, 16 dry rhizomes, 260 g for analysis.

8.2.16 *D. odontoloma*. CRFJ-9304–13, 9316, 25-XII-1978, S. India, Tamil Nadu, Nilgiri Hills, Western Ghats, 2200 m, scattered woods by stream, 1 mile along Lovedale road, S. side of Ootacamund, 11 dry rhizomes = 150 g, tripl. apomict. – CRFJ-9389–91, 9393–9409, 24-XII-1978, S. India, Tamil Nadu, Nilgiri Hills, Western Ghats, roadside, stream-banks in forest, 2250 m alt., 16 km W. of Kotagiri, on Ootacamund road, with *D. juxtaposita*, 20 dry rhizomes, 150 g for analysis.

8.2.17 *D. arguta*. F. A. Lang 384, 12-IV-1970, Oregon (Canada), Jackson Co: 4 km S. of Ashland, Emigrant Lake, 1/4 mile N. of dam on N. slope of hill above Emigrant Lake, 6 rhizomes, 43.5 g (Widén & Britton 1971 c: 1590). – L. Bonnell, s.n. 20-VII-1970 Canada, Brit. Col., Hornby Island, Haron Rock Point, 150 ft. alt., 10 rhizomes, 415 g. (Widén & Britton 1971 c: 1590). – A. Gerber A-1, 14-IX-1971, USA California, surrounding of San Francisco, S. of San Anselmo, c. 8 miles. N. of the Golden Gate Bridge, in redwood forest, obtained living (14-X-1971) and cult. as TR-3290 in Basel, later in Agarone (S. Switzerland) and propagated from spores. Two pieces of rhizomes separated and dried (3-I-1976) = 31.1 g. – W. Bennert s.n. 29-VIII-1976, USA, Calif. Mixed evergreen forest on the Page Mill road, between Skyline Drive and Foothill Park, Palo Alto, Santa Clara Co. c. 550 m alt., 504 g of dry rhizomes. Plant propagated from spores.

8.2.18 *D. fragrans*. D. M. Britton 1758–61, 3-VII-1969, Canada, Ontario, see Widén & Britton 1971 b: 989–992. Four dry rhizomes, 3 g used for analysis. – D. M. Britton 1857–9, 3-VII-1969, Canada, Ontario, Nipissing Distr. Algonquin Park, see Widén & Britton 1971 b: 989–992 three dry rhizomes, 3.5 g used for analysis. – P. Kallio s.n. 1966–68, Newfoundland, Canada, Churchill falls, seven rhizomes dried 37.2 g. – P. Kallio s.n. 1966–69, Finland, Utsjoki, Puksalskaidi, W. of Kenesjärvi, seven dry rhizomes, 85.9 g, see v. Schantz & Widén (1967), Widén & Britton (1971 b: 989–992). – J. Pintér s.n. 19-VI-1983, Mongolia Bogd Ul, near Ulan Bator mountain on rocks one dried rhizome = 3 g. – J. Pintér, July 1974, USSR, Irkutsky at the lake Baikal, near the village Bolshie Koty, on wet rocks, along a small stream, one rhizome dried, 3 g.

### 8.3 Chemical analysis

Extraction and further analysis was performed as mentioned in chapter 4.4 and described by v. Euw et al. (1980: 301, 1985: 1266, see also chapter 8.4 of this paper).

Table 7 gives the amounts of crude extracts obtained and used for analysis by TLC or by preparative chromatography. This was performed in the first part of this work (European, some Asiatic and N. American species) on columns of unbuffered SiO<sub>2</sub> gel while in the later part of this work (the bulk of Asiatic species) buffered SiO<sub>2</sub> gel was used, avoiding deterioration of sensitive compounds.

8.3.1 Yield of crude extracts and crude phenolic is given in Table 7.

Tab. 7. Giving amounts of dry rhizome used for analysis, yields of crude or "cation-free" ether extract for each batch in g (in % of dry rhizome or stipe bases). This is partly falsified when rhizomes contain soil or other impurities. Whenever the crude acyl-phloroglucinols (= "phenolics") were further concentrated by the MgO or Ba(OH)<sub>2</sub> procedure the corresponding amounts of product are given in g (in % of crude ether extract). n.p. = not prepared. Other abbreviations and footnotes as in Table 5

Name of plant and collectors number		Origin	Dry rhizome in g	Ether extract in g (in %) crude cationfree		Phenolics in g (in %)	
						MgO	Ba(OH) <sub>2</sub>
<i>D. villarii</i> subsp. <i>villarii</i>	TR-2747	It <sup>14</sup>	77	10.44 (13.5)	n.p.	2.20 (2.86)	n.p.
	TR-4829	CH	50	6.1 (12.2)	n.p.	1.20 2.40	n.p.
<i>D. submontana</i>	ACJ s.n.	GB <sup>14</sup>	85	5.2 (6.12)	n.p.	0.49 (0.5)	n.p.
	TR-2862 = prog. GV s.n.	Rm	3	n.p.	n.p.	n.p.	n.p.
	TR-3023	Sp	5.3	0.2 (3.77)	n.p.	0.11 (2.08)	n.p.
	TR-3629	Ga	112.0	13.4 (11.96)	n.p.	2.6 (2.32)	1.86 (1.66)
	TR-3241	Ju	3.8	0.15 (3.86)	n.p.	0.06 (1.58)	n.p.
<i>D. pallida</i> subsp. <i>pallida</i>	TR-116	Sd <sup>14</sup>	1	n.p.	n.p.	n.p.	n.p.
	TR-116	Sd	20.4	2.2 (10.78)	n.p.	0.24 (1.17)	0.37 (1.81)
	TR-545	It	55.3	3.9 (7.04)	n.p.	1.57 (2.8)	0.43 (0.78)
	TR-3244	JU	1.5	0.05 (3.33)	n.p.	0.02 (1.33)	n.p.
<i>D. pallida</i> subsp. <i>balearica</i>	TR-3253	Mal <sup>14</sup>	7.5	0.3 (5.2)	n.p.	0.032 (0.43)	n.p.
	TR-3253	Mal <sup>14</sup>	35.7	2.29 (6.4)	n.p.	0.079 (0.22)	n.p.
<i>D. pallida</i> subsp. <i>libanotica</i>	TR-1825	Cy	3.3	0.09 (2.73)	n.p.	n.p.	n.p.
	CRFJ-4574 = TR-3926	Tk	325.0	19.8 (6.09)	n.p.	2.0 (0.62)	0.13 (0.09)
<i>D. pallida</i> subsp. <i>raddeana</i>	TR-1545	Ir	221.0	8.7 (3.94)	n.p.	1.66 (0.75)	0.26 (0.12)
	TR-2742	Ir	29.7	0.6 (2.02)	n.p.	0.67 (2.26)	n.p.
	CRFJ-6034-6;	Ir	153	7.1 (4.64)	n.p. }	2.16 (0.86)	0.24 (0.10)
	CRFJ-6041-2	Ir	97	4.4 (4.54)			
	PU-19351	Ir	3.3	0.07 (2.12)	n.p.	n.p.	n.p.

Tab. 7 (continuation)

Name of plant and collectors number		Origin	Dry rhizome in g	Ether extract in g (in %) crude cationfree		Phenolics in g (in %)	
						MgO	Ba(OH) <sub>2</sub>
<i>D. nigropaleacea</i>	IK-6697	Pak	6.0	0.49 (8.17)	n.p.	n.p.	0.02 (0.33)
	CRFJ-6230-3	Pak	45.0	2.25 (5.0)	n.p.	0.67 (1.49)	0.39 (0.87)
	CRFJ-7377	Pak	3.7	0.14 (3.78)	n.p.	n.p.	n.p.
	CRFJ-8027-41	Pak	107.0	2.74 (2.56)	2.60 (2.43)	n.p.	n.p.
	CRFJ-8235	N.In	20.4	0.36 (1.76)	n.p.	n.p.	n.p.
<i>D. ramosa</i>	CRFJ-6280-3	Pak	29.0	1.2 (4.14)	n.p.	0.63 (2.17)	0.16 (0.55)
	CRFJ-7373; 7379-83	Pak	16.7	1.1 (6.6)	n.p.	n.p.	n.p.
	CRFJ-7418-25	N.In	33.5	1.87 (5.58)	n.p.	0.38 (1.13)	0.45 (1.34)
	CRFJ-7843-51	N.In	25.0	0.37 (1.48)	n.p.	n.p.	n.p.
<i>D. stewartii</i>	PU-17447	Pak	1.7	0.13 (7.65)	n.p.	n.p.	n.p.
	PU-17514	Pak	0.7	0.06 (8.57)	n.p.	n.p.	n.p.
	CRFJ-6298	Pak	7.2	0.32 (4.44)	n.p.	0.03 (0.4)	0.09 (1.25)
	CRFJ-6329	Pak	10.2	0.71 (6.96)	n.p.	0.2 (2.1)	0.02 (0.20)
	CRFJ-7370-2; 7374-6; 7378; 7384-8	Pak	15.2	0.84 (5.53)	n.p.	n.p.	n.p.
	CRFJ-7426-9	N.In	21.8	0.78 (3.58)	n.p.	n.p.	n.p.
	CRFJ-7457	N.In	11.2	0.54 (4.82)	n.p.	n.p.	n.p.
	CRFJ-7614; 7616; 7651	N.In	15.1	0.39 (2.57)	n.p.	n.p.	n.p.
	CRFJ-7852-9	N.In	236.0	4.71 (2.02)	3.07 (1.30)	1.16 (0.49)	1.43 (0.61)
	CRFJ-7917-21	Pak	15.8	0.18 (1.14)	n.p.	n.p.	n.p.
	CRFJ-7938-9	Pak	13.2	0.47 (3.56)	n.p.	n.p.	n.p.
	CRFJ-8421-33	N.In	27.8	3.5 (11.32)	n.p.	0.26 (0.95)	0.31 (1.12)
<i>D. marginata</i>	CRFJ-8609-12; 8614-6; 8618-23	N.In	12.6	1.51 (11.98)	n.p.	n.p.	n.p.
	CRFJ-8795-8	N.In	17.0	1.26 (7.41)	n.p.	n.p.	n.p.

Tab. 7 (continuation)

Name of plant and collectors number		Origin	Dry rhizome in g	Ether extract in g (in %) crude cationfree		Phenolics in g (in %)	
						MgO	Ba(OH) <sub>2</sub>
<i>D. barbiger</i>	CRFJ-7433	N.In	64.0	2.28 (3.56)	n.p.	n.p.	n.p.
	CRFJ-7450	N.In	47.0	2.33 (4.97)	n.p.	n.p.	n.p.
	CRFJ-7715-20	N.In	305.0	4.52 (1.48)	n.p.	1.56 (0.51)	0.23 (0.08)
<i>D. blanfordii</i>	CRFJ-7410-15	N.In	366.0	6.60 (1.80)	n.p.	0.78 (0.21)	0.26 (0.07)
	CRFJ-7430	N.In	12.7	0.93 (7.3)	n.p.	n.p.	n.p.
	CRFJ-7401-6	N.In	106.0	3.58 (3.38)	n.p.	0.78 (0.74)	0.26 (0.25)
	CRFJ-7431	N.In	20.0	1.70 (8.51)	n.p.	0.26 (1.30)	0.06 (0.3)
<i>D. parachrysocoma</i>	CRFJ-7681-8	N.In	319.0	13.6 (4.24)	n.p.	4.82 (1.51)	0.35 (0.11)
<i>D. woodsiiisora</i>	CRFJ-8754-69; 8771-4	N.In	199.0	10.0 (5.02)	n.p.	1.91 (0.96)	0.27 (0.14)
	CRFJ-8779	N.In	73.0	4.30 (5.89)	n.p.	0.60 (0.82)	0.05 (0.07)
<i>D. juxtaposita</i>	CRFJ-7595; 7607; 7615; 7621-9; 7642; 7653	N.In	23.3	0.89 (3.82)	n.p.	n.p.	n.p.
	CRFJ-8163-9	N.In	23.5	1.13 (4.81)	n.p.	0.30 (1.20)	0.02 (0.09)
	CRFJ-8210-16; 8218-9; 8221-8	N.In	200.0	5.02 (2.51)	4.59 (2.30)	n.p.	n.p.
	CRFJ-8616-9	N.In	23.1	0.71 (3.07)	n.p.	n.p.	n.p.
	CRFJ-9215-8	S.In	25.0	0.78 (3.12)	n.p.	n.p.	n.p.
	CRFJ-9222-7	S.In	13.5	0.67 (4.96)	n.p.	n.p.	n.p.
	CRFJ-9278; 9280-2	S.In	17.9	0.46 (2.57)	n.p.	n.p.	n.p.
	CRFJ-9365-76; 9379; 9381-3	S.In	23.1	1.01 (4.37)	n.p.	n.p.	n.p.
	CRFJ-9378; 9380	S.In	7.3	0.33 (4.5)	n.p.	n.p.	n.p.
	CRFJ-9304-13; 9316	S.In	100.6	6.02 (5.98)	5.68 (5.65)	n.p.	n.p.
<i>D. odontoloma</i>	CRFJ-9389-91; 9393-9409	S.In	22	1.90 (8.64)	n.p.	0.55 (2.50)	0.03 (0.14)



Tab. 7 (continuation)

Name of plant and collectors number		Origin	Dry rhizome in g	Ether extract in g (in %) crude cationfree		Phenolics in g (in %)	
						MgO	Ba(OH) <sub>2</sub>
<i>D. arguta</i>	FL-384	Og <sup>15</sup>	43.5	6.0 (13.8)	n.p.	1.03 (2.37)	n.p.
	L. Bonnell s.n.	BC <sup>15</sup>	415.0	18.6 (4.5)	n.p.	3.10 (0.75)	n.p.
	Gerber A-I = TR-3290	Cal	31.1	1.3 (4.18)	n.p.	0.33 (1.06)	0.20 (0.64)
	W. Bennert s.n. (= TR-4043)	Cal	504.0	34.9 (6.92)	n.p.	5.60 (1.11)	1.0 (0.20)
<i>D. fragrans</i>	DMB-1758-61	Ont. <sup>16</sup>	3.0	0.354 (11.18)	n.p.	0.007 (0.23)	n.p.
	DMB-1857-9	Ont. <sup>16</sup>	3.5	0.362 (10.8)	n.p.	0.057 (1.62)	n.p.
	P. Kallio s.n.	NF <sup>16</sup>	37.2	2.08 (5.6)	n.p.	0.39 (1.06)	n.p.
	P. Kallio s.n.	FS <sup>16</sup>	85.5	2.58 (3.0)	n.p.	0.287 (0.34)	n.p.
	J. Pintér s.n.	USSR	3.0	n.p.	n.p.	n.p.	n.p.
	J. Pintér s.n.	Mong.	3.0	n.p.	n.p.	n.p.	n.p.

8.3.2 Estimation of homologues. The total amount of homologues in representative samples of each taxon was estimated after mild reductive cleavage of a small part of ether extract or crude "MgO-filicin" and subsequent analysis of the resultant mixture of monocyclic compounds in buffered paper chromatography (PC). The homologous filicinic acids (36 and homologues) separate well at pH 4.0 and the aspidinols (2) at pH 8.6 (Widén et al. 1973: 2132-33, 2141). As pointed out by these authors the variation of the acyl side chains (A-V) is mainly restricted to the geminally substituted filicinic acid rings (36). Results of semiquantitative measurements of the filicinic acids in our material are given in Table 8.

#### 8.4 Description of analysis

The procedure is mentioned in chapter 4.4. We give here a short description for one batch as example. All other taxa were treated in a similar way. Amounts of crude extracts are given in Table 7 of final results in Table 5. Wherever preparative isolation of pure, crystalline compounds has been performed, their identity and amounts are given in Table 10.

8.4.1 Analysis of 221 g dry rhizome powder of *D. pallida* subsp. *raddeana* TR-1545. Using the standard method (v. Euw et al. 1980: 302) gave 8.7 g crude ether extract from which 1.66 g "MgO-filicin" and 0.26 g "BaOH<sub>2</sub>-filicin" were obtained. The 1.82 g of the combined filicins were chromatographed on a column of 5.5 g silica gel (Widén et al. 1973) prepared with hexane, using 10 ml solvent for each fraction. The following abbreviations are used: An=acetone, Be=benzene, Chf=chloroform, Et=abs. ethanol,

Tab. 8. The semiquantitative composition of the acylfilicinic acid mixture (36 and homologues) obtained after reductive cleavage of the crude "filicins" (v. Euw et al. 1980). B = butyryl, P = propionyl, A = acetyl. Abbreviation for origin and footnotes as in Table 5

Name of plant and collectors number		Origin	Acylfilicinic acid in % of total amount		
			B	P	A
<i>D. villarii</i> subsp. <i>villarii</i>	TR-2747 <sup>14</sup>	CH	90	5	5
<i>D. pallida</i> subsp. <i>pallida</i>	TR-116 <sup>14</sup>	Sd	66	26	8
	TR-545	It	90	8	2
<i>D. submontana</i>	ACJ s.n. <sup>14</sup>	GB	90	5	5
	GV s.n. <sup>14</sup>	Rm	90	5	5
	TR-3023	Sp	75	20	5
	TR-3629	Ga	96	2	2
	TR-3241	JU	90	7	3
<i>D. pallida</i> subsp. <i>libanotica</i>	TR-3926 = CRFJ-4874	Tk	60	28	2
<i>D. pallida</i> subsp. <i>raddeana</i>	TR-1545	Ir	60	28	2
	TR-2742	Ir	60	25	5
	PU-19351	Ir	93	5	2
	CRFJ-6034-6; 6041-2	Ir	95	5	0
<i>D. nigropaleacea</i>	JK-6697	Pak	45	5	50
	CRFJ-8027-41	Pak	88	2	10
	CRFJ-6230-33	Pak	88	2	10
<i>D. ramosa</i>	CRFJ-7418-25	N.In	50	30	20
	CRFJ-6280-3	Pak	40	40	20
<i>D. stewartii</i>	CRFJ-6298	Pak	85	5	10
	CRFJ-6329	Pak	35	30	35
	CRFJ-7457	N.In	90	5	5
	CRFJ-7852-9	N.In	70	15	15
	PU-17447	Pak	85	5	10
	PU-17514	Pak	85	5	10
<i>D. marginata</i>	CRFJ-8421-33	N.In	90	8	2
<i>D. barbigera</i>	CRFJ-7715-20	N.In	60	20	20
<i>D. blanfordii</i>	CRFJ-7431	N.In	40	20	40
	CRFJ-7841-2	N.In	35	30	35
<i>D. para-chrysocoma</i>	CRFJ-7681-8	N.In	50	40	10
<i>D. juxtaoposita</i>	CRFJ-8163-9	N.In	70	5	25
	CRFJ-8816-9	N.In	60	5	35
	CRFJ-9215-8	S.In	70	5	25
<i>D. odontoloma</i>	CRFJ-9389-91; 9393-9409	S.In	70	10	20
<i>D. arguta</i>	FL-384 <sup>15</sup>	Og	14	43	43
	A. Gerber A-I = TR-3290	Cal	14	43	43
	W. Bennert s.n. = TR-4043	BC	10	45	45
<i>D. fragrans</i>	DMB-1758-61 <sup>16</sup>	On	84	8	8
	Kallio s.n. <sup>17</sup>	FS	89	10	1
	Kallio s.n. <sup>17</sup>	NF	98	1	1

Fr=fraction, He=hexane, Me=methanol, Mixtures=Vol/vol. Results are given in Table 9.

Tab. 9. Chromatography of 1.82 g crude "filicians" of *D. pallida* subsp. *raddeana* (TR-1545) on 55 g SiO<sub>2</sub> (Silica Gel "Merck" für Säulenchromatographie Korngröße 0.05–0.2 mm)

Fraction No.	Solvent for elution	Eluate		
		Amount in mg	Spots in TLC	Further treatment and solvent for crystallisation
1–7	He-Be 1:1	201	albaspidin (10) aspidin (6)	83.4 mg cryst. Albaspidin-BB, PB, PP (10-BB, PB, PP) from An
8–17	He-Be 1:1	743	aspidin-BB (6-BB) para-aspidin (7) (aspidinol (2))	422 mg cryst. para-aspidin-BB(PB) (7-BB, PB) from An
18–52	He-Be 1:1	94	aspidin AB (6-AB) para-aspidin (7)	Fr 18–77 united (218 mg) and and rechromatographed on 7 g SiO <sub>2</sub> gave 3 mg cryst. Tris- para-aspidin-BBB (20) from An
68–77	Be	124	trispara-aspidin (20) (para-aspidin (7)) trispara-aspidin (20) (aspidinol (2))	
78–107	Be	281	aspidinol (2)	
108–197	Be-Chf 1:1 pure Chf	159	Flavaspidic acid (5) butyryl-filicinic acid (36)	No crystals obtained

8.4.2 Pure crystalline compounds. The amounts of pure crystalline compounds (if any) which were isolated in each batch are given in Table 10.

Tab. 10. Pure crystalline compounds isolated from each of the analysed taxa. In compounds consisting of several homologues the main homologue is marked with fat letters and trace homologues omitted. Abbreviations and footnotes as in Table 5

Name of plant and collectors number		Amount of rhizome used (g)	Origin	Compounds isolated in crystals (mg)
<i>D. villarii</i> ssp. <i>villarii</i>	TR-2747 <sup>14</sup>	(77)	It	para-aspidin-BB = 7BB (9), desaspidin-BB = 8-BB (23), filixic acid-BBB = 19-BBB (151)
	TR-4829	(50)	CH	filixic acid-BBB = 19-BBB (24)
<i>D. pallida</i> ssp. <i>pallida</i>	TR-545	(55)	It	albaspidin-BB, PB, PP = 10-BB, PB, PP (2.3), para-aspidin-BB, PB = 7-BB (PB) (206), trispara- aspidin-BBB = 20-BBB (3)
	TR-116	(20)	Sd	albaspidin-BB, PB, PP = 10-BB, PB, PP (6)

Tab. 10 (continuation)

Name of plant and collectors number		Amount of rhizome used (g)	Origin	Compounds isolated in crystals (mg)
<i>D. submontana</i>	ACJ s.n. <sup>14</sup>	(85)	GB	albaspidin-BB = 10-BB (21), para-aspidin-BB = 7-BB (5.5)
	TR-3629	(112)	Ga	albaspidin-BB = 10-BB (32.7), filixic acid-BBB = 19-BBB (98), para-aspidin-BB = 7-BB (315), aspidin-BB = 6-BB (25), desaspidin-BB = 8-BB (9.5), trisdesaspidin-BBB = 21-BBB (12.3), aspidinol-B = 2-B (2.3)
<i>D. pallida</i> <i>ssp. libanotica</i>	CRFJ-4574 = TR-3926	(325)	Tk	albaspidin-BB, PB, PP = 10-BB, PB, PP (91), para-aspidin-BB, PB = 7-BB, PB (365), trispara-aspidin-BBB = 20-BBB (2)
<i>D. pallida</i> <i>ssp. raddeana</i>	TR-1545	(221)	Ir	albaspidin-BB, PB, PP = 10-BB, PB, PP (23), para-aspidin-BB, PB = 10-BB, PB (22), trispara-aspidin-BBB = 20-BBB (2), aspidinol-B, P = 2-B, P (43)
	CRFJ-6034-36; 6041-2	(250)	Ir	albaspidin-BB, PB, PP = 10-BB, PB, PP (15), para-aspidin-BB, PB = 7-BB, PB (161), trispara-aspidin-BBB, PBB, PBP = 20-BBB, PBB, PBP (13)
<i>D. nigropaleacea</i>	TR-6230-33	(45)	Pak	albaspidin-BB, PB, PP, AB, AP = 10-BB, PB, PP, AB, AP (0.5), filixic acid-BBB = 19-BBB (4.7), flavaspidic acid-BB = 5-BB (24.5)
	CRFJ-8027-41	(107)	Pak	albaspidin-BB = 10-BB (3), flavaspidic acid-BB = 5-BB (98), trisflavaspidic acid-ABB = 23-ABB, (16, not quite pure)
<i>D. ramosa</i>	CRFJ-6280-83	(29)	Pak	filixic acid-PBB, PBP, ABB = 19-PBB, PBP, ABB (13.6), flavaspidic acid-BB, PB = 5-BB, PB (20)
	CRFJ-7843-51	(25)	N.In	flavaspidic acid-BB, PB = 5-BB, PB (12), filixic acid-BBB, PBB, PBP = 19-BBB, PBB, PBP (13)
<i>D. stewartii</i>	CRFJ-7852-59	(236)	N.In	filixic acid-BBB, PBB, PBP, ABB, ABP = 19-BBB, PBB, PBP, ABB, ABP (3), flavaspidic acid-BB, PB = 5-BB, PB (20)
<i>D. marginata</i>	CRFJ-8421-33	(101)	N.In	filixic acid-BBB, PBB, PBP, ABB = 19-BBB, PBB, PBP, ABB (7), albaspidin-BB, PB = 10-BB, PB (37)

Tab. 10 (continuation)

Name of plant and collectors number		Amount of rhizome used (g)	Origin	Compounds isolated in crystals (mg)
<i>D. barbigera</i>	CRFJ-7715-20	(305)	N.In	filixic acid-BBB, PBB = 19-BBB, PBB(27), filixic acid-BBB, PBB, PBP, ABB = 19-BBB, PBB, ABB(58), trisflavaspidic acid-BBB, PBB, ABB = 23-BBB, PBB, ABB (14, not quite pure)
<i>D. blanfordii</i>	CRFJ-7410-15	(366)	N.In	filixic acid-PBB, PBP, ABA, ABP = 19-PBB, PBP, ABB, ABP(16.5), flavaspidic acid-BB, PB = 5-BB, PB(21)
<i>D. para-chrysocoma</i>	CRFJ-7681-88	(319)	N.In	albaspidin-BB, PB = 10-BB, PB(3), filixic acid-BBB, PBB = 19-BBB, PBB(9), filixic acid-BBB, PBB, PBP, ABB = 19-BBB, PBB, PBP, ABB(55)
<i>D. juxtaposita</i>	CRFJ-8210-16; 8818-19; 8821-28	(200)	N.In	albaspidin-BB, PB = 10-BB, PB(5), albaspidin-BB, PP, AB = 10-BB, PP, AB(3), filixic acid-BBB, ABP(16), filixic acid-BBB, PBB, PBP, ABB, ABP = 19-BBB, PBB, PBP, ABB, ABP(3), flavaspidic acid-BB = 5-BB(74). In addition JU-1, JU-2, JU-3, (amorphous, not quite pure)
<i>D. odontoloma</i>	CRFJ-9304-13; 9316	(101)	S.In	albaspidin-BP, PP, AB, AP = 10-PB, PP, AB, AP(4), filixic acid-BBB, PBB, ABB, PBP = 19-BBB, PBB, ABB, PBP(7), flavaspidic acid-BB, PB = 5-BB, PB(82)
<i>D. arguta</i>	L. Bonnell s.n. <sup>15</sup>	(415)	BC	filixic acid-BBB, PBB, PBP = 19-BBB, PBB, PBP(70), flavaspidic acid-BB, PB = 5-BB + PB(435), flavaspidic acid-AB = 5-AB(166)
	TR-4043 = W. Bennert s.n.	(504)	Cal	filixic acid-PBB, PBP, ABB, ABP = 19-PBB, PBP, ABB, ABP(23), flavaspidic acid BB, PB = 5-BB, PB(90), flavaspidic acid-AB = 5-AB(97)
<i>D. fragrans</i>	P. Kallio s.n. <sup>17</sup>	(48)	FS	aspidin-BB = 6-BB(18)



### 8.5 Remark on close relation of *D. pallida* subsp. *pallida* with subsp. *raddeana* and correction of former incorrect statement

We have the following reason to assume that the chromosomes of subsp. *raddeana* are homologous to those of subsp. *pallida*. When working on *D. tyrrhena* Fraser-Jenkins, Vida & Reichstein (1975) these authors showed that *D. tyrrhena* is most probably an allo-tetraploid species arisen by chromosome doubling in a diploid hybrid *D. pallida*  $\times$  *D. oreades* (= *D. abbreviata* sensu Manton). *D. tyrrhena* should therefore have the genome formula (PaPaOrOr) if we denote one genome of *D. pallida* as (Pa) and one genome of *D. oreades* as (Or). In order to prove this assumption the meiosis of the two back crosses, the naturally occurring *D.  $\times$  sardoa* (= *oreades*  $\times$  *D. tyrrhena*) and the experimentally produced hybrid *D. pallida*  $\times$  *D. tyrrhena* were examined. The pairing behaviour of the chromosomes of both hybrids was in agreement with the assumed parentage, both produced c. 40<sup>II</sup>, the remaining c. 43 chromosomes as univalents.

We only realised much later that the spores (TR-949) which we had used for experimental hybridisation were incorrectly assumed to be spores of subsp. *pallida*; in fact they were spores of subsp. *raddeana*. This has the following reasons. The plant (TR-949) from which the spores and part of the rhizome were taken (on 12th Sept. 1963) was growing in the garden of E. Hauser near Gargnano (Lago di Garda, N. Italy). It was diploid ( $n=41^{\text{II}}$ , G.V. 17-VI-1969) and E. Hauser believed to remember (incorrectly) to have collected it some years before on Mt. Bondone (near Trento, N. Italy) at c. 450–500 m alt. This place was revisited on 12-13-VII-1970 by A. Sleep, G. & K. Vida, T. Reichstein led by E. Hauser. In spite of many hours of careful search we failed to find a single specimen of *D. pallida* nor any other member of the *D. villarii* group at c. 400–700 m alt. (*D. villarii* diploid, grows at c. 1600 and c. 1800 m alt. on dolomitic scree on M. Bondone). *D. pallida* obviously does not grow on Mt. Bondone, this is in agreement with the distribution map of E. Nardi (1976:14) for *D. pallida* on which the point for M. Bondone in N. Italy is far outside the area of *D. pallida*, which in Italy is confined to S. Italy. Careful examination of plant TR-949 by C.R.F.J. and T.R. revealed that it was subsp. *raddeana* which E. Hauser had obviously collected some years before 1963, in Persia on the W. shore of the Caspian Sea.

We still believe that *D. oreades* (OrOr) and *D. pallida* subsp. *pallida* (PaPa) are the true ancestors of *D. tyrrhena* (OrOrPaPa). The true first parent of the experimental triploid hybrid *D. pallida*  $\times$  *D. tyrrhena* is, however, subsp. *raddeana* (not subsp. *pallida*). This triploid hybrid was therefore formed by fusion of one genome (Ra) of subsp. *raddeana* with one genome (OrPa) of *D. tyrrhena* and must possess the formula (OrPaRa). The formation of c. 40 pairs at meiosis of this hybrid shows that it must contain two homologous genomes. This can best be understood by assuming that subsp. *pallida* (PaPa) and subsp. *raddeana* (RaRa) contain homologous genomes and form pairs with formula (PaRa) which for better understanding may be written as (PaPa'). This would mean that subsp. *pallida* and subsp. *raddeana* can exist as distinct taxa only due to their geographical and perhaps some ecological separation. For a rigid proof of this assumption further experimental hybridisation work would be desirable.

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