Ann. Bot. Fennici 34: 21–26
Helsinki 7 March 1997
ISSN 0003-3847


# New phloroglucinol derivatives in *Dryopteris subimpressa* (Pteridophyta, Dryopteridaceae)

Carl-Johan Widén, Christopher R. Fraser-Jenkins & Tadeus Reichstein†

*Widén, C.-J., Department of Pharmacognosy, Viikinkari 5, FIN-00560 University of Helsinki, Finland**
*Fraser-Jenkins, C. R., Newcastle House, Bridgend, Glamorgan, CF31 4HD, United Kingdom*
*Reichstein, T.†, Institut für Organische Chemie, Universität Basel, St. Johanns Ring 19, CH-4056 Basel, Switzerland*

*Received 2 August 1996, accepted 20 September 1996*

The phloroglucinol composition of *Dryopteris subimpressa* Loyal from North India has been investigated for the first time. This diploid sexual species contains almost exclusively propionyl (P) homologues of flavaspidic acid (*5*), aspidin (*6*), desaspidin (*8*) and trisdesaspidin (*21*). The following homologues have been isolated in crystalline form for the first time: aspidin PP (*6*-PP); desaspidin PP (*8*-PP); desaspidin AP/PA (*8*-AP/PA) and trisdesaspidin PBP (*21*-PBP). These preparations contain only minor amounts of acetyl (A) and butyryl (B) homologues as found by mass spectrometry (MS) and reductive degradation.

Key words: *Dryopteris subimpressa*, propionyl (P) derivatives of phloroglucinols, Pteridophyta

## INTRODUCTION

Continuing our studies on anthelmintically active phloroglucinol derivatives in ferns on a worldwide basis (Widén *et al.* 1991–1993, 1996, Fraser-Jenkins & Widén 1993) we have investigated *Dryopteris subimpressa* Loyal from North India. According to the classification of Fraser-Jenkins it belongs to subgenus Dryopteris, section Marginatae (Fraser-Jenkins 1986).

*D. subimpressa* is a diploid species (2n = 82) that occurs in India (eastern parts of W. Himalaya and E. Himalaya in Sikkim), Nepal, China (Yunnan) (Gibby 1985, Fraser-Jenkins 1989). It is a somewhat uncommon Sino-Himalayan species although widespread. So far there are no published records on phloroglucinols in *D. subimpressa*.

## MATERIAL AND METHODS

### Plant material

The material of *D. subimpressa* was collected by Christopher Fraser-Jenkins during his expedition in 1978 and treated in

* Corresponding address: Pharmacy of Maunula, Metsäpurontie 23, FIN-00630 Helsinki, Finland.


Fig. 1. A fertile frond of *Dryopteris subimpressa*.

the same way as described in previous papers (e.g. Widén *et al*. 1992, 1993). Voucher specimens are deposited at the Botanical Museum, University of Helsinki (H). The collection data are: N. India, Uttar Pradesh, Chamoli, below Kedarnath, Jangal Chatti, 2 450 m, 26.XI.1978, C. R. Fraser-Jenkins 8391, 8393, 8400, 8403, 8405-08. In Fig. 1 we reproduce a frond of *D. subimpressa*.

## Extraction procedure and analysis of rhizomes

This was performed by the methods described in detail by von Euw *et al.* 1980, 1985. Amounts of crude extractives are listed in Table 1. The residue of the Me-Phase (2.45 g) was separated on 73.5 g silica gel buffered at pH 4 and that of the He-Phase (1.50 g) on 45 g silica gel buffered at pH 6, see below.

### *Me-phase*

The fractions 27–81 eluted with He-benzene 1:1 contained minute amounts of albaspidin, no crystals obtained. This compound was presumably an artefact that was formed in the work-up conditions by rottlerone change (cf. Widén *et al.* 1996). No albaspidin was detected when crude ether ex-

tract/filicin was chromatographed on TLC. The fractions 82–86 (benzene) gave 40 mg desaspidin, m.p. 137–138°C from Me (not investigated in more detail). The fractions 87–93 eluted with benzene-chloroform 3:1 afforded the following three different crystallates of desaspidin when crystallised from Me: 1) 152.2 mg, m.p. 105–108°C; 2) 39 mg, m.p. 135–139°C and 3) 2.0 mg, m.p. 139°C. Sample 2 was studied in detail, see Results and Discussion.

Fractions 94–101 (benzene-chloroform 3:1) gave 51.7 mg desaspidin, m.p. 119°C, from methanol. In TLC it separated into two spots (not studied in more detail). Fractions 102–120 (benzene-chloroform 3:1) contained desaspidin and trisdesaspidin (TLC). They afforded 0.5 mg desaspidin, m.p. 172–175°C (Me). It was studied by MS, see Results and Discussion. Fractions 121–134 (benzene-chloroform 3:1) gave 2.9 mg trisdesaspidin, m.p. 118–123°C from Me. It was studied by MS, see Results and discussion. The fractions 135–170 (benzene-chloroform 3:1) contained flavaspidic acid, no crystals obtained.

### *He-phase*

The fractions 1–15 eluted with hexane contained small amounts of albaspidins (artefact), no crystals obtained. The fractions 16–26 (He) gave two crystallates:

1) 12.5 mg of aspidin, m.p. 123–127°C, containing traces of albaspidin, not studied in detail and
2) 30.3 mg of aspidin, m.p. 135–137°C, for closer examination see Results and Discussion.

Both samples were crystallised from acetone. The last fractions 27–132, eluted with mixtures He, benzene and chloroform, contained albaspidin and aspidin as well as traces of desaspidin. No uniform crystallates were obtained.

For final identification of individual phloroglucinols we had a complete set of pure reference substances (for structure and numbering, see Widén *et al.* 1991, 1993). The total amount of homologues in *D. subimpressa* was estimated after mild reductive cleavage of 10 mg "MgO-filicin" and subsequent analysis of the resultant mixture of acylfilicinic acids (*36*) by paper chromatography (PC) at pH 4.0 (Widén *et al.* 1973, 1992). It was found that *D. subimpressa* contained almost exclusively propionylfilicinic acid (*36*-P, 85%), accompanied by some acetylfilicinic acid (*36*-A, 10%) and traces of butyrylfilicinic acid (*36*-B, 5%).

## RESULTS AND DISCUSSION

The analytical results for *D. subimpressa* are summarized in Table 2. It contained new homologues of aspidin (*6*), desaspidin (*8*), and trisdesaspidin (*21*), the structures of which could be elucidated by MS and by analysis of the monocyclic phloroglucinols formed by mild reductive degradation (Widén *et al.* 1973, 1992). Detailed discussion of the isolated compounds follow below.

### Aspidin-PP (*6*-PP)

IUPAC name: 3,5-dihydroxy-4,4-dimethyl-2-(1-oxopropyl)-6-[2,6-dihydroxy-4-methoxy-3-methyl-5-(1-oxopropyl)-phenyl]-methyl-2,5-cyclohexadien-1-one.

In MS in the molecular ion region peaks at m/z 446 (weak), 432 (main peak), 418 (weak) and 404

Table 1. Amounts of crude extractives in *Dryopteris subimpressa*, n.s. = not studied/processed, Me = methanol, He = hexane.

| | Sample no. | Dried rhizomes, g | Crude ether extract, g (%) | Cation-free ether extract[1], g (%) | Residue of Me-phase, g (%) | Residue of He-phase, g (%) | Crude MgO-filicin, g (%) | Crude $Ba(OH)_2$-filicin, g (%) |
|---|---|---|---|---|---|---|---|---|
| CRFJ-8391, 8393, 8400, 8403, 8405–08 | 1 | 257 | 5.46 (2.12) | 4.75 (1.85) | 2.45 (51.6) | 1.54 (32.4) | n.s. | n.s. |
| | 2 | 10.6 | 0.41 (3.88) | n.s. | n.s. | n.s. | 0.072 (0.75) | 0.096 (1.01) |

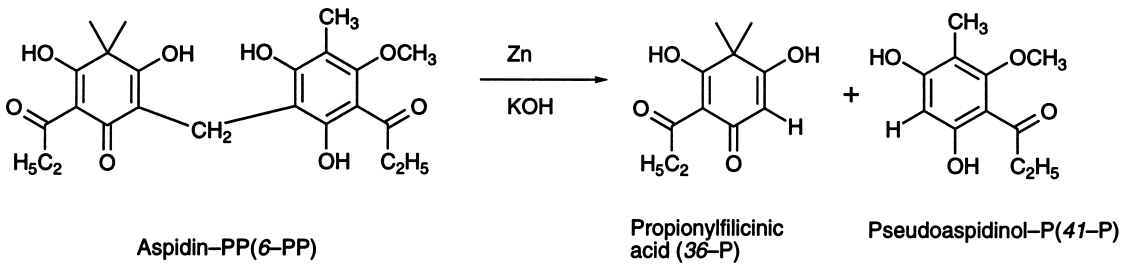



Fig. 2. Main products formed by reductive alkaline cleavage of aspidin (*6*), m.p. 134–135°C, isolated from *Dryopteris subimpressa.* This sample mainly consisted of aspidin-PP (*6*-PP) = $C_{23}H_{28}O_8$ (432) containing only minute amounts of higher and lower homologues as impurities (see text).

(small) were found. They correspond to homologues PB/BP (*6*-PB/BP), PP/AB (*6*-PP/AB), AP/PA (*6*-AP/PA) and AA (*6*-AA), respectively.

After reductive alkaline cleavage propionylfilicinic acid (*36*-P) as well as small spots of acetylfilicinic acid (*36*-A) and butyrylfilicinic acid (*36*-B) were detected on papers buffered at pH 4.0, and pseudo-aspidinol B (*41*-B) and pseudoaspidinol P (*41*-P, main spot) on papers buffered at pH 8.6 (Fig. 2). Unfortunately, no pseudo-aspidinol P (*41*-P) was available for our chromatographic studies. However, the coloration with fast blue B (light brown) was exactly the same as that of pseudo-aspidinol B (*41*-B) and the mobilities on paper chromatography (PC) (Rf – 0.38 and Rf – 0.63, respectively) were exactly the expected ones. See also corresponding results on the homologous aspidinols (*2* A–V) and desaspidinols (*42* A–V) in PC (Widén *et al.* 1973, 1975).

In the light of the above results, the sample melting at 134–135°C mainly consists of aspidin-PP (*6*-PP) containing minute amounts of A and B homologues as impurities.

In thin-layer chromatography the mobility of aspidin-PP (*6*-PP) is similar to that of aspidin-BB (*6*-BB).

## Desaspidin-PP (*8*-PP)

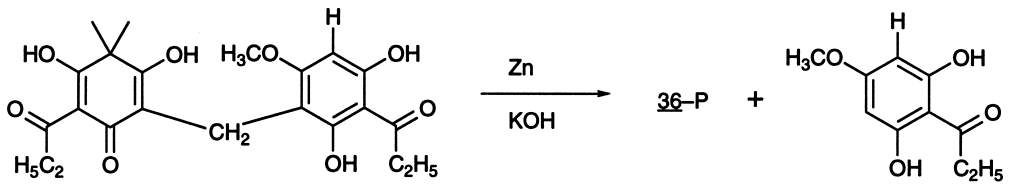
IUPAC name: 3,5-dihydroxy-4,4-dimethyl-2-(1-oxopropyl)-6-[2,4-dihydroxy-6-methoxy-3-(1-oxopropyl)-phenyl]-methyl-2,5-cyclohexadien-1-one.

In MS in the molecular ion region peaks at m/z 446 (*8*-BB, weak), 432 (*8*-PB, BP, small), 418 (*8*-PP, AB, BA, main peak), 404 (*8*-AP, PA, small) were found.

Table 2. Semiquantitative composition of the phloroglucinol derivatives of *Dryopteris subimpressa*. Estimated from intensity of spots in TLC and yield in preparative column chromatography. +++ = 20% or more of the crude filicin mixture, ++ = 10–20%, + = 5–10%, (+) = 1–5%, – = < 1%.

| Taxon and ploidy | Coll. no. | Origin | Flavaspidic acid PP (*5*-PP) [1] | Flavaspidic acid AP/PA (*5*-AP/PA) | Aspidin PP (*6*-PP) [1] | Aspidin AP/PA (*6*-AP/PA) | Desaspidin PP (*8*-PP) [1] | Desaspidin AP/PA (*8*-AP/PA) | Trisdesaspidin PBP (*21*-PBP) [2] |
|---|---|---|---|---|---|---|---|---|---|
| 2x, s | CRFJ-8391, 8393, 8400, 8403, 8405–08 | N. India | ++ | + | ++ | + | ++ | + | + |

1) These compounds also contain traces of B homologues (see Results and discussion).
2) *21* also contains minute amounts of B and A homologues (see Results and discussion).




Desaspidin–PP(*8*–PP)

Desaspidinol–P(*42*–P)

Fig. 3. Main products formed by reductive alkaline cleavage of desaspidin (*8*), m.p. 135–137°C, isolated from *Dryopteris subimpressa*. This sample mainly consisted of desaspidin-PP (*8*-PP) = $C_{22}H_{26}O_8$ (418) containing minor amounts of higher and lower homologues as impurities (see text). Another sample of desaspidin, m.p. 172–175°C, proved most likely to be a mixture of the lower homologues of desaspidin AP (*8*-AP) and PA (*8*-PA) = $C_{21}H_{24}O_8$ (404).

After reductive alkaline cleavage desaspidinol A (*42*-A), desaspidinol P (*42*-P, main spot) and desaspidinol B (*42*-B, trace) were detected on papers buffered at pH 8.6, and acetylfilicinic acid (*36*-A), propionylfilicinic acid (*36*-P) and butyrylfilicinic acid (*36*-B, trace) on pH 4.0 papers (Fig. 3).

Accordingly this sample of desaspidin (*8*) mainly consisted of desaspidin-PP (*8*-PP) (Fig. 3) containing minor amounts of the A and B homologues listed under MS results above.

In TLC desaspidin-PP (*8*-PP) has the same mobility as that of desaspidin-BB (*8*-BB).

### Desaspidin AP/PA (*8*-AP/PA)

This minor sample was only investigated with MS. One major peak at m/z 404 corresponding to homologues *8*-AP/PA was traced. Moreover, weak peaks at m/z 432, 418, 404 and 390 were found consisting of the homologues considered under desaspidin-PP (*8*-PP) except for m/z 390, which may be the homologue *8*-AA. Although no degradation was made this sample may consist of the homologues *8*-AP and PA with only acetyl and propionyl side chains in their molecules.

In TLC the mobility of *8*-AP/PA is slightly slower than that of *8*-PP.

### Trisdesaspidin PBP (*21*-PBP)

IUPAC name: 3,5-dihydroxy-4,4-dimethyl-2-(1-oxopropyl)-6-{2,4,6-trihydroxy-3-(1-oxobutyl)-5-[2,4-dihydroxy-6-methoxy-3-(1-oxopropyl)phenyl]-methyl-phenyl}-methyl-2,5-cyclohexadien-1-one.

In MS the parent peak in the molecular ion region was recorded at m/z 626 corresponding to trisdesaspidins PBP/ABB (*21*-PBP/ABB). Minor peaks at m/z 612 and 598 were found as well. They correspond to homologues *21*-ABP/PBA and *21*-ABA, respectively. Also noteworthy is a relative abundant peak at m/z 640 corresponding to homologues *21*-PBB/BBP. However, we consider the peak at m/z 640 and probably that at m/z 598 to be artefacts due to the easily occurring rottlerone change in the MS of 3 to 6 ring compounds (cf. Lounasmaa *et al.* 1973, Richter *et al.* 1987, Widén *et al.* 1994).

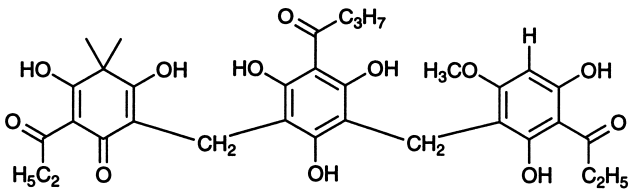
Analogous to the above results on aspidin (*6*) and desaspidin (*8*), we consider that our sample of trisdesaspidin (*21*) mainly consists of the homologue PBP (*21*-PBP, Fig. 4) containing minute amounts of the homologues discussed above in connection of the MS results.

### Flavaspidic acid PP (*5*-PP)

IUPAC name: 3,5-dihydroxy-4,4-dimethyl-2-(1-oxopropyl)-6-[2,4,6-trihydroxy-3-methyl-5-(1-oxopropyl)-phenyl]-methylcyclohexadien-1-one.

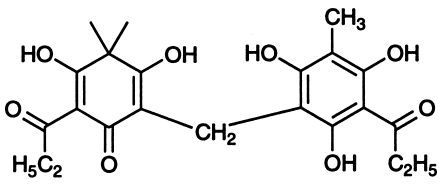
No flavaspidic acid (*5*) could be isolated in the present work. It was only traced by TLC. However, by analogy with the findings on aspidin PP (*6*-PP) and desaspidin PP (*8*-PP) the flavaspidic acid (*5*) may consist mainly of the homologue *5*-PP (Fig. 5).

*D. subimpressa* studied in the present work is unique among the species of *Dryopteris* investigated so far as it contains almost exclusively propionyl side chains (P homologues) in both rings




Trisdesaspidin–PBP(*21*–PBP)

Fig. 4. Chemical structure of trisdesaspidin (*21*), m.p. 118–123°C, isolated from *Dryopteris subimpressa*. This sample presumably consisted of trisdesaspidin-PBP (*21*-PBP) = $C_{33}H_{38}O_{12}$ (626) containing small amounts of trisdesaspidin ABP/PBA (*21*-ABP/PBA) = $C_{32}H_{36}O_{12}$ (612).




Flavaspidic acid–PP(*5*–PP)

Fig. 5. Chemical structure of flavaspidic acid PP (*5*-PP) = $C_{22}H_{26}O_8$ (418).

(substances *5, 6, 8*) or in case of *21* in both terminal rings.

For the biosynthetic formation of the acyl side chains in *Dryopteris* phloroglucinol derivatives, see discussions by Huhtikangas *et al.* 1978, Penttilä 1967, 1969 and references therein.

*Acknowledgements.* The authors thank Mrs Helly Rissanen, Kuopio, for technical aid in the laboratory.

## REFERENCES

Fraser-Jenkins, C. R. 1986: A classification of the genus Dryopteris (Pteridophyta: Dryopteridaceae). — Bull. Br. Mus. Nat. Hist. (Bot.) 14: 183–218.

Fraser-Jenkins, C. R. 1989: A monograph of Dryopteris (Pteridophyta: Dryopteridaceae) in the Indian subcontinent. — Bull. Br. Mus. Nat. Hist. (Bot.) 18: 323–477.

Fraser-Jenkins, C. R. & Widén, C.-J. 1993: Phloroglucinol derivatives in Dryopteris ardechensis and D. corleyi (Pteridophyta, Dryopteridaceae). — Ann. Bot. Fennici 30: 43–51.

Gibby, M. 1985: Cytological observations on Indian subcontinent and Chinese Dryopteris and Polystichum (Pteridophyta: Dryopteridaceae). — Bull. Br. Mus. Nat. Hist. (Bot.) 14: 1–42.

Lounasmaa, M., Widén, C.-J. & Reichstein, T. 1971: Die Massenspektren von dreikernigen pflanzlichen Phlorogluciden. — Helv. Chim. Acta 44: 2850–2857.

Penttilä, A. 1967: On the biosynthesis of Dryopteris acylphloroglucinols. — Acta Polytechn. Scand. CH. 64: 1–73.

Penttilä, A. 1969: Dryopteris – asylphloroglusinolien biosynteesistä. — Suomen Kemistilehti A 42: 27–33.

Richter, W. J., Raschdorf, F., von Euw, J., Reichstein, T. & Widén, C.-J. 1987: Field-desorption mass spectra of fern phloroglucinols containing three to six ring constituents. — Helv. Chim. Acta 70: 881–893.

von Euw, J., Lounasmaa, M., Reichstein, T. & Widén, C.-J. 1980: Chemotaxonomy in Dryopteris and related fern genera. — Studia Geobotanica 1: 275–311, Trieste.

von Euw, J., Reichstein, T. & Widén, C.-J. 1985: The phloroglucinols of Dryopteris aitoniana Pichi-Serm. (Dryopteridaceae, Pteridophyta). — Helv. Chim. Acta 68: 1251–1275.

Widén, C.-J., Faden, R. B., Lounasmaa, M., Vida, G., von Euw, J. & Reichstein, T. 1973: Die Phloroglucide von neun Dryopteris-Arten aus Kenya sowie der D. oligodonta (Desv.) Pic.-Serm. und D. "dilatata" von den Canarischen Inseln. — Helv. Chim. Acta 56: 2125–2151.

Widén, C.-J., Lounasmaa, M., Jermy, A., von Euw, J. & Reichstein, T. 1976: Die Phloroglucide von zwei Farnhybriden aus England und Schottland, von autentischem "Aspidium remotum" Braun A. und von Dryopteris aemula (Aiton) Kuntze O. aus Irland. — Helv. Chim. Acta 59: 1725–1744.

Widén, C.-J., Widén, K., Vida, G. & Reichstein, T. 1991: The phloroglucinols of the D. villarii complex and some related ferns. (Pteridophyta, Dryopteridaceae). — Bot. Helv. 101: 77–120.

Widén, C.-J., Äyräs, P. & Reichstein, T. 1992: The phloroglucinols of Dryopteris stenolepis (Pteridophyta, Dryopteridaceae). — Ann. Bot. Fennici 29: 41–54.

Widén, C.-J., Äyräs, P., Neuvonen, K. & Reichstein, T. 1993: New phloroglucinol derivatives in Dryopteris pulvinulifera and D. subtriangularis. (Pteridophyta, Dryopteridaceae). — Ann. Bot. Fennici 30: 285–297.

Widén, C.-J., Pyysalo, H. & Reichstein, T. 1994 : Fast-atom-bombardment mass spectra of phloroglucinols from Dryopteris ferns. — Helv. Chim. Acta 77: 1985–1998.

Widén, C.-J., Fraser-Jenkins, C. R., Reichstein, T., Gibby, M. & Sarvela, J. 1996 : Phloroglucinol derivatives in Dryopteris sect. Fibrillosae and related taxa. — Ann. Bot. Fennici 33: 69–100.