Phytochemistry, 1973, Vol. 12, pp. 683 to 687. Pergamon Press. Printed in Enlgand.

PHLOROGLUCINOL DERIVATIVES IN *DRYOPTERIS PARALLELOGRAMMA* AND *D. PATULA*

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(*Received* 5 *September* 1972. *Accepted* 15 *October* 1972)

Key Word Index—*Dryopteris parallelogramma*, *D. patula*; Polypodiaceae; phloroglucinol derivatives; albaspidin-*AA*; filixic acid-*ABA*.

Abstract—The phloroglucinol composition in two tropical American ferns, *Dryoperis parallelogramma* and *D. patula* has been investigated. *Dryopteris parallelogramma*, a member of the *D. filix-mas* complex, contains filixic acids, which are a characteristic of the group. *Dryopteris patula* contains aspidin, as do its relatives in the *D. dilatata* complex, and albaspidin-*AA*.

INTRODUCTION

CONTINUING our studies[1,2] on anthelmintically active phloroglucinol derivatives in European and North American *Dryopteris* ferns, we have now investigated two species of *Dryopteris* from Veracruz, Mexico. *Dryopteris parallelogramma* (Kunze) Alston [*D. paleacea* (Sw.) C. Chr.] is a morphologically uniform species distributed from the Greater Antilles to Mexico, south to Venezuela, Bolivia, Argentina and southeastern Brazil. It is the tropical representative of the *D. filix-mas* complex in America. The other American member, *D. filix-mas* (L.) Schott, has a boreal-montane distribution in the United States and Canada. In Europe the complex is represented by *D. filix-mas*, *D. borreri* Newm. (closest to *D. parallelogramma*), and *D. abbreviata* (DC.) Newm. Several additional species are recognized in the Himalayas and eastern Asia, for example, *D. crassirhizoma* Nakai, *D. fibrillosa* (Clarke) Hand.-Mazz., *D. barbigera* (Hook.) O.Ktze., and *D. wallichiana* (Spreng.) Hylad. [*D. paleacea* (Don) Hand.-Mazz.]. The last species also occurs in Rhodesia, Java and New Guinea. *Dryopteris patula* (Sw.) Underw. is distributed from the southwestern United States to Bolivia and southeastern Brazil. It is a variable species, divided by Christensen[3] into three varieties, each of which is variable. Mexican specimens are referred to *D. patula* var. *Rossii* C. Chr., and have been reported as diploid ($n = 41$).[4] The immediate allies of *D.*

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[1] C.-J. WIDÉN, G. VIDA, J. VON EUW and T. REICHSTEIN, *Helv. Chim. Acta* **54**, 2824 (1971).
[2] C.-J. WIDÉN and D. M. BRITTON, *Can. J. Bot.* **49**, 1589 (1971).
[3] C. CHRISTENSEN, *Danske Vid. Selsk. Skrift.* **10**, 7 (1913).
[4] J. T. MICKEL, W. H. WAGNER, JR. and K. L. CHEN, *Caryologia* **19**, 97 (1966).

patula are other Mexican species such as *D. glandulifera* (Liebm.) C. Chr., *D. cinnamomea* (Cav.) C. Chr. and *D. mexicana* (Presl) C. Chr. All of these are southern relatives of the boreal *Dryopteris dilatata* (sens. latis.) group.

Rhizomes for chemical analysis were obtained by Rolla Tryon. *Dryopteris parallelogramma*: Pedregal Las Vigas, 22 km north north-west of Jalapa, Veracruz, Mexico (Voucher: 23 Dec. 1971, *J. Dorantes* MEXU). *Dryopteris patula*: Pedregal Esquilón, near Jilotepec, 10 km north of Jalapa, Veracruz, Mexico (Voucher: *D. Barrington* 429 GH).

RESULTS AND DISCUSSION

As in analoguous cases[1,2] the phloroglucinol mixtures from *D. parallelogramma* and *D. patula* were separated by column chromatography on silica gel.

Two phloroglucinol derivatives were isolated from *D. parallelogramma* in crystalline form: filixic acid-*ABA* (I), and flavaspidic acid-*AB*. The former has been isolated from *D. dickinsii* (Fr. et Sav.) C. Chr.,[5] and the latter from *D. abbreviata* Newman[1] and *D. polylepis* (Fr. et Sav.) C. Chr.[6] Filixic acid-*ABB* and flavaspidic acid-*BB* were also present in the samples in trace quantities as indicated by TLC and MS. The semiquantitative composition of the ploroglucinol derivatives in *D. parallelogramma* is given in Table 1. There was no detectable variability in the different rhizomes investigated. The alkaline cleavage of the crude filicin from *D. parallelogramma* and the investigation of the acylfilicinic acids formed[1] indicated that this taxon contains virtually only acetyl homologues of the phloroglucinol derivatives (98%) with only traces of butyryl homologues (2%). Propionyl homologues could not be detected.

TABLE 1. COMPOSITION OF THE PHLOROGLUCINOL DERIVATIVES IN *D. parallelogramma* AND *D. patula*

	Dried rhizomes (g)	Ether extract g (%)	Crude filicin (aspidin) g (%)	Albaspidin-*BB*	Albaspidin-*BA*	Albaspidin-*AA*	Aspidin-*BB*
D. parallelogramma	172	10·5 (6·1)	1·88 (1·1)	−	−	−	−
D. patula (cum desaspidin)	181	12·5 (6·9)	3·62 (2·0)	+	++	+	++
D. patula (sine desaspidin)	182	13·6 (7·5)	4·62 (2·5)	+	++	+	++

	Aspidin-*AB*	Filixic acid-*ABB*	Filixic acid-*ABA*	Desaspidin-*BB*	Flavaspidic acid-*BB*	Flavaspidic acid-*AB*
D. parallelogramma	−	(+)	+	−	(+)	+++
D. patula (cum desaspidin)	++	−	−	+*	+	+
D. patula (sine desaspidin)	++	−	−	−	+	+

* In addition a small spot of desaspidin-*AB* was detected.

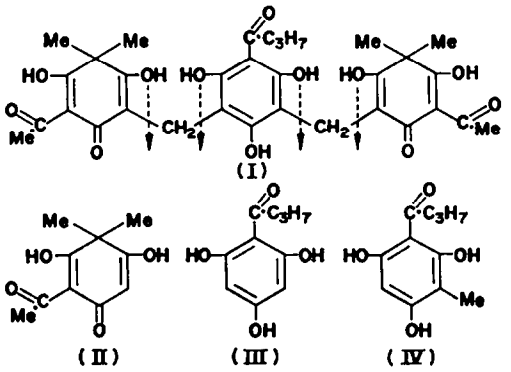
Key. − absent; (+) present in trace amounts (<5%); + present in small amounts (5–10%); ++ present in moderate amounts (10–20%); +++ present in large amounts (≧25%).

Filixic acid-ABA (I) ($C_{32}H_{36}O_{12}$). The MS shows the parent peak at *m/e* 612 corresponding to $C_{32}H_{36}O_{12}$. Other important peaks at *m/e* 417, 404, 209, 208, 196, 193, 181, 165 and 153 can be explained on the basis of a fragmentation pattern similar to that found earlier in connection of filixic acid-*BBB*.[7] The peak at *m/e* 404 and some lower peaks are

[5] S. HISADA, K. SHIRAISHI and I. INAGAKI, *Phytochem.* **11**, 1850 (1972).
[6] S. HISADA, K. SHIRAISHI and I. INAGAKI, *Phytochem.* **10**, 2451 (1971).
[7] M. LOUNASMAA, C.-J. WIDÉN and T. REICHSTEIN, *Helv. Chim. Acta* **54**, 2850 (1971).

partly due to albaspidin-*AA*, formed in the ionization chamber by rottlerone rearrangement. A weak peak at *m/e* 640 suggests the presence of filixic acid-*ABB* ($C_{34}H_{40}O_{12}$) in the sample in trace quantities. The NMR spectrum ($CDCl_3$) showing the following signals is in complete agreement with the proposed structure (see also Ref. 5): δ 1·01 (3H, *t*, *J* 7 Hz, $-CO-CH_2-CH_2-CH_3$), δ 1·44, 1·54 (12 H, each *s*, two *gem.* dimethyl groups), δ 1·78 (2 H, *m*, $-CO-CH_2-\overline{CH_2}-CH_3$), δ 2·72 (6 H, *s*, two $-CO-CH_3$ groups), δ 3·21 (2 H, *t*, *J* 7 Hz, $-CO-\overline{CH_2}-CH_2-CH_3$), δ 3·54, 3·58 (4 H, each *s*, two $\geqslant C-CH_2-C \leqslant$ groups). The signals at δ 10·10 (2 H, *s*), δ 11·50 (1 H, *s*), δ 12·93 (1 H, *s*), δ 16·06 (1 H, *s*) and δ 18·33 (2 H, *s*) may be attributed to hydrogen bonded OH-groups. Their presence is also indicated by the IR spectrum (KBr) which presents a broad band at 3160 cm^{-1}.

The alkaline cleavage leading to acetylfilicinic acid (II), phlorobutyrophenone (III) and methylphlorobutyrophenone (IV), is in agreement with the proposed structure (I) (Scheme 1). Traces of butyrylfilicinic acid could also be detected. This supports the suggestion that the sample contained small amounts of filixic acid-*ABB*.



SCHEME 1. ALKALINE CLEAVAGE OF FILIXIC ACID-*ABA* (I).

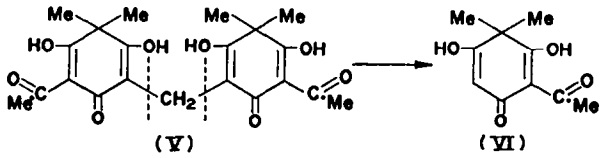
TLC of filixic acid-ABA (I). The chromatographic behaviour of the butyryl (B), propiony (P) and acetyl (A) homologues of the filixic acids has been recently studied by one of us on thin-layers buffered at different pHs.[8] According to these studies the filixic acids could only be partially separated at the pH values tested. However, the homologues *BBB*, *PBB* and *PBP* formed an elongated spot readily separating from the homologues *ABB*, *ABP* and *ABA* (I) forming another elongated spot. A fairly good separation of filixic acids *ABB* and *ABA* (I) could be obtained when the homologue *ABP* was lacking as in *D. parallelogramma*. The R_fs were 0·30 and 0·23, respectively, at pH 6·0, in *n*-hexane–$CHCl_3$ (1:1).

Five substances were isolated in crystalline form from *D. patula* and identified by comparison with authentic materials (Table 1). These were albaspidin-*AA* (V) and -*BB*, aspidin-*BB*, desaspidin-*BB* and flavaspidic acid-*BB*. This is the first time that albaspidin-*AA* (V) has been isolated from *Dryopteris* species, although its existence has been noticed previously in several taxa.[9] In addition to these compounds, aspidin-*AB* and desaspidin-*AB* were detected with TLC by comparison with authentic substances. There was a slight variability in the phloroglucinol compositions of the different rhizomes analyzed. Some samples were totally lacking desaspidin-*BB* and -*AB*, other ones contained small amounts

[8] L. HAAPALAINEN and C.-J. WIDÉN, *Farm. Aikak.* **79**, 161 (1970).
[9] C.-J. WIDÉN and D. M. BRITTON, *Can. J. Bot.* **49**, 1141 (1971).

of those compounds. All the plants were from one locality. *D. patula* contains about equal percentages of acetyl and butyryl homologues (46 and 54%, respectively). Also this taxon was totally lacking propionyl homlogues.

Albaspidin-AA (V) ($C_{21}H_{24}O_8$). The MS, which shows the parent peak at *m/e* 404 corresponding to $C_{21}H_{24}O_8$, is identical with that found earlier for synthetical albaspidin-*AA*.[10] Just visible peaks at *m/e* 446 and 432 in the spectrum of the natural product suggest the presence of higher homologues in trace quantities. The NMR spectrum ($CDCl_3$) of the natural product, which is identical with that of the synthetic sample, shows the following signals: δ 1·44, 1·54 (12 H, each *s*, two *gem.* dimethyl-groups), δ 2·72 (6 H, *s*, two $-CO-CH_3$ groups), δ 3·34, 3·36 (2 H, each *s*, one $\geqslant C-CH_2-C \leqslant$ group). The signals at δ 10·70 (1 H, *s*), δ 12·16 (2 H, *s*) and 12·93 (1 H, *s*) are due to hydrogen bonded OH-groups. The IR spectrum (see Experimental) is also completely identical with that of synthetic albaspidin-*AA*. After alkaline cleavage of the natural product, the only identifiable monocyclic compound from the reaction mixture was acetylfilicinic acid (VI) (Scheme 2). TLC of albaspidin-*AA* (V), see previous report.[9]



SCHEME 2. ALKALINE CLEAVAGE OF ALBASPIDIN-*AA* (V).

As shown in Table 1, *D. parallelogramma* contains large amounts of flavaspidic acid-*AB* as well as some filixic acid-*ABA* (V). It is thus chemotaxonomically related to the taxa of the *D. filix-mas* complex.[1,2] However, it differs from all other taxa hitherto investigated by having only acetyl homologues of the phloroglucides. It is chemically probably most closely related to European *D. borreri*, which usually contains much acetyl homologues. This relation agrees with the morphological similarities of the two species. *D. patula*, on the other hand, containing aspidin-*BB* and -*AB*, is more closely related to the *D. dilatata* complex. This conclusion is also supported by the morphological similarities.

EXPERIMENTAL

Extraction of rhizomes. The powdered rhizomes were macerated 3× with peroxide-free Et_2O and the Et_2O was evaporated in vacuum. The crude filicins (aspidins) were prepared with MgO using Na_2SO_3 as an antioxydant as previously described.[1] The yields of the Et_2O extracts and crude filicins (aspidins) from the taxa investigated are listed in Table 1.

Phloroglucinol derivatives of D. parallelogramma. The crude filicin (1·78 g) was suspended in benzene and chromatographed on a column containing 45 g silica gel as previously described.[1,2] The fractions 16–20 (10 ml fractions) eluted with benzene contained only filixic acid. After removal of the solvent, the residue was treated with acetone to yield 24 mg of filixic acid-*ABA* (V), m.p. 161–162°. The fractions 21–45 (benzene) gave only mixed crystals of flavaspidic acid and filixic acid. The fractions 46–90 (benzene–$CHCl_3$, 1:1) gave 58 mg flavaspidic acid-*AB*, m.p. 201–203° (cryst. from MeOH). IR, MS and TLC, were identical with those of flavaspidic acid-*AB* from *D. abbreviata*.[1] The MS showed the parent peak at *m/e* 418 ($C_{22}H_{26}O_8$). However, a just visible peak at *m/e* 446 was indicating the presence of trace amounts of flavaspidic acid-*BB* ($C_{24}H_{30}O_8$). The fractions 91–130 ($CHCl_3$) gave from $MeCO_2H$ 122 mg of flavaspidic acid-*AB*, m.p. 196–198°.

Filixic acid ABA (V) ($C_{32}H_{36}O_{12}$). M.p. 161–162°(cryst. from acetone). IR (KBr), 3160, 2985, 2965, 2935, 2880, 1638, 1630, 1608, 1560, 1548, 1542, 1534, 1476, 1456, 1438, 1398, 1366, 1318, 1256, 1194, 1156,

[10] M. LOUNASMAA, A. KARJALAINEN, C.-J. WIDÉN and A. HUHTIKANGAS, *Acta Chem. Scand.* **26**, 89 (1972).

1044, 1020, 996, 946, 930, 920, 896, 868, 840, 800, 790, 780, 740, 724, 718 and 702 cm^{-1}. For the MS and NMR spectra, see Results.

Alkaline cleavage of filixic acid ABA (V). This was performed as previously described for trispara-aspidin.[11] For the monocyclic phloroglucinol derivatives found, see Results.

Phloroglucinol derivatives of D. patula. The crude aspidin (4·5 g from rhizomes lacking desaspidin) was suspended in benzene and chromatograpehd on a column containing 102 g of silica gel. The fractions 1–2 eluted with benzene contained only albaspidin-*BB*. The residue was cryst. from acetone to give 20 mg albaspidin-*BB*, m.p. 149–150°. IR spectrum and TLC were identical with those of authentic albaspidin-*BB*. Fractions 11–25 (benzene) contained albaspidin-*BB* and -*AB*, and aspidin-*BB*. After fractional cryst. from *n*-hexane, 181 mg of aspidin-*BB*, m.p. 119–120°, was collected. Fractions 26–60 (benzene) gave only mixed crystals of aspidin and albaspidin. Fractions 61–110 (benzene–$CHCl_3$, 1:1) contained aspidin-*BB* and -*AB*, and albaspidin-*AA* (V). After fractional cryst. from acetone, 8 mg of albaspidin-*AA* (V), m.p. 162–164° (acetone) was recovered. IR and NMR spectrum and TLC were identical with those of a synthetic sample (see below). Fractions 111–185 ($CHCl_3$) contained albaspidin, aspidin and flavaspidic acid. No uniform crystals were obtained from MeOH. Fractions 186–240 ($CHCl_3$–EtOH, 28:1) gave 84 mg flavaspidic acid-*BB*, m.p. 83–85°/157–158° (MeOH). IR spectrum and TLC were identical with those of an authentic specimen of flavaspidic acid-*BB*. From the rhizomes containing desaspidin-*BB* and -*AB*, 12 mg of fairly pure desaspidin-*BB*, m.p. 138–139°, could furthermore be isolated after column chromatography on silica gel. IR spectrum and TLC were identical with those of authentic desaspidin-*BB*.

Albaspidin-AA (V)($C_{21}H_{24}O_8$), m.p. 162–164° (cryst. from acetone). IR (KBr), 3100, 2998, 2942, 2882, 1644, 1580, 1550 (shoulder), 1490, 1440, 1384, 1360, 1334, 1300, 1268, 1204, 1164, 1050, 1030, 1002, 950, 936, 880, 840, 804, 792, 746, 700 cm^{-1}. For the MS and NMR spectra see Results.

Alkaline cleavage of albaspidin-AA (V). This was performed in the same way as for filixic acid-*ABA* (I). For the monocyclic compounds formed see Results.

Acknowledgements—The authors thank Mrs. M. L. Lehtonen for technical assistance with the chromatography.

[11] C.-J. Widén, J. von Euw and T. Reichstein, *Helv. Chim. Acta* **53**, 2176 (1970).