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Phloroglucinol Derivatives of *Dryopteris crassirhizoma* from Japan

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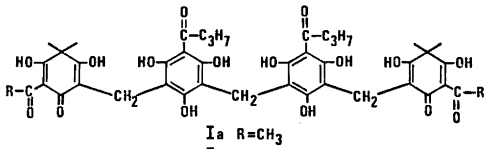
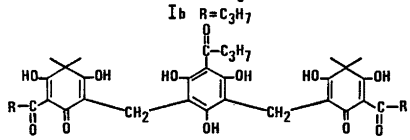
The phloroglucinol composition in *Dryopteris crassirhizoma* Nakai, a Japanese member of the *D. filix-mas* complex, has been investigated. The results are compared with those of European *D. filix-mas* s. str.. *D. crassirhizoma* contains large amounts of dryocrassins of which the homologue ABBA (Ia) predominates and some flavaspidic acid. Filixic acid, one of the main compounds in *D. filix-mas* s. str., has not been detected in *D. crassirhizoma*.

Dryopteris crassirhizoma Nakai is a diploid sexual taxon, which occurs in Japan, Sakhalin, southern Kuriles, Korea and northern China.[1] It is a member of the *Dryopteris filix-mas* complex. The oleoresin of *D. crassirhizoma* has been listed in the Japanese Pharmacopoeia as an anthelminticum.[2] According to chemical investigations of Hisada and Noro,[3] *D. crassirhizoma* contains flavaspidic acid, albaspidin (not in every specimen), filixic acid, and a substance similar to filixic acid (substance F).* This has later been identified as dryocrassin (Ia) (dryocrassin-ABBA) (methylene-bis-norflavaspidic acid-ABBA), a tetracyclic phloroglucinol derivative.[4] It also occurs in *D. polylepis* (Fr. & Sav.) C. Chr.[5,6] The corresponding tricyclic compound, filixic acid-ABA (IIa), has previously been isolated from *D. dickinsii* (Fr. & Sav.) C.Chr.[7,8] and *D. parallelogramma* (Kunze) Alston.[9] Another tetracyclic phloroglucinol, methylene-bis-norflavaspidic acid-BBBB (Ib), has been isolated from *D. austriaca* s. lat. by Penttilä and Sundman.[10]** The corresponding tricyclic compound filixic acid-BBB (IIb), is widely distributed in taxa of the *D. villarii* and *D. filix-mas* complex.[11–12] It has also been isolated in trace amounts from *D. austriaca* s.l. lat.,[13] and from *D. dickinsii*.[7,8]

Material. Rhizomes of *D. crassirhizoma* for chemical analysis were kindly collected by professor K. Iwatsuki, Kyoto University, and Dr. T. Seki, Hiroshima University, Japan. The collection data follow: Japan: Mt. Hiba, alt. *ca.* 1250 m, Hiba-gun, Hiroshima Pref., Aug. 28, 1973, leg. T. Seki (2 rhizomes), Seryntoye, north of Kyoto, alt. *ca.* 400 m, Honshu, Kyoto Pref., July 6, 1973, leg. K. Iwatsuki (*ca.* 10 rhizomes).



Ia R=CH_3
Ib R=C_3H_7



IIa R=CH_3
IIb R=C_3H_7

RESULTS AND DISCUSSION

As in previous investigations [11–12] the phloroglucinol mixture (crude filicin) from *D.*

* For the chemical formulae not presented here, see Refs. 11 and 14.

** The correct botanical source is most probably *D. assimilis* S. Walker or *D. spinulosa* Watt.

Table 1. Composition of the phloroglucinol derivatives in *D. crassirhizoma* and *D. filix-mas*.

Taxon and ploidy	*D. crassirhizoma* 2×	*D. filix-mas* 4×
Source	Japan	Finland
Dryocrassin	+++[a]	−
Filixic acid	−	+++[b]
Flavaspidic acid	+[a]	+++[b]
Albaspidin	−	−
Para-aspidin	−	+[b]
Desaspidin	−	+[b]
Trisdesaspidin	−	+[b]

[a] Mainly consisting of acetylhomologues (see text). [b] Mainly consisting of butyryl- and propionyl-homologues.[11,15]

crassirhizoma was separated by column chromatography on silica gel. Several samples of dryocrassin (I) with slightly different melting points were isolated by us. In addition, flavaspidic acid was detected by thin-layer chromatography. The albaspidin and the filixic acid, detected by Hisada and Noro,[3] were not found. It is possible that these compounds are not natural substances in *D. crassirhizoma*, but have been formed by the so-called rottlerone change during the MgO-treatment in the isolation of crude filicin (*cf.* Ref. 14). The semiquantitative composition of the phloroglucinol derivatives from *D. crassirhizoma* is given in Table 1. The corresponding results for European *D. filix-mas* taken from previous investigations [15] are also included in the table. This taxon is related to *D. crassirhizoma* morphologically, but is known to be tetraploid. Dryocrassin-ABBA (Ia) is the main compound in *D. crassirhizoma*. There are also small amounts of flavaspidic acid present. The phloroglucinol composition of *D. filix-mas* is quite different; flavaspidic acid predominates and much filixic acid is present also. In addition, there are small amounts of para-aspidin, desaspidin and traces of trisdesaspidin. No dryocrassin has been found in *D. filix-mas*. There was no detectable variability in the different rhizomes of *D. crassirhizoma* investigated. The reductive alkaline cleavage of the crude filicin of *D. crassirhizoma* and the investigation of the acylfilicinic acids formed, indicated that this taxon contains virtually only acetyl (A) homologues (95 %) with traces of propionyl (P) homologues (5 %). In distinct contrast to *D. filix-mas* and all other *Dryopteris* species so far investigated,[11] no butyryl (B) homologues were detected.

The mass spectrum of dryocrassin (m.p. 210–215 °C) (Fig. 1) shows a molecular peak at *m/e* 820 corresponding to $C_{48}H_{68}O_{16}$ (dryocrassin-ABBA (Ia)). Weak peaks at *m/e* 848, 834, and 806, combined with the results of chromatography (*cf.* below), suggest the presence of PBBP, PBBA and/or PPBP, and APBA homologues in trace quantities. The fragmentation pattern is very similar to that found earlier for filixic acid-BBB (IIb) and -ABA (IIa).[9,16,17] On analogy with the earlier cases [16,17] the thermic rottlerone change has to be taken into consideration in the present case, too.

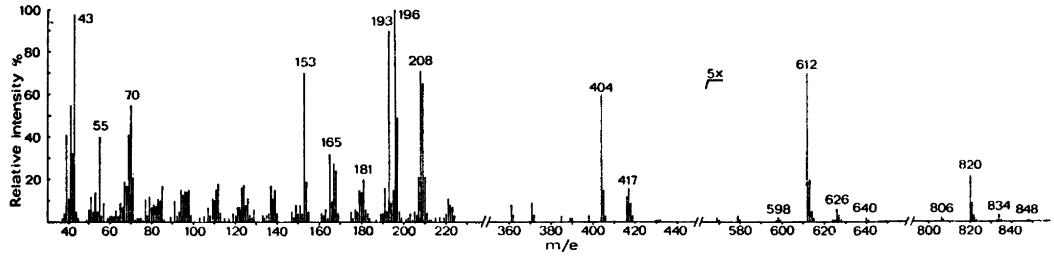


Fig. 1. Mass spectrum (70 eV, *T* 220 °C) of dryocrassin (m.p. 210–215 °C).

Thus, it can be expected *inter alia* that the peaks at *m/e* 612 and 404 are partly due to filixic acid-ABA (IIa) and albaspidin-AA, respectively, thermally formed in the ionization chamber of the mass spectrometer before ionization.

The NMR spectrum ($CDCl_3$) of dryocrassin (m.p. 210–215 °C), showing the following signals, is in full agreement with the proposed structure (Ia). δ 0.98 (6 H, t, $J=7$ Hz, two $-CO-CH_2-CH_2-CH_3$ groups), 1.42 and 1.52 (12 H, each s, two *gem.* dimethyl groups), 1.72 (4 H, m, two $-CO-CH_2-CH_2-CH_3$ groups), 2.74 (6 H, s, two $-CO-CH_3$ groups), 3.18 (4 H, m, two $-CO-CH_2-CH_2-CH_3$ groups), 3.54 (4 H, s, two $C-CH_2-C$ groups), and 3.84 (2 H, s, one $C-CH_2-C$ group). The OH signals are omitted (*cf.* also ref. 4).

Reductive alkaline cleavage of dryocrassin. (*cf.* Ref. 18, Cleavage A). Two main products, namely acetylfilicinic acid and butyrylphloroglucinol were identified by paper and thin-layer chromatography. These were the same monocyclic compounds which were found by Noro *et. al.*[4] However, in agreement with the results from mass spectrometry (see above) and thin-layer chromatography (see below) trace amounts of propionylfilicinic acid were detected too. On the other hand, due to the very similar thin-layer chromatographic behavior of butyrylphloroglucinol and propionylphloroglucinol,[14] the latter could not be detected with certainty.

Thin-layer chromatography. All samples of dryocrassin gave one large spot, R_F 0.30, dryocrassin-ABBA (Ia), when chromatographed on thin-layers buffered to pH 6.0 in hexane-chloroform 1:1.[14,15] However, just visible or small spots due to homologous dryocrassins were present, too. These were tentatively identified as the dryocrassins-PBBP (R_F 0.40), -PBBA and/or -PPBP (R_F 0.38), and -APBA (R_F 0.27) according to the results from mass spectrometry and reductive alkaline cleavage.

The thin-layer chromatographic behaviours of dryocrassin-ABBA (Ia) and -PBBA and/or -PPBP and the corresponding tricyclic and bicyclic compounds, filixic acid-ABA (IIa) and albaspidin-AA, respectively, were also studied on thin-layers buffered to pH 4–8 using gradient technique.[19] As seen from Fig. 2. dryocrassin-ABBA (Ia)* is running slower than filixic acid-ABA (IIa) at every pH tested. Both substances show distinct pH-dependence. However, it appeared that the R_F-value of dryocrassin-PBBA and/or -PPBP* was identical with that of filixic acid-ABA (IIa). Therefore, an accurate identification of these substances and their higher homologues cannot be achieved without isolating crystalline compounds and submitting these to mass spectrometry and reductive alkaline cleavage.

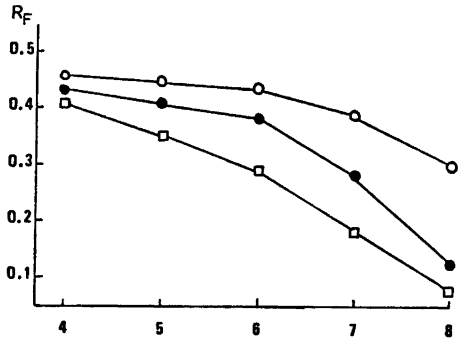


Fig. 2. Gradient TLC of dryocrassin-ABBA (□, brown), dryocrassin-PBBA and/or -PPBP and filixic acid-ABA (●, brown), and albaspidin-AA (○, red). Solvent: hexane-$CHCl_3$, 50:50. Colour with Fast Blue Salt B in parenthesis. Chromatographed 3 × 15 cm.

As apparent from Table 1. *D. crassirhizoma* contains large amounts of dryocrassin-ABBA (Ia) and traces of its homologues. There is most probably no filixic acid-ABA (IIa) present. These facts suggest interesting differences in the biosynthetic pathways of these two substances. So far *D. crassirhizoma* is unique among the representatives of the *D. filix-mas* complex in lacking filixic acid.

The morphology of *D. crassirhizoma* and *D. filix-mas* indicate that these two taxa are closely related. However, our chromatographic results do not suggest that these taxa are conspecific. Their ploidy levels are also different as already pointed out.

* Noteworthy, a slight decomposition of the dryocrassins were observed on the alkaline side of the plates.

EXPERIMENTAL

General. The mass spectrum was recorded on an A.E.I. MS-9 mass spectrometer at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, through the courtesy of Dr. B. C. Das. The NMR spectrum was recorded on an I.E.F. 240 B spectrometer (240 MHz) at the Institut d'Électronique Fondamentale, Faculté des Sciences d'Orsay, France, through the courtesy of Drs. S. K. Kan and G. Massiot. The melting points have been determined on a Kofler micro hot stage and are uncorrected. The chromatographic methods were those previously reported.[11,13,19]

Preparation of ether extract and crude filicin of D. crassirhizoma. These were prepared from air-dried rhizomes (275 g) according to previously reported methods,[14,15] yielding 21.1 g (7.7 %) of ether extract (oleoresin) from which 5.224 g (1.9 %) of crude Mg-filicin and 6.620 g (2.4 %) of crude Ba-filicin were obtained.

Column chromatography of crude Mg-filicin. The crude Mg-filicin (5.124 g) was suspended in benzene and chromatographed on a column containing 265.5 g SiO_2 as previously described.[15] The fractions eluted with benzene or benzene/chloroform 1:1 (10 ml each), all contained dryocrassin as found by TLC. The fractions 1 – 35 (benzene) were combined, evaporated *in vacuo*, and the residue was crystallized from ether, yielding several subsequent fractions of dryocrassin with slightly different melting points. Those with the melting points 205 – 207 °C (94.5 mg) and 210 – 215 °C (33.1 mg) consisted of almost pure dryocrassin-ABBA (Ia) as found by TLC, alkaline cleavage, MS, and NMR spectra (see theoretical section). The last fractions eluted with chloroform/ethanol (95/5) consisted of impure flavaspidic acid (TLC). No crystals were obtained from methanol.

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