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On Natural and Synthetic Homologues of *Dryopteris* Phloroglucinol Derivatives

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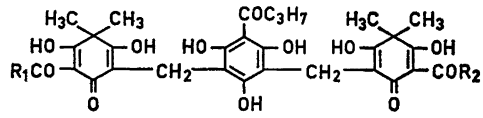
It has been shown that in *Dryopteris filix mas* the naturally occurring phloroglucinol derivatives albaspidin, flavaspidic acid and desaspidin do not occur as chemically uniform compounds but consist of a mixture of homologues. As previously shown by us in the case of the naturally occurring homologous filixic acids isolated from *Dryopteris filix mas*,[1] the homologues of albaspidin, flavaspidic acid and desaspidin differ from each other in regard to the acyl side chains of the filicinic acid rings. A total of six albaspidins, three flavaspidic acids and three desaspidins have been identified, in these the acyl side chains of the filicinic acid rings are either butyryl, propionyl, or acetyl.

The existence of homologous series is most prominent in *Dryopteris filix mas*, in which the relation between the butyryl, propionyl, and acetyl groups is found to be about 60:30:10.

In *Dryopteris austriaca subsp. dilatata* the great majority of the side chains are butyryl groups, only traces of acetyl groups have been found and propionyl groups are totally lacking.

Homologous series of some phloroglucinol derivatives with acyl groups varying from acetyl to caproyl have been synthesized. The chemical and chromatographic data of the synthetic compounds are given. The homologous desaspidins were tested toxicologically on mice, and it was found that the toxicity increases with increasing length of the acyl side chain from acetyl to butyryl-valeryl. Further lengthening of the acyl side chain decreases the toxic properties of the homologous desaspidins.

In a previous paper[1] the naturally occurring filixic acid isolated from *Dryopteris filix mas* has been shown to exist as a mixture of homologues which differ from each other only in regard to the acyl side chains attached to the filicinic acid rings. Depending on the degree of purity the filixic acid mixture of natural origin consists of three or more compounds. In total, six homologous filixic acids have been detected by paper chromatography and the three main compounds have been isolated in a pure state. These are called filixic acid BBB (I), filixic acid PBB (II) and filixic acid PBP (III), in which the acyl side chains attached to the filicinic acids are butyryl-butyryl, propionyl-butyryl and propionyl-propionyl, respectively. In the three remaining homologues,

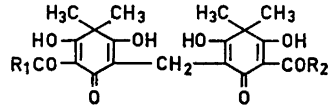


I $R_1 = R_2 = C_3H_7$
II $R_1 = C_2H_5$; $R_2 = C_3H_7$
III $R_1 = R_2 = C_2H_5$

which are present in the naturally occurring mixtures in quite small amounts, one or both of the butyryl or propionyl groups are replaced by acetyl groups.

The occurrence of homologous series has now been established not only for filixic acid but also for other phloroglucinol derivatives in *Dryopteris filix mas.*

Albaspidin. Corresponding to the filixic acids, six homologous albaspidins can be postulated to exist, theoretically. According to the nomenclature used



IV $R_1 = R_2 = C_3H_7$
V $R_1 = C_2H_5$; $R_2 = C_3H_7$
VI $R_1 = R_2 = C_2H_5$
VII $R_1 = CH_3$; $R_2 = C_3H_7$
VIII $R_1 = CH_3$; $R_2 = C_2H_5$
IX $R_1 = R_2 = CH_3$

for filixic acids, the homologues of albaspidin should be called albaspidin BB, PB, PP, AB, AP and AA (IV—IX), respectively.

In order to study the paper chromatographic behaviour of the albaspidin homologues the symmetrical compounds albaspidin BB, PP and AA were synthesized. When examined by our previously reported chromatographic method,[2] based on buffered and formamide impregnated papers with cyclohexane-chloroform (1:1) as solvent, albaspidin BB, PP and AA proved to have the R_F values listed in Table 1. However, when two of these symmetrical albaspidins are chromatographed together from the same starting point, three different spots are always obtained, the third new spot being located between the two original spots. Similarly, all three albaspidins chromatographed together yield a total of six spots. This is due to the fact that the symmetrical albaspidins disproportionate under alkaline conditions according to the scheme: BB + PP $\rightleftharpoons$ 2 BP. The alkalinity of the paper buffered to pH 8.6 is sufficient to cause this reaction during chromatography. The same disproportionation reaction has previously been observed in filixic acids [1] with the only difference that the symmetrical filixic acids do not disproportionate until exposed to strongly alkaline conditions. Filixic acids, therefore, never show disproportionation under the conditions prevailing during chromatography on buffered papers [2] as do the albaspidins.

A similar disproportionation can also be achieved by heating; *e.g.* two symmetrical albaspidins heated together to melting point yield a mixture of

Table 1. R_F-values of homologous albaspidins, desaspidins and flavaspidic acids chromatographed on papers buffered to pH 8.6, 8.8 and 4.0, respectively, and the R_F values obtained for the homologous albaspidins by the method of Hegnauer.

Compound	Chromatography on buffered papers			Chromatography by the method of Hegnauer
	R_F values			R_F values
	pH 8.6	pH 8.8	pH 4.0	
Albaspidin BB	0.88	0.55		0.17
» PB	0.78	0.41		0.21
» PP	0.66	0.35		0.27
» AB	0.53	0.20		0.33
» AP	0.37	0.11		0.40
» AA	0.15	0.06		0.54
Desaspidin BB	0.75	0.49		
» PB	0.59	0.36		
» AB	0.30	0.13		
Flavaspidic acid BB			0.80	
» » PB			0.71	
» » AB			0.45	

three albaspidins. A similar reaction was also observed in the case of the filixic acids.[1]

When the crude albaspidin isolated from *Dryopteris filix mas* is chromatographed on papers buffered to pH 8.6, five of the six possible albaspidins can be detected, the amounts decreasing in the following sequence: albaspidin BB, PB, PP, AB and AP. Because of the ready occurrence of disproportionation during chromatography on alkaline papers no conclusions could be drawn as to the presence in the original extract of all the albaspidins detected by this method. It was found, however, that by the method reported by Hegnauer [3] using paraffine-impregnated papers and 85 % acetic acid as solvent, very good separation of the homologous albaspidins could be achieved (Table 1). By this method, too, five albaspidins could be identified in the crude albaspidin mixture. Because no disproportionation was ever observed under acid conditions, the unrefined albaspidin mixture isolated from *Dryopteris filix mas* was thus proved to contain five homologues of albaspidin. The sixth possible compound, albaspidin AA, could not be detected with certainty.

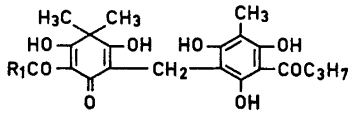
Through recrystallizations of the crude albaspidin mixture the minor components containing an acetyl group are easily removed. After the first recrystallization from acetone a pure white substance melting at 142—144° was obtained. By paper chromatography, however, all five albaspidins could still be indentified. Further recrystallizations from the same media yielded a product with a melting point of 147—148°. This natural albaspidin was composed of the albaspidins BB, PB, and PP; the homologues with acetyl groups could no longer be detected. In contrast to the homologous filixic acids which by purification reached a constant melting point as a mixture of the three filixic acids BBB, PBB, and PBP,[1] the mixture of the three albaspidins could be

further purified through recrystallizations from acetone. The melting point was gradually raised towards the final melting point of the pure albaspidin BB, which was found to be 153—154°. This melting point was always observed for albaspidin BB isolated from *Dryopteris austriaca*. The previously reported melting points for albaspidin are usually about 147—149°; an exception is reported by Maizite,[4] who isolated albaspidin from "*Nephrodium austriacum*" and "*Nephrodium eu-spinulosum*" with melting points of 152—153° and 152—154°, respectively.

All methods used for the isolation of phloroglucinol derivatives from *Dryopteris* species involve alkaline conditions in some form, usually as MgO treatment of the extracts or ground rhizomes. These treatments, naturally, cause the disproportionation of the albaspidins. If reliable knowledge of the homologues of albaspidin actually present in the rhizomes is wanted, chromatography should be performed on extracts never exposed to alkali. The method of Hegnauer [3] was not suitable for this purpose because the fatty material of the extract prevented contact between the solvent and the phloroglucinol derivatives and hence elution was poor. To remove the bulk of the interfering fat and non-phloroglucinol substances from the extract, column chromatography was carried out on silica gel. When benzene was used as solvent the first fractions obtained removed most of the lipids present and the following fractions contained a mixture of filixic acids and albaspidins. Chromatography of these fractions on paraffine-impregnated papers with 90 % acetic acid [5] as solvent revealed all the possible albaspidins except albaspidin AA, which could not be detected.

A peculiar phenomenon observed by Klevstrand [6] and frequently referred to by Zwimpfer and Büchi,[7] could possibly be explained by the albaspidin homologues. According to Klevstrand, a sample of albaspidin melting at 141—144° produced five different spots in one of the paper chromatographic methods used.[6] In this method the papers were impregnated with a mixture of formamide and dimethylformamide (1:2) and cyclohexane was used as solvent. Our efforts to separate pure samples of albaspidin BB, PP and AA by this method were only partly successful; albaspidin AA could well be separated from the other two. However, as pointed out by Zwimpfer and Büchi, the pH value of formamide shows great variations from batch to batch owing to decomposition during storage. Although Klevstrand gave full details about the purity of the formamide used, no information concerning the purification of the dimethylformamide was given, and it is therefore well within the bounds of possibility that the dimethylformamide had an alkaline reaction. If now the sample of albaspidin used was isolated from *Dryopteris filix mas* and not very highly purified, as indicated by the relatively low melting point of the sample, the spots obtained might well have originated from the homologues of albaspidin which, owing to the slightly alkaline reaction of the impregnated paper, may have been separated during chromatography.

Flavaspidic acid. In connexion with our studies on the homologous filixic acids it was established that the variations of the acyl side chains were limited to the filicinic acid rings alone. Thus, when the methylene bridges of the homologues of filixic acid were ruptured and the mixture of one-ring compounds obtained was analysed,[1] butyrylphloroglucinol was present, but not even traces

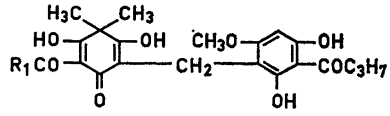


X $R_1 = C_3H_7$
XI $R_1 = C_2H_5$
XII $R_1 = CH_3$

of either propionyl- or acetylphloroglucinol could be detected. On account of these results only three homologous flavaspidic acids, BB, PB and AB (X—XII), were to be expected. This also proved to be true. When chromatographed on papers buffered to pH 4.0 the flavaspidic acid isolated from *Dryopteris filix mas*, as well as the *Dryopteris filix mas* extract itself, clearly showed, in addition to the major spot of flavaspidic acid BB, two minor spots having R_F values identical with specimens of the synthetic flavaspidic acids PB and AB (Table 1).

This observation was further confirmed by alkaline cleavage of the crude flavaspidic acid mixture.[1] When the one-ring compounds obtained were chromatographed on papers buffered to pH 4.0 and 5.0, butyryl-, propionyl- and acetylfilicinic acids could be identified by comparison with the R_F values of the corresponding synthetic compounds (Table 2). The alkaline decomposition mixture was also chromatographed with tetraline-acetic acid-water as solvent, but only butyryl-3-methylphloroglucinol could be identified.[1]

Desaspidin. On analogy to the flavaspidic acids three desaspidins were also to be expected. *Dryopteris filix mas* extract contains only relatively small amounts of desaspidin, and therefore the homologues of desaspidin could not be detected by paper chromatography of the extract itself, mainly because of overlapping of the homologues of filixic acid. To remove the bulk of the interfering substances, the extract was chromatographed through a column



XIII $R_1 = C_3H_7$
XIV $R_1 = C_2H_5$
XV $R_1 = CH_3$

packed with silica gel, benzene being used as solvent. The fractions obtained after the bulk of the filixic acids and albaspidins had been removed from the column were chromatographed on papers buffered to pH 8.6. All three desaspidins expected, BB, PB and AB (XIII—XV), could then be clearly separated and identified by comparison with the synthetic desaspidins BB, PB and AB. The R_F values of these compounds are listed in Table 1.

The homologous series of the phloroglucinol derivatives mentioned above have been detected and identified in the extracts of *Dryopteris filix mas*, as

already pointed out. The existence of acetyl and propionyl groups can easily be verified in the extracts without isolation of the individual compounds. This is achieved by carrying out an alkaline cleavage [1] directly on the extracts. Hereby all the methylene bridges present are ruptured and the acylfilicinic acids originating from the different phloroglucinol derivatives are liberated and can easily be identified by paper chromatography on papers buffered to pH 4.0 and 5.0.[1] Semiquantitative estimation of the acylfilicinic acid spots revealed the relative amounts of butyryl, propionyl and acetyl groups to be about 60, 30, and 10, respectively.

The phenomenon of the homologous series was first assumed to apply only to the compounds of *Dryopteris filix mas* as briefly mentioned in our previous paper. However, in the technical isolation of flavaspidic acid from *Dryopteris austriaca* rhizomes, batches of flavaspidic acid were repeatedly obtained which, when chromatographed on papers buffered to pH 4.0, revealed an additional compound very difficult to remove by recrystallization. This compound was later identified as flavaspidic acid AB, which, owing to its poor solubility in most solvents, crystallized together with the flavaspidic acid BB. The presence of acetyl compounds in *Dryopteris austriaca* was further confirmed by alkaline decomposition of extracts from authentic specimens of *Dryopteris austriaca subsp. dilatata* rhizomes. A small amount, about 1—3 %, of acetyl groups could hereby be detected, besides butyryl groups, whereas not even traces of propionyl groups could be found.

In an attempt to explain the biosyntheses of natural phenolic compounds Collie worked out the acetate theory, which was later supported by Birch on the basis of his biological experiments, using acetic acid with isotopically labelled carbon. According to these studies the biosyntheses of the natural acyl derivatives of phloroglucinol can be supposed to proceed through "head-to-tail" linkage of acetic acid units and subsequent ring closure, as shown in Fig. 1.

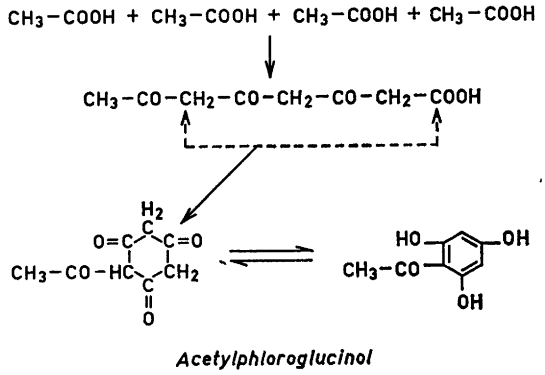


Fig. 1. The biosynthesis of acetylphloroglucinol through the "head-to-tail" linkage of acetic acid units according to Collie-Birch acetate theory.

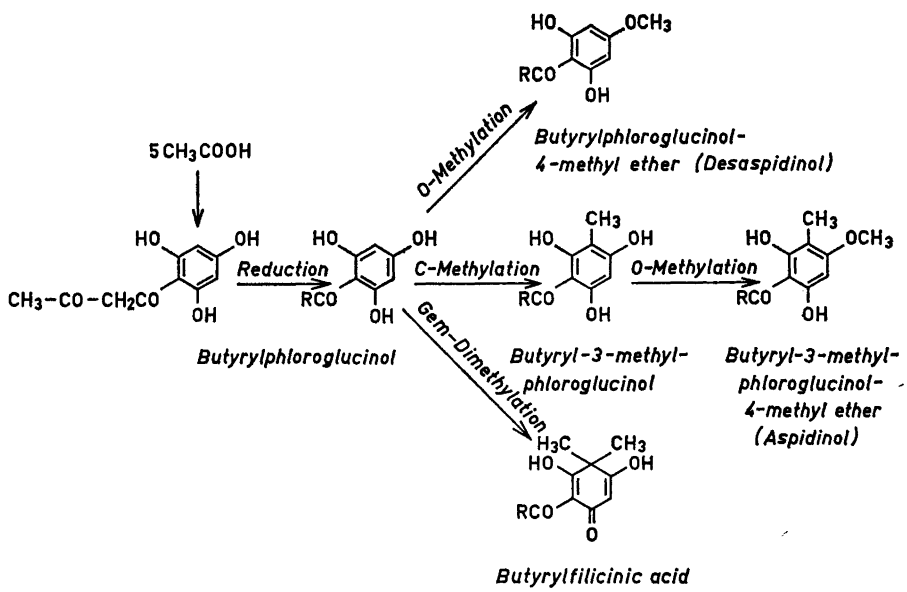


Fig. 2. The pathways of the assumed biosyntheses of the most frequently occurring one-ring compounds building up the natural *Dryopteris* phloroglucinol derivatives. R = $CH_3 \cdot CH_2 \cdot CH_2-$

It may be postulated that five molecules of acetic acid linked together in this way and thereafter undergoing reduction and C- or O-alkylation (Fig. 2) give rise to all the one-ring compounds building up the natural phloroglucinol derivatives. The existence of butyryl and acetyl groups in the phloroglucinol derivatives is in full agreement with these findings, and therefore the biosyntheses in the rhizomes of *Dryopteris austriaca* can well be thought to take place according to the Collie-Birch theory.

The propionyl groups of *Dryopteris filix mas*, on the other hand, seemingly do not fit into this theory. According to Birch, however, any acid known to appear in nature could conceivably participate in the biosyntheses; the fatty acids, particularly, appear to be frequently involved. Incorporation of propionic acid units in addition to acetic acid units offers a possible explanation of the propionyl side chains of the phloroglucinol derivatives in *Dryopteris filix mas*.

The phloroglucinol derivative homologues used as standard substances in this study were synthesized from the corresponding acylfilicinic acids * (Table 2). Two molecules of the individual acylfilicinic acids condensed with formaldehyde produced the homologous albaspidins; to obtain the homologous flavaspidic acids and desaspidins one molecule of acylfilicinic acid and one molecule of butyryl-3-methylphloroglucinol or butyrylphloroglucinol-4-methyl ether, respectively, were condensed with formaldehyde.

* Synthesized in this laboratory by Dr. Lars Andersen.[8]

Table 2. Melting points and R_F values of the synthetic acylfilicinic acids chromatographed on papers buffered to pH 4.0 and 5.0.

Compound	Melting point	R_F values	
		pH 4.0	pH 5.0
Acetylfilicinic acid	177–178°	0.27	0.07
Propionylfilicinic acid	114–115°	0.54	0.23
Butyrylfilicinic acid	98– 99°	0.70	0.36
Isobutyrylfilicinic acid	153–154°	0.76	0.42
Valerylfilicinic acid	97– 98°	0.84	0.55
Caproylfilicinic acid	92– 93°	0.92	0.72

In addition to the acetyl and propionyl homologues, valeryl, caproyl and isobutyryl derivatives of albaspidin and desaspidin were also prepared. The melting points and the analytical data of the synthesized compounds are listed in Tables 3 and 4.

Table 3. Melting points and analytical data of the homologous albaspidins.

Compound	Melting point	Found		Calculated		Formula
		C	H	C	H	
Albaspidin AA	170–171°	62.47	6.07	62.38	5.94	$C_{21}H_{24}O_8$
Albaspidin PP	135–137°	63.98	6.63	63.89	6.48	$C_{23}H_{28}O_8$
Albaspidin iBiB	168–169°	65.17	7.08	65.22	6.96	$C_{25}H_{32}O_8$
Albaspidin VV	121–122°	66.53	7.48	66.39	7.38	$C_{27}H_{36}O_8$
Albaspidin CC	126–128°	67.61	7.70	67.44	7.75	$C_{29}H_{40}O_8$

The homologues of desaspidin were tested toxicologically on mice. The LD_{50} values obtained are given in Fig. 3. It appeared that the toxicity increased with the length of the acyl side chain from acetyl to butyryl or valeryl, which were the most toxic of the desaspidins tested. Further lengthening of the side chain resulted in a sharp reduction of the poisonous qualities.

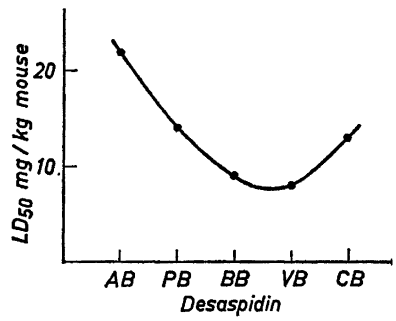


Fig. 3. The LD_{50} values of the synthetic homologous desaspidins with acyl side chains varying from acetyl to caproyl.

Table 4. Melting points and analytical data of the homologous desaspidins.

Compound	Melting point	Found C	Found H	Calculated C	Calculated H	Formula
Desaspidin AB	145–147°	63.26	6.38	63.16	6.22	$C_{22}H_{26}O_8$
Desaspidin PB	141–142°	63.80	6.42	63.89	6.48	$C_{23}H_{28}O_8$
Desaspidin iBB	133–135°	64.57	6.79	64.57	6.73	$C_{24}H_{30}O_8$
Desaspidin VB	123–125°	65.79	7.11	65.22	6.96	$C_{25}H_{32}O_8$
Desaspidin CB	105–106°	66.07	7.09	65.82	7.17	$C_{26}H_{34}O_8$

EXPERIMENTAL

Syntheses of the homologous albaspidins. Albaspidin AA: Acetylfilicinic acid (3.92 g) was dissolved in aqueous potassium hydroxide (120 ml, 1 %) and formaldehyde (0.75 ml, 40 %) was added. The mixture was kept at room temperature for 1 min and made acid with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. After recrystallizations from acetone albaspidin AA had a m.p. of 170–171°.

Albaspidin PP, albaspidin BB, albaspidin iBiB, albaspidin VV and albaspidin CC were correspondingly synthesized from propionylfilicinic acid, butyrylfilicinic acid, isobutyrylfilicinic acid, valerylfilicinic acid and caproylfilicinic acid, respectively, and formaldehyde. The synthetic products were recrystallized from acetone and yielded the homologous albaspidins with the analytical data and melting points listed in Table 3.

Syntheses of the homologous flavaspidic acids. Flavaspidic acid AB: Acetylfilicinic acid (1.96 g) and butyryl-3-methylphloroglucinol (2.10 g) were dissolved in aqueous potassium hydroxide (200 ml, 1 %) and formaldehyde (0.75 ml, 40 %) was added. The mixture was kept at room temperature for 1 min and acidified with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. After several recrystallizations from methanol and cyclohexane, flavaspidic acid AB was obtained in a pure state with a m.p. of 210–212°. (Found: C 63.15; H 6.24. Calc. for $C_{22}H_{26}O_8$: C 63.16; H 6.22.)

Flavaspidic acid PB was correspondingly synthesized from propionylfilicinic acid and butyryl-3-methylphloroglucinol. The purified product had a m.p. of 170–171°. (Found: C 64.26; H 6.42. Calc. for $C_{23}H_{28}O_8$; C 63.89; H 6.48.).

Syntheses of the homologous desaspidins. Desaspidin AB: Acetylfilicinic acid (1.96 g) and butyrylphloroglucinol-4-methyl ether (2.10) were dissolved in aqueous potassium hydroxide (200 ml, 1 %) and formaldehyde (0.75 ml, 40 %) was added. The procedure was continued as described for the synthetic flavaspidic acids. Repeated recrystallizations from cyclohexane yielded pure desaspidin AB with a m.p. of 144–146°.

Desaspidin PB, desaspidin BB, desaspidin iBB, desaspidin VB and desaspidin CB were correspondingly synthesized from propionylfilicinic acid, butyrylfilicinic acid, isobutyrylfilicinic acid, valerylfilicinic acid and caproylfilicinic acid, respectively, and butyrylphloroglucinol-4-methyl ether condensed with formaldehyde. The synthetic products were recrystallized from cyclohexane and yielded the homologous desaspidins with the melting points and analytical data listed in Table 4.

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