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The Structures of Filixic Acids

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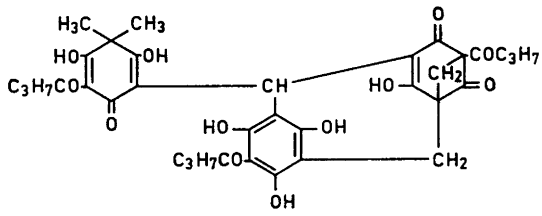
Naturally occurring filixic acid, the characteristic phloroglucinol derivative from *Dryopteris filix mas*, has been shown to be a mixture of six homologues, which differ from each other in regard to the acyl side chains of the filicinic acid nuclei. For the main three compounds of these filixic acid homologues the following names are proposed: filixic acid BBB (V), filixic acid PBB (VI) and filixic acid PBP (VII), in which the acyl side chains attached to filicinic acids are butyryl-butyryl, propionyl-butyryl or propionyl-propionyl, respectively. These three compounds, all of which have been isolated in a pure state, are shown to be the principal components of the natural filixic acid which after recrystallizations from ethyl acetate has a melting point of 183–185°. The structures of the symmetrically composed filixic acids BBB and PBP have been proved by syntheses.

When raw filixic acid isolated from male fern extracts is purified by treatments with methanol but without recrystallizations from ethyl acetate, a product is obtained which seems to contain three additional homologues with an analogous composition but with acetyl groups in the side chains. For these compounds the names filixic acids ABB, ABP and ABA are proposed.

Filixic acid was first isolated by Luck [1,2], who obtained it as a solid precipitate in male fern extracts. The author reported a m.p. of 161° for his filixic acid; later the m.p. was corrected to 184.5° and the lower m.p. was explained by the fact that a previously fused material had been used for the determination [3]. Alkaline decomposition of filixic acid yielded butyric acid.

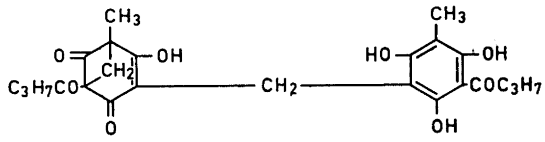
About 20 years later Grabowsky [4] showed filixic acid to be a derivative of phloroglucinol. Daccomo [5] isolated filixic acid by a selective extraction of male fern extracts; his product had a m.p. of 179–180°. These authors, besides Luck [3] and Schiff [6], proposed different structures for filixic acid but none of these has been confirmed later.

In 1892 Poulsson [7] reported that filixic acid appeared in two modifications of which the amorphous one was biologically active whereas the crystalline form with a m.p. of 184.5° was ineffective. The latter was claimed to be an anhydride of the amorphous filixic acid, but no exact chemical structures were given.



I

(Boehm)



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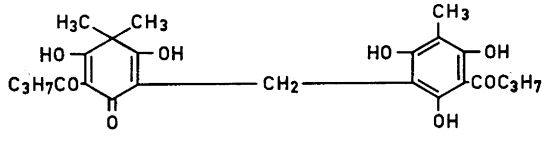
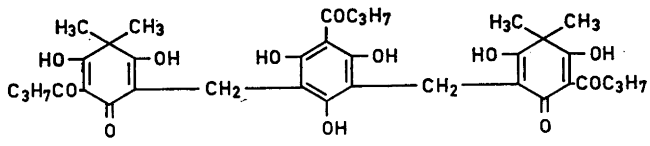
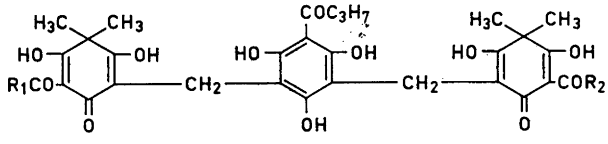


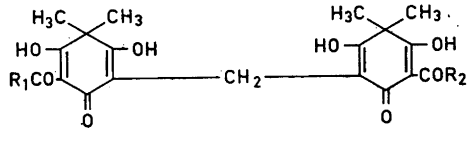
III



IV



V $R_1=R_2=C_3H_7$; VI $R_1=C_2H_5$; $R_2=C_3H_7$; VII $R_1=R_2=C_2H_5$



VIII $R_1=R_2=C_3H_7$; IX $R_1=C_2H_5$; $R_2=C_3H_7$; X $R_1=R_2=C_2H_5$

Kraft [8] later argumented against the theory of an anhydrous form of filixic acid, considering both filixic acids of Poulsson chemically identical and explaining their dissimilarities to stem from different physical states.

Through the extensive investigations of Boehm much light was thrown upon the chemistry in this field. Boehm proved the *Dryopteris* phloroglucinol derivatives generally to be composed of butyrylfilicinic acid and phlorobutyrophenones by means of a methylene bridge [9,10]. Boehm performed elementary analyses and quantitative decompositions of filixic acid, showing it to be composed of three nuclei; on the basis of his results filixic acid could be given the structure (I) [10].

According to Boehm the molecular formula of filixic acid is $C_{35}H_{38}O_{12}$ and the melting point 184—185°. The *endo*-methylene bridge in (I) was enforced on the basis that alkaline decomposition yielded trimethylphloroglucinol which could be identified. For the same reason an analogical *endo*-methylene structure was also assigned to flavaspidic acid (II) [10].

The formation of trimethylphloroglucinol in the alkaline decompositions of both flavaspidic acid and filixic acid was later explained by the Rottleron-change *i.e.* a disproportionation of unsymmetrical polyhydroxydiarylmethanes yielding two symmetrical compounds [11–13], and the theory of *endo*-methylene structures was then abandoned. After the structure of flavaspidic acid (III) was resolved and proved by synthesis [13,14] Riedl proposed an analogous structure for filixic acid (IV) [14,15] corresponding to the molecular formula $C_{36}H_{44}O_{12}$. The structure (IV) was also proposed by Chan and Hassall [16] on the basis of their experimental results and ultraviolet absorption studies.

Attempts have been made by Riedl [15] to prove the proposed structure of filixic acid by synthesis, but only albaspidin (VIII) and highmelting polymers could thereby be isolated.

In our earlier studies on the phloroglucinol derivatives of *Dryopteris* ferns, several syntheses of these compounds have been performed in order to confirm the structures of phloropyron [17], phloraspin [18] and para-aspidin [19]. In continuation of these studies we undertook to prove the earlier proposed structure for filixic acid (IV) in the same way. The synthesis was carried out by allowing 3-butyrylfilicinic acid and phlorobutyrophenone to react with formaldehyde in dilute alkaline solution. Among a number of undefined condensation products albaspidin, methylene-bis-phlorobutyrophenone, butyrylfilicinic acid and a compound with the R_F values of filixic acid could be identified by paper chromatographic separations [20] of the reaction mixture. For isolation of the synthetic filixic compound, successive treatments with methanol and recrystallizations from acetone and ethyl acetate were performed. The purified compound showed all the typical filixic acid properties [10]: it was very slightly soluble in methanol and acetone, freely in warm ethyl acetate and acetic acid as well as in cold benzene and chloroform. The ferric chloride reaction in alcoholic solution was redbrown. Diazoaminobenzene produced a scarlet red derivative with a melting point of 235° identical with the corresponding derivative of natural filixic acid [10]. The analytical data of the synthetic filixic compound agreed with the formula $C_{36}H_{44}O_{12}$ and a molecular weight determination confirmed it as 668. Only this synthetic compound did not show the melting point of filixic acid: it melted at 172—174° whereas natural filixic acid has a melting

point of 183—185°. By mixing these two compounds an indistinct melting point of 172—181° was observed.

These results indicated that the synthetic compound, hereafter called filixic acid BBB, and natural filixic acid were extremely alike but not identical. This opinion was supported by improved paper chromatographic studies.

Various chromatographic methods for separating the phloroglucinol derivatives from *Dryopteris* ferns have been suggested [21–27]. All these methods report uniform spots for filixic acid. Using our earlier published chromatographic method [20] based on buffered filter papers impregnated with formamide, uniform spots for natural filixic acid showed at pH 8.8 (R_F 0.10) and at pH 8.4 (R_F 0.75). It appeared, however, that chromatography of 50—100 mμg amounts of natural filixic acid done in papers buffered to pH 8.6 resulted in somewhat elongated spots with the R_F values of about 0.45. Then too, the treatment with diazo reagent [20] did not produce uniform colouring all over the outstretched spot but rather showed a zone formation of the colour intensity. When only 20—40 mμg amounts of filixic acid was chromatographed in the same way three different spots with the R_F values 0.45, 0.36 and 0.20, respectively, separated fairly well.

The synthetic filixic acid BBB gave, by this technique, a uniform spot which was identical with one of the spots obtained from the natural filixic acid, actually with the one having the R_F value of 0.45.

All attempts to isolate the three different compounds from the natural filixic acid using ordinary laboratory techniques such as recrystallizations, extractions or column chromatography, were unsuccessful. A special countercurrent apparatus according to Hietala [28] consisting of 200 extractors was employed and the separation was performed between benzene and a buffer solution of pH 9.3. By this technique the three compounds of natural filixic acid could be separated. Each compound was recrystallized from ethyl acetate and obtained in a pure state showing the melting points of 172—174°, 184—186° and 192—194°, respectively. A mixed melting point determination of the first mentioned natural compound with the synthetic filixic acid BBB showed no depression and melted sharply at 172—174°. The analytical data of the three isolated compounds were in good agreement with the formulas $C_{36}H_{44}O_{12}$, $C_{35}H_{42}O_{12}$ and $C_{34}H_{40}O_{12}$, respectively. These natural filixic acids, for which we propose the names filixic acid BBB, filixic acid PBB and filixic acid PBP, apparently form a homologous series and consequently their chemical properties are extremely alike.

The structure of filixic acid BBB could be considered as proved by the synthesis of the same, which is mentioned above, and could be given as (V). In order to find out the structural differences between the three filixic acids, alkaline cleavages of the compounds under very mild conditions were carried out. Hereby only the methylene bridges were expected to be broken up whereas any loss of the acyl groups ought to be prevented. When the decomposition mixtures were chromatographed in papers buffered to pH 5.0 and 4.0 [20] the following acylfilicinic acids could be identified: filixic acid BBB yielded only butyrylfilicinic acid, filixic acid PBB yielded an equal mixture of propionyl- and butyrylfilicinic acids, and filixic acid PBP finally yielded only propionylfilicinic acid. The acylfilicinic acids were identified by comparison with

Table 1. R_F-values of acylfilicinic acids on papers buffered to pH 4.0 and 5.0.

	R_F-values	
	pH 4.0	pH 5.0
Butyrylfilicinic acid	0.70	0.35
Propionylfilicinic acid	0.54	0.22
Acetylfilicinic acid	0.27	0.07

corresponding synthetic compounds, the R_F values of which are shown in Table 1 [20].

When the alkaline decomposition mixtures were chromatographed with tetraline-acetic acid-water solution, an eluate we have used to separate pholoroacylophenones, only pholorobutyrophenone and methylphlorobutyrophenone could be identified in each mixture. Not even traces of phloropropiophenone could be detected in any case.

The results of these experiments can be interpreted as follows: each of the three filixic acids is composed of three nuclei of which the middlemost one in each case is phlorobutyrophenone and the two outermost ones are either butyrylfilicinic acid (BBB) or propionylfilicinic acid (PBP) or, finally, the molecule has one nucleus of each kind (PBB). The structures of filixic acids PBB and PBP can be given as (VI) and (VII), respectively.

Filixic acid PBP was synthesized from propionylfilicinic acid and phlorobutyrophenone in the same manner as filixic acid BBB. The synthetic compound had a melting point of 192—194° showing no depression on mixing with the isolated filixic acid PBP.

No attempts have been made to synthesize filixic acid PBB. A corresponding synthesis naturally should yield a mixture of all three filixic acids.

A reverse disproportionation of pure filixic acids PBP and BBB in alkaline solution was observed. The reaction proceeds according to the scheme: PBP + BBB $\rightleftharpoons$ 2 PBB. The equilibrium is pressed markedly towards the right in consequence of which an alkaline solution of the two symmetrical compounds on acidification always yields a mixture of all three filixic acids.

A similar reaction was also observed on heating filixic acids BBB and PBP together to melting temperature. A paper chromatographic analysis of the fused material showed a mixture of the three filixic acids.

Besides the interchange reactions also a slight decomposition of filixic acids on application of heat was observed. Since Boehm's investigations, the formation of albaspidin by boiling the natural filixic acid in alcohol has been known [29]. Later Widén [30] reported the formation of crystalline albaspidin by sublimation of filixic acid.

Our studies confirmed these observations only with the distinction that instead of albaspidin a mixture of three homologous albaspidins was obtained.

When natural filixic acid was heated to and kept at the melting temperature for 5 min and the fused material was paper chromatographed by a method of Hegnauer [25], the formed albaspidins separated nicely. They were identified as albaspidin BB (VIII), albaspidin PB (IX) and albaspidin PP (X) by comparison with the R_F values of corresponding synthetic compounds [31].

On mixing equal amounts of the three filixic acids, the mixture should be expected to have the melting point of natural filixic acid, 183—185°. This could, however, not be verified; instead an unsharp melting point of 177—189° was observed. But when equal amounts of the same filixic acids were dissolved in warm ethyl acetate and the solution was cooled to room temperature the crystals so obtained melted sharply at 183—185° and were shown to consist of all three filixic acids. This behaviour probably is explainable by mixed crystal formation for which the three filixic acids constitute good qualifications, all having equal molecule skeletons which show differences only in the lengths of the hydrocarbon side chains.

In addition to the ordinary filixic acid melting at 183—185° another kind of natural filixic acid, earlier reported by Aho [32], was observed. This filixic acid could easily be obtained by mixing male fern extract with acetone and storing the mixture at —20° for some weeks. The crystalline precipitate was purified by dissolving it in methanol containing some ammonia and precipitating it with hydrochloric acid. After treatments with boiling methanol this filixic acid had a constant melting point of about 150°, earlier reported 148—149° [32]. Paper chromatographic studies of this product indicated the presence of the three mentioned filixic acids and, furthermore, three additional filixic acids could be verified with the R_F values 0.12, 0.07 and 0.02, respectively. By alkaline decomposition, besides butyryl- and propionylfilicinic acids, also acetylfilicinic acid could be identified.

On the grounds of these facts it seems most probable that the new filixic acids are analogically composed, with variations derived from an acetyl side chain. They ought, therefore, to be called filixic acid ABB, ABP and ABA. These filixic acids were not isolated in a pure state but a concentrate of the said could be obtained by recrystallizing the natural filixic acid melting at 150° from ethyl acetate and recovering the mother liquids. Then too, the melting point of the filixic acid could gradually be raised to the final melting point of 183—185° as the amounts of the filixic acids ABB, ABP and ABA reduced.

In regard to the chemical composition there is only a gradual difference between the both natural filixic acids. Though a mixture of pure filixic acids BBB, PBB and PBP recrystallized from ethyl acetate has a melting point of 183—185°, as mentioned above, the same melting point also can be observed for samples of natural filixic acid which clearly contain small amounts of the three additional filixic acids or some of them. Because ethyl acetate as recrystallization media has a tendency to concentrate the filixic acids BBB, PBB and PBP at the expense of the filixic acids ABB, ABP and ABA the final product melting at 183—185° always contains smaller amounts of the three last mentioned acids than does the natural filixic acid melting at 150°. This estimation is based on semiquantitative paper chromatographic analyses; more exact determinations based on hydrolytic liberation of the fatty acids

from filixic acid acyl side chains are being made and the results will be published later [31].

According to preliminary observations, in addition to filixic acid at least some of the other naturally occurring phloroglucinol derivatives from *Dryopteris filix mas* seem to exist as a mixture of homologues. We have never found such homologous series in *Dryopteris austriaca* extracts [31], which fact can be made use of in identifying *Dryopteris* extracts of unknown origin [31].

EXPERIMENTAL

Synthesis of filixic acid BBB. Butyrylfilicinic acid (4.48 g) and phlorobutyrophenone (1.96 g) were dissolved in potassium hydroxide (400 ml, 1 %) and formaldehyde (1.5 ml, 40 %) was added. The mixture was kept at room temperature for 15 min and made acidic with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. Treatment with cold methanol yielded an insoluble portion which was repeatedly recrystallized from ethyl acetate and acetone. The pure product had a m.p. of 172—174°. (Found: C 64.47; H 6.54. Calc. for $C_{36}H_{44}O_{12}$: C 64.67; H 6.59).

Synthesis of filixic acid PBP. Propionylfilicinic acid (4. 20 g) and phlorobutyrophenone (1.96 g) were dissolved in potassium hydroxide and the synthesis continued as above. The purified product had a m.p. of 192—194°. (Found: C 63.34; H 6.17. Calc. for $C_{34}H_{40}O_{12}$: C 63.75; H 6.25).

Diazoaminobenzene derivative of filixic acid BBB. To filixic acid BBB (334 mg) dissolved in chloroform (1 ml) was added diazoaminobenzene (200 mg) in ethanol (15 ml). The mixture was heated on a steam bath for some minutes and then kept at room temperature overnight. The precipitate recrystallized from ethanol had a m.p. of 235° and showed no depression when mixed with the analogical compound from natural filixic acid.

Isolation of natural filixic acids BBB, PBB and PBP. Counter-current extractor according to Hietala [28] was filled with benzene and borate buffer solution of pH 9.3. Natural filixic acid, m.p. 183—185° (10 g) was dissolved in benzene and the first ten tubes were filled with the solution. Extraction with the buffer solution was continued until a satisfactory separation could be verified by paper chromatography. The extractor was emptied by counter-current flow of benzene making use of a fraction collector. The fractions were analyzed by paper chromatography and each separated compound was recrystallized from ethyl acetate (Found: C 64.57; H 6.72. Calc. for $C_{36}H_{44}O_{12}$ (filixic acid BBB): C 64.67; H 6.59. Found: C 64.14; H 6.44. Calc. for $C_{35}H_{42}O_{12}$ (filixic acid PBB): C 64.22; H 6.42. Found: C 63.71; H 6.30. Calc. for $C_{34}H_{40}O_{12}$ (filixic acid PBP): C 63.75; H 6.25).

Alkaline cleavage of filixic acids: Filixic acid (30 mg) was dissolved in aqueous sodium hydroxide (5 %, 40 ml), zinc dust (700 mg) was added and the mixture heated on a steam bath for 5 min. The solution was diluted with water, filtered, acidified with hydrochloric acid (10 %) and extracted with ether. The ethereal solution was chromatographed [20] and butyrylfilicinic acid, m.p. 98°, propionylfilicinic acid, m.p. 110—112° and acetylfilicinic acid, m.p. 175—177°, were used as standards.

For identifying phloroacylophenones in the decomposition mixture, chromatography was made in unbuffered papers with tetraline-acetic acid-water (10:10:1) solution using phlorobutyrophenone, m.p. 185—186°, and phloropropiophenone, m.p. 179—181°, as standard substances.

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