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REVIEW

# The chemistry of dryopteris acylphloroglucinols

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The ancient remedies effective against helminthiasis caused by fish tape worm, *Diphyllobothrium latum*, include the powdered rhizomes and extracts of dryopteris ferns which have had, and still have, a widespread use among the parasite infested populations.

Extensive investigations in this field were started around the turn of the century by Boehm (1897, 1898, 1901a,b, 1903a–d) and resulted in the isolation of several dryopteris fern constituents as well as the resolution of the chemical structure of some of them. These studies have served as a basis for the current knowledge of the chemistry of dryopteris acylphloroglucinols.

The basic chemical structure of the dryopteris constituents may be presented as a two-ring construction in which a butyrylfilicinic acid moiety (A-ring) is linked either to another similar moiety or to a *C*- or *O*-methylated butyrylphloroglucinol moiety (B-ring), by means of a methylene bridge. Naturally occurring variations of this basic pattern include substitutions of the A-ring by an acylphloroglucinolic nucleus, enlargement of the molecule by additional acylphloroglucinol units to yield trimer or tetramer structures, substitution of the butyryl side-chain by an acetyl or propionyl homologue, and insertion of a pyrone ring structure instead of a phloroglucinolic one.

## CHEMICAL REACTIONS OF THE ACYLPHLOROGLUCINOLS

The general chemical reactions of the dryopteris acylphloroglucinols are those logically derived from their instability under alkaline or oxidative conditions, or both. Thus, the methylene bridge between the two rings is usually easily broken up by alkalis and the acyl side-chain can be removed by the action of either alkalis or strong acids. As phenols these compounds are readily oxidized and especially under alkaline conditions decomposition due to autoxidation is apt to follow if not prevented.

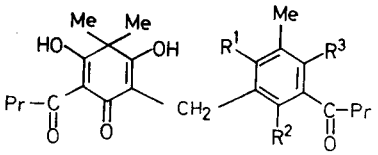
The easily occurring rupture of the methylene linkage leads to a reaction typical for asymmetric polyhydroxydiphenylmethanes of the type $R_1$–$CH_2$–$R_2$. It involves an interchange of the rings and results in an equilibrium between the original asymmetric dimer and the two symmetric dimers, $R_1$–$CH_2$–$R_1$ and $R_2$–$CH_2$–$R_2$. This reaction, known as the rottlerone change, was originally studied on the closely related phenolic constituents of kamala, an Indian colouring matter and anthelmintic drug (Backhouse, McGookin & others, 1948; McGookin, Robertson & Simpson, 1951). In the dryopteris series the rottlerone change was first reported by Birch (1951), and soon it was observed that e.g. flavaspidic acid (I) when heated in carbonate solution undergoes rottlerone change yielding albaspidin (XI) (McGookin, Robertson & Simpson, 1953).

### *Flavaspidic acid*

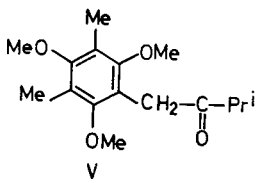
Flavaspidic acid (I) is the methylene linked dimer of butyrylfilicinic acid and methylbutyrylphloroglucinol. This structure was discussed by Boehm (1901b), but

to explain certain decomposition products of flavaspidic acid, Boehm (1901b, 1903b) preferred a slightly modified structure. The latter was commonly accepted until McGookin & others (1953) and Riedl (1954) practically simultaneously synthesized flavaspidic acid and thereby confirmed its structure as (I).

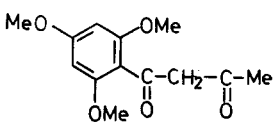
Flavaspidic acid exhibits two tautomeric forms: the enolic α-form is obtained through crystallization from ethanol and has a melting point of 92–95°, while the ketonic β-form, obtainable from benzene or glacial acetic acid, has a m.p. of 156°



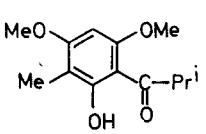
I $R^1$ = OH $R^2$ = OH $R^3$ = OH
II $R^1$ = OH $R^2$ = OH $R^3$ = OMe
III $R^1$ = MeO $R^2$ = OH $R^3$ = OH
IV $R^1$ = OH $R^2$ = MeO $R^3$ = OH



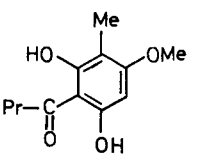
V



VI



VII



VIII

(Boehm, 1903b; Aho, 1958). Since the α-form is convertible into the β-form by heat, under gradual increase of the temperature the former actually shows two melting points separated from each other by a crystalline phase.

*Aspidin*, para-*aspidin and related compounds*

When the B-ring of flavaspidic acid is replaced by its monomethoxyl derivative, three isomeric compounds, aspidin (II), *para*-aspidin (III) and iso-aspidin (IV) are obtained. Aspidin was isolated by Boehm (1897, 1898, 1903c) but the chemical structure then ascribed to it was later shown to be that of *para*-aspidin (III), which was isolated, characterized, and synthesized by Penttilä & Sundman (1962a). Riedl & Mitteldorf (1956) reinvestigated the naturally occurring aspidin and its decomposition products and proved the structure (II) by synthesis. Independently, the same structure was proposed by Aebi, Kapoor & Büchi (1957b).

The third isomer of this group, iso-aspidin (IV), is a synthetic compound (Penttilä & Sundman, 1964b), so far not found in dryopteris ferns.

It would be possible to consider the B-ring to be constituted of a two- or three-methoxyl derivative of methylbutyrylphloroglucinol. Since such related polymethoxyl compounds are common in nature—e.g. torquatone (V), eugenone (VI) and baeckeol (VII)—their occurrence in *Dryopteris* species could well be considered. However, this is not so; the methoxylated constituents are composed of monomers carrying only one methoxyl group.

The mixtures of acylphloroglucinols from *Dryopteris* species almost always contain variable amounts of the mononucleous aspidinol (VIII), first isolated by Boehm (1897, 1901a, 1903a) and synthesized by Karrer & Widmer (1920) and Robertson &

Sandrock (1933a). However, the actual occurrence of aspidinol in nature has seemed questionable and careful investigations have led to the opinion that aspidinol is an artifact arising chiefly from *para*-aspidin (III) (Penttilä & Sundman, 1966). In fact, only those *Dryopteris* species that are devoid of *para*-aspidin, have been shown to yield aspidinol-free concentrates of acylphloroglucinols (Penttilä & Sundman, 1966; Wieffering, Fikenscher & Hegnauer, 1965; Widén, 1967a, 1968). The easily occurring decomposition of *para*-aspidin may also explain why this dimer has escaped detection for so long, in spite of its widespread occurrence in various *Dryopteris* species.

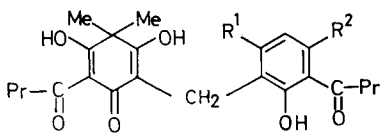
The occurrence in nature of other monomeric acylphloroglucinols, especially methylbutyrylphloroglucinol, has been reported by Stahl & Schorn (1963) but not confirmed.

*Desaspidin and* ortho-*desaspidin*

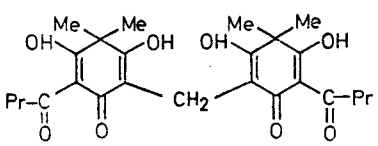
The corresponding isomers derived from butyrylphloroglucinol acting as the B-ring are desaspidin (IX) and *ortho*-desaspidin (X). The former, an analogue of *para*-aspidin (IV), was first detected and isolated by Büchi, Aebi & Kapoor (1957). The structure of desaspidin (IX), based on decomposition analyses and spectral studies was later confirmed by synthesis (Penttilä & Sundman, 1962b, 1964a).

Of the variety of acylphloroglucinol derivatives isolated from dryopteris, desaspidin has won the most widespread interest among investigators: it has been shown to be superior in anthelmintic activity to other dimers from the same source (Östling, 1961; Mühlemann & Tatrai, 1969), and its capability of acting as a powerful uncoupler in oxidative phosphorylations has been intensively studied in recent years (Runeberg, 1963; Hind, 1966; Gromet-Elhanan & Avron, 1966).

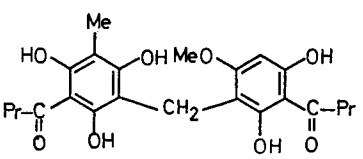
The occurrence in nature of the dimer corresponding to aspidin, *ortho*-desaspidin (X), was to be expected on analogical grounds. It was synthesized and its chemical and chromatographic properties were examined. Guided by this knowledge, Penttilä & Sundman (1964b) detected and isolated it from natural sources, where it occurs only in trace quantities.



IX $R^1$ = MeO, $R^2$ = OH  X $R^1$ = OH, $R^2$ = OMe



XI



XII

*Albaspidin*

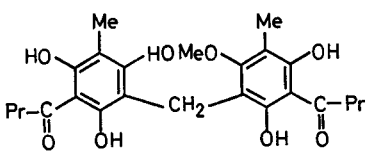
The dimers presented hitherto have been composed of a butyrylfilicinic acid moiety and a methylated butyrylphloroglucinol moiety. If two butyrylfilicinic acid moieties are combined *via* a methylene bridge, the symmetrical albaspidin (XI) is obtained.

It was isolated and characterized by Boehm (1897, 1901b) who also synthesized albaspidin by condensing two molecules of butyrylfilicinic acid with formaldehyde.

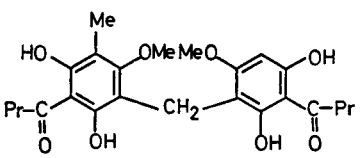
*Phloraspin and margaspidin*

When the butyrylfilicinic acid rings of desaspidin (IX) and *para*-aspidin (III) are substituted by a methylbutyrylphloroglucinol unit, phloraspin (XII) and margaspidin (XIII), respectively, are obtained. Phloraspin is almost insoluble in benzene and therefore can be easily isolated from dryopteris extracts even though it can only be detected occasionally and in relatively small quantities. Boehm (1903d) proposed the structure (XII) for phloraspin, and this was later confirmed by Penttilä & Sundman (1961c) who synthesized it.

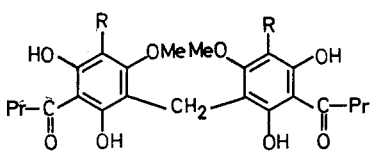
Margaspidin (XIII) is the major compound of *Dryopteris marginalis*, the American substitute for *Dryopteris filix-mas* (Penttilä & Kapadia, 1965). *D. marginalis* is known in commerce as American Aspidium or Marginal Fern, and has been the source of the official drug in the United States. In contrast to the other common acylphloroglucinols, margaspidin has so far been found only in the North American *D. marginalis*, while all the other usual acylphloroglucinols have been reported to occur in both the European and American species (Hegnauer, 1961; Fikenscher & Gibson, 1962; Fikenscher & Hegnauer, 1963a; Wieffering & others, 1965; Penttilä & Kapadia, 1965).



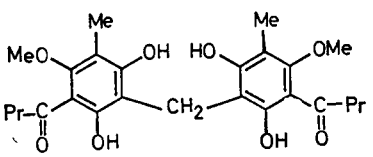
XIII



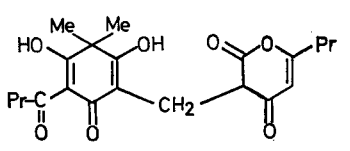
XIV



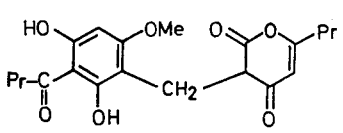
XV R=H XVI R=Me



XVII



XVIII



XIX

*Phloraspidinol and methylene-bis-desaspidinol*

From dryopteris ferns only two dimers have been isolated with a methoxyl group in each ring: phloraspidinol (XIV) and the symmetrical methylene-bis-desaspidinol (XV) (Penttilä & Sundman, 1963b). The closely related and likewise symmetrical homologue methylene-bis-aspidinol (XVI) (Boehm, 1903a) could well be expected to be a naturally occurring dimer, but so far it has not been detected (Penttilä &

Sundman, 1963b). Neither has $\psi$-aspidin (XVII), a dimer easily obtained from aspidin in alkaline conditions (Boehm, 1903c; Riedl & Mitteldorf, 1956).

*Phloropyron and phloraspyron*

At the first glance it may seem surprising that the structurally rather homogenous group of acylphloroglucinols from *Dryopteris* species is disordered by compounds where an acylphloroglucinol moiety is substituted by a pyronic one as is the case in phloropyron (XVIII) and phloraspyron (XIX) (Penttilä & Sundman, 1961b, 1963b). Biogenetically, this coexistence seems much less confusing, since the two ring structures may be considered to arise from a common precursor: butyrylphloroglucinol from acetate and four malonate units *via* *C*-acylation, and pyronone from acetate and three malonate units *via* *O*-acylation (Sundman & Penttilä, 1964; Penttilä, 1967).

Further relations between acylphloroglucinols and pyrones have been examined on a different basis: some pyrones and polypyrones have been proposed as stabilized intermediates in the biosynthesis of acylphloroglucinols and other phenolic compounds (Money, Qureshi & others, 1965; Douglas & Money, 1967; Comer, Money & Scott, 1967). On the other hand, successful biogenetic-type syntheses *in vitro* of both acylphloroglucinols and pyrones have been recently effected by cyclization of polyketoacids or their esters (Harris & Carney, 1967; Harris & Combs, 1968).

An interesting connection between acylfilicinic acid and pyrone structures has recently been reported by Young & Hart (1967a,b). In a study of the photochemical behaviour of acetylfilicinic acid these authors showed that a ready photoisomerization occurred yielding a pyrone compound analogous to the pyrone moieties of dryopteris.

In 1897, Boehm isolated from *Dryopteris* species a colourless crystalline substance, aspidinin. A year later Poulsson (1898) described a similar compound which he called polystichinin. No attempts were made to resolve the chemical structure of either of them until 1961 when phloropyron (Penttilä & Sundman, 1961b) was isolated, characterized and its properties found to be similar to those of aspidinin and polystichinin.

The usual method for resolving the structure of unknown dryopteris constituents comprises a reductive cleavage of the methylene bridge and identification of the two monomeric acylphloroglucinols as themselves or as their one-higher homologues, depending on which side of the methylene bridge the cleavage has occurred. The identification is then completed by synthesis where the two monomers are condensed with formaldehyde and the expected dimer isolated and proved identical with the natural compound.

With phloropyron the major difficulties were connected to the non-phloroglucinolic B-ring: its identification as 2,3-dihydropyran-2,4-dione was preceded by chemical and spectral analyses and partial syntheses (Penttilä & Sundman, 1961b).

*Filixic acids*

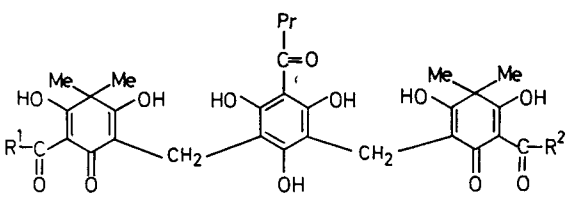
Investigations of filixic acid during the fifty years following its detection (Luck, 1845) revealed little concerning its chemical structure. Luck (1851) found that when filixic acid was treated with acids or alkalies, it liberated butyric acid, and Grabowski (1867) found it to be a derivative of phloroglucinol. Several different structures for filixic acid were proposed and discussed, but the first decisive contribution was made by Boehm (1901b) who found out that filixic acid was composed of three rings of which two were butyrylfilicinic acids and the third was butyrylphloroglucinol. These

fragments were combined by means of methylene bridges to yield the molecule of filixic acid. Riedl (1954, 1955), and later Chan & Hassall (1957), suggested minor modifications in this structure, but an attempted synthesis to prove it, failed (Riedl, 1955).

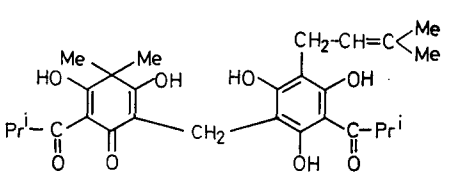
Recent studies have shown that the naturally occurring filixic acid is not an unambiguous compound but rather a mixture of six homologues which vary in the length of the acyl side-chains of their filicinic acid nuclei (Penttilä & Sundman, 1963a). The major constituents of the mixture are the filixic acids BBB (XXa), PBB (XXb) and PBP (XXc), in which the acyl side-chains attached to the filicinic acid rings are butyryl-butyryl, propionyl-butyryl or propionyl-propionyl, respectively. All of these have been isolated in pure form and their chemical characterization completed by the syntheses of the symmetrical filixic acids BBB and PBP (Penttilä & Sundman, 1963a).

Probably because of mixed crystal formation, the individual filixic acids cannot be separated by the usual methods although a partial concentration of the butyryl and butyryl-propionyl homologues at the expense of the acetyl homologues, filixic acid ABB (XXd), ABP (XXe) and ABA (XXf), may be attained. Most chromatographic methods also fail to separate the homologues; a fairly good separation can be achieved on buffered papers (Penttilä & Sundman, 1961a, 1963a) and with other special methods (Widén, 1968).

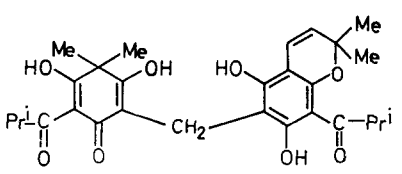
The occurrence of homologous mixtures is not limited to filixic acids but is rather a general phenomenon within certain *Dryopteris* species. Neither are the variations of the side-chain lengths limited to the filicinic acid nucleus, they may also concern the phloroglucinolic ring but those on the side-chains of the filicinic acid nuclei appear to predominate.



XXa $R^1$ = Pr $R^2$ = Pr
XXb $R^1$ = Et $R^2$ = Pr
XXc $R^1$ = Et $R^2$ = Et
XXd $R^1$ = Me $R^2$ = Pr
XXe $R^1$ = Me $R^2$ = Et
XXf $R^1$ = Me $R^2$ = Me



XXI



XXII

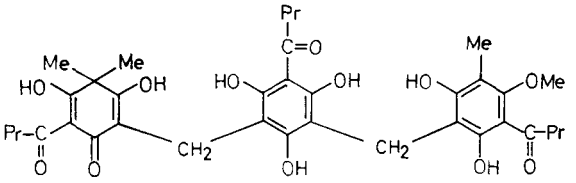
The distribution of the acetyl, propionyl and butyryl side-chains within certain species is constant and conversely offers a valuable tool in chemotaxonomic studies

of *Dryopteris* species and hybrids (Penttilä & Sundman, 1964a; Wieffering & others, 1965; Widén, 1968, 1969).

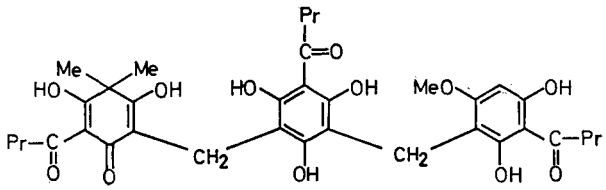
An analogical case of homologous mixtures has recently been reported by Parker & Johnson, 1968; Parker, Flynn & Boer, 1968). The uliginosins A (XXI) and B (XXII) isolated from *Hypericum uliginosum* are composed of phloroglucinol and filicinic acid residues carrying isobutyryl side-chains. A homologous impurity present in the isolated crystals of these compounds is assumed to carry valeroyl groups instead of isobutyryl groups.

*Trisaspidin, trisdesaspidin and trisflavaspidic acid*

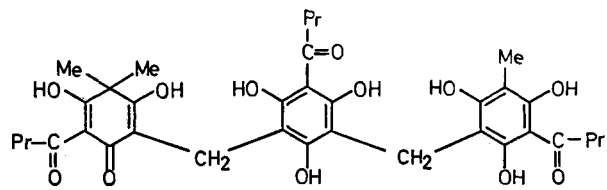
Besides filixic acids some other three-ring compounds have been isolated. They can be derived from known two-ring components by adding a butyrylphloroglucinol unit between their two rings. Thus trisaspidin (XXIII) has a butyrylphloroglucinol nucleus between the two rings of aspidin (II), correspondingly trisdesaspidin and trisflavaspidic acid have the structures (XXIV) and (XXV), respectively (Penttilä & Sundman, 1963c). These compounds are found only in trace quantities in *Dryopteris* ferns, but according to Widén (1967a) the trimer trisdesaspidin can always be detected in certain species when the corresponding dimer, desaspidin, is present in higher amounts.



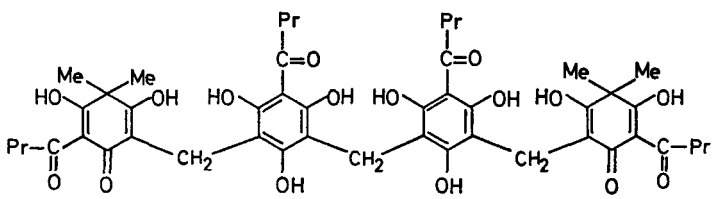
XXIII



XXIV



XXV



XXVI

*Methylene-bis-norflavaspidic acid*

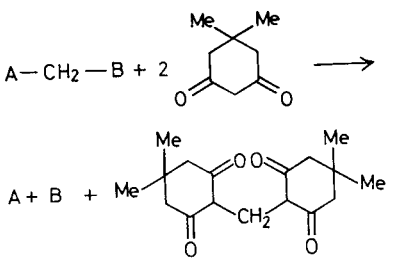
The only four-ring compound isolated from dryopteris is composed of two butyryl-filicinic acid residues separated by two butyrylphloroglucinol residues and is known as methylene-bis-norflavaspidic acid (XXVI) (Penttilä & Sundman, 1963d). Owing to its symmetric structure, methylene-bis-norflavaspidic acid can be synthesized by condensing with formaldehyde two molecules of nor-flavaspidic acid, a dimer derived from flavaspidic acid by omitting its ring methyl group.

Nor-flavaspidic acid is a synthetic compound.

## DETECTION AND IDENTIFICATION OF DRYOPTERIS ACYLPHLOROGLUCINOLS

The ferric chloride reaction of 1,3-diketones has been widely used for the detection of dryopteris acylphloroglucinols. A variety of crystalline derivatives with distinct melting points, especially the reaction products with diazoaminobenzene, phenylhydrazine and aniline, have been prepared and have proved useful in the identification of unknown compounds (Poulsson, 1898; Boehm, 1901a,b, 1903c).

Besides the formation of the usual derivatives of phenols the different decomposition products of acylphloroglucinols have been examined. The mild alkaline reductive cleavage on either side of the methylene bridge theoretically results in the liberation of the two rings of which either one may carry the reduced bridge methyl group. This has been developed into the method of choice for identifying unknown dimers, as already mentioned on page 397. However, complications arise because the cleavage occurs preferably on one side of the bridge and because the one higher homologue carrying the bridge methyl group is not always stable and identifiable. A modification of the method has been developed in which an excess of dimedone is added to the alkaline solution of the dimer. Since dimedone has proved capable of substituting the two rings its reaction results in quantitative removal of the methylene bridge whereby the two rings are liberated and can be identified (Scheme 1). This method




Scheme I

has been used with advantage in biosynthetic studies of labelled dimers (Penttilä, 1967).

Under more destructive alkaline treatments the dimers are deprived of their acyl side-chains as well as their methoxyl groups. This reaction yields a mixture of homologous methyl derivatives of phloroglucinol and filicinic acid (Boehm, 1898, 1903a).

Finally, spectral analyses have been applied in structure studies of fern constituents (Aebi, 1956; Aebi, Kapoor & Büchi, 1957b; Aho, 1958), but the most comprehensive progress in the detection, identification and structural studies of dryopteris acylphloroglucinols has been made with chromatography.

## CHROMATOGRAPHY

The first paper chromatographic attempts to separate dryopteris constituents were made by Büchi & others (1957) and Klevstrand (1957, 1960). The former method was based on reversed-phase chromatography using a formamide–water mixture as the mobile phase on ether–pyridine impregnated paper. Klevstrand achieved separation on formamide or formamide-dimethylformamide impregnated paper developed by hexane–chloroform or benzene–chloroform mixtures. Godin (1958) separated some acylphloroglucinols by using sodium carbonate solution as an eluent.

A thorough evaluation and comparison of these methods has been published by Zwimpfer & Büchi (1963a,b, 1964) who improved the technique of Godin to allow the separation of all known acylphloroglucinols and alkaline decomposition products.

Hegnauer (1961) described a two-solvent system, to run in parallel, for identifying phloroglucinols. Later he and his colleagues successfully used a method based on varying concentrations of acetic acid as eluent (Fikenscher, 1962; Fikenscher & Gibson, 1962; Fikenscher & Hegnauer, 1963a,b; Wieffering & others, 1965). Penttilä & Sundman (1961) used buffered papers treated with formamide and developed by benzene–chloroform or cyclohexane-chloroform. All known acylphloroglucinols and their monomeric decomposition products can be separated by this method according to the pH of the buffered papers. Furthermore, at the correct pH the homologous filixic acids migrated yielding distinctly separate spots; other homologues could also be separated on buffered papers (Penttilä & Sundman, 1963a, 1964a). In the study of homologous compounds the method was complemented by the paraffin-acetic acid method of Fikenscher & Hegnauer (*loc. cit.*) which also can be used to separate homologous mixtures.

Thin-layer chromatography was first applied on dryopteris constituents by Stahl & Schorn (1962). v. Schantz (1962a) modified the thin-layer method by buffering the plates to pH 6·0 and this method has later been used as base for the quantitative or semiquantitative estimation of acylphloroglucinols (v. Schantz, 1962b; Widén, 1967a,b; v. Schantz & Widén, 1967).

Further modifications of the thin-layer technique have been reported by Blakemore, Bowden & others (1964), v. Schantz, Ivars & others (1964) and Fish & Kirk (1968).

Column chromatography was applied on dryopteris phloroglucinols by Fichter as early as 1938 but without success. A few years later Mühlemann & Käsermann (1942) likewise attempted separation of raw filicin on a column of alumina, but real progress was made only when acylphloroglucinol mixtures were chromatographed on silica gel, $CaSO_4$, or polyamide powder (Aebi & others, 1957a,c; Penttilä & Sundman, 1963b,c,d; 1964b; Penttilä & Kapadia, 1965; Carelli & Petrangeli, 1961; Widén, 1967a,b, 1968).

Several colour reactions have been employed to identify acylphloroglucinols on paper or thin-layer chromatograms. Zwimpfer & Büchi (1963b) have given a detailed survey of the variety of reagents used for this purpose and they conclude that ferric chloride-potassium ferricyanide and the Folin-Ciocalteu reagent are the most sensitive. Penttilä & Sundman (1961a), however, have found the fast blue salt B (tetrazotized di-*o*-anisidine) superior to all reagents tested.

## SYNTHESES OF DRYOPTERIS ACYLPHLOROGLUCINOLS

After deducing the structures of unknown isolated compounds from their chemical and spectral analyses, the task is preferably completed by an unambiguous synthesis.

With symmetrical dimers this could be readily done by condensing two molecules of the monomer with formaldehyde in slightly alkaline solution. Theoretically, the synthesis of any unsummetrical dimer is quite similar, difficulties may arise only from the fact that the reaction product is made up of a mixture of three compounds: two symmetrical ones and the wanted unsymmetrical compound. In certain instances it has been possible to increase the yield of the wanted dimer at the expense of either of the other two (Penttilä & Sundman, 1963b; Penttilä & Kapadia, 1965). This basic method and modifications of the same have been adapted for industrial production of anthelmintically active acylphloroglucinols, especially desaspidin (Andersen, Pentillä & others, 1967a, b, 1968a).

In a broader sense the synthesis of acylphloroglucinols comprises the synthetic preparation of the individual monomers. Thus, Robertson & Sandrock (1933b), Angus, Clark & others (1954) and Riedl & Risse (1954) have synthesized filcinic acid, the latter two authors have also prepared the butyryl derivative of filicinic acid. Recently, the syntheses of acylfilicinic acids have been thoroughly studied (Andersen, 1968; Andersen, Laurén & others, 1968b).

The aromatic moieties of the natural dimers have been synthesized by several investigators, i.e. aspidinol has been prepared by Karrer & Widmer (1920), Riedl & Mitteldorf (1956), and Robertson & Sandrock (1933a). A general method for the syntheses of *para-O*-alkylated polyphenolketones has been reported by Andersen, Penttilä & others (1967a).

## BIOSYNTHESIS OF DRYOPTERIS ACYLPHLOROGLUCINOLS

Since Birch & Donovan (1953) presented their study on some possible biosynthetic routes to derivatives of phloroglucinol, the intramolecular *C*-acylation of the four-unit head-to-tail acetate chain to yield acetylphloroglucinol has become a classical example of biosynthetic principles. Both acetylphloroglucinol, as well as its homologue, butyrylphloroglucinol, may be considered as precursors of the naturally occurring dryopteris fern constituents. Results from a biosynthetic study of living *D. marginalis* plants, using a radioactive labelling technique, indicated that butyric acid was the source of the butyryl side-chain, and that the *C*- and *O*-methyl groups were derived from methionine (Penttilä, Kapadia & Fales, 1965; Penttilä, 1967). Furthermore, the methylene link between the two rings was labelled equally with the methyl groups, which suggested that an aromatic methyl was the precursor of the methylene bridge. This aspect was confirmed by enzymatic *in vitro* studies in which appropriate monomers were combined with peroxidase and hydrogen peroxide to yield the naturally occurring dimers (Penttilä & Fales, 1966; Penttilä, 1967).

## BIOLOGICAL EVALUATION OF DRYOPTERIS CONSTITUENTS

In the search for effective drugs against the fish tape worm *Diphyllobothrium latum* and related parasites, a variety of techniques and test animals have been applied. The screening of dryopteris extracts and isolated phloroglucinol derivatives has been recently reviewed (Zwimpfer & Büchi, 1962; Fish & Calderwood, 1966) and will not be repeated here.

In spite of countless attempts it appears not to have been possible to create an entirely reliable method by which the anthelmintic activity could easily be evaluated. In the screening work recently performed, the dwarf tape worm (*Hymenolepis nana*) has been used for *in vitro* and *in vivo* tests of mice (Blakemore & others, 1964; Bowden,

Broadbent & Ross, 1965; Steward, 1955; Sen & Hawking, 1960). Unfortunately, results obtained with these methods have proved inconsistent with controlled clinical tests (Rosengård, S., Hackman, C. R., Räsänen, T. A. & Hiltunen, R. A., personal communication). Attempts made so far to cultivate the fish tape worm *in vitro* have shown little promise. Work on this line is being continued, however, and it is to be hoped that a reliable biological evaluation method allowing an easy screening of new anthelmintics will be available soon.

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