CHEMOTAXONOMY OF EUROPEAN PTERIDOPHYTA

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Summary.

The biochemical systematics in the European Pteridophyta is reviwed. The main biochemical characters (flavonoids and related compounds, acylphloroglucinol derivatives, terpenoids) and other biochemical characters (amino acids, fatty acids, alkaloids, proteins, nucleic acids) are discussed mainly in connection with taxonomic and phylogenetic relationships. The utility of chemical analysis for solving the origin of hybrids is discussed.

Key words: European pteridophyta; chemotaxonomy; phylogeny.

Riassunto.

I costituenti di maggior interesse chemotassonomico delle Pteridofite Europee (flavonoidi e composti ad essi correlati, derivati dell'acilfloroglucina, terpenoidi) ed altri gruppi di costituenti chimici (amminoacidi, acidi grassi, alcaloidi, proteine, acidi nucleici) sono discussi principalmente in funzione di correlazioni tassonomiche e filogenetiche. Inoltre é descritto il contributo fornito dall'analisi dei costituenti chimici alla determinazione dell'origine degli ibridi.

Parole chiave: Pteridofite europee, chemotassonomia, filogenia.

INTRODUCTION.

In 1962 data on chemotaxonomy of Pteridophyta were reviwed for the first time (HEGNAUER, 1962); six years later a review dealing with the chemical constituents of ferns was published (BERTI and BOTTARI, 1968). These two reviews gave information of limited value from the systematic point of view. Subsequently many interesting works on the distribution of chemical constituents of Pteridophyta have appeared and these results have been reviewed (SWAIN and COOPER-DRIVER, 1973; GIANNASI, 1974; COOPER-DRIVER, 1980).

In the present review, data of chemotaxonomic and phylogenetic interest on European Pteridophyta are emphasized. The most interesting biochemical characters (flavonoids and related compounds, acylphloroglucinol derivatives, terpenoids) as well as other biochemical characters (amino acids, fatty acids, alkaloids, proteins, nucleic acids) are discussed mainly in relation to the possible utility in systematic problems.

FLAVONOIDS AND RELATED COMPOUNDS.

It is well known that plant flavonoids are easly isolated even from herbarium tissue; these compounds are widespread in Pteridophyta and may be identified by not expensive instruments. Flavonoid evolution in plants shows two steps: elaboration of structure and, secondarily, reduction of structure (Tab. 1). Hence these compounds have a considerable structure diversity which may be of interest in the study of taxonomic and phylogenetic relationships.

The skeletons for the classes of flavonoids and related compounds discussed in this review are shown (I-VIII) in Fig. 1.

Flavonoid chemistry of fern allies is of interest from the systematic point of view. Biflavonyl compounds (e.g. amentoflavone (IX)) have been found (VOIRIN, 1970) in *Selaginella* ; these compounds (which are considered primitive biochemical character) are absent (VOIRIN, 1970) from *Lycopodium, Isoetes* and *Equisetum* ; these results show that *Selaginella* may not be closely related to other members of the *Lycopsida* (SWAIN and COOPER-DRIVER, 1973). *Lycopodium* and *Isoetes* have (VOIRIN et al., 1976; VOIRIN and JAY, 1978a and b) the rare flavones chrysoeriol (X), selgin (XI) and tricin (XII). In addition *Isoetes* contain (VOIRIN et al., 1975) luteolin (XIII) bearing an extra hydroxyl group in 6-, 8- or 2'- position and it is of interest that these compounds are absent from *Lycopodium*.

Among fern allies flavonols have been found (VOIRIN, 1970) only in *Equisetum* ; the presence of these compounds represents an advancement from the evolutionary point of view since it has been suggested (SWAIN, 1979) that flavonols may be protectective agents against phytopatogens and predators. Table 2 shows that flavonol glycosides of some European species of *Equisetum* have differences in their glycosylation patterns.

Flavonol glycosides based on kaempferol (XIV) and quercetin (XV) are widespread in *Equisetum*. However some species have (COOPER-DRIVER, 1980) gossypetin (XVII) and herbacetin (XVIII) which are flavonols with an hydroxyl group in position 8; in addition the flavanone naringenin (XIX) has been isolated by Russian workers (SYRCHINA et al., 1975; 1978). In ferns flavonols (III) and proanthocyanidins (IV) are common and flavones (I) are also present. Among flavonols kaempferol (XIV) and quercetin (XV) are common (VOIRIN, 1970) but myricetin (XVI) is near absent as it has been identified only in two species one of which is the European fern *Cheilanthes fragrans* (IMPERATO,1986). By contrast, myricetin is present in 10% of angiosperms (VOIRIN, 1970) and it has been suggested (BATE-SMITH, 1969) that the presence of the B-ring vicinal trihydroxyl grouping may be considered a primitive biochemical character whereas the absence of this grouping may be regarded as an advanced biochemical character. In addition the B-ring vicinal trihydroxyl grouping (XVI) may be (BATE-SMITH and SWAIN, 1965; WILLIAMS and SWAIN, 1971) a remnant of a primitive biosynthetic patway different from patway leading to flavonoids with an hydroxyl group in 4' position or two hydroxyl groups in 3' and
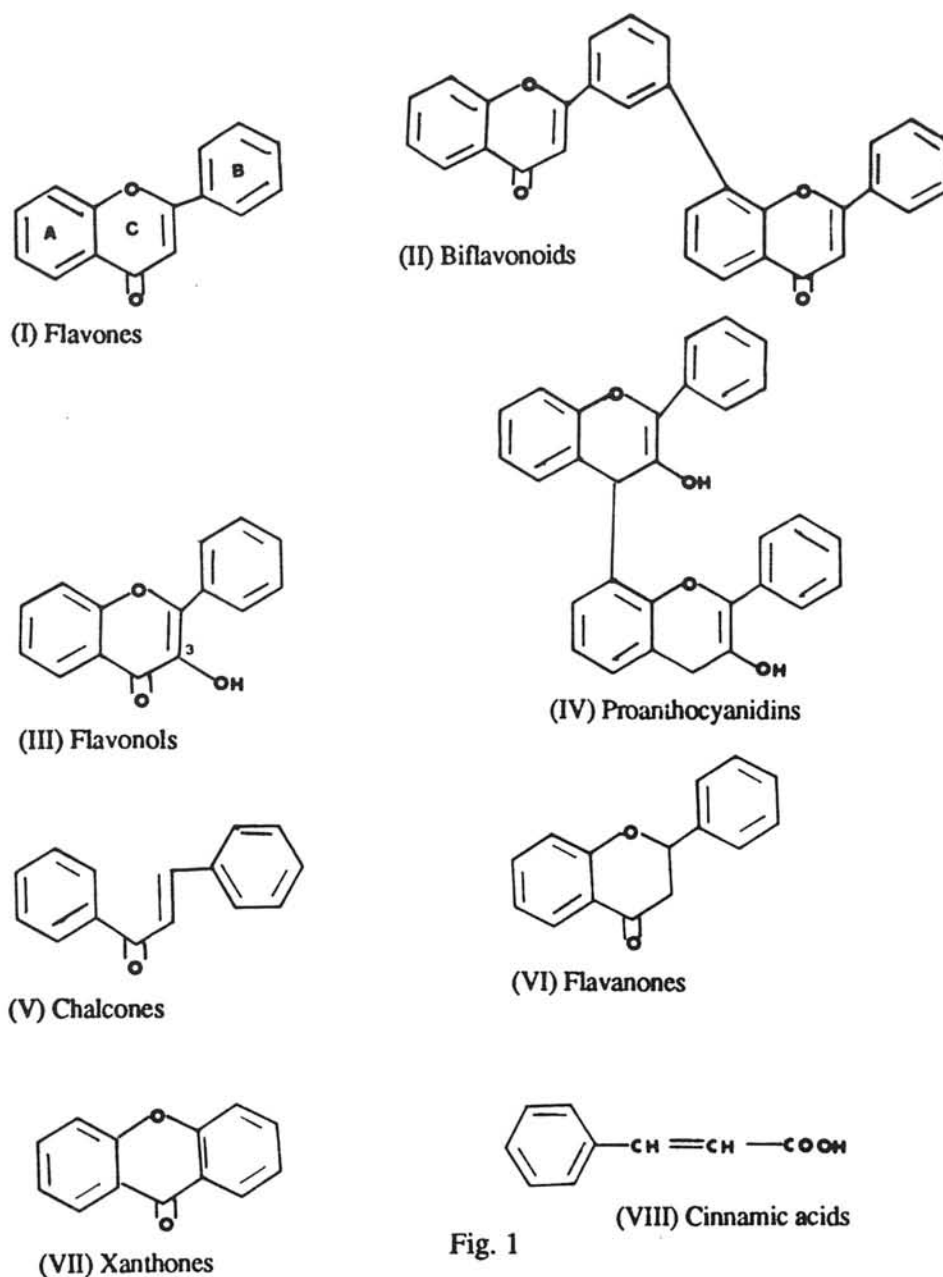

Elaboration of structure
- Oxidation in 3-position
- Change from C-glycosylation to O-glycosylation
- 6-, 8- and 2' -Hydroxylation

Reduction of structure
- Replacement of flavonols by flavones
- Elimination of trihydroxylation in flavonol B-ring
- Presence of xanthones, chalcones and flavanones

Table 1.   Flavonoid Evolution within the Pteridophyta

(I) Flavones

(II) Biflavonoids

(III) Flavonols

(IV) Proanthocyanidins

(V) Chalcones

(VI) Flavanones

(VII) Xanthones

(VIII) Cinnamic acids

Fig. 1

Species	Kaempferol Glycosides			Quercetin Glycosides
	3-glycosides	7-glycosides	3, 7-diglycosides	3, 7-diglycosides
Equisetum telmateia[1]	+	+	+	
E. ramosissimum[2]	+		+	
E. palustre[3]			+	
E. silvaticum[4]			+	+

Table 2. Variations in Glycosylation Patterns of Flavonol Glycosides from some European *Equisetum* species.
[1]Data from Geiger et al., 1978; [2] Data from Saleh and Abdalla, 1980; [3] Data from Beckmann and Geiger, 1963; [4] Data from Aly et al., 1975.
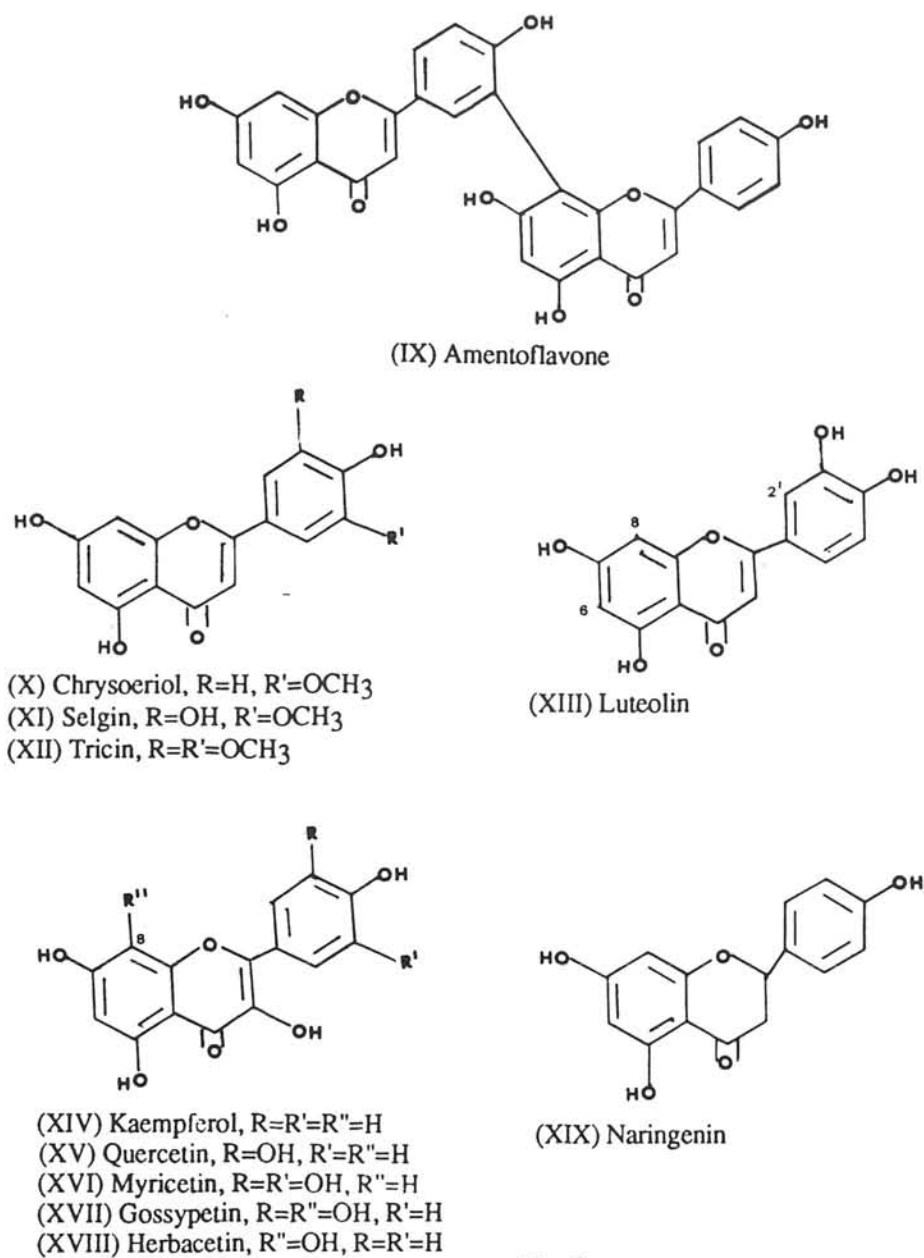
(IX) Amentoflavone

(X) Chrysoeriol, R=H, R'=OCH$_3$
(XI) Selgin, R=OH, R'=OCH$_3$
(XII) Tricin, R=R'=OCH$_3$

(XIII) Luteolin

(XIV) Kaempferol, R=R'=R"=H
(XV) Quercetin, R=OH, R'=R"=H
(XVI) Myricetin, R=R'=OH, R"=H
(XVII) Gossypetin, R=R"=OH, R'=H
(XVIII) Herbacetin, R"=OH, R=R'=H

(XIX) Naringenin

Fig. 2

Species	Kaempferol Glycosides			Quercetin Glycosides	
	3, 7-diglycosides	3-glycosides	3-(sulphate)-glycosides	3-glycosides	3-(sulphate) glycosi
Asplenium trichomanes	+				
A. septentrionale	+	+	+	+	+
A. filix-foemina Bernh	+		+		
A. fontanum Bernh		+	+		

Table 3. Differences in the Glycosylation Patterns of Flavonol Glycosides from some European *Asplenium* species. Data from Imperato, 1989.

4' positions of B-ring.

Biflavonyl compounds (II) are completely absent from ferns and it has been suggested (GEIGER and QUINN, 1975) that the ability to form these substances was lost during evolution leading to ferns. Chemical investigation (HARBORNE, 1966) on red pigments of the juvenile fronds of several fern species has led to isolation of 3-deoxyanthocyanidins (e.g. apigenidin 5-O-glucoside (XX) and luteolinidin 5-O-glucoside (XXI)) from ferns including *Osmunda regalis* and *Pteris vittata* ; these substances may be regarded (HARBORNE, 1966) as primitive biochemical character since they are simpler than 3-hydroxylated anthocyanins and have been found in mosses and are of rare occurrence in higher plants.

According to COOPER-DRIVER, the most ancient fern families accumulate only flavonols (III) and proanthocyanidins (IV) whereas the most advanced families have also flavanones (VI), chalcones (V), xanthones (VII) and often flavones (I). In this connection it is of
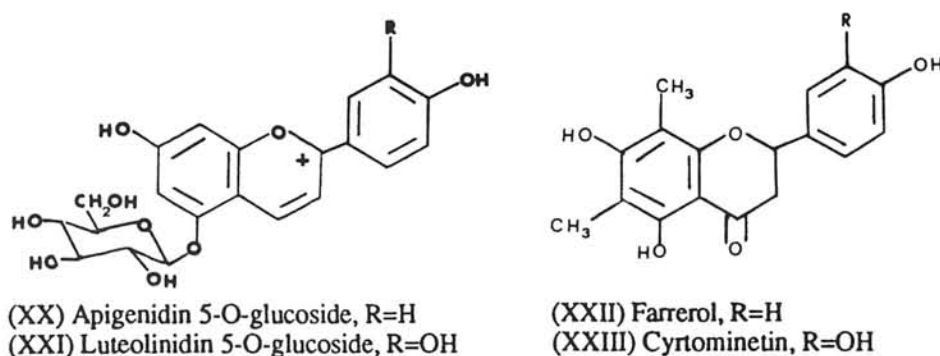

(XX) Apigenidin 5-O-glucoside, R=H
(XXI) Luteolinidin 5-O-glucoside, R=OH

(XXII) Farrerol, R=H
(XXIII) Cyrtominetin, R=OH

Figura 3.-

Aglycone	European species	Species from New World
Kaempferol	+	+
Quercetin	+	
Kaempferol 4'-methyl ether		+
Kaempferol 3, 4'-dimethyl ether		+

Table 4.   Geographical Distribution of Aglycones of Flavonol Glycosides from *Asplenium* species. Data from Imperato, 1989.

interest that two rare flavanones (farrerol (XXII) and cyrtominetin (XXIII)) bearing C-methyl substituents in 6- and 8- position have been isolated (ARTHUR and KISHIMOTO, 1956; KISHIMOTO, 1956a, b and c) from *Cyrtomium falcatum* ; since these rare substances have been found in *Pinus* and *Rhododendron* their presence in the above fern suggests a link between ferns and higher plants.

Flavonol glycosides of ferns may provide species-specific markers for taxonomic purposes; for example, differences at the species level exist (Table 3) in the glycosylation as well as in the aglycone patterns of four European species belonging to the genus *Asplenium*.
It is also of note that flavonol glycosides of European *Asplenium* ferns show differences (IMPERATO, 1989) from those of *Asplenium* ferns of the New World as some of these (*A. platyneuron* and *A. bulbiferum*) contain glycosides based on O-methylated flavonols (Table 4); in addition complex or rare glycosylation patterns have been encountered in some *Asplenium* ferns of the New World (*A. nidus, A. rhyzophyllum* and *A. platyneuron*) but such patterns are absent (Table 5) from European *Asplenium* ferns so far examined (IMPERATO, 1989).
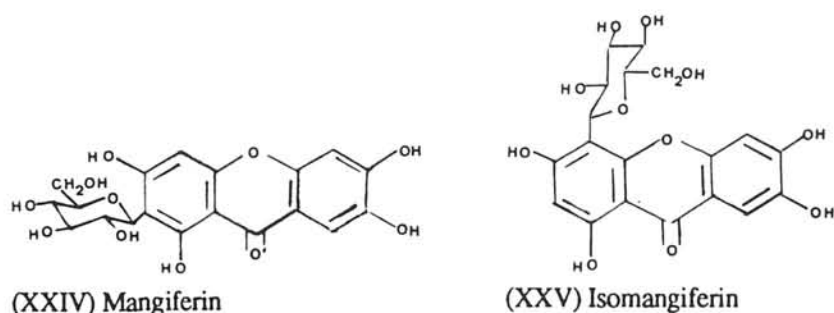

(XXIV) Mangiferin   (XXV) Isomangiferin

Fig. 4

(XXVI) Sinapic acid   (XXVII) Siringic acid

Fig. 5

(XXVIII) I-p-Coumarylglucose 3-sulphate
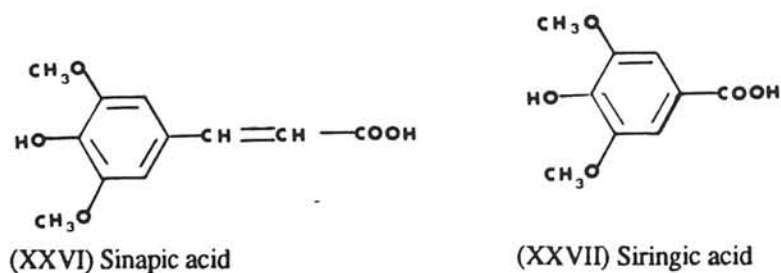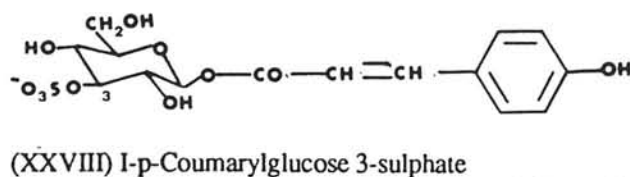
Fig. 6

The distribution of flavonoids in ferns may show taxonomic relationships between various genera. In a survey of a number of ferns belonging to six genera of the Athyriaceae, it has been shown (HIRAOKA, 1978) that the flavonoid composition separates onocleoid ferns (e.g. *Matteuccia, Onoclea*) from athyreoid ferns (e.g. *Athyrium, Diplazium*); the former group contains complex flavonol glycosides and flavone C-glycosides and flavanones (from *Matteuccia*) whereas the latter group has kaempferol and quercetin 3-O-glycosides. In spite of the fact that onocleoids have morphologically advanced characters (green spores and dimorphic leaves) and biochemical advanced character (methylated flavanones), Hiraoka claimed that they are more primitive than athyreoids since this group of ferns has lost the capacity to synthetize complex flavonol glycosides and a great range of flavonoid structures.

A further example of the utility of flavonoid chemistry is the study (COOPER-DRIVER, 1976) of many varieties of *Pteridium aquilinum* collected word-wide; since no variation has been observed in flavonoid patterns of these varieties it has been suggested that this is a monospecific genus.

Xanthones (e.g. mangiferin (XXIV) and isomangiferin (XXV)) may be considered advanced biochemical characters since the biosynthesis of these compounds involves only two malonate units. Xanthones are not of considerable interest from the taxonomic point of view at higher level as they have an erratic occurrence in ferns and are absent from fern allies; however these substances are of interest in the study of relations between diploids and allopolyploids (RICHARDSON, 1984).

Xanthone analysis has shown (RICHARDSON and LORENZ-LIBURNAU, 1982) that *Asplenium adiantum-nigrum* L. is an allotetraploid derived from *Asplenium cuneifolium* Viv and *A. onopteris* L.: in addition *Asplenium balearicum* Shivas is a tetraploid whose diploid parents are *Asplenium onopteris* L. and the other diploid *Asplenium obovatum* Viv. It is interesting to note that a parallel situation exists in the chemistry of a group of Nord American *Asplenium* ferns (SMITH and LEVIN, 1963) which provide a classic example of additive inheritance of chemical characters. Common hydroxycinnamic acids and hydroxybenzoic acids are widespread in ferns as in angiosperms (BOHM and TRYON, 1967; BOHM, 1968; GLASS and BOHM, 1969); however sinapic acid (XXVI) and siringic acid (XXVII) are of rare occurrence in ferns whereas these compounds are widespread in angiosperms. This observation is of interest since a relation has been found between the hydroxylation-methoxylation pattern of cinnamic acids (and benzoic acids) and that of benzaldeide obtainable (by milde oxidation) from lignin of plant in which these acids occurr (IBRAHIM et al., 1962).

| Glycosylation Pattern	European species	Species from New World
3, 7-Diglycosides	+	+
3-Glycosides	+	+
3, 7, 4'-Triglycosides		+
4'-Glycosides		+
7-Glycosides		+

Table 5.   Geographical Distribution of Glycosylation Patterns of Flavonol Glycosides from *Asplenium* species. Data from Imperato, 1989.

In recent years sulphate esters of hydroxycinnamic acid-sugar derivatives (with sulphate attached to the carbohydrate moiety) have been isolated (COOPER-DRIVER and SWAIN, 1975) from *Pteridium aquilinum* and some *Adiantum* species; subsequently these substances have been isolated from ferns belonging to the family Aspleniaceae (*Asplenium septentrionale* (IMPERATO, 1984), *A. filix-foemina Bernh* and *Ceterach officinarum* (IMPERATO, 1981)). These compounds (e.g. XXVIII) are advanced biochemical characters; in addition they are useful taxonomic markers because they may easly be detected by colour reactions and paper electrophoresis.

ACYLPHLOROGLUCINOL DERIVATIVES.

From the chemical point of view, these substances are characterized by the presence of two or more rings joined by methylene groups; at least one of these rings (which in most cases carry an acetyl, propionyl or butyryl group) has the phloroglucinol hydroxylation pattern. Examples are aspidin (XXIX), paraspidin (XXX), flavaspidic acids (XXXI-XXXIII), and phloropyrone (XXXXIV).

Acylphloroglucinol derivatives have been intensively investigated because some of these compounds are of pharmacological interest (antielmintic action). Acylphloroglucinol derivatives have been found in angiosperms (SWAIN and COOPER-DRIVER, 1973); however they are of restricted distribution in ferns and recent work (WIDEN et al., 1983) has shown that they are present exclusively in species belonging to the family Aspidaceae always in connection with typical unicellular internal or external secretory glands; among European Pteridophyta these substances have been found only in the genus *Dryopteris*.
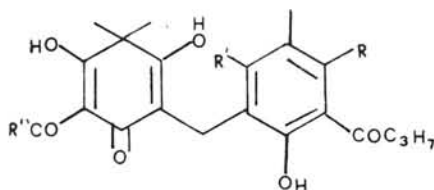
(XXIX) Aspidin, R=OCH3; R'=H; R"=C3H7
(XXX) Paraspidin, R=H; R'=OCH3; R"=C3H7
(XXXI) Flavaspidic acid AB, R=R'=OH; R"=CH3
(XXXII) Flavaspidic acid BB, R=R'=OH; R"=C3H7
(XXXIII) Flavaspidic acid PB, R=R'=OH; R"=C2H5
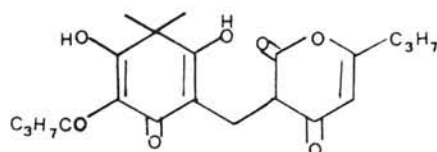
(XXXIV) Phloropyrone

Fig. 7

Species	Aspidin	Paraspidin	Phloropyrone	Flavaspidic acids AB	BB	PB
Dryopteris cristata		+	+	+	+	+
D. spinulosa	+	+	+		+	+
D. assimilis	+	+	+		+	
D. dilatata	+	+	+	+	+	
D. intermedia	+			+	+	

Table 6. Differences in acylphloroglucinol patterns of European species of the *Dryopteris spinulosa* complex. Data from Wieffering et al., 1965.

The chemotaxonomic value of these compounds is shown by the following examples. The five European species of *Dryopteris spinulosa* complex (Table 6) may be identified by their phloroglucinol derivatives (WIFFERING et al., 1965).

Examination of acylphloroglucinol derivatives has led to the conclusion (WIDEN et al., 1971) that the alloteraploid *Dryopteris filix-mas* s. str. originated from a hybrid of *D. abbreviata* (DC.) Newm. with *D. villarii* (Bellardi) Woynar (with doubling of chromosomes), the triploid *D. borreri* is a hybrid of diploid *D. borreri* and *D. abbreviata* whereas the apogamous triploid *D. remota* (A. Br.) Druce originated from a hybrid of *D. assimilis* S. Walter and diploid *D. borreri* Newm. These conclusions do not agree with genome analysis with a single exception (the origin of *D. remota*).

Analysis of phloroglucinols of species and synthesized hybrids of the *Dryopteris carthusiana* complex has shown (WIDEN et al., 1978) that hybrids apparently show the influence of either, or both, of the parental species.

ISOPRENOIDS.

In spite of the fact that monoterpenes are widespread in bryophytes and gymnospermes, data relating to the presence of these compounds in Pteridophyta are scanty and there is no report dealing with the presence of monoterpenes in European Pteridophyta. However some monoterpenes have been identified (SWAIN and COOPER-DRIVER, 1973) in *Thelypteris patens*, *Pteris strammea* Mett and *Ctenitis* species; in addition a sesquiterpene alcohol has been found (BERTI and BOTTARI, 1968) in *Paesia scaberula*. Among diterpenes, gibberellin-like compounds have been found in ferns including the European species *Dryopteris spinulosa, D. linneana* and *Athyrium filix-foemina* (BERTI and BOTTARI, 1968); in addition gibberellic acid (XXXV) is present in *Anemia phyllitidis* (SCHRAUDOLF, 1966).

Very recently (MURAKAMI, 1987) examination of a large number of Pteropsida has shown that sesqui- and diterpenes are widely distributed in Copeland's Pteridaceae but are absent from other families with the exception of a single species; these results agree with Copeland's suggestion that Pteridaceae are an unified natural group.

A number of triterpenes and sterols have been found in ferns and many of such compounds (BERTI and BOTTARI, 1968) are absent from angiosperms and gymnosperms.
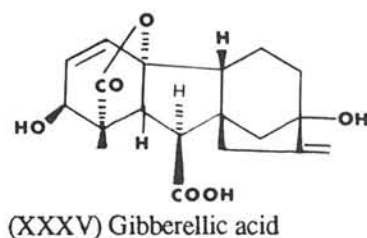
(XXXV) Gibberellic acid

Fig. 8

Species	% of total triterpenoids		
	Hop-22(29)-ene (XXXVII)	Fern-9(11)-ene (XXXVIII)	Other types
Marsilea quadrifolia	66	25	9
Blechnum spicant	16	13	66
Asplenium adiantum-nigrum	90	0	5
A. trichomanes	100	0	0
Phyllitis scolopendrium	89	0	9
Dryopteris filix-mas	82	0	7

Table 7.   Distribution of triterpenoid hydrocarbons in some European ferns. Data from Bottari et al., 1972.

Typical fern triterpenoids are hydrocarbon derivatives of hopane (XXXVI) (or its rearrangement products) generally with one double bond. Examples are hop-22(29)ene (XXXVII) and fern-9(11)ene (XXXVIII).

These triterpene hydrocarbons occur in a variety of ferns (BERTI and BOTTARI, 1968) including some European species (e.g. *Scolopendrium vulgare* and *Adiantum capillus-veneris*) and may be considered a primitive character since they are widespread in mosses (MARSILI et al., 1968; 1970; 1971) and have been identified in algae and bacteria (BIRD et al., 1971). Quantitative examination of these compounds (BOTTARI et al., 1972) has shown that differences of taxonomic interest exist at the species level (Table 7).
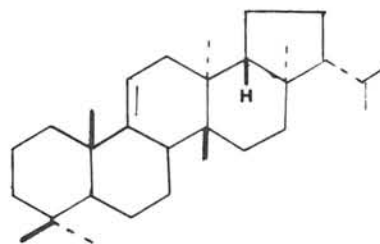
Other triterpenoids isolated from European ferns include 3α, 4α-epoxyfilicane (XXXIX) (BERTI et al., 1963a), 29-nor-22-hopanone (XL) (BERTI et al., 1963b), 21β-hydroxy-29-nor-22-hopanone (XLI) (ZAMAN et al., 1966) which are present in *Adiantum capillus-veneris* and cyclolaudenol (XLII) (BERTI et al., 1964) which has been found in *Ceterach officinarum*,
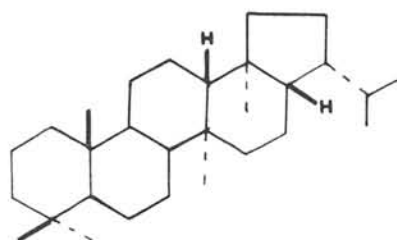
(XXXVI) Hopane
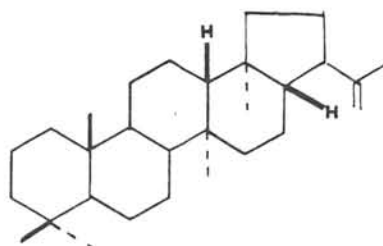
(XXXVII) Hop-22(29)ene

(XXXVIII) Fer-9(11)ene

Fig. 9

Asplenium trichomanes, A. adiantum-nigrum, Dryopteris filix-mas, Blechnum spicant and *Marsilia quadrifolia.* Cyclolaudenol (XLII), cycloartanol (XLIII) and the corrisponding nor-compounds (without methyl group at position 4) are fern terpenoids of biosynthetic interest because they are precoursors of ecdysone and related compounds (XLVIII-LIII) which are moulting hormones isolated from ferns.

Chemotaxonomical investigation (TSUDA et al., 1974) of a number of *Lycopodium* plants (including some European species) has shown that examined plants contained triterpenoids of the serratane (XLIV) group or its assumed precursor, α-onocerin (XLV). These plants may be divided into three groups according to the major triterpenoids: α-onocerin (XLV) group (including *L. clavatum* and *L. inundatum*), serratenediol (XLVI) group (including *L. cernuum*) and 21-episeratriol (XLVII) group (including *L. annotinum*).

An interesting group of fern sterols are ecdysone and related compounds (XLVIII-LIII) which have been isolated also from European species (e.g. *Pteridium aquilinum* and *Onoclea sensibilis*) and may be ubiquitous in ferns (IMAI et al., 1968); these compounds may induce metamorphosis in insects and crustacea (HEROUT, 1970). It is not known if ecdysones are important in the growth of ferns but it has been shown that they are a defence against insect attack (REES, 1971).
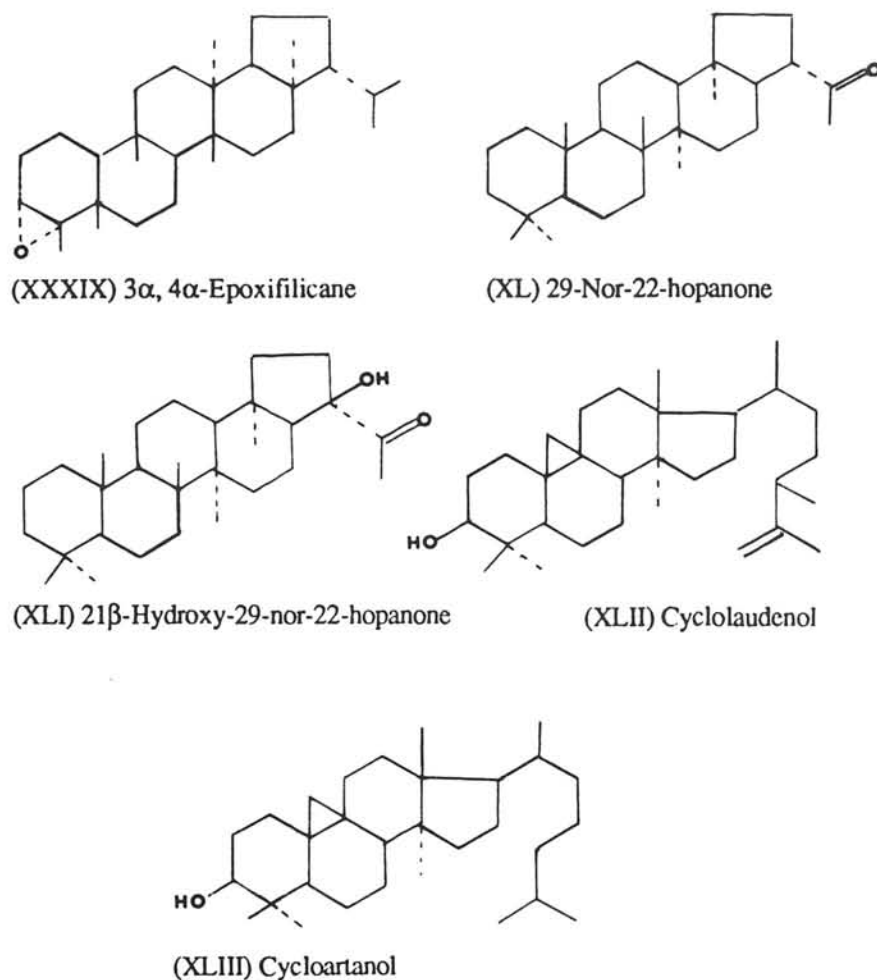
(XXXIX) 3α, 4α-Epoxifilicane

(XL) 29-Nor-22-hopanone

(XLI) 21β-Hydroxy-29-nor-22-hopanone

(XLII) Cyclolaudenol

(XLIII) Cycloartanol

Fig 10

Species	24-Ethylcholest -5-enol		24-Ethylcholest-5-, 22-dienol2		24-Methylcholest-5-ol	
	24-α	24-β	24-α	24-β	24-α	24-β
Lycopodium cernuum	100	0	100	0	40	60
L. clavatum	100	0	100	0	40	60
L. complanatum	100	0	100	0	30	70
Equisetum arvense	100	0	100	0	80	20
E. ramisissimum	100	0	100	0	80	20
Blechnum orientale	100	0	100	0	90	10

Table 8.   C-24 Epimeric Composition of 24-Ethylcholest-5-enol, 24-ethylcholest-5, 22-dienol and 24-methylcholest-5-enol in some European Pteridophyta Data from Chiu et al., 1988.
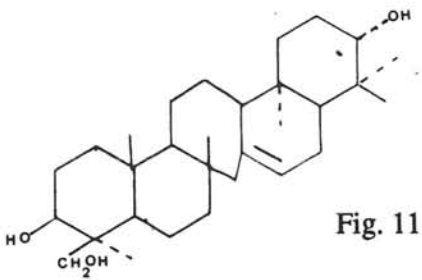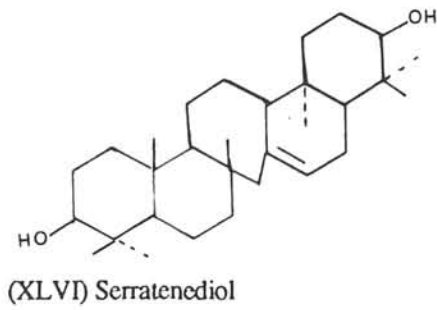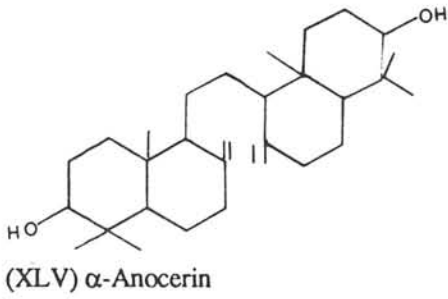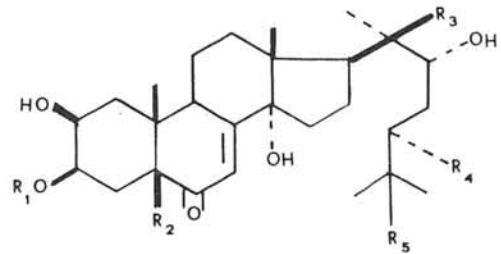
(XLV) α-Anocerin

(XLVI) Serratenediol

(XLVIII) Ecdysone, R₁=R₂=R₃=R₄=H; R₅=OH

(XLVIII) Ecdysone, $R_1=R_2=R_3=R_4=H$; $R_5=OH$
(XLIX) Ecdysterone, $R_1=R_2=R_4=H$, $R_3=R_5=OH$
(L) Ponasterone A, $R_1=R_2=R_4=R_5=H$; $R_3=OH$
(LI) Ponasteride A, $R_2=R_3=R_4=R_5$; $R_1=$glucose
(LII) Pterosterone, $R_1=R_2=R_5=H$; $R_3=R_4=OH$
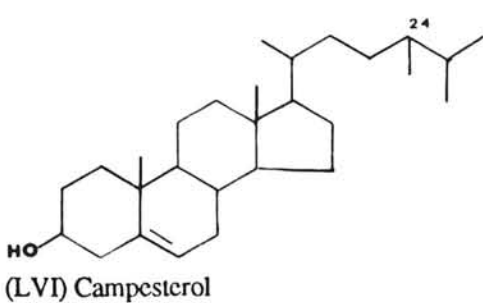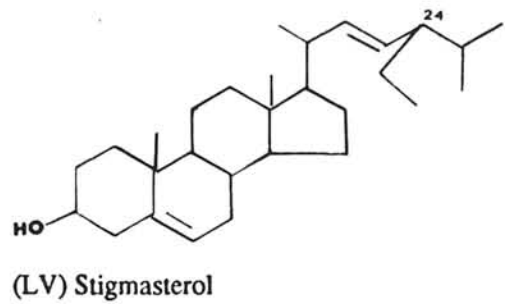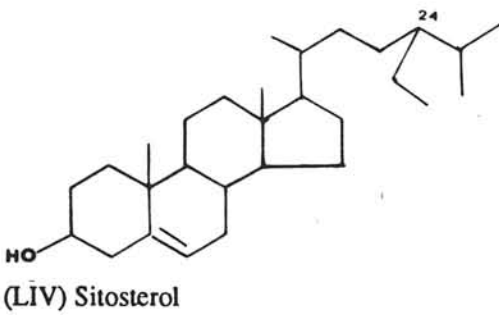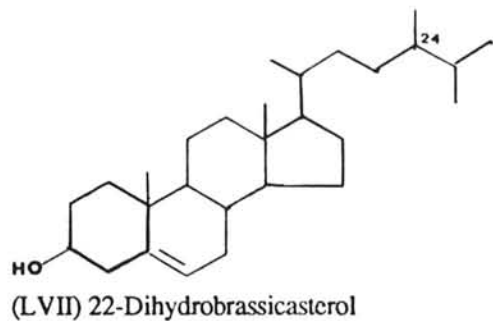(LIII) Polypodine B, $R_1=R_4=H$; $R_2=R_3=R_5=OH$

Fig 12

Fig. 11

(XLVII) 21-Episcratriol

(LIV) Sitosterol

(LV) Stigmasterol

(LVI) Campesterol

Fig 13

(LVII) 22-Dihydrobrassicasterol

Very recently (CHIU et al., 1988), investigation of the configuration of substituents at C-24 of sterols from a number of Pteridophyta (including (Table 8) some European species) has led to results of phylogenetic interest. C-24 Epimeric composition of 24-ethylcholest-5-enol, 24-ethylcholest-5, 22-dienol and 24-methylcholest-5-enol shows that the 24-ethylsterols are present exclusively as the 24-α-epimers sitosterol (LIV) and stigmasterol (LV) whereas the 24-methylsterols are present as epimeric mixture in which the 24-α-epimer (campesterol) (LVI) dominates with the exceptions of the genera *Lycopodium* and *Selaginella* in which the 24-β-epimer (22-dihydrobrassicasterol) (LVII) dominates. Data relating to 24-ethylsterols show that Pteridophyta are similar to Spermatophyta and different from Bryophyta; in addition results on the 24-methylsterols show that true ferns resemble Spermatophyta whereas *Lycopodium* and *Selaginella* are similar to mosses.

Data relating to carotenoids of Pteridophyta are scanty. The amount of β-carotene in bracken fronds at different stages of growth has been studied (SMITH and FENTON, 1944); in addition it has been shown that the content of carotenoids increases with the maturation of reproductive organs in ferns (SAMORODOVA-BIANKI, 1960). Recently (CZECZUGA, 1985) 27 carotenoids have been detected in a survey of 66 representatives of Pteridophyta; the carotenoids characteristic of club moss and horsetail species are β-carotene, β-cryptoxanthin, lutein epoxide and zeaxanthin whereas the carotenoids characteristic of fern species are β-cryptoxanthin, lutein epoxide, zeaxanthin, violaxanthin and rhodoxanthin.

OTHER BIOCHEMICAL CHARACTERS.

Investigation of free protein amino acids has shown that these compounds may be of interest in systematic problems. Ferns belonging to the genus *Thelypteris* (including the European species *T. palustris* Schott) have differences (PANVISAVAS et al., 1968) in free protein amino acid patterns at the species level. Examination (KHANDELWAL and GOSWAMI, 1976) of ferns of the genus *Ophioglossum* (including *O. vulgatum*) has shown that cysteine and ornithine are present in rhizome but are absent from trophophyllum, petiole and spike; arginine is the main amino acid of the rhizome of *Pteridium aquilinum* and *Aspidium aculeatum* (BERTI and BOTTARI, 1968) while the amido amino acids predominate in aerial parts.

$$
\begin{array}{ll}
\begin{array}{l}
COOH \\
| \\
CHNH_2 \\
| \\
CHOH \\
| \\
CHOH \\
| \\
COOH
\end{array}
&
\begin{array}{l}
COOH \\
| \\
CHNH_2 \\
| \\
CH_2 \\
| \\
CH_2 \\
CH_2NHCOCH_3
\end{array}
\end{array}
$$

(LVIII) 3, 4-Dihydroxyglutamic acid   (LIX) δ-N-acetylornithine

Fig. 14
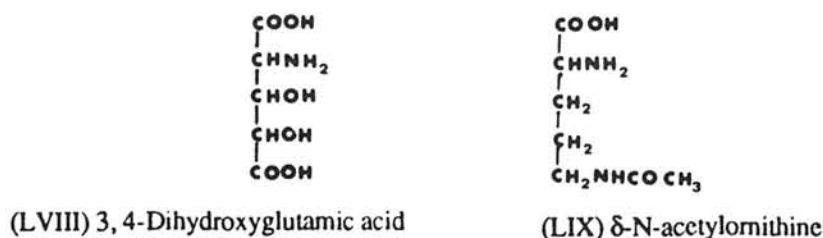
$$CH_3(CH_2)_4 CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$$

(LX) Arachidonic acid

Fig. 15

Many of free non protein amino acids of ferns are substituted glutamic acids; some of these compounds have unusual structures (e.g. 3,4-dihydroxyglutamic acid (LVIII) from *Struthiopteris filicastrum* (VIRTANEN and ETTALA, 1957)). Several species of the genus *Asplenium* (including *A. septentrionale* and *A. trichomanes*) have δ-N-acetylornithine (LIX) as one of the main constituents but this compound is absent from other genera of Polypodiadeae (e.g. *Dryopteris, Pteridium, Woodsia* and *Athyrium*) (VIRTANEN and LINKO, 1955).

Some species of ferns contain diastereoisomeric 4-substituted acidic amino acids in characteristic association (MEIER and SORENSEN, 1979). For example, several *Asplenium* ferns (including *A. septentrionale, A. trichomanes*) have (Table 9) 2(S), 4(R)-4-methylglutamic acid, the two diastereoisomers of 2(S)-4-hydroxy-4-methylglutamic acid and the two diastereoisomers of 2(S)-4-hydroxy-2-aminopimelic acid in a typical concentration ratio. In addition all of the Aspidiaceae examined also have 4-substituted acidic amino acids with the exception of a single species. However these ferns (including *Polystichium munitum* Pr and *P. arostichoides* Schott) contain only one of the two diastereoisomers of 2(S)-4-hydroxy-4-methylglutamic acid and of 2(S)-4-hydroxy-2-aminopimelic acid whereas both diastereoisomers of 2(S)-4-methylglutamic acid are absent.

Spore proteins of ferns may be examined by serological and electrophoretic techniques (PETERSON and FAIRBROTHER, 1971); by using these techniques it has been shown that *Osmunda cinnammomea* L. is closer to *O. claytoniana* L. than to *O. regalis*. Very recently (CONANT and DE MAGGIO, 1987), analysis of spore storage proteins of aspidioid and cyatheoid ferns have revealed the existence of strong similarities among genera within these groups; in addition it has been shown that similarity between groups is little.

A comparative study of isozymes has led to new results on the evolution of *Dryopteris spinulosa* complex (WERTH, 1987): *D. cristata* and *D. carthusiana* seem to have in common an extinct ancestral genome although such result is limited by "orphan" alleles, multiple origins and apparent silencing of duplicate gene expression. The presumed antiquity of these two allotetraploids is confirmed by the substantial level of gene silencing which may have an interest from the evolutionary point of view.

Examination of fatty acids of the lipids of Pteridophyta has led to results of phylogenetic interest. *Equisetum arvense* and some fern species (e.g. *Matteuccia struthiopteris* and *Cyrtomium falcatum*) have some polyunsaturated fatty acids (HITCHCOCK and NICHOLS, 1971) which are absent from angiosperms (Table 10); in addition one o these compounds (arachidonic acid (LX)) may be considered a primitive character since it is present in the lipids of red and brown algae (NICHOLS, 1970).

| Plant	Aspartic acid	Glutamic acid	4-Methyl-glutamic acid		4-Hydroxy-4 methylglutamic acid		4-Hydroxy-2amino-pimelic acid	
			1	1'	2	2'	3	3'
Asplenium septentrionale	+	+++			(-)	++	+	(-)
A. trichomanes	+	++	++		++	+	+	(-)
Polystichium munitum Pr.	++	++++			(-)	(-)	(-)	+
P. acrostichoides Schott	++	+++			(-)	++	(-)	+

Table 9. Acidic Amino Acids of some European Ferns. Data from Meier and Sorensen, 1979;
(-)=not detectable; +=weak; ++=medium; +++=strong; ++++=very strong.
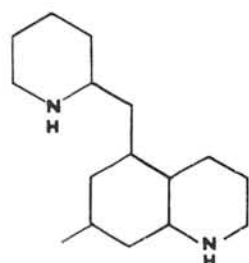1=2(S), 4(R)-4-methylglutamic acid. The absolute configuration at C-2 in 2, 2', 3 and 3' is 2(S). The absolute configuration of 2, 2',3 and 3' is unknown.
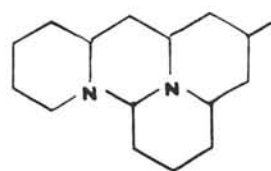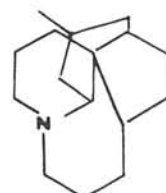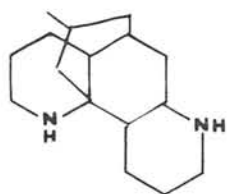
(LXI) Cernuane
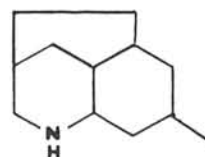
(LXII) Lucidulane

(LXIII) Phlegmarane

(LXIV) Fawcettidane

(LXV) Lycodane
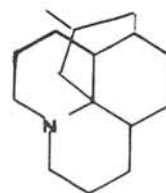
(LXVI) Lycopodane
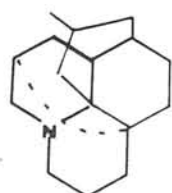
(LXVII) Inundatane
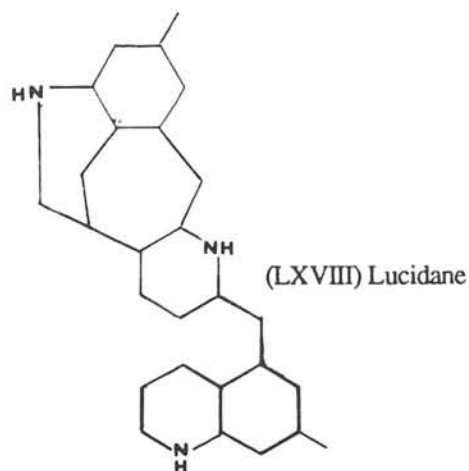
Fig. 16

(LXVIII) Lucidane

Species	Fatty Acids (% total)[2]				
	16:0	16:3	18:2	18:3	20:4
Equisetum arvense	28.2	9.4	11.5	43	2.1
Matteuccia struthiopteris	17.6	4.1	6.8	54.2	8.2
Onoclea sensibilis	21.1	5.3	5.4	47.9	8.0
Cyrtomium falcatum	24.9		31.2	14.9	5.2

Table 10.   Major Fatty Acids Composition of some European Pteridophyta[1]

[1] Data from Hitchcock and Nichols, 1971.

[2] The numbers indicate the number of carbon atoms and the number of double bonds; for example 20:4 has 20 carbon atoms and 4 double bonds and is arachidonic acid.

In spite of the fact that only about 10% of the extant *Lycopodium* species have been examined for alkaloids, a large number of these substances (about 100) have been found in these plants.

It has been shown (BRAECKMAN et al., 1980) that lysine-derived alkaloids may be used as markers for the classification of species belonging to the genus *Lycopodium* (s. l.); these authors suggest that the distribution of the above compounds separate this genus in three groups: *Hupertia* (A), *Lycopodiella* (B) (with the exclusion of *L. volubile* and *L. deuterodensum*) and *Lycopodium* s. str. (C).

The first group (*Hupertia*) (A) has skeletons LXII-LXVII (Fig. 16); in addition most species contain skeleton LXVIII. The second group (*Lycopodiella* s. l.) (B) has skeletons LXIII, LXIV and LXVI; in addition this group is characterized by the presence of skeleton LXI or LXVII. The third group (*Lycopodium* s. str.) (C) is characterized by the presence of a large number of alkaloids having skeleton LXVI. This group may be divided in three sections. In the first section (*Lycopodium*) (C-1) alkaloid skeleton LXVI is present; moreover skeletons LXIII-LXV may be present. The second section (*Fastigiatum* group) (C-2) also is characterized by the presence of skeleton LXVI; in addition skeleton LXIV may be present. The third section (*Complanata* section sensu Wilce) (C-3) is characterized by the presence of large quantities (90%) of lycopodine with two exceptions (*L. thyoides* and *L. fawcettii*).

It is interesting to note that flavonoid analysis (VOIRIN, 1978b) has led to conclusion which agree with the above results based on the alkaloid distribution. Data relating to alkaloid skeletons of some European species are collected in Table 11.

A GC/MS method for the rapid screening of *Lycopodium* extracts for their alkaloids has been used (GERARD and MACLEAN, 1986) in the examination of four *Lycopodium* species (including *Lycopodium clavatum*). New alkaloids have been discovered by this method; in addition alkaloids which has not been previously observed, have been revealed. Data relating to *Lycopodium clavatum* var. *borbonicum* are reported in Table 12. In a previous examination of alkaloids of this species by conventional method nine alkaloids (B, C, D, F, G, J, K, L (Table 12) and lycodiflexine) were identified. By GC/MS, lycodiflexine may not have been detected because of its involatility;

Species	Skeleton of alkaloid present							
	LX I	LXII	LXIII	LXIV	LXV	LXVI	LXVII	LXVIII
A *Lycopodium selago* L.					2	3		
B *L. cernuum* L.	4		1			1		
L. inundatum L.				2		2	3	
C-1 *L. annotinum* L. var. *annotinum*					2	4		
L. clavatum var. *borbonicum* Bory			1	1		4		
L. clavatum var. *clavatum*				1	1	4		
var. *megastachyum* Fern. & Bissel						4		
C-3 *L. alpinum* L.					1	5		
L. issleri (Rouy) Lawalrée						5		
L. thyoides Humb. & Bompl. ex Willd					1	4		
L. tristachyum Pursh					1	5		

Table 11. Distribution of major skeletons of lysine-derived alkaloids in some European *Lycopodium* species. Data from Braekman et al., 1980.

Table 12. Alkaloids of *Lycopodium clavatum* variety *borbonicum* [1]

Component	Alkaloid	% of total
A	Lycodine	} 3.5
B	Anhydrolycodiline	
C	Lycopodine	} 80.5
D	Dihydrolycopodine	
E	Flabelliformine	0.4
F	Acetyldihydrolycopodine	10.0
G	Lycodoline	1.6
H	L 20	0.7
I	Unknown	0.9
J	Lycoflexine	0.2
K	Borbonicine	0.5
L	N α-acetyl-N β-methylphlegmarine	1.4

[1] Data from Gerard and MacLean, 1986.

Table 12. Alkaloids of *Licopodium clavatum* var. *borbonicum*. Data from Gerard and MacLean, 1986.

by contrast lycodine, flabelliformine, alkaloid L 20 and alkaloid of m/z 279 (whose identity has not been firmly established) have been found for the first time in *Lycopodium clavatum* var. *borbonicum* by GC/MS method.

In recent years some interesting results have been obtained using RNA or DNA from Pteridophyta.

Determination of nucleotide sequences of 5S rRNA from *Equisetum arvense* and *Dryopteris acuminata* has shown that these sequences are more related to those of Bryophyta than to those of seed plants (HORI et al., 1984).

Analysis of chloroplast DNA fragments produced by restriction endonuclease has been used (STEIN and YATKIEVIYCH, 1987) to examine evolutionary relationship between closely related genera (including *Polystichium* and *Cyrtomium*) and to assess levels of divergences among members of each group.

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