Chapter-II

Section A : A short review on the chemical constituents of ferns of Polypodiaceae family.

The exceptional revival of interest in phytochemistry in recent years, which has accompanied the development of powerful modern techniques for structural investigations and has been stimulated by the potential usefulness of plants as a source of new therapeutic agents, has only touched in a marginal way the class of ferns (Pterophyta or Filicinae). Of the fourteen families and the many thousands of species into which this botanically very interesting class is subdivided, very few have been investigated by the phytochemist. With the exception of some genera of Polypodiaceae, only occasional and very incomplete attention has been paid to the various families, some of which, such as the Dipteridaceae and Salviniaceae, are completely unknown from the chemical point of view. However, recent results, such as the discovery of many new acylphloroglucinol derivatives in Dryopteridoideae and of several characteristic triterpenoids, and the availability of efficient methods for their analysis, indicate that the phytochemical investigation of ferns could be of great interest both to the taxonomist, as an aid in the very difficult task of their

classification, and to the natural products chemist in his search for novel structures.

We have undertaken, in this chapter, to give an up-to-date description of all organic compounds that have been isolated from ferns of Polypodiaceae family.

Polypodium vulgare L : The constituents of the fern Polypodium vulgare L. Growing in Europe, Asia and America were many times investigated. The outstanding sweet taste of the rhizomes of the plant in question in particular attracted the interest of chemists and pharmacists. Guignet[2] in 1885 reported that this property of rhizomes was caused by glycyrrhizine like in papilionaceous plant Glycyrrhiza glabra L. Fisher and Goodrich[3] agreed with Guignets[2] opinion, but shortly afterwards Fisher and Lynn[4] rejected this assertion and stated only that sweet taste of rhizomes was caused by relatively high content of sugars and that mainly by saccharose besides fructose and glucose. The named authors isolated besides less defined compounds the glycoside polydine, characterized by melting point 188-89°C. Volmer and Reebe[5] reported another substance of m.p. 150-51°C which they called polypodine. Freise[6] found in the rhizomes also a compound of a bitter taste which he named samambaine. The latter compound is a glycoside which on enzymatic hydrolysis

affords glucose, rhamnose, benzoic acid and resins. Later, Jermstad and co-workers[7] corroborated the absence of glycyrrhizine in P.vulgare and reported the presence of a series of compounds besides polydine, among others also caffeic acid chlorogenic acid. However, through investigation of the rhizomes of P.vulgare was mainly due to Jizba and Herout[8]. These named authors mainly paid their attention on the hydrophilic part of the extract. The ethanolic extract of the rhizomes from which the lipophilic material was removed by extraction with light petroleum was chromatographed on polyamide powder.

Fraction which was eluted first contained relatively pure saccharose in a yield of about 2.5% on the weight of dry drug. The mother liquors contained small amount of glucose and fructose by paper chromatography. Eluates which followed gave a crystalline substance, m.p. 229-33°C in about 1.8% yield was found to be the mixture of two compounds. They separated the two constituents by chromatography on silica gel. One compound is polypodine crystallised as hydrate, m.p. 150-51°C. The second compounds they isolated, showed m.p. 244-46°C with the same molecular formula as the first one (Chart I) Jizba and Herout[8] named these compounds as polypodine A and polypodine B. Next eluates furnished a crystalline compound in about 0.6% yield which was identified as glucocaffeic acid. The following eluate they found, was a mixture of two compounds. These compounds were separated by partition chromatography on solica gel.

The compound eluted first was not obtained crystalline, the other was polydine, m.p. 191-93°C, $(\alpha)_D^{20}$ -121.6° (C 0.244; MeOH). The mother-liquors after polydine contained a series of compounds of glycoside character. Their isolation was carried out by chromatography on polyamide powder. Aqueous methanol eluted "saponin I" m.p., 199-201°C, the aqueous solution of which formed a rich foam. This compound differed from "Saponin II" by the presence of methoxyl group in its molecule.

After separation of saponin I, they isolated the sweet principle, the interesting constituent, by partition chromatography on kieselguhr. The yield of the compound, m.p. 201-203° (hydrated form) or 252-254°C (anhydrous form) was about 0.03% of the weight of the dry drug. These authors assumed that this compound was the true sweet principle of polypody rhizome. This compound affords unstable aglycone and rhamnose and glueose and they proposed the name osladine for the compound.

The eluates which yielded osladine on rechromatography on silicagel and elution by a mixture of chloroform containing 15% ethanol they isolated a small amount of crystalline substance of bitter taste. This compound, according to them, has a relation to samambaine already described by Frese[6] and also to the compound osladine by their preliminary results. These authors have also isolated a small amount of crystalline "saponin II", m.p. 213-14°C from the plant collected in another season of the year.

Chart - I

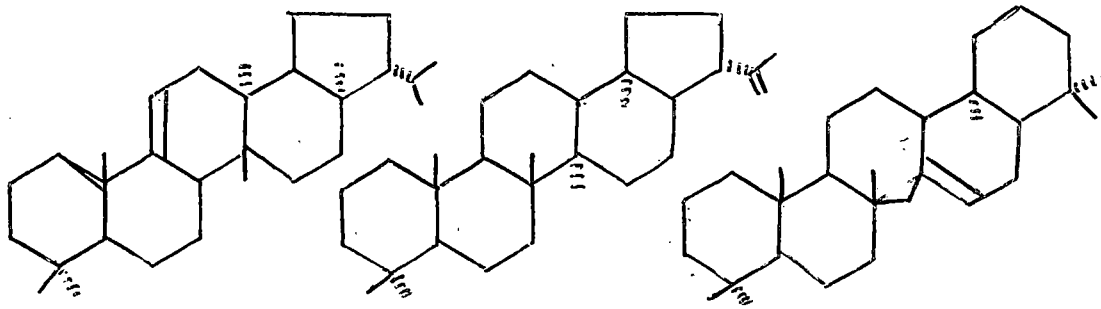
Compounds isolated from Polypodium vulgare L.

Name	M.P., °C	$[\alpha]_D^{20}$	Composition
Saccharose	184-85	+ 67.5	$C_{12}H_{20}O_{11}$
Polypodine A	150-51	+ 61.8	$C_{27}H_{44}O_7 \cdot 3H_2O$
Polypodine B	244-46	+ 92.8	$C_{27}H_{40}O_8 \cdot \frac{1}{2} H_2O$
Glucocaffeic acid	133-35	- 80.4	$C_{15}H_{18}O_9 \cdot H_2O$
Polydine	191-93	-121.6	$C_{20}H_{22}O_{10} \cdot H_2O$
Osladine	201-203		a
Saponin I	199-201		a
Saponin II	213-14		a
Samambaine	251-52		a

The authors have also reported the isolation of fernene from the lipophilic part of the extract.

Berti and co-workers[9-11] investigated the fern by means of modern methods and found the presence of several triterpenic hydrocarbons in the lipophilic part. The rhizomes of polypodium vulgare contain 0.4% of a mixture of triterpene hydrocarbons; gas liquid chromatographic analysis indicated the presence of three components - 9(11) fernene 1, m.p., 170-71° $(\alpha)_D$-16.5, 22(29) hopene 2, m.p., 211-12°, $(\alpha)_D$+61° and serratene 3, m.p. 237-39° $(\alpha)_D$-13.4°. The third component was isolated in a pure state by column

chromatography over silica gel impregnated with silver nitrate. This compound had been obtained before as the dehydroxylation product of serratene diol, a triterpenoid from Lycopodium serratum. 17,21- Epoxyhopane 4, m.p. 268-70°, $(\alpha)_D^{28}$ +47° ($CHCl_3$)



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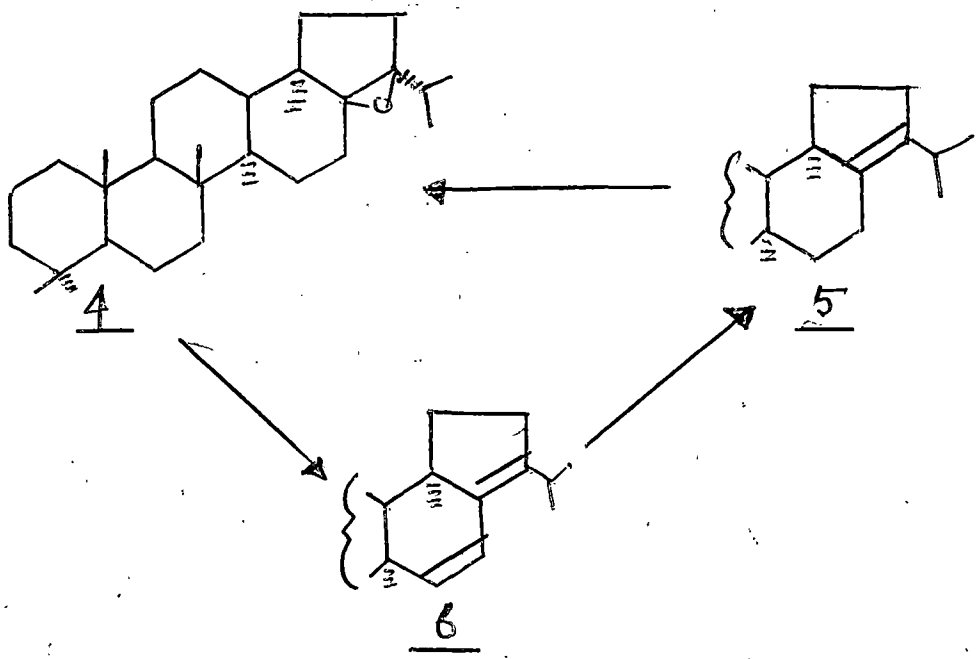
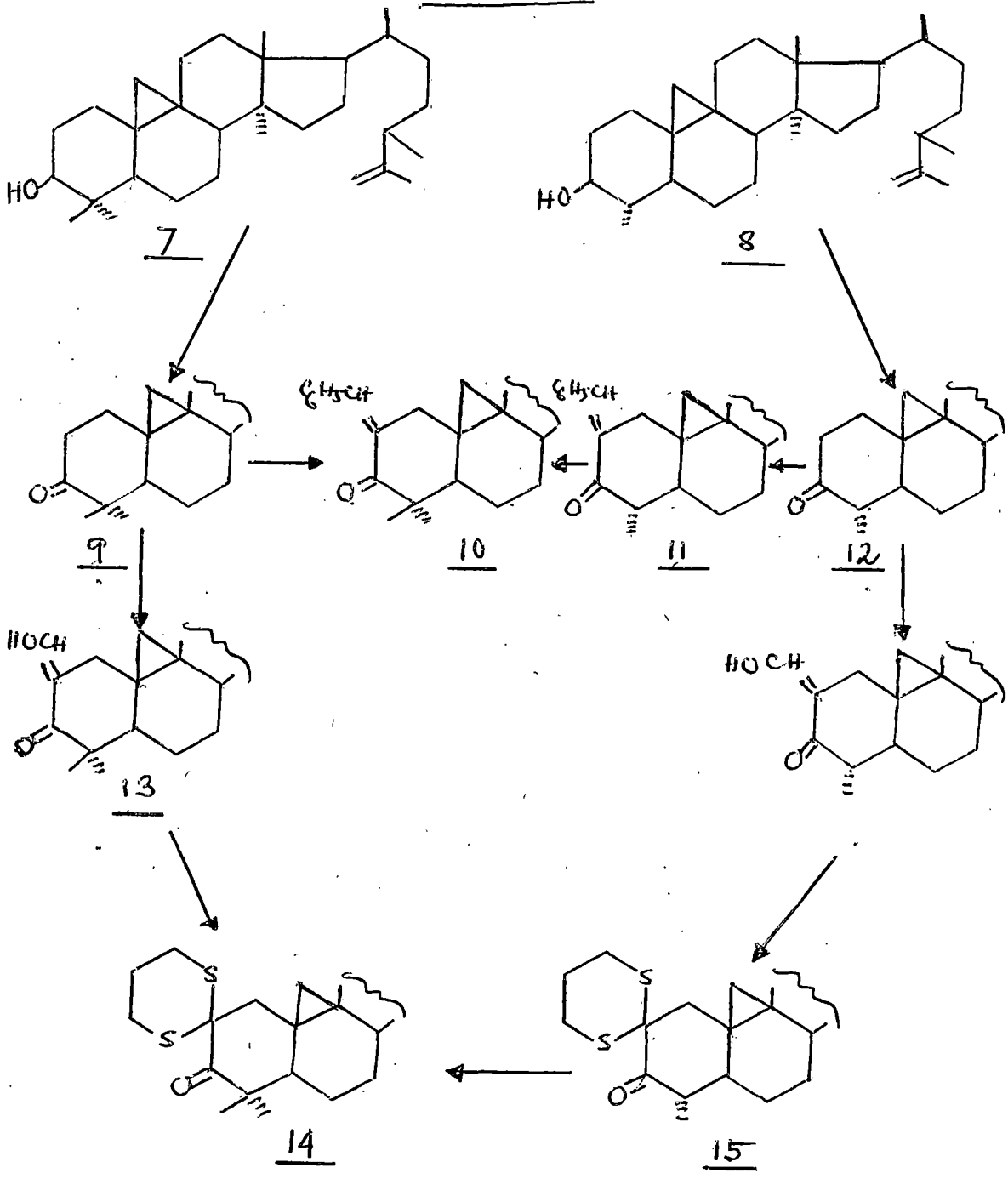
is one of the minor triterpenoids of Polypodium vulgare.[10] Treatment with hydrogen chloride in ethanol, or chromatography on alumina, converts it into the heteroannular diene 6 which is partially reduced to the known 17(21)-hopene (hopene-1) 5 by catalytic hydrogenation. The latter compound transformed back into the epoxide 4 wth p-nitroperoxybenzoic acid; therefore, the stereochemistry of the epoxide ring of 4, although not rigorously proved, is very probably β, since peroxy acids usually attack double bonds from less hindered side.



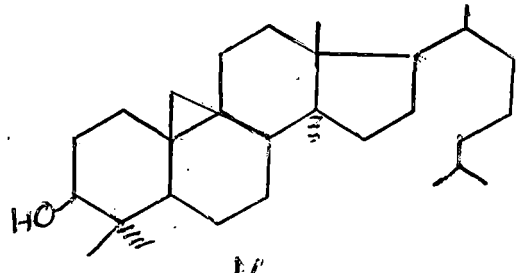
Triterpenoids of the 9,19- cyclolanostanol group has been reported from Polypodium vulgare. About 1.4% of the dry weight of P. Vulgare rhizomes consists of a mixture of alcohols, the main constituent of which is cycloläudenol 7,[9] m.p. 123-25°, $(\alpha)_D$ + 45°, a compound that had previously been found in Papaver somniferum[12] and in Manikara bidentata.[13] A second constituent was identified as 31-norcycloläudenol 8, m.p. 139-40°, $(\alpha)_D$ +44°, on the basis of its i.r., n.m.r., and mass spectra[9]. The relation between 8 and 7 was established (scheme 1) by converting the corresponding ketone 12 into the benzal derivative 11 and methylating the latter to 10, which turned out to be identical with benzalcycloläudenone. Another link

was established through the dithiane derivative 15, m.p. 200°, the methylation of which produced 14, m.p. 162', also prepared from 7 through 9 and 13.[14]

Scheme - 1



Two more 9,19 cyclolanostanol derivatives were found as minor constituents in Polypodium vulgare: cycloartanol 16, m.p. 101-2° $(\alpha)_D$ +51° and 31-norcycloartanol 17, m.p. 128-32° $(\alpha)_D$ +49°. The former had been tentatively identified as a trace constituent of rice brain oil[15]; the latter had never been found in nature but had been obtained as an intermediate in the structural elucidation of cycloeucalenol[16].



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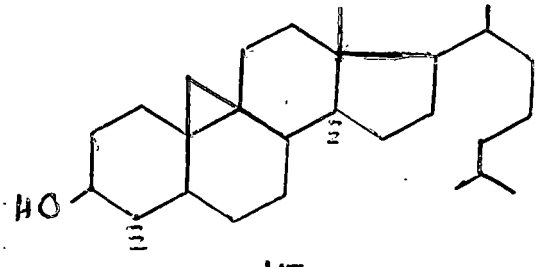

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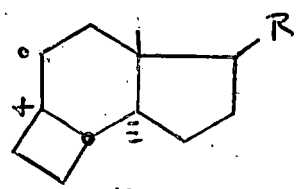
Compounds 7, 8, 16, and 17, were easily analysed by gas-liquid chromatography of their trimethylsilyl ethers on a neopentylglycol succinate column. The order of increasing retention times is : 17 < 16 < 8 < 7. The preparative separation is not satisfactory, since only an incomplete separation into mixtures of cycloaudenol and cycloartanol, and of 31-norcycloaudenol and 31-norcycloartanol is achieved. A much better method is based on the chromatography of the benzoates over

silver nitrate impregnated silica gel. Elution with petroleum-ether containing 2% benzene gives a mixture of the benzoates of cycloartanol and 31-norcycloartanol, while higher amounts of benzene are required for the elution of those of cyclolaudenol and 31-norcyclolaudenol. The 31-norderivatives cycloartanol and 31-norcycloartanol are then separated from the corresponding parent compounds cyclolaudenol and 31-norcyclolaudenol by conversion into the 3-ketones and chromatography over alumina; after separation, the ketones can be reconverted into the alcohols.

9,11 - cyclolanostanol derivatives are easily identified in the n.m.r. spectrum by an AB quartet (J=4 cps) at very high field (between 9.40 and 9.80τ), which is due to the magnetically nonequivalent cyclopropane protons. The quartet for 4-monomethyl derivatives is at higher field than for 4,4-dimethyl derivatives[9]. Further evidence for the cyclolanostane structure is provided by the presence in the mass spectrum of the alcohols of a strong peak at $M^+-C_9H_{16}O$ for compounds with two methyl groups in $C_{(4)}$ such as cyclolaudenol and 31-norcycloartanol and at $M^+-C_8H_{14}O$ for those with one such as 31-norcyclolaudenol and cycloartanol. This peak is due to the loss of ring A and $C_{(19)}$, and probably corresponds to the ion 18 [11,17]. Mass spectra also provide useful information on the type of side chain, by the presence

of a peak due to fragementation between $C_{(17)}$ and $C_{(20)}$, with the charge remaining on the larger fragment. The methylene side chain of cyclolaudenol and 31-norcyclolaudenol has also been readily distinguished from the saturated one of cycloartanol and 31-norcycloartanol by the strong $=CH_2$ i.r. bands at 6.10 and 11.22 μ.



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9,19 - cyclolanostanol derivatives have recently been receiving much attention, because they could be more important than lanosterol as intermediates in the biosynthetic transformation of squalene into phytosterols[18]. It is a well known fact that cycloartenol is widely distributed in plants but it is becoming more and more evident that other compounds of this class, such as cycloeucalenol, 22-methylene cycloartanol, cyclolaudenol, etc, are also very common, even if usually present only in small amounts. The hypothesis has been put forward that squalene can cyclize directly to cycloartenol and this compound gives rise to sterols through a series of steps : elimination of the methyl groups $C_{(30)}$, $C_{(31)}$, and $C_{(32)}$, opening of the cyclopropane ring, methylation and reduction of the side chain, etc.

The isolation from the same plant of cyclolaudenol, 31-norcyclolaudenol, artanol and 31-norcycloartanol may indicate that they are metabolic intermediates of two different pathways in the transformation of cycloartenol into sterols.

Fischer et al isolated a sterol, m.p. 132-33°, from P. vulgare[4] which was assumed to be β-sitosterol[14]. The presence in the same plant of another, unknown sterol, m.p. 162-63°, benzoate, m.p. 165-66° has been reported[7].

Polypodium aureum L. Jizba et al[19], in 1974, studied this fern, Polypodium aureum L. and in this connection they also investigated the fern Polypodium vulgare L.[8] The latter gave steroid compounds of cholestane type in form of Glycosides[20,21], phytoecdysones[22,23] and polyphenolic compounds[8,24].

The ethanolic extract obtained from rhizomes and leaves of P. aureum L. was separated by partitioning between light petroleum and water. From the unpolar fraction triterpenic hydrocarbons fernene and vallichiene were isolated. Both hydrocarbons were identified on the basis of their melting points and their identity was confirmed by IR spectra, mass spectra as well as by oxidation of fernene and vallichiene with chromium trioxide to fernenone[25] and vallichienone[26] respectively. The more polar fractions gave β-glucoside of β-sitosterol, benzoic acid, saccharose, glucose, and three

phytoecdysones of which one was identical with ecdysterone and the other two remained unidentified.

Some other species of polypodiaceae family have also been investigated. Suzuki[27] reported the isolation of coumarin from Polypodium hastatum. In a recent[28] quantitative analysis of free sugars by gas-liquid chromatography on their trimethylsilyl ethers, fructose, galactose, glucose and sucrose were detected from Polypodium polypodioides. 3-Desoxyanthocyanidin glucosides, which at one time were erroneously identified as 6-hydroxypelargonidin and 6-hydroxycyanidin glucosides[29], are present in Polypodium rhodoleuron.