COMPONENTS OF THE FERN *Polypodium aureum* L.*

J.Jizba, S.Vašíčková and V.Herout

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received June 27th, 1973

During the study of the fern *Polypodium aureum* L. of the *Polypodiaceae* family the triterpenic hydrocarbons fernene and vallichiene were isolated as well as 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.

In connection with the study of the components of some *Polypodiaceae* we also investigated the fern *Polypodium vulgare* L[1]. In the ethanolic extract of its rhizomes steroid compounds of the cholestane type in the form of glycosides[2,3] were found, further phytoecdysones[4,5] and polyphenolic compounds[1,6]. In the light petroleum fraction triterpenic hydrocarbons[7,8] were identified.

For comparison we now investigated the components of the ethanolic extracts from rhizomes and leaves of *P. aureum* L.

The extract obtained from the rhizomes was separated by partitioning between light petroleum and water. From the unpolar fraction triterpenic hydrocarbons fernene and vallichiene were isolated by chromatography on silver nitrate impregnated silica gel, known from other species of *Pteridophyta*. Both hydrocarbons were identified on the basis of their melting points and their identity was confirmed by IR spectra and mass spectra, as well as by oxidation with chromium trioxide. After such oxidation of fernene fernenone[9] was isolated from the reaction mixture, and the oxidation of vallichiene gave vallichienone[10]. From the reaction mixture we isolated another product of the composition $C_{30}H_{44}O_3$ (according to mass spectrometry) even when the oxidation was carried out with sodium chromate under the conditions described by Fazakerley and coworkers[11]. The formation of this compound has not yet been observed.

When chromatographing the more polar fraction of the ethanolic extract of leaves on silica gel benzoic acid was isolated from the early fractions. From subsequent

* Part XXXVII in the series Plant Substances; Part XXXVI: This Journal *38*, 3637 (1973).

* This fern was obtained from the Botanical garden in Prague and other hothouses in Czechoslovakia; for its identification our thanks are due to Dr J. Toman.

fractions β-glucoside of β-sitosterol was isolated and identified as acetate[12,13]. On hydrolytic cleavage with a mixture of β-glycosidases from *Aspergillus wentii* of the glycoside bond a reaction mixture was obtained containing glucose and β-sitosterol. Therefore it may be concluded that glucose is bound by a β-glucosidic bond. The same glucoside of β-sitosterol was also isolated from *P. vulgare* L. and identified in the same manner.

A further compound isolated from the same chromatography was an unknown phytoecdysone, m.p. 244–247°C, as we concluded from the ecdysone-like activity tested on the last instar of *Dermestes vulpin* larvae, which were previously submitted to ligature behind their heads and contaminated with an active analogue of juvenile hormone. As the most polar compound saccharose was eluted from the silica gel column.

When working up the ethanolic extract of the rhizomes by chromatography on polyamide and silica gel an additional two phytoecdysones were isolated; one of them was identified as ecdysterone on the basis of its mass spectra, PMR spectrometry and CD measurements. The second newly obtained phytoecdysone, to which we gave the name polypodoaurein, had mol. weight 476 according to mass spectrometry. This compound when dissolved in 50% ethanol and applied to the ligatured rear parts of the larvae of the blow fly (*Calliphora erythrocephala*) in a dose of 1 μg per specimen causes a 90% pupariation. In the rhizomes a larger amount of glucose is present the identity of which was confirmed by paper chromatography and gas chromatography of its pertrimethylsilyl ether.

A common feature of *P. vulgare* L. and *P. aureum* L. is the presence of phytoecdysones and the β-glucoside of β-sitosterol. From the point of view of chemosystematics the finding of both triterpenic hydrocarbons fernene and vallichiene the occurrence of which is limited just to *Pteridophyta* is also very interesting.

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Silica gel G for thin-layer chromatography was from Merck & Co. For column chromatography silica gel (0·05—0·1 mm) from the Hermann firm was used. The polyamide used was a product of Woelm & Co. Gas chromatographic measurements were carried out on a Perkin Elmer model F 11 apparatus with FID and a stainless steel capillary column 50 m, 0·15 mm diameter, wetted with Apiezon L, at 180°C, carrier gas nitrogen. The IR spectra were measured on a Zeiss UR-10 (Jena) apparatus, the PMR spectra on a Varian HA-100 machime, and the mass spectra on an AEI MS 902 spectrometer; optical rotatory dispersion was measured on a JASCO ORD-UV 5 apparatus and circular dichroism on a Jouan 185-II apparatus.

Isolation and Identification of Substances from Rhizomes

Fresh rhizomes of *P. aureum* L. (3·25 kg) were grated and extracted with ethanol (12 l). After evaporation of the solvent the residue (180 g) was partitioned between light petroleum and water. After evaporation of the light petroleum 25 g of residue A were obtained. Aqueous ethanolic solution was concentrated and polar substances were isolated from it (fraction B). After 14 days standing fraction A crystallised. The isolated mixture of crystals (1·2 g) was shown to contain

two components on chromatography on a thin layer of silica gel impregnated with 20% of silver-nitrate, with pentane as eluent. A larger amount of this substance (0·4 g) was then separated on a silica gel column (50 g, impregnated with a solution of 20% silver nitrate). Pentane (70 ml) eluted 150 mg of the triterpenic hydrocarbon fernene; in subsequent pentane fractions (150 ml) a mixture of hydrocarbons was eluted, while the next 45 ml of pentane eluted the triterpenic hydrocarbon vallichiene (100 mg).

Fernene: (m.p. 171—172°C: methanol–acetone 1 : 2) melted underpressed on admixture of an authentic specimen. The IR spectrum contains the following bands (KBr): 1634, 1359, 1370 and 1385 cm^{-1}.

Oxidation of fernene: To a solution of fernene (0·2 g) in 20 ml of benzene acetic acid (10 ml) was added followed by a solution of chromium trioxide in 15 ml of acetic acid. The oxidation course was followed by thin-layer chromatography in a mixture of light petroleum and acetone (90 : 10). Already after 15 minutes the oxidation product appeared. After one hour the reaction was terminated, the solvent evaporated *in vacuo* and the reaction mixture extracted with benzene. The benzene extract was freed from acidic material by extraction with an aqueous sodium hydrogen carbonate solution, then dried and evaporated. The residue was chromatographed on a silica gel column (20 g). Elution was started with light petroleum and acetone was then added. After collection of 120 ml of eluate a fractions was obtained from which fernenone was isolated, m.p. 217—220°C (ethanol), in agreement with the literature[9].

Vallichiene: m.p. 196—197°C (ethanol–acetone), mass spectrum contains the molecular peak at mass 410 (10%), base peak at mass 41 (100%), and other characteristic peaks at masses 395 (1·9%), 229 (11%), 218 (28%), 206 (9·0%), 205 (19%), 204 (16·3), 203 (14%), 192 (15%), 191 (44%), 189 (16%). IR-spectrum (KBr) displays bands at 1357, 1366, 1377 cm^{-1}.

Oxidation of vallichiene: 0·2 g of vallichiene were oxidised and the product isolated as described in the case of fernene oxidation. After oxidation the product was chromatographed on a silica gel column. Two fractions were obtained. From the first one vallichienone crystallised, m.p. 248—251°C, in agreement with the literature[10]. From the second one a compound of m.p. 259—261°C (acetone–methanol) was obtained. Its high resolution mass spectrum, with the molecular peak at 452 mass units, indicates the composition $C_{30}H_{44}O_3$ (m.w. 452·3299; theoretical value 452·3290). IR spectrum (KBr) displays bands at 1608, 1675, 1705 cm^{-1}. CD measurement (in methanol) gave the following values: $\Delta\varepsilon_{360} = 0$, $\Delta\varepsilon_{294} = -5{\cdot}72$, $\Delta\varepsilon_{276} = -4{\cdot}33$, $\Delta\varepsilon_{255} = -8{\cdot}55$, $\Delta\varepsilon_{237} = 0$.

Ecdysterone: Extract B extracted with light petroleum (10 g) was chromatographed on a polyamide column (250 g). Elution with 175 ml of water gave 0·48 g of material which was rechromatographed on silica gel with chloroform–methanol (80 : 20). The first fraction, obtained by elution with 260 ml of the solvent, crystallised out after one week's standing. After crystallisation from methanol 23 mg of ecdysterone, m.p. 237—239°C were obtained which melted undepressed on admixture of an authentic sample. In its mass spectrum M^+ 480 was found, as well as other characteristic peaks at *m/e* 462, 444, 426, 408, 99 and 81. PMR spectrometry gave the following values in hexadeuteriodimethyl sulfoxide and deuteriochloroform (1 : 1): 0·81 s, methyl on $C_{(18)}$; 0·90 s, methyl on $C_{(19)}$; 1·03 bs, 3 methyls on $C_{(21,26,27)}$; 5·69 bs, hydrogen on $C_{(6)}$. CD measurement gave the following values for ecdysterone $\Delta\varepsilon_{376} = 0$; $\Delta\varepsilon_{327} = 1{\cdot}62$; $\Delta\varepsilon_{287} = 0$; $\Delta\varepsilon_{253} = -3{\cdot}30$; $\Delta\varepsilon_{236} = 0$; and ecdysterone from *P. aureum:* $\Delta\varepsilon_{375} = 0$; $\Delta\varepsilon_{327} = 1{\cdot}65$; $\Delta\varepsilon_{287} = 0$; $\Delta\varepsilon_{252} = -3{\cdot}16$: $\Delta\varepsilon_{235} = 0$.

Ecdysterone diacetonide: Ecdysterone (10 mg) dissolved in excess acetone was refluxed in the presence of 100 mg of anhydrous copper(II) sulfate. After 4 hours heating the reagent was

filtered off and the product isolated from the filtrate by thin-layer chromatography (benzene–acetone 8 : 3). The mass spectrum contained the molecular peak at 560 *m/e*.

Polypodoaurein: From the same chromatography of the extract B on polyamide elution with further 210 ml of water gave a fraction which on rechromatography on silica gel (10 g) afforded 5 mg of a pure compound the mass spectrum of which contained the molecular peak at 476 mass units.

Glucose: From the first fraction of polyamide chromatography a mixture of sugars was obtained in which we proved glucose by paper chromatography (paper Whatman No 3; pyridine–ethyl acetate–water 1 : 3·6 : 1·15 as solvent). Applying the known procedure the sugars were converted to their pertrimethylsilyl ethers and glucose was also proved gas chromatographically.

Isolation of Substances from Leaves

Benzoic acid: 6 g of extract B from leaves were chromatographed on a column of silica gel (600 g) with chloroform–methanol (8 : 2). Elution with 2·1 l of the mixture gave a fraction which gave on sublimation benzoic acid (7 mg), m.p. 119–121°C (water), undepressed with an authentic specimen. The mass spectrum was identical with that of an authentic sample[11].

β-*Glucoside of* β-*sitosterol:** From the more polar fractions from the chromatography of fraction B (6·5 l of solvent) β-glucoside of β-sitosterol was obtained. After its separation (it precipitates on concentration) the rest of the fraction was chromatographed on a polyamide column (20 g). Elution with 200 ml of water gave phytoecdysone, m.p. 244–247°C (1 mg). Elution with methanol gave another fraction of β-sitosterol β-glucoside of m.p. 278–285°C. Mass spectrometry gave M^+ 576 and other characteristic peaks at masses 414, 396, 381, 329, 303, 255, 213. The acetate of the β-sitosterol β-glucoside was prepared in the usual manner (by the pyridine method) and it melted at 169–171°C (methanol). Literature[13] gives m.p. 166–167°Ce

Saccharose: From the most polar fractions from the chromatography of fraction B saccharose was isolated, m.p. 181–183°C.

We thank Mr J. Drábek for technical assistance, Mrs M. Vokáčová for the measurements and Dr L. Dolejš for the interpretation of the mass spectra. For help during the collection of plant material our thanks are due to Dr J. Toman. For the determination of ecdysone activity we thank Dr K. Sláma and Dr J. Žďárek, Entomological Institute, Czechoslovak Academy of Sciences. For the gas chromatographic analysis we thank Dr V. Lukeš and Mrs S. Holubová.

REFERENCES

1. Jizba J., Herout V.: This Journal *32*, 2867 (1967).
2. Jizba J., Dolejš L., Herout V., Šorm F., Felhaber H. W., Snatzke G., Tschesche R., Wulff G.: Chem. Ber. *104*, 837 (1971).
3. Jizba J., Dolejš L., Herout V., Šorm F.: Tetrahedron Letters *1971*, 1329.
4. Jizba J., Herout V., Šorm F.: Tetrahedron Letters *1967*, 1689.
5. Jizba J., Herout V., Šorm F.: Tetrahedron Letters *1967*, 5139.
6. Uvarova N. I., Jizba J., Herout V.: This Journal *32*, 3075 (1967).
7. Berti G., Bottari F., Marsili A., Morelli I.: Tetrahedron Letters *1966*, 979.

* This glycoside was also isolated from the fern *P. vulgare* L. and identified in the same manner.

8. Berti G., Bottari F., Macchia B., Marsili A., Ourisson G., Piotrowska H.: Bull. Soc. Chim. France *1964*, 2359.
9. Ageta H. A., Iwata K., Natori S.: Tetrahedron Letters *1963*, 1447.
10. Pandey G. N., Mitra C. R.: Tetrahedron Letters *1967*, 4683.
11. Fazakerley H., Halsall T. G., Jones E. R. H.: J. Chem. Soc. *1959*, 1877.
12. M. S. D. C. Series Mass Spectral Data.
13. Salway A. H.: J. Chem. Soc. *1913*, 1022.

Translated by Ž. Procházka.