PONASTERONE A AND 22-HOPANOL, CHARACTERISTIC COMPONENTS OF THE FERN *Blechnum spicant* L.*

J.JIZBA and V.HEROUT

In.titute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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In the fern *Blechnum spicant* L. (*Polypodiaceae*) the presence of the following substances has been established: 22-hopanol, phytoecdysone ponasterone A and 10-nonacosanol in addition to waxy substances (alkanes, wax alcohols and cerides derived predominantly from palmitic acid) and β-sitosterol and its glucoside.

In connection with the study of the chemical composition of ferns[1,2] we also gave attention to the single representative of the genus *Blechnum* occurring in Czechoslovakia, *i.e. B. spicant* L. (*Polypodiaceae*). For the analysis we took plants collected as a rule in autumn in Jizera Mountains.

This plant was cursorily studied by Bottari and co-workers[3] who mention the presence of triterpenic hydrocarbons of the hopane type, n-alkanes and other components, detected and identified by gas chromatography.

On analysing the extractive substances from its infertile leaves we found that in its typical components this species is analogous with the East Asian species *B. amabile*[5] and *B. niponicum*[4]. In both mentioned species the presence of ponasterone A has been established. This substance may be considered as characteristic of this genus. For the first time ponasterone A was isolated[6] from *Podocarpus Nakaii Hay*. Its identity was proved on the basis of its mixture melting point and mass spectrum. Another component was a triterpenic alcohol which was identified of the basis of its mass spectrum and mixture melting point as 22-hopanol. Although hopane derivatives are components typical of ferns, 22-hopanol has not yet been obtained from natural material and an authentic sample was prepared synthetically[7].

In the waxy part of the extract[8,9] we proved the presence of n-alkanes $C_{19}-C_{31}$, accompanied by a mixture of homologous branched alkanes, by gas chromatography. In contrast to the commonly occurring n-alkane this pattern mixture did not show the usual predominance of odd-numbered homologues over the even-numbered ones. A further typical group in the plant was the homologous series of primary n-alkanols (C_{22} to C_{32}). The occurrence of the secondary alcohol 10-nonacosanol

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is exceptional. Its occurrence in *Brassica oleracea* L. was demonstrated by Purdy and Truter[10], and in several *Rosaceae* plants by Wollrab[11]. We haved identified it after oxidation to a ketone on the basis of its IR and mass spectrum. We also found true cerides in the waxy fraction, which were identified after re-esterification with methanol in the presence of hydrogen chloride. In the methyl esters obtained palmitic acid was the principal component, in addition to a smaller amount of lignoceric, behenic and stearic acids; the alcoholic part of the waxes consisted of n-alkanols C_{24}, C_{26} and C_{28} in addition to β-sitosterol. In the more polar part of the extract β-sitosterol glucoside was detected in the presence of the ubiquitous saccharose and the already mentioned ponasterone A.

EXPERIMENTAL

The melting points were measured on a Kofler block. Silica gel G (Merck) was used for thin-layer chromatography and silica gel (Hermann) (0·05—0·1 mm) for column chromatography. The polyamide used was a product of Woelm and Co. Gas chromatographic measurements were carried out on a PYE Series 104 Chromatograph, Model 24, provided with a dual column and detector (FI) system and the possibility of programmed heating. Columns of 0·4 × 150 cm size were filled with 2·5—3% of SE — 30G. C. Grade on Gas Chrom P (100—120 mesh). The IR spectra were measured on a Zeiss UR-10 (Jena) instrument, the mass spectra on an AEI MS 902 spectrometer.

Isolation. Dry, ground leaves (2·98 kg) of *Blechnum spicant* were percolated with ethanol; the residue weighed 232 g, and it was stirred with 5% aqueous ethanol. The non-polar part was extracted with light petroleum and chromatographed on 5 kg of silica gel (overloaded column), taking 2 l fractions. Fractions 1—4 were eluted with light petroleum, fractions 5—15 with light petroleum containing 10% of ether, fractions 16—25 with a light petroleum–ether mixture (1 : 1), fractions 26—35 with pure ether.

n-*Alkanes and branched alkanes*: The combined fractions 2—6 (15·6 g) were rechromatographed on a silica gel column (2 kg) with light petroleum (3 l). The first fractions contained 1·8 g of hydrocarbons. These were compared gas chromatographically with a mixture of standard n-alkalnes. The presence of C_{19}—C_{31} hydrocarbons, with a maximum at C_{23}, was proved. Some hydrocarbons were isolated and their identity was confirmed by mass spectrometry. Simultaneously mass spectrometry demonstrated that the mixture of aliphatic straight-chain alkanes is accompanied by a lesser fraction (in a 1 : 20 ratio) of homologous branched iso- and anteisoparafinic hydrocarbons.

10-*Nonacosanol*: From fractions 7—12 of the rechromatography procedure on 2 kg of silica gel, which were obtained by elution with approximately 4 l of light petroleum containing 1% of acetone 10-nonacosanol was isolated, m.p. 81·5°C (ethanol)[10,11] the mass spectrum of which contained a molecular peak $M^+ - 1$ at 423. For the mass M — 18 high resolution measurement gave the value 406.4514 (calculated value 406.4538). IR spectrum ($CHCl_3$) 3 620 cm^{-1}.

Nonacosan-10-*one*: 10-Nonacosanol (28 mg) was oxidized with chromium trioxide according to Jones[12]. The reaction mixture was extracted with chloroform and separated on a silica gel column. A product was obtained melting at 75°C (acetone); in accordance with the literature[13] its IR spectrum ($CHCl_3$) contained a band at 1 708 cm^{-1} and its mass spectrum had peaks at 422 (molecular peak), 404, 411, 310, 295, 171, 170 and 155 mass units.

2-*Hopanol*: From the fractions 8—9 (of the first chromatography) 10-nonacosanol crystallized out and it was filtered off. The residue of the filtrate (0·1 g) was chromatographed on 20 g of silica

gel to afford another alcohol of m.p. 254—255°C (acetone–methanol). Mass spectrum of hopanol: M^+ 428 (7%), 410 (16·22), 231 (8·2), 207 (24·8), 191 (100). The mass spectrum is identical with the mass spectrum of an authentic specimen, and the mixture melting point is undepressed.

n-*Alkanols*: The combined fractions 17—18 of the first chromatography gave 0·6 g of a mixture of n-alkanols which were identified by gas chromatography on comparison with a mixture of standards. The chromatogram indicated a mixture of C_{22}—C_{30} alkanols in which C_{24} and C_{26} compounds prevailed; the natural mixture was acetylated and gas chromatography of the obtained acetylated mixture corroborated the above finding.

Cerides

The fraction 20 from the first chromatography (0·7 g) was crystalline and it was reesterified according to Streibl and co-workers[14] to a mixture of methyl esters and free alcohols. The mixture was separated to esters and alcohols and fractions were analyzed by gas chromatography by comparison with known standards (methyl esters and alcohols). The dominant component was palmitic acid, minor components were lignoceric, behenic, arachidonic, stearic and myristic acids. Among alcohols β-sitosterol and C_{24} and C_{26} alcohols were most abundant.

β-Sitosterol

Fraction 23 from the first chromatography gave a substance of m.p. 138—139°C (acetone) which was identified by mass spectrometry as β-sitosterol.

Polar fraction: The ethanolic extract (20 g) was extracted with light petroleum, and the polar phase chromatographed on silica gel (2 kg) with a chloroform–ethanol mixture (8 : 2). Elution with 13 l of the eluent gave glucoside of β-sitosterol, m.p. 278—282°C. Mass spectrometry gave M^+ 576 and further characteristic peaks at masses 414, 396, 381, 329, 303, 255, and 213. From the last fractions of the chromatography, obtained by elution with methanol, saccharose was obtained, m.p. 181—183°C.

Ponasterone A

Another part of the ethanolic extract (20 g) freed from lipophilic material by extraction with light petroleum was purified by chromatography on an alumina column (Brockmann, neutral, activity III). A chloroform–ethanol mixture (1 : 1, the fractions were 1 l each) eluted in the 6th and the 7th fractions 0·35 g of a mixture of substances in which according to thin-layer chromatography (in chloroform–methanol–water 65 : 20 : 10) and a biological test phytoecdysones were present. This mixture was separated on a column of polyamide (10 g). Elution was carried out first with water (80 ml) and then with aqueous methanol (up to 10%). From the fractions obtained by elution with 250 ml of the eluent a substance was obtained, m.p. 262—264°C (acetone) which melted undepressed on admixture of an authentic sample of ponasterone A. Mass spectrum: 446 ($M - H_2O$); 428 ($M - 2\,H_2O$); 345, 327, 309, 301, 285, 269, 276, 145, 109, 83. The mass spectrum of our ponasterone A was identical with that of an authentic sample. The ecdysone activity was determined on the last instar of *Dermestes vulpinus larvae* which were ligatured behind their head after preliminary contamination with the active juvenile hormone analogue.

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REFERENCES

1. Jizba J., Herout V.: This Journal *32*, 2867 (1967).
2. Jizba J., Vašíčková S., Herout V.: This Journal *39*, 501 (1974).
3. Bottari F., Marsili A., Morelli I., Pacchiani M.: Phytochemistry *11*, 2519 (1972).
4. Takemoto T., Hikino T., Okuyama T., Arihara S., Hikino H.: Tetrahedron Letters *1968*, 6095.
5. Takemoto T., Okuyama T., Arihara S., Hikino Y., Hikino H.: Chem. Pharm. Bull (Tokyo) *17*, 1973 (1969).
6. Nakanishi K., Koreeda M., Sasaki S., Chang M. L., Hsu H. Y.: Chem. Commun. *1966*, 915.
7. Jones E. R. H.: Croatica Chim. Acta *29*, 173 (1957).
8. Chibnall A. C., Piper S. H., Pollard A., Williams E. F., Sahai P. N.: Biochem. J. *28*, 2189 (1934).
9. Eglinton G., Hamilton R. J.: *Chemical Plant Taxonomy* (T. Swain, Ed.), p. 187. Academic Press, London and New York 1963.
10. Purdy S. J., Truter E. V.: Proc. Roy Soc. B *158*, 553 (1963).
11. Wollrab V.: Phytochemistry *8*, 623 (1969).
12. Bowers A., Halsall T. G., Jones E. R. H., Lemin A. J.: J. Chem. Soc. *1953*, 2548.
13. Bentley H. R., Henry J. A., Irvine D. S., Mukerji D., Spring F. S.: J. Chem. Soc. *1955*, 596.
14. Streibl M., Jirošová J., Stránský K.: Fette, Seifen, Anstrichmittel *73*, 301 (1971).

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