

ISOLATION OF ECDYSTERONE (CRUSTECDYSONE) FROM POLYPODIUM VULGARE L. RHIZOMES

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As a part of study (1) of constituents of polypody rhizomes (Polypodium vulgare L.) we isolated recently, besides other compounds, also two crystalline substances and proposed the names polypodine A and polypodine B for them.\* We have found now (3) polypodine A to be identical with ecdysterone (hydroxyecdysone, crustecdysone) which, as has been shown, is the moulting hormone isolated from Crustaceans (4) and later from *Bombyx mori* (5) and has the structure of  $2\beta,3\beta,14\alpha,20\beta,22\beta,25$ -hexahydroxy- $5\beta$ -cholest-7-en-6-one; ecdysterone possesses higher physiological activity in comparison with ecdysone isolated from insects. The proof of identity of polypodine A with ecdysterone has been carried out by comparison of physical constants of both compounds, chemical properties and biological activity. The physical constants are summarized in Table I. Ecdysterone crystallizes from an aqueous solution with three molecules of water (like ecdysone which crystallizes with two molecules of water) and as a hydrate has m. p.  $150 - 151^{\circ}\text{C}$ . The elemental analysis corresponds to the molecular formula  $\text{C}_{27}\text{H}_{44}\text{O}_7 \cdot 3\text{H}_2\text{O}$  (534.7) calculated 60.65% C, 9.43% H; found 60.28% C, 9.23% H.

The molecular weight has been determined by mass spectrometry ( $M = 480$ ); this is in accordance with the molecular weight of tetra(trimethylsilyl)ether prepared from polypodine A ( $M = 768$ ) as well.

The character of fragments in the mass spectra was entirely identical in both compounds and the NMR spectra as well are in full accordance. The infra-

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\* A substance called polypodine (m. p.  $150 - 151^{\circ}\text{C}$ ) was, for the first time, reported by Volmar and Reebe (2). The authors took polypodine for a glycoside and did not characterize it in detail.

TABLE I  
Physical Constants of Polypodine A and Ecdysterone

	Polypodine A	Ecdysterone
M. p.	243°C	237.5 - 239.5°C
$[\alpha]_D$	+61.8°C	*
UV (methanol)	244 nm (log $\epsilon$ 4.09)	242 nm (log $\epsilon$ 4.09) (5) 240 nm (log $\epsilon$ 4.13) (6)
IR (KBr disc)	1642, 1608 $\text{cm}^{-1}$	1650, 1610 $\text{cm}^{-1}$ (5) 1645, 1612 $\text{cm}^{-1}$ (6)

\*For ecdysone found  $[\alpha]_D +58.5^\circ\text{C}$

red spectra of our specimen, measured in KBr pelet (dried at 80°C), exhibited maxima of the main frequencies, particularly of carbonyl group and conjugated double bond, identical with the published data. In other parts of the spectra there were observed certain differences. After drying the sample at higher temperatures, the infra-red spectrum was entirely identical with that (6) of ecdysterone. This phenomenon is quite frequent and has been observed already many times.

Polypodine A on treatment with acetone in the presence of anhydrous cupric sulfate yielded a characteristic diisopropylidene derivative, m. p. 229-234°C. For  $\text{C}_{33}\text{H}_{52}\text{O}_7$  (560.8) calculated : 70.68% C, 9.35% H, 0.36%  $\text{H}^+$ ; found : 70.45% C, 9.44% H, 0.38%  $\text{H}^+$ . The formation of diacetonide corroborates the presence of two pairs of vicinal hydroxyl groups, as well as the result of potassium periodate oxidation (determined polarographically).

Polypodine A exhibits enormous ecdysone-like activity in insect. The effect has been tested on freshly moulted larvae of Pyrrhocoris apterus. The microcrystals of the compound (about 50  $\mu\text{g}$ ) implanted in to the haemolymph cause precocious moulting after two and a half days whereas the normal moulting does not take place before the seventh day.

All the facts discussed prove the identity of polypodine A with ecdysterone. The presence of a considerably high amount of ecdysterone (over 1% of dry drug) in Polypodium vulgare rhizomes indicates that compounds of the character of

moulting hormone may be exogenous factors and that the insect receives them with the food. Indirectly this assumption is in agreement with the fact that compounds of ecdysone activity were isolated from adult insect specimens, in which the phoracic glands had already degenerated.

## REFERENCES

1. J. Jizba and V. Herout, Coll. Czech. Chem. Commun., in press.
2. J. Volmar and E. Reebe, J. Am. Pharm. Assoc., 22, 1225 (1933).
3. J. Jizba, V. Herout and F. Šorm, Czechoslovak patent application.
4. F. Hampshire and D.H.S. Horn, Chem. Comm., 1966, 37.
5. P. Hocks and R. Wiechert, Tetrahedron Letters, 1966, 2989.
6. H. Hoffmeister and H.F. Grützmacher, Tetrahedron Letters, 1966, 4017.