

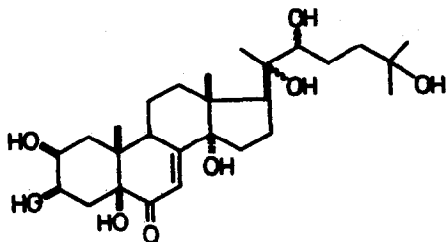
POLYPODINE B - A NOVEL ECDYSONE-LIKE SUBSTANCE FROM PLANT MATERIAL

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An increasing number of compounds showing insect-moulting effect is being found in vegetable material in recent years. In a preliminary communication (1) we have shown that polypodine A (2), isolated from rhizomes of *Polypodium vulgare* L., is identical with ecdysterone (= 20 β -hydroxyecdysone) while Nakanishi and his colleagues found ponasterol in *Podocarpus nakai* HAY (3). Ecdysterone has been further found in *Vitex megapotamica* (SPRENG) MOIDENKE (4), together with inocosterone in *Achyranthes fauriei* (5) and *Morus* sp. (6), and it is probably also contained in *Podocarpus elatus* R.Br. (7); cyanosterone has been isolated from *Cyatula capitata* MOQUIN-TANDON (8) and the presence of moulting - hormone active substances has been noted in other plants (9), too. We now wish to present evidence that yet another compound isolated by us from this fern (i.e. *Polypodium vulgare* L.), polypodine B, is an ecdysone-like substance, i.e. 5 β ,20 β -dihydroxyecdysone (I). This compound therefore is /together with the recently reported 20 β ,26-dihydroxyecdysone in Tobacco Hornworm-*Manduca sexta* (JOHANNSSON) (10)/yet another highly hydroxylated (i.e. a heptahydroxy-) derivative of cholest-7-en-6-one.



The properties of polypodine B closely resemble those of polypodine A. The compounds co-crystallize and can be separated by chromatography only with difficulty. Polypodine B crystallizes with water of crystallization which it keeps tenaciously; a sample crystallized from a dioxane still contained one molecule of water, which even at elevated temperatures it lost only partially. The monohydrate has m.p. 254-257°, $C_{27}H_{44}O_8 \cdot H_2O$ (514.7), calculated 63.01% C, 9.01% H; found 62.86% C, 9.15% H. The diacetonide of polypodine B has m.p. 249-251° (aqueous methanol) $C_{33}H_{52}O_8$ (576.8), calculated 68.72% C, 9.09% H, 0.52% H act.; found 68.52% C, 9.10% H and 0.48% H act. The mass spectrum of polypodine B did not contain any molecular ion but exhibited ions of $m/e = 478, 460, 442, \text{ and } 424$ resulting from successive splitting off of four molecules of water. If the highest peak $m/e = 561$ in the spectrum of the diacetonide is taken to be an $M-15$ fragment, a value $M = 576$ for the molecular weight of this derivative meets the above mentioned structural requirements. Further significant peaks in the mass spectrum together with characteristic absorptions in the infrared and ultraviolet of polypodine B and ecdysterone are summarized in Table 1. As can be seen,

Table 1. Characteristic data of i.r., u.v. and mass spectra

Compound	UV	IR (cm^{-1})	Mass spectrum
Ecdysterone (Polypodine A)	327 nm	1655	81, 99, 327
	(log $\epsilon = 2.07$)	1055	328, 344, 345
	244 nm	3400	363, 426, 444
	(log $\epsilon = 4.11$)		
Polypodine B	317 nm	1687	81, 99, 343, 344,
	(log $\epsilon = 2.21$)	1657	360, 361, 424
	243 nm	1636	442, 460, 478
	(log $\epsilon = 4.07$)	3340	

the u.v. spectra of both polypodine A and B are almost identical. The shift of the infrared absorption band of the carbonyl group conjugated with the double bond in polypodine B by 32 cm^{-1} in comparison with ecdysterone is consistent with the presence of substituent in the neighbourhood of this chromophore. The nature of the further hydroxyl group in polypodine B molecule can be largely explained on the basis of analysis of its p.m.r. spectrum, the main features of which are shown in Table 2.

Table 2. Chemical shifts of some protons in p.m.r. spectra of ecdysterone and polypodine B*

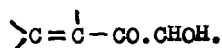
Compound	C-18, C-19	O-C-CH ₃	tert.OH	sec.OH	H-7
Ecdysterone	9.23 (3)	8.92 (9)	5.48 (1)	5.71 (2)	4.39
(Polypodine A)	9.15 (3)		5.96 (1)	5.84 (1)	
			6.55 (1)		
Polypodine B	9.21 (6)	8.93 (9)	4.58 (1)	5.19 (1)	4.32
			5.34 (1)	5.66 (2)	
			5.94 (1)		
			6.47 (1)		

* In τ -values. The spectra were taken on Varian HA-100 instrument in hexadeuterodimethylsulphoxide containing deuteriochloroform using hexamethyldisiloxane (HMDS) as an internal reference (HMDS = 9.948 TMS/tetramethylsilane/).

The close resemblance of the chemical shifts of all the important groups in the p.m.r. spectra of ecdysterone and polypodine B suggests that the skeleton of both compounds is identical. Moreover, the p.m.r. spectrum of the latter showed that in this compound a further tertiary hydroxy group must be present and to fulfill structural requirements of the skeleton it can be attached only to the positions 5, 9 or 17 in ecdysterone. On oxidation with sodium periodate in buffer solution at pH 6.8 (followed polarographically) polypodine B consumed 2.44 mole of the reagent after 24 hours at room temperature whereas ecdysterone at the same conditions

showed a consumption of 2.05 mole only. Evidently the oxidation in the case of polypodine B continues, although rather slowly, which excludes the position 9 as the site of the new tertiary hydroxy group. Attempts were made to oxidize polypodine B with sodium periodate on a preparative scale with a view to look for acetic acid. Acetic acid, however, could not be detected even in traces in the reaction mixture thus showing that the location of this hydroxy group in position 17 is improbable. The remaining possibility, i.e. that it is located in position 5, was further confirmed by an exhaustive deuteration of polypodine B in alkaline solution. Mass spectrometrical investigation of the reaction products showed that in the case of polypodine B two, and in some molecules even three deuterium atoms were introduced whereas ecdysterone cleanly exchanged three (and only in very small extent also four) hydrogen atoms for deuterium.

A circular dichroism curve* of ecdysterone (polypodine A) exhibits a positive Cotton effect with a characteristic fine structure with a principal maximum (R-band) at 339 nm (+1.60); an analogous band in the c.d. curve of polypodine B showed a simple structure characterized by a maximum of dichroic absorption at 329.5 nm (+2.91). A hypsochromic shift of the R-band, accompanied by disappearance of its fine structure of this type, have been observed (11) in some eneones and their α -hydroxyderivatives



Summarizing the above results we have reason to believe that the structure I can be assigned unambiguously to polypodine B, yet a further ecdysone-like natural product. It is about four times more active in the test on pupae of Calliphora fly than synthetic ecdysone (Synthex Co., Inc.), thus being the most active moulting hormone-like substance so far. The details of this investigation will be published in full in Coll. Czech. Chem. Commun. in the near future.

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REFERENCES

- (1) J.Jizba, V.Herout, and F.Šorm, Tetrahedron Letters, 1967, 1689.
- (2) J.Jizba and V.Herout, Coll. Czech. Chem. Commun., 32, 2867 (1967).
- (3) K.Nakanishi, M.Koreeda, S.Sasaki, M.L.Chang, and H.Y.Hsu, Chem. Commun., 1966, 915.
- (4) H.Rimpler and G.Schulz, Tetrahedron Letters, 1967, 2033.
- (5) T.Takemoto, S.Ogawa, and N.Nishimoto, Yakugaku Zasshi, 87, 325 (1967).
- (6) T.Takemoto, S.Ogawa, N.Nishimoto, H.Hirayama, and S.Taniguchi, Yakugaku Zasshi, in press.
- (7) M.N.Calbraith and D.H.S.Horn, Chem. Commun., 1966, 905.
- (8) T.Takemoto, Y.Hikino, K.Nomoto, and H.Hikino, Tetrahedron Letters, 1967, 3191.
- (9) T.Takemoto, S.Ogawa, N.Nishimoto, and H.Hoffmeister, Z.Naturforsch., 22b, 681 (1967).
- (10) M.J.Thompson, J.N.Kaplanis, W.E.Robbins, and R.T.Yamamoto, Chem. Commun., 1967, 650.
- (11) J.Polonsky and J.L.Fourrey, Tetrahedron Letters, 1967, 2033.