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POLYPODOAUREIN, A NEW PHYTOECDYSONE FROM *POLYPODIUM AUREUM* L.

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Key Word Index—*Polypodium aureum*; Polypodiaceae; phytoecdysone; insect hormone; polypodoaurein.

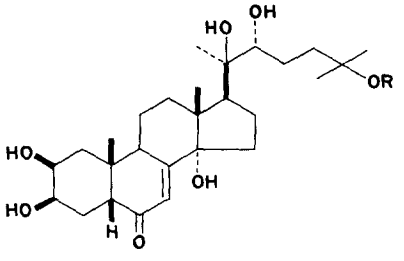
Abstract—A new phytoecdysone was isolated from *Polypodium aureum* L. and its structure determined as 25-*O*-methylecdysterone.

INTRODUCTION

THE TERPENOID components of *Polypodium aureum* L. (Polypodiaceae) have been investigated[1] and, in addition to ecdysterone two other undescribed ecdysones have been isolated from this fern. The structure of one of these is described in this paper. This compound belongs to the group of phytoecdysones with a substituted 25-hydroxyl group. Similar compounds are already known, where the hydroxyl group is either glucosylated (ponasteroside[2]), bound in a lactone ring (cyasterone,[3] sengosterone,[4] capitasterone[5]) or found in the form of a cyclic ether (stachysterone D[6]).

RESULTS AND DISCUSSION

Only a few mg of the compound under investigation (polypodoaurein (**1**)) m.p. 251–253°, were isolated from the rhizomes of *Polypodium aureum* L. Its UV spectrum showed a maximum at 244 nm, well in agreement with the data found in compounds similar to ecdysone; the oxo group in the position 6 is conjugated with the Δ^7 double bond in the cholestane skeleton.



(**1**) R = H ecdysterone
(**2**) R = Me polypodoaurein

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The mass spectrum of polypodoaurein (**1**) does not contain the molecular ion; however, the peak M-18 at mass 476 is clearly visible. The peaks in the highest mass range of the spectrum belong to the fragments arising by further elimination of H_2O or methanol: *m/e* 458 ($C_{28}H_{42}O_5$, M-2H_2O), 440 ($C_{28}H_{40}O_4$, M-3H_2O), 426 ($C_{27}H_{38}O_4$, M-(2H_2O + CH_3OH)).

The cleavage of the C_{20}–C_{22} and C_{17}–C_{20} bonds gives rise to the characteristic peaks at 363 ($C_{21}H_{31}O_5$), 345 ($C_{21}H_{29}O_4$), 327 ($C_{21}H_{27}O_3$) and 300 ($C_{19}H_{24}O_3$); the same fragmentation pattern was observed in ecdysterone[7] (**2**) and makisterone A.[8] Prominent peaks at *m/e* 113 ($C_7H_{13}O$) and 99 ($C_6H_{11}O$) are due to the side chain fragments (fission of the C_{20}–C_{22} bond and elimination of a molecule of H_2O or methanol).

From the above data it follows that the methoxy group is probably located in the side chain on either C_{22} or C_{25}. Reaction of polypodoaurein with excess acetone in the presence of anhydrous cupric sulfate gave a diacetonide, as shown by mass spectrometry of the product. This indicates that the methoxy group is on C_{25} rather than on C_{22}. The MS of diacetonides of polypodoaurein[7] (**1**) and ecdysterone[9] (**2**) were also compared and found to be analogous.

In contrast to the corresponding ecdysterone derivative the MS of polypodoaurein diacetonide does not show the molecular ion or the M-15 fragment. The peak of the highest mass *m/e* 542 corresponds to the M-methanol fragment. In the middle and lower mass range the spectrum shows the same peaks (*m/e* 403, 385, 369, 368, 341, 340, 311, 310, 283, 282, 125) as the ecdysterone diacetonide. The differences in relative abundance may be attributed to the different stability of molecular ions.

In view of the fact that ecdysterone has also been isolated from *P. aureum*[1], it seems probable on the basis of biogenetical considerations that polypodoaurein (**1**) is an *O*-methyl analogue. Ecdysterone-type activity was shown using the ligatured hind parts of the blow fly larvae (*Calliphora erythrocephala*) into which a solution polypodoaurein was injected (1 μg/larvae); this causes a 90% pupariation in the *Calliphora* test*.

EXPERIMENTAL

The MS were run on a mass spectrometer AEI-MS 902, the precise mass measurements were within 5 ppm.

Polypodoaurein isolated from the rhizomes of *Polypodium aureum* L. (3 mg) was crystallized from MeOH (m.p. 251–253°); UV spectrum λ_{max} 244 nm (log ε = 4·0; EtOH); MS: *m/e* 476 (M-18; 0·08), 458 (0·8), 440 (3·8), 426 (4·3), 363 (42), 345 (75), 327 (50), 301 (19), 300 (20), 285 (20), 269 (30), 267 (22), 213 (17), 113 (91), 99 (67), 95 (81), 81 (100%).

Polypodoaurein diacetonide. Polypodoaurein (1·5 mg) was heated with excess acetone in the presence of anhyd. $CuSO_4$ (dried at 230° *in vacuo* for 8 hr) for 3 hr, and the filtrate evaporated to dryness. The residue was separated by preparative TLC on silica gel-G. The main fraction was scraped off and extracted, and its MS measured: *m/e* 542 (M-32, 0·2), 527 (0·8), 509 ($C_{32}H_{45}O_5$, 1·4), 466 ($C_{30}H_{42}O_4$, 1·2), 403 (3), 385 (2·4), 368 ($C_{24}H_{32}O_3$, 7), 341 ($C_{22}H_{29}O_3$, 14), 340 (9), 311 (3), 283 (8), 282 (8), 267 (7), 225 (8), 125 (26), 97 (24), 95 (15), 93 (15), 43 (100%).

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