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THE STRUCTURE OF OSLADIN - THE SWEET PRINCIPLE OF THE RHIZOMES OF POLYPODIUM VULGARE L.

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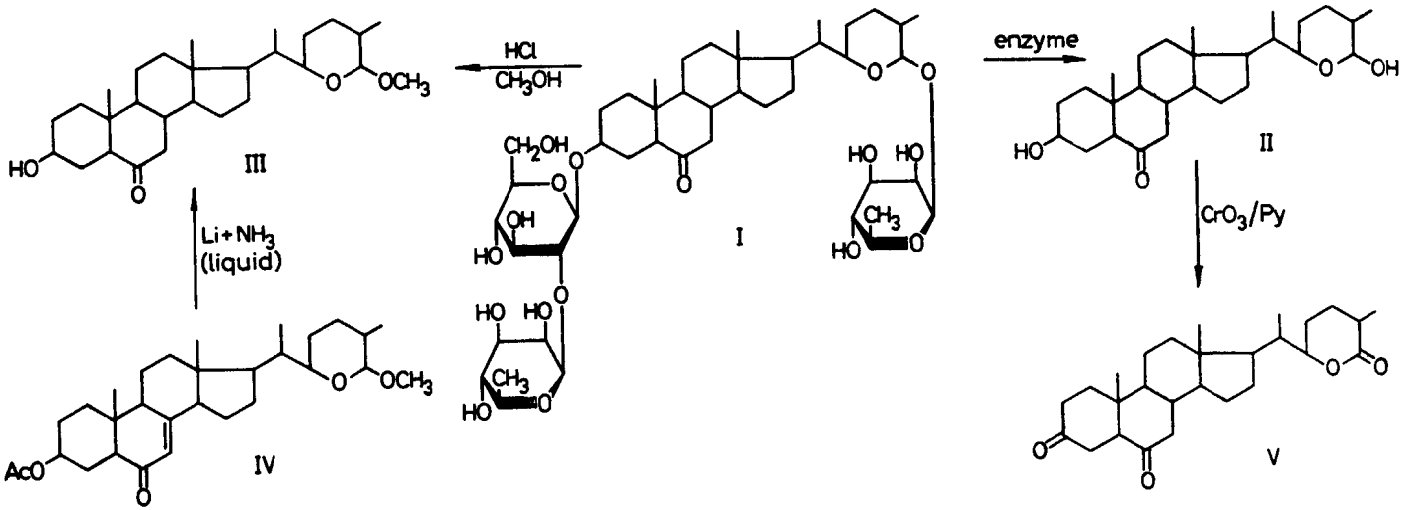
Recently we published[1] a paper on the structure of saponin glycosides isolated from the rhizomes of Polypodium vulgare L. It was demonstrated that polypodosapogenin (3β-hydroxy-26ξ-methoxy-22ξ,26ξ-epoxy-6-oxo-5α--cholest-7-ene) represent a new type of steroid saponins. We isolated[2] another glycosidic compound to which we gave the name osladin (I); this substance is the carrier of the strong sweetness of the rhizomes of Polypodium vulgare L. Osladin is related to the glycosides of the saponin type and is a bis-desmosidic glycoside of 3β,26ξ-dihydroxy-22ξ,26ξ-epoxy-6-oxo--5ξ-cholestane (II).

For the isolation of osladin from the mixture of glycosides we modified the former method of separation[2]. The enriched mixture of steroid glycosides was freed from compounds of phenolic character by partition between butanol--xylene mixture and water. Further separation was carried out on a column of silica gel with chloroform-methanol-water 65:35:10. Osladin (I) had m.p. 198--199°c (methanol). IR spectrum in nujol: 1704 cm^{-1}, 3400 cm^{-1}. On acid hydrolysis it gave glucose and rhamnose. Under the influence of a crude enzymic preparation from Aspergillus wentii[3], containing mainly β-glucosidase, liberation of the mentioned monosaccharides could also be demonstrated. Simultaneously we obtained the aglycone which after chromatography on a silica gel column (chloroform-methanol 9:1) and crystallisation from methanol had m.p. 193-195°C; IR spectrum in chloroform: 1697 cm^{-1}, 3600 cm^{-1}. NMR-spectrum: d_6-DMSO, 100MHz, HMDS ($\delta_{TMS} = \delta_{HMDS} + 0.05$ ppm) 0.64 s, 2x CH_3-$\overset{|}{C}$-; 0.71 d, J = 6 Hz, 3 H, CH_3-CH; 0.81 d, J = 6 Hz, 3 H, CH_3CH; 3.33 mt, 2 H,

2x CH-O; 6.10 d, J = 6.3 Hz, exchangeable, 1 H, OH; 4.49 d, J = 4.6 Hz, 1 H, exchangeable, OH. Mass spectrum: m/e 432 (1.8% M^+); 414 (61%, M-18); 345 (26%, with the assigned structure "a"). Fission of the $C_{(20)}$-$C_{(22)}$ bond particles 317 (63%, "b"); 115 (56%, "c"; R=H) are formed. According to metastable transitions both of them eliminate a molecule of water and give fragments of m/e 299 (38%, m^x 282.0) and m/e 97 (100%, m^x 81.8). Fission of the $C_{(17)}$-$C_{(20)}$ bond afforded ions 287 (84%; transfer of two hydrogen atoms to the departing particle of the side chain) and 126 (77%, structure "d").

Treatment of osladin with methanolic hydrogen chloride leads to aglycone III methoxylated at $C_{(26)}$, m.p. 162-163°C (methanol). For $C_{28}H_{46}O_4$ (446.7) OCH_3 found 6.61%, calculated 6.94%. Mass spectrum: the mass of the molecular ion M^+ 446 (0.4%) and the mass of the characteristic fragment "c" m/e 129 (100%; R=CH_3) are shifted by 14 mu. as compared with the analogous fragments of substance II. Ions 386 (16%, M-60; elimination of $HCOOCH_3$) and 344 (19%, M-102; product of retro-Diels-Alder reaction of olefin M-CH_3OH) characterise the acetal grouping. Cleavage of the $C_{(20)}$-$C_{(22)}$ bond brings about a shift of the hydrogen atom from the steroid nucleus to the eliminated unit, under formation of fragment 316 (17%). Ion of mass 287 (40%) arises in the same manner as in the case of substance II. The mass spectrum of the methylated aglycone III is identical with the spectrum[1] of the methylated dihydropolypodosapogenin; this substance m.p. 161-162°C obtained in the preceding work[1] on reduction of acetyl derivative IV by lithium in liquid ammonia does not give a depressed mixture melting point with the methylated genin of osladin (III).

When oxidizing the aglycone of osladin (II) with chromium trioxide in pyridine compound V is formed in which the hydroxyl at $C_{(3)}$ is oxidized as well as the acetal group in the side chain (to a δ-lactone). IR spectrum: 1713 cm^{-1} (superimposed oxo and δ-lactone groups), 1085 cm^{-1}, 1187 cm^{-1} (six-membered lactone). The mass spectrum of lactone V contains an intense molecular peak M^+ 428 (98%). Characteristic ions 285 (17%; M-(side chain + 2 H)), 286 (19%), 245 (32%; M-(side chain + 42)), 113 (100%; analog of ion "c"), and 85 (53%, "c" -28).


HCl
CH3OH
enzyme
CrO3/Py
Li+NH3
(liquid)
III
I
II
IV
V
HO
OCH3
AcO
CH2OH
OH
CH3
„a"
„b"
„c"
„d"
OR

During the quantitative determination of monosaccharides in osladin (I), using gas chromatography of permethylsilylated methyl glycosides of monosaccharides, we were able to prove one molecule of glucose and two molecules of rhamnose. We never isolated osladin methylated at $C_{(26)}$ as we were able to do in the analogous case of polypodosaponin[1], when we isolated methylated polypodosaponin. We were unable to prepare this derivative either, unless the glycosidic bonds were hydrolysed. From this fact, and on the basis of the biogenetical relationship of osladin with polypodosaponin we draw the conclusion that one molecule of rhamnose is bound to glucose in the form of a diglycoside bound to the $C_{(3)}$ hydroxyl of the genin. The second rhamnose molecule is evidently bound to the hydroxy group of the hemi-acetal in the position $C_{(26)}$ of the genin side chain.

In agreement with this assumption we were able to prove by thin-layer chromatography (chloroform-methanol 10:1) and paper chromatography (butanol--water-carbon tetrachloride 40:40:30), using authentic samples for comparison, that after the hydrolysis of permethylated osladin the reaction mixture contained 3,4,6-tri-O-methyl-D-glucose and exclusively 2,3,4-tri-O-methyl-L-rhamnose. Anilide of 2,3,4-tri-O-methyl-L-rhamnose (m.p. 125-127°C) melted undepressed on admixture of the corresponding derivative prepared from permethylosladin. The obtained 3,4,6-tri-O-methyl-D-glucose had $[\alpha]_D^{25}$ +72.6° (c=0.35, water). In view of the fact that glucose was split off by β-glucosidase we can assume that its link with the aglycone is β-glycosidic. The configuration of the glycosidic bond of L-rhamnose has not as yet been determined.

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