FURTHER STERoidal AND FLAVONOID CONSTITUENTS OF THE SWEET PLANT, POLYPodium GLYCRRHIZA*

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Abstract—Two novel steroidal glycosides, polypodiosides B and C, as well as three known compounds, polydione B, (+)-catechin and (+)-afzeliechin, were isolated from the rhizomes of the sweet plant, Polypodium glycyrrhiza. By the application of various spectral methods, polypodiosides B and C were assigned the structures, 26-O-α-L-rhamnopyranosyl-polypodogenin-3-O-β-D-glucopyranoside and 26-O-α-L-acroiofranopyranosyl-polypodogenin-3-O-β-D-glucopyranoside, respectively. Polypodioside B tasted sweet, although polypodioside C was devoid of this effect.

INTRODUCTION

In previous work, we have established that the sweetness of the rhizomes of the North American fern, Polypodium glycyrrhiza D.C. Eaton (Polypodiaceae), is mainly due to the presence of the steroidal glycoside, polypodioside A [1, 26-Ω-α-L-rhamnopyranosyl-polypodogenin-3-O-β-D-rhamnopyranosyl(1→2)-β-D-glucopyranoside] [1]. The structure and stereochemistry of polypodogenin (2), the known aglycone of 1, were established as [2S, 25R, 26R]-3β, 26-dihydroxy-22, 26-epoxy-6-dom-5a-cholest-7-ene by Czechoslovakian workers [2-4]. Compound 1 was converted to its aglycone, 2, using the enzyme, hesperidinase, and polypodogenin (2) was fully characterized using modern spectroscopic methods [1]. Polypodioside A (1) was found to be nonmutagenic and not acutely toxic to mice, and was rated by a taste panel as being 600 times sweeter than a 5% w/v sucrose solution [1]. Other documented constituents of this plant part are the flavonoids, (+)-afzelicin-7-O-β-D-apioside and polydine, which proved to be bitter and neutral-tasting, respectively [5], as well as sucrose [6] and several aromatic acids [7]. In the present communication, we wish to report the isolation of additional steroidal and phenolic constituents of P. glycyrrhiza rhizomes, inclusive of two novel analogues of polypodioside A, which we have named polypodioside B (3) and polypodioside C (4), and the known compounds, polydione B, (+)-catechin and (+)-afzeliecin. A preliminary account of structure-sweetness relationships within the polypodogenin glycoside class of intense sweeteners is presented.

RESULTS AND DISCUSSION

As a result of the observation of a lithium-catalysed molecular ion occurring at m/z 745 in its low-resolution FABMS, the M+ of polypodioside B (3) was determined as 738. The molecular formula of this isolate was confirmed as C36H44O13 by high-resolution FABMS. The 13C NMR chemical shifts of this compound were assigned with the assistance of a 1H-13C heteronuclear chemical shift correlated (HETCOR) NMR experiment, and, after comparison with the 13C NMR spectrum of polypodioside A (1) obtained in our earlier investigation [1], it was apparent that polypodioside B (3) was also a glycoside of polypodogenin (2), that varied structurally from I only its saccharide moieties, and that these sugar units were also affixed to the C-3 and C-26 positions.

The sugar units obtained after acid hydrolysis of compound 3 were identified by GC-MS and TLC as D-glucose and L-rhamnose. The linkages of the saccharide units to the aglycone unit of 3 were conveniently established using the selective INEPT NMR technique [1, 5, 8, 9]. The signals appearing at δ 5.05 and 5.64, which in turn were found to correspond to carbon signals appearing at δ 102.19 and 101.87, were assignable, respectively, to the anomeric protons of the glucose and rhamnose units of polypodioside B (3). Thus, when the proton at δ 5.05 was irradiated (JCH = 6 Hz), only C-3 (δ 76.89) in the aglycone moiety was enhanced, although an enhancement was also observed for C-3’ (δ 78.44) of the glucose unit. Similarly, when the C-1’ proton of the rhamnose unit of 3 was irradiated at δ 5.64 (JCH = 6 Hz), C-2’ (δ 107.25) of the aglycone was enhanced, as were C-2” (δ 77.20), C-3” (δ 72.04), and C-5” (δ 70.36) of the rhamnose unit. In this manner, it was established that the glucose and L-rhamnose units were affixed, respectively, to positions C-3 and C-26 in the molecule of 3. The configurations of the anomeric protons of the sugar units of polypodioside B (3) were determined as β-D and α-L, by comparison of the 13C NMR spectrum of this isolate with analogues data for sugars of known configurations [10, 11]. Therefore, polypodioside B (3) was assigned the structure, 26-O-α-L-rhamnopyranosyl-polypodogenin-3-O-β-D-glucopyranoside.

The M+ of polypodioside C (4) was proposed as 752, as protonated and sodium-cationized molecular ions were...
apparent at m/z 753 and 775, in its low-resolution FABMS; and the elemental formula of this compound was confirmed as C_{30}H_{42}O_{12} by high-resolution FABMS. After the $^1$H and $^{13}$C NMR spectra of 4 were correlated by a $^1$H-$^{13}$C HETCOR experiment, it was apparent that this compound was again based on the aglycone, polydopodigenin (2), and differed from 3 in only one of its saccharide moieties.

Enzymatic hydrolysis of compound 4 with β-glucosidase afforded compound 3 and D-glucose, with this sugar being identified by GC-MS and TLC. The D-glucose unit was determined as being attached to C-3 in 4, as a result of the observation of an upfield shift of δ 76.83 in the C-3 resonance in the $^{13}$C NMR spectrum of 4 to δ 70.67 in that of 5. This was confirmed by a selective INEPT experiment, in which irradiation of the anomeric proton at C-1' (δ 5.07, J_{C1'CH} = 6 Hz) of 4 resulted in the enhancement of C-3 (δ76.83). Therefore, polydopodigenin C (4) was identical to polydopodigenin B (3) in terms of the sugar unit attached to C-3.

That the other sugar unit of polydopodigenin C (4) was attached to the C-26 position was confirmed by a selective INEPT NMR experiment. When the anomeric proton at C-1" was irradiated (δ 5.65, J_{C26CH} = 6 Hz), carbon C-26 was the only carbon on the aglycone of 4 to be enhanced. In the $^1$H NMR spectrum of compound 4, a methoxy group signal appeared at δ 3.56, and the position of this functionality was investigated by a further selective INEPT experiment. Irradiation of the methoxy group at δ 3.56 (J_{C26CH} = 8 Hz) led to the enhancement of C-3" (δ82.62) on the C-26-attached saccharide unit, and showed unambiguously that the methoxy group was not directly attached to the aglycone of 4, as in the case of the known compound 26-O-methylpolydopodogenin [2]. The methoxy group of this sugar unit was confirmed as occurring at the 3"-position, since the $^{13}$C NMR data observed for this sugar unit closely correlated with those obtained for the L-acrofriose (3-O-L-rhamnose) unit of the compound, digoxigenin α-L-acrofriose [12]. The identity of this sugar was confirmed after hydrolysis of compound 5 using methanolic HCl and direct comparison with an authentic sample of methyl L-acrofriose. Acrofriose is of apparently limited distribution in the plant kingdom [13]. The configuration of this sugar unit at C-26 was assigned as α on the basis of $^{13}$C NMR chemical shift comparison of the respective anomeric carbons of compounds 1 [1] and 3. The structure of compound 4 was therefore assigned as 26-O-α-L-acrofriopyranosyl-polydopodigenin-3-O-β-D-glucopyranoside.

Three nonglycosidic isolates were also obtained in this investigation. The ecdyasterol, polydopine B, was identified by interpretation of its spectral characteristics, and confirmed direct by comparison with the authentic substance. Also isolated were the flavonoids, (+)-catechin and (+)-afzelatin, which were both identified by comparison with authentic standards. Herout and coworkers obtained polydopine B and (+)-catechin as constituents of another species in the genus Polydorum, namely, P. vulgare [14, 15].

In the present investigation, polydoposides B (3) and C (4) were obtained as minor polydopodigenin glycoside constituents of P. alcyrenitza rhizomes. During the fractionation procedure that led to the isolation of polydoposide A (1) as the major intensely sweet principle of this plant part, it was found that polydoposide B (3) was slightly sweet, while the corresponding 3"-O-methyl derivative, polydopodigenin C (4) was devoid of this effect. Although a detailed sensory evaluation of polydopodogenin B (3) was not carried out, it was apparent that it was less intensely sweet than polydopodigenin A (1). It may be pointed out that the known monodesmosidic polydopogenin analogue, polydoposapin (6), was not associated with the sweetness of its plant of origin, P. vulgare, since this taste sensation was attributed entirely to the steroidal saponin, osaladin, a compound based on a different aglycone to polydoposides A and B [1-4, 15]. Therefore, these observations indicate that sweet polydopogenin glycosides must be bidesmosidic to exhibit a sweet taste, with saccharide substitution occurring at the C-3 and C-26 positions. Any alteration in either of these sugar units apparently profoundly affects sweetness.

**EXPERIMENTAL.**

Mps uncorr. UV: EtOH; IR: KBr disc. $^1$H and $^{13}$C NMR spectra were recorded on 300 or 360 MHz instruments with TMS as int. std. EIMS (70 eV) data were measured with a direct probe.

**Plant material.** The rhizomes of Polydorum alcyrenitza D.C. Eaton (Polydoraceae) were collected in south Oregon in the autumn of 1983. Specimens documenting these collections have been deposited in the herbarium of the Field Museum of Natural History, Chicago, Illinois.

**Extraction and isolation procedure.** The extraction of the air-dried, milled plant material with 80% MeOH-H$_2$O, partition of the residue on drying between n-BuOH and H$_2$O, and CC of the n-BuOH extract over silica gel, have been described previously [1]. A portion (1.38 g) of a fraction eluted by CHCl$_3$-MeOH (7:1) was further purified by gel filtration chromatography over Sephadex LH-20 (100 g) using MeOH as eluent. An initial fraction (0.97 g) was purified by low-pressure CC (silica gel, 100 g, 230-400 mesh), eluent CHCl$_3$-MeOH-H$_2$O (13:7:2, lower layer) to afford polydopodogenin B (3, 280 mg, 0.055% w/w), while a second fraction (0.34 g) yielded (+)-catechin (15 mg, 0.0038% w/w) and (+)-afzelatin (5 mg, 0.0010% w/w) after prep. TLC using CHCl$_3$-MeOH-H$_2$O (13:7:2, lower layer) as developing solvent. A portion (1.53 g) of a fraction eluted by CHCl$_3$-MeOH (8:1) after gel titration chromatography was
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Further purified by low-pressure CC (silica gel, 300 g, 230–400 mesh, eluent, CHCl₃–MeOH–H₂O (6:3:1, lower layer), and reversed-phase CC. (RP–8, Merck, size 6, gradient elution from H₂O to H₂O–MeOH mixture) to provide polypodioside C (4, 12.5 mg, 0.06% w/w) and polypodioside D (3, 300 mg, 0.02% w/w).

Polypodioside C (3). Colourless needle-shaped crystals from MeOH; mp 207–209°; [α]D = 270° (MeOH, c 0.1); UV λmax nm: 244 (log e 4.08); IR, νmax cm⁻¹: 240, 1666, 1382, 1142, 1094, 1062, 1031, 984; 1H NMR (360 MHz, pyridine-δ₆): δ 5.85 (1H, br, s, H-7), 5.64 (1H, br, s, H-1), 5.05 (1H, d, J = 7.6 Hz, H-6), 4.47 (1H, d, J = 6 Hz, H-6), 3.46 (1H, m, H-22), 1.68 (1H, d, J = 6 Hz, H-6), 1.02 (3H, d, J = 6 Hz, H-21), 0.91 (3H, d, J = 6 Hz, H-21), 0.74 (31H, s, H-11), 0.49 (31H, s, H-18); 13C NMR (90.8 MHz, CDCl₃): δ 199.58 (s, C-6), 163.33 (s, C-8), 123.11 (d, C-7), 106.99 (d, C-26), 99.59 (d, C-11), 81.21 (d, C-3), 78.00 (d, C-22), 71.60 (d, C-4), 70.67 (d, C-3), 68.15 (d, C-3), 66.83 (d, C-2'), 56.95 (q, 3-O-Me), 55.23 (d, C-14), 53.32 (d, C-5), 52.73 (d, C-20), 50.03 (d, C-9), 44.70 (t, C-13), 40.12 (d, C-20), 38.80 (t, C-12), 38.19 (t, C-10), 36.82 (t, C-1), 35.87 (d, C-25), 31.20 (t, C-24), 30.40 (t, C-22), 26.89 (t, C-16), 23.53 (t, C-23), 22.62 (t, C-15), 21.78 (t, C-11), 17.27 (q, C-6'), 16.47 (q, C-27), 13.62 (q, C-21), 13.20 (q, C-19), 12.20 (q, C-18); EIMS, 70 eV, m/z (rel. int.): 590 (M⁺) (1), 430 [aglycone] (34), 413 [aglycone-H₂O] (70); HRMS, mass measurement, found, 590.3811, calcd. for C₃₈H₄₀O₈, 590.3819. Compound 5 (3 mg) was dissolved in 1 M MeOH–HCl soln (3 ml), and hydrolysed for 4 hr at 100°. The reaction mixture was neutralized with Ag₂CO₃, and partitioned between CHCl₃ and H₂O. By GC-MS and TLC, the sugar fraction (1 mg) was found to contain only methyl α-L-acetoside.

Polypodiosine B (2) was recrystallized from MeOH as colourless needles, mp undepressed on mixing with polypodiosine B isolated from P. vulgare. This compound exhibited closely comparable physical and UV, IR, 1H NMR, 13C NMR and EIMS data to those published previously [14, 16, 17]. (+)-Catechin was identified by comparison of its physical and spectral characteristics with those published data [18, 19], and confirmed by direct comparison with an authentic sample purchased from Aldrich Chemical Co., Milwaukee, Wisconsin.

(+)-Afzelechin was identified by comparison of its physical and spectral data with published values [20, 21], and confirmed by direct comparison with an authentic sample isolated from Saxifraga ligulata Wall.

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