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ISOLATION AND STRUCTURES OF TWO NEW p-HYDROXYSTYRENE GLYCOSIDES, PTELATOSIDE-A AND PTELATOSIDE-B FROM BRACKEN, PTERIDIUM AQUILINUM VAR. LATIUSCULUM, AND SYNTHESIS OF PTELATOSIDE-A

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Two new p-hydroxystyrene glycosides, ptelatoside-A and ptelatoside-B were isolated from the carcinogenic fraction of aqueous extracts of bracken, Pteridium aquilinum var. latiusculum, and their structures were established to be p-β-primeverosyloxystyrene and p-β-neohesperidosyloxystyrene, respectively by chemical and spectral means. A synthesis of ptelatoside-A was achieved.

The edible plant, bracken (Pteridium aquilinum var. latiusculum: Warabi in Japanese) has been known to show carcinogenicity to various experimental animals.[1] Recently we have examined the constituents of the aqueous extracts of this plant, isolated a novel norsesquiterpene glucoside, ptaquiloside (9) from the fraction exhibiting strong carcinogenicity, and revealed the carcinogenic property of ptaquiloside (9).[2] In order to examine whether or not this carcinogenic fraction contains another type of carcinogen(s), we have performed further scrutiny of this same fraction, resulting in the isolation of two new p-hydroxystyrene glycosides, ptelatoside-A (1) and ptelatoside-B (2). Herein we wish to describe the structural elucidation of these two new p-hydroxystyrene glycosides, 1 and 2, and the unambiguous synthesis of ptelatoside-A (1).

The dried powdered bracken (3 kg) was extracted with boiling water (3 x 30 l, 10 min each). The combined aqueous extracts were concentrated and treated with the resin Amberlite XAD-2. The portion adsorbed on the resin was eluted with methanol and repeatedly partitioned (n-BuOH - H_2O). The n-BuOH fraction (ca. 0.3 %) exhibiting strong carcinogenicity to rats was separated by chromatography on silica gel [$CHCl_3$ - MeOH (4:1)] and then alumina [MeOH - H_2O (4:1)] to give a mixture of glycosides. Further purification by preparative HPLC[3] afforded two new glycosides, ptelatoside-A (1) (120 mg, 0.004%) and ptelatoside-B (2) (90 mg, 0.003%), respectively.

Ptelatoside-A (1): $C_{19}H_{26}O_{10}$;[4] mp 183-185 °C (H_2O - acetone); $[\alpha]_D^{22}$ -104° (c 0.68, H_2O); UV (MeOH) λ_{max} (ε) 254 nm (19 500), 288 (shoulder) (1 700), 299 (shoulder) (1 000); IR (KBr) 3410, 1628, 1606, 1511 cm^{-1}; ^1H NMR (CD_3OD, 90 MHz) δ 4.33 (1H, d, J = 7.0 Hz, H-1"), 5.11 (1H, dd, J = 10.8, 1.1 Hz, H-8), 5.64 (1H, dd, J = 17.6, 1.1 Hz, H-8), 6.68 (1H, dd, J = 17.6, 10.8 Hz, H-7), 7.07 and 7.37 (total 4H, AA'BB' system, aromatic protons); ^{13}C NMR (Table 1).

Ptelatoside-B (2): $C_{20}H_{28}O_{10}$;[4] amorphous powder; $[\alpha]_D^{23}$ -94.8° (c 1.00, H_2O); UV (MeOH) λ_{max} (ε) 254 nm (17 800), 288 (shoulder) (1 900), 299 (shoulder) (1 100); IR (KBr) 3430, 1628, 1605, 1512 cm^{-1}; ^1H NMR (CD_3OD, 90 MHz) δ 1.29 (3H, d, J = 6.2 Hz, H-6"), 5.01 (1H, d, J = 7.0 Hz, H-1'), 5.11 (1H, dd, J = 10.8, 1.1 Hz, H-8), 5.28 (1H, d, J = 1.5 Hz, H-1"), 5.62 (1H, dd, J = 17.6, 1.1 Hz, H-8), 6.67 (1H, dd, J = 17.6, 10.8 Hz, H-7), 7.00 and 7.35 (total 4H, AA'BB' system, aromatic protons); ^{13}C NMR (Table 1).

In the ^1H NMR spectrum of 1, the signals at δ_H 7.37, 7.07, 6.68, 5.64, and 5.11 strongly suggested the presence of p-O-substituted styrene moiety in 1, which was further supported by the ^{13}C NMR spectrum (δ_C 157.0, 117.7, 128.4, 133.5, 136.7, and 114.0) and the UV spectrum of 1. On acidic methanolysis [H_2SO_4 - MeOH (1:200), reflux, 3.5 h], 1 gave a mixture of methyl glycosides of D-xylose[5a] and D-glucose[5b] together with a phenol 5[6] [mp 98-100.5 °C (Et_2O - hexane)], a methanol adduct of the aglycone, p-hydroxystyrene. Acetylation of 1 (Ac_2O - Py, room temp, 14 h) gave the corresponding hexaacetate 3[7] [mp 97.5-100 °C (MeOH), $[\alpha]_D^{20}$ -54.4° (c 1.0, $CHCl_3$)]. In the ^{13}C NMR spectrum of 1, the signals at δ_C 69.1, 100.9, and 104.1 suggested the sugar moiety of 1 to be represented as β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl (β-primeverosyl), which was further supported by the detailed analysis of ^1H NMR spectrum of 3. Consequently ptelatoside-A was determined to be p-β-primeverosyloxystyrene (1).[8] The structure of 1 was confirmed by the unambiguous synthesis described below.

Comparison of the ^1H NMR and ^{13}C NMR spectra of 2 with those of 1 revealed the presence of the same aglycone, p-hydroxystyrene in 2 as in 1. On acidic methanolysis [H_2SO_4 - MeOH (1:200), reflux, 3.5 h], 2 gave a mixture of methyl glycosides of D-glucose[5b] and L-rhamnose[5c] together with the phenol 5.[6] Acetylation of 2 (Ac_2O - Py, room temp, 14 h) gave the corresponding hexaacetate 4[9] [amorphous powder, $[\alpha]_D^{24}$ -29.4° (c 0.47, $CHCl_3$)]. The signals at δ_C 61.4, 76.8, 99.2, and 102.1 in the ^{13}C NMR spectrum of 2 and the signals at δ_H 3.87, 4.60, and 5.43 in the ^1H NMR spectrum of 4 suggested the sugar moiety to be represented as α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl (β-neohesperidosyl). Ptelatoside-B was thus determined to be p-β-neohesperidosyloxystyrene (2).

In order to confirm the structure of the glycoside 1 unambiguously and secure a large amount of 1 for examining carcinogenicity of 1, the synthesis of 1 was attempted and executed as follows. p-Ethylphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6)[10] was converted to p-ethylphenyl 2,3,4-tri-O-acetyl-β-D-glucopyranoside (7)[11] [mp 138-139 °C (EtOH - hexane), $[\alpha]_D^{22}$ -13.2° (c 1.35, $CHCl_3$)] in 65% overall yield by the sequence: (i) methanolysis (NaOMe - MeOH, room temp, 1 h); (ii) tritylation [$(Ph)_3CCl$ - Py, 100 °C, 12 h]; (iii) acetylation (Ac_2O - Py, room temp, 3 h); (iv) detritylation (AcOH - H_2O, 100 °C, 50 min). Condensation of 7 with α-acetobromo-D-xylose [$Hg(CN)_2$ - $HgBr_2$, MeCN, room temp, 1 h][12] gave the desired disaccharide 8[11] [mp 84.5-87 °C (EtOH - hexane), $[\alpha]_D^{23}$ -46.4° (c 1.0, $CHCl_3$)] in 86% yield. Photobromination[13] of 8 (hν, Br_2 - $NaHCO_3$, $CHCl_3$, room temp, 30 min) followed by dehydrobromination[13] (AcONa, AcOH - Ac_2O, reflux, 40 h) gave the hexaacetate 3[11] [mp 97.5-100 °C (MeOH)] in 73% yield, which was identical to the hexaacetate 3 derived from natural 1 in all respects (mp, mmp,

$[\alpha]_D$, ^1H NMR, and chromatographic properties). Finally methanolysis of 3 (NaOMe - MeOH, room temp, 1.5 h) afforded p-β-primeverosyloxystyrene (1) [mp 182-184 °C (H_2O - acetone)] in 88% yield. Spectral properties (^1H and ^{13}C NMR, IR, and UV) and physical properties (mp, mmp, and $[\alpha]_D$) of synthetic 1 were identical to those of natural 1.

So far, reports on the natural occurrence of p-hydroxystyrene and its derivatives are quite few: whereas p-hydroxystyrene itself was isolated from Papaver somniferum L.[14] in 1945, isolation of its β-D-glucoside from Cheilanthes kuhnii[15] was described rather recently in 1980. Isolation of p-hydroxystyrene glycosides (1 and 2)[16] from the carcinogenic fraction of the bracken extracts is significant, because styrene and styrene oxide are known to be the mutagens in Salmonella typhimurium.[17] Carcinogenicity of ptelatoside-A (1) to rats is currently under investigation.

We are grateful to Dr. K. Matsushita, JEOL Ltd., for measurements of the ^1H NMR (270 MHz) spectra.

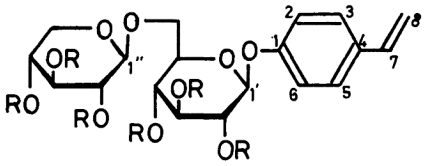
Table 1. ^{13}C NMR Spectral Data[a)]

No	1	2
1	157.0 (s)	156.8 (s)
2	117.7 (d)	117.2 (d)
3	128.4 (d)	128.5 (d)
4	133.5 (s)	133.3 (s)
5	128.4 (d)	128.5 (d)
6	117.7 (d)	117.2 (d)
7	136.7 (d)	136.7 (d)
8	114.0 (t)	114.0 (t)
1'	100.9 (d) (163)*	99.2 (d) (164)*
2'	73.7 (d)[b)]	76.8 (d)[c)]
3'	76.4 (d)[b)]	80.0 (d)[c)]
4'	70.1 (d)[d)]	70.1 (d)[d)]
5'	76.1 (d)[b)]	77.3 (d)[c)]
6'	69.1 (t)	61.4 (t)
1"	104.1 (d) (158)*	102.1 (d) (173)*
2"	73.7 (d)[b)]	70.4 (d)[d)]
3"	76.1 (d)[b)]	71.1 (d)[d)]
4"	70.1 (d)	72.8 (d)
5"	65.9 (t)	69.8 (d)
6"	-	17.6 (q)

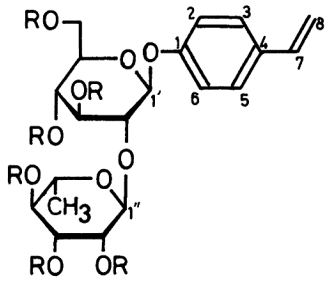
a) Spectra were taken at 22.5 MHz in D_2O. Chemical shifts were relative to TMS: δ(TMS) = δ(dioxane) - 67.4.

b,c,d) Values bearing the same superscript may be interchanged.

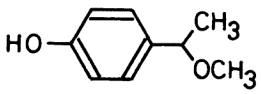
* This value is $^1J_{C-H}$ (Hz).



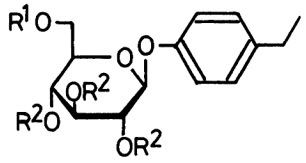
1 R = H (Ptelatoside-A)
3 R = Ac



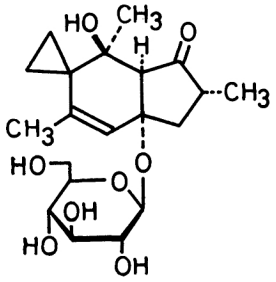
2 R = H (Ptelatoside-B)
4 R = Ac



5



6 R^1 = R^2 = Ac
7 R^1 = H, R^2 = Ac
8 R^1 = 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl
R^2 = Ac



9 (Ptaquiloside)

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2) H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, I. Hirono, and K. Matsushita, Tetrahedron Lett., 24, 4117 (1983); H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, S. Ohba, Y. Saito, I. Hirono, and K. Matsushita, Tetrahedron Lett., 24, 5371 (1983); I. Hirono, K. Yamada, H. Niwa, Y. Shizuri, M. Ojika, S. Hosaka, T. Yamaji, K. Wakamatsu, H. Kigoshi, K. Niiyama, and Y. Uosaki, Cancer Lett., in press.
3) A Column of 22 mm x 30 cm of Fuji Gel ODS-Q3; H_2O - EtOH (80:20), flow rate 2 ml/min.
4) The molecular formulas of these glycosides, 1 and 2 were determined based on the molecular ion peaks in SIMS [1, m/z 437 $(M + Na)^+$; 2, m/z 451 $(M + Na)^+$] and ^{13}C NMR spectra, and on the consideration of their components (the aglycone derivative 5 and sugar components).
5) Identified as: a) methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside (mp 82-83 °C); b) methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (mp 65-66 °C); c) methyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside (mp 87-88 °C).
6) 5: ^1H NMR ($CDCl_3$, 90 MHz) δ 1.42 (3H, d, J = 6.5 Hz), 3.20 (3H, s), 4.24 (1H, q, J = 6.5 Hz), 4.85 (1H, s), and 6.80-7.19 (total 4H, AA'BB' system); MS m/z 152 (M^+), 137, and 120; IR ($CHCl_3$) 3620, 3300, 1613, 1599, 1515 cm^{-1}. cf. F. Bohlmann, U. Fritz, and R. M. King, Phytochemistry, 18, 1403 (1979).
7) 3: ^1H NMR (C_6D_6, 270 MHz) δ 1.56, 1.62, 1.67, 1.71 (3H each, s each, 4 x Ac), 1.73 (6H, s, 2 x Ac), 2.87 (1H, dd, J = 11.7, 9.0 Hz, H-5"), 3.48 (2H, m, H-5', H-6'), 3.80 (1H, m, H-6'), 3.85 (1H, dd, J = 11.7, 5.1 Hz, H-5"), 4.36 (1H, d, J = 6.9 Hz, H-1"), 4.87 (1H, d, J = 7.6 Hz, H-1'), 5.04 (2H, m, H-4', H-4"), 5.09 (1H, dd, J = 10.9, 1.0 Hz, H-8), 5.24 (1H, dd, J = 8.9, 6.9 Hz, H-2"), 5.32 (1H, dd, J = 8.9, 8.2 Hz, H-3"), 5.41 (1H, dd, J = 9.6, 9.2 Hz, H-3'), 5.54 (1H, dd, J = 9.6, 7.6 Hz, H-2'), 5.68 (1H, dd, J = 17.5, 1.0 Hz, H-8), 6.67 (1H, dd, J = 17.5, 10.9 Hz, H-7), 7.00 and 7.40 (total 4H, AA'BB' system, aromatic H).
8) Recently, miyaginin was isolated from Lespedeza thunbergii, the assigned planar structure of which was the same one as that of 1: M. Kanao and H. Matsuda, Yakugaku Zasshi, 98, 366 (1978). However, miyaginin was found to be different from ptelatoside-A (1) by direct comparison of spectral and physical data, and miyaginin was shown to possess a different aglycone from that of 1 (unpublished result).
9) 4: ^1H NMR (C_6D_6, 270 MHz) δ 1.36 (3H, d, J = 6.3 Hz, H-6"), 1.58, 1.63, 1.65, 1.70, 1.72, 2.08 (3H each, s each, 6 x Ac), 3.27 (1H, ddd, J = 9.9, 5.1, 2.5 Hz, H-5'), 3.87 (1H, dd, J = 9.6, 7.9 Hz, H-2'), 3.98 (1H, dd, J = 12.4, 2.5 Hz, H-6'), 4.24 (1H, dd, J = 12.4, 5.1 Hz, H-6'), 4.45 (1H, dq, J = 9.7, 6.3 Hz, H-5"), 4.60 (1H, d, J = 7.9 Hz, H-1'), 5.08 (1H, dd, J = 10.9, 1.0 Hz, H-8), 5.14 (1H, dd, J = 9.9, 9.6 Hz, H-4'), 5.22 (1H, d, J = 1.7 Hz, H-1"), 5.43 (1H, dd, J = 3.3, 1.7 Hz, H-2"), 5.43 (1H, t, J = 9.6 Hz, H-3'), 5.55 (1H, dd, J = 17.5, 1.0 Hz, H-8), 5.56 (1H, dd, J = 10.2, 9.7 Hz, H-4"), 5.73 (1H, dd, J = 10.2, 3.3 Hz, H-3"), 6.59 (1H, dd, J = 17.5, 10.9 Hz, H-7), 7.00 and 7.23 (total 4H, AA'BB' system, aromatic H).
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16) During our investigation on these glycosides, 1 and 2, isolation of two p-hydroxystyrene glycosides similar to 1 and 2 from Dicranopteris dichotoma and Microlepia obtusiloba has been reported: T. Kuraishi, Y. Mitadera, T. Murakami, N. Tanaka, Y. Saiki, and C.-M. Chen, Yakugaku Zasshi, 103, 679 (1983).
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