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STUDIES ON *PTERIDIUM AQUILINUM* VAR. *LATIUSCULUM*, IV.[1] ISOLATION OF THREE *p*-HYDROXYSTYRENE GLYCOSIDES AND AN EFFICIENT METHOD FOR THE ISOLATION OF PTAQUILOSIDE, AN UNSTABLE BRACKEN CARCINOGEN

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Bracken, *Pteridium aquilinum* Kuhn var. *latiusculum* Underw. (Pteridaceae), has been shown to be carcinogenic to various experimental animals (1-3). Recently we have performed fractionation of the aqueous extract of bracken by means of the assay based on carcinogenicity and isolated a new, unstable, norsesquiterpene glucoside, ptaquiloside (**1**) from the carcinogenic fraction (4,5). Then, the carcinogenicity of ptaquiloside (**1**) has been proved (6). Subsequent examination of this carcinogenic fraction led to the isolation of two *p*-hydroxystyrene glycosides, ptelatoside-A (**2**) and ptelatoside-B (**3**) (7). The original procedure for isolating ptaquiloside (**1**) was, however, inefficient and complicated and led to only ca. 0.02% yield based on the dry weight of bracken (4-6).

We report here the development of an efficient and convenient method for the isolation of the bracken carcinogen (**1**) in a yield five times greater than the original procedure (4-6). In the course of the isolation of **1** by the new procedure, three *p*-hydroxystyrene glycosides, *p*-hydroxystyrene β-D-glucoside (**4**) (8), Glycoside A (**5**) (9), and a new compound ptelatoside-C (**6**) were isolated in addition to ptelatoside-A (**2**) and ptelatoside-B (**3**).

In our new procedure, the dried, powdered bracken was extracted with H_2O at room temperature and the aqueous extract was treated with the resin Amberlite XAD-2. The resin adsorbate was eluted with MeOH. The aqueous solution of the MeOH eluate residue was extracted with *n*-BuOH repeatedly and the combined *n*-BuOH extracts were chromatographed on silica gel with EtOAc, EtOAc-MeOH (92:8), and MeOH, successively. Separation of the EtOAc-MeOH (92:8) eluate by reverse-phase column chromatography followed by reverse-phase hplc afforded ptaquiloside (**1**). The fractions eluted with MeOH were separated by chromatography first on alumina and then on silica gel and subsequently by reverse-phase hplc to give Glycoside A (**5**) and ptelatoside-C (**6**) together with ptelatoside-A (**2**) and ptelatoside-B (**3**). *p*-Hydroxystyrene β-D-glucoside (**4**) was obtained by chromatography of the *n*-BuOH extracts (see above) on silica gel with $CHCl_3$-MeOH (9:1) and subsequent reverse-phase hplc.

The molecular formula $C_{19}H_{26}O_{10}$ of ptelatoside-C (**6**) was determined from the molecular ion peak in fabms and the ^{13}C-nmr spectrum, and by consideration of the components [the aglycone derivative (**8**) and two sugar components]. The presence of the *p*-*O*-substituted styrene moiety in **6** was deduced by the spectral data: ^1H nmr δ 7.06 and 7.38 (4H, m, AA'BB' type); ^{13}C nmr δ 157.1 (s), 133.4 (s), 128.5 (d, 2×C), 117.8 (d, 2×C); uv (see Experimental section). Acidic methanolysis of ptelatoside-C (**6**) gave a mixture of methyl glycosides of D-glucose and L-arabinose together with the phenol (**8**) (7), a MeOH adduct of the aglycone, *p*-hydroxystyrene. Acetylation of **6** gave the corresponding hexaacetate (**7**). In the ^{13}C-nmr spectrum of **6**, the signals at δ 68.7 (t, C-6'), 100.9 (d, $^1J_{CH}$ = 164

[1]The article by Ojika *et al.* (7) can be considered as Part III of the series.

Hz, C-1′), and 104.0 (d, $^1J_{CH}$=164 Hz, C-1″) revealed the sugar moiety of **6** to be represented as α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl, which was further supported by the detailed analysis of the ^{1}H-nmr spectrum of the hexaacetate (**7**). Thus, the structure of ptelatoside-C was determined to be **6**.

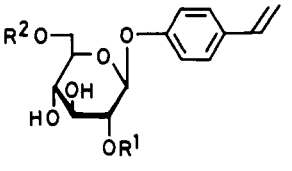
So far, reports on the natural occurrence of *p*-hydroxystyrene and its glycosides are rare. While *p*-hydroxystyrene and its β-D-glucoside were isolated from *Papaver somniferum* (10) in 1945 and *Cheilanthes kuhnii* (8) in 1980, respectively, isolation of four *p*-hydroxystyrene glycosides from *Dicranopteris dichotoma* (9), *Microlepia obtusiloba* (9), and *P. aquilinum* var. *latiusculum* (7) was only recently described.

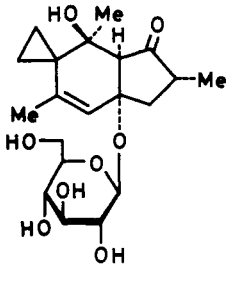
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir and uv spectra were recorded on a JASCO Model IRS spectrophotometer and a JASCO UVIDEC-510 spectrophotometer, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were measured at 90 MHz and 22.5 MHz, respectively, with a JEOL JNM-FX90QE spectrometer. The ^{1}H-nmr spectrum at 270 MHz was obtained on a JEOL JNM-GX270 spectrometer. Mass spectra were recorded on a Hitachi RMU-6C spectrometer and a JEOL JMS-DX300 instrument. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. For column chromatography, Fuji-Davison silica gel BW-820MH, Fuji-Davison 5D ODS (ODS-silica gel), and Merck neutral alumina (Aluminiumoxid 90, Activity II-III) were used. Preparative tlc was conducted on Merck silica gel PF_{254}. Hplc was performed with a JASCO TRI ROTAR-II apparatus using a reverse-phase column of Develosil ODS-5 (250×10 mm id).

PLANT MATERIAL.—Plants of *P. aquilinum* var. *latiusculum* were collected in the Nayoro area of Hokkaido, Japan, in July 1983, and were identified by botanist H. Wakita. A voucher specimen, KY-N-83, has been deposited at the Herbarium of the Laboratory of Organic Chemistry, Department of Chemistry, Nagoya University.

EXTRACTION PROCEDURE AND PREPARATION OF THE *n*-BuOH EXTRACT.—The dried, finely powdered, plant materials (3.0 kg) were stirred in H_2O (30 liters) at room temperature for 2 h and filtered. After the resin Amberlite XAD-2 (wet volume, 12 liters) was added to the filtrate, the mixture was stirred at room temperature for 1 h and filtered. The filtrate was again treated with fresh Amberlite XAD-2 (wet volume, 12 liters). MeOH (30 liters) was added to the combined





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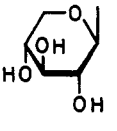
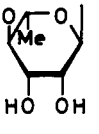
2 R^1=H, R^2=



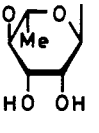
3 R^1=

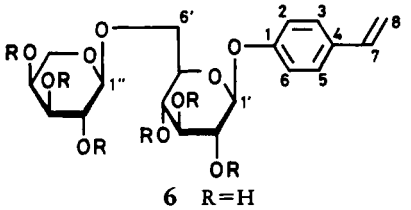


, R^2=H

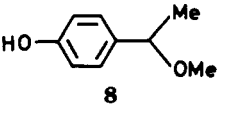
4 R^1=H, R^2=H

5 R^1=H, R^2=





6 R=H
7 R=Ac



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resin XAD-2 and the mixture was stirred for 3 h at room temperature. The mixture was filtered and the resin was washed with MeOH (30 liters). The combined MeOH solution was concentrated under reduced pressure, and the residue (90 g) was dissolved in H_2O saturated with *n*-BuOH (1 liter). The aqueous solution was extracted with *n*-BuOH saturated with H_2O (5×500 ml). Concentration of the combined organic solution under reduced pressure afforded the *n*-BuOH extract as a dark-brown, amorphous solid (33 g). By the procedure described above, another 1.2 g of the *n*-BuOH extract was prepared from 100 g of the dried, finely powdered, plant materials.

ISOLATION OF PTAQUILOSIDE (**1**), GLYCOSIDE A (**5**), AND PTELATOSIDE-C (**6**).—The *n*-BuOH extract (33 g) (see above) was chromatographed on silica gel (600 g) with EtOAc, EtOAc-MeOH (92:8), and MeOH, successively. Concentration of the fractions eluted with EtOAc-MeOH (92:8) under reduced pressure gave a residue (9 g), which was chromatographed on ODS-silica gel (180 g) with H_2O-MeOH (60:40). The resulting crude **1** was further purified by hplc with H_2O-MeOH (50:50) and freeze-dried to give pure **1** (3 g, 0.1%) as a colorless amorphous powder. Concentration of the fractions eluted with MeOH under reduced pressure afforded a residue (18 g), which was chromatographed on alumina (300 g) with MeOH and MeOH-H_2O (9:1, 8:2, 7:3). Evaporation of the fractions eluted with MeOH-H_2O (9:1, 8:2, 7:3) under reduced pressure yielded a residue (2.4 g), which was chromatographed over silica gel (70 g) with $CHCl_3$-MeOH (4:1). Further separation by hplc with EtOH-H_2O (25:75) afforded Glycoside A (**5**) (28 mg, 0.0009%, amorphous powder), $[\alpha]^{22}D$ −66.5° (c 1.00, H_2O), Lit. $[\alpha]^{20}D$ −65.5° (c 2.55, MeOH) (9), and ptelatoside-C (**6**) (62 mg, 0.002%, amorphous powder) together with ptelatoside-A (**2**) (135 mg, 0.005%) (7) and ptelatoside-B (**3**) (200 mg, 0.007%) (7). Glycoside A (**5**) was identified by comparison (uv, ^1H nmr, ^{13}C nmr) with an authentic sample. Ptelatoside-C (**6**): $[\alpha]^{22}D$ −67.3° (c 0.797, H_2O); uv λ max (MeOH) 254 (ε 15800), 288 (shoulder, 1800), 298 (shoulder, 1100) nm; ir ν max (KBr) 3420, 1628, 1605, 1510, 1170 cm^{-1}; ^1H nmr (90 MHz, CD_3OD) δ 5.11 (1H, dd, J=11.0, 1.1 Hz, H-8), 5.64 (1H, dd, J=17.6, 1.1 Hz, H-8), 6.68 (1H, dd, J=17.6, 11.0 Hz, H-7), 7.06 and 7.38 (4H, m, AA'BB' type, H-2, H-3, H-5, H-6); ^{13}C nmr (22.5 MHz, D_2O) δ 66.9 (t, C-5″), 68.7 (t, C-6′), 69.0 (d), 70.2 (d), 71.6 (d), 73.1 (d), 73.7 (d), 76.3 (d), 100.9 (d, $^1J_{CH}$=164 Hz, C-1′), 104.0 (d, $^1J_{CH}$=164 Hz, C-1″), 114.1 (t, C-8), 117.8 (d, C-2, C-6), 128.5 (d, C-3, C-5), 133.4 (s, C-4), 136.7 (d, C-7), 157.1 (s, C-1); fabms *m/z* 437 $(M+Na)^+$.

ISOLATION OF *p*-HYDROXYSTYRENE β-D-GLUCOSIDE (**4**).—The *n*-BuOH extract (1.2 g) (see above) was chromatographed on silica gel (55 g) using $CHCl_3$-MeOH (9:1, 4:1, 3:1). Evaporation of the fractions eluted with $CHCl_3$-MeOH (9:1) afforded a residue (136 mg), which was further separated by hplc with MeOH-H_2O (45:55) to give **4** (28 mg, 0.028%), mp 193-194° (H_2O), $[\alpha]^{17}D$ −62.3° (c 0.278, MeOH). Compound **4** was identified by comparison (uv, ir, and ^1H nmr) with an authentic sample, mp 193-194° (H_2O), $[\alpha]^{11}D$ −69.4° (c 0.274, MeOH), synthesized by the known procedure (11).

METHANOLYSIS OF PTELATOSIDE-C (**6**).—A mixture of **6** (107 mg) in MeOH (6 ml) containing H_2SO_4 (0.09 ml) was refluxed for 4 h and then neutralized by addition of $NaHCO_3$. Concentration of the mixture under reduced pressure afforded a residue, which was diluted with H_2O (10 ml) and extracted with $CHCl_3$ (3×10 ml). Concentration of the aqueous layer under reduced pressure gave a residue (335 mg), which was separated by chromatography first on silica gel (10 g) with EtOAc-MeOH (20:1) and then on silica gel (4 g) with EtOAc-MeOH (40:1) and finally by preparative tlc with EtOAc-MeOH (15:4). There was obtained a mixture of anomeric methyl L-arabinosides (18 mg) and of anomeric methyl D-glucosides (51 mg), the former being identified as methyl 2,3,4-tri-*O*-acetyl-β-L-arabinopyranoside (mp 75.5-76.0°) and the latter as methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (mp 64.5-65.0°) after acetylation and purification [preparative tlc, CCl_4-Me_2CO (5:1)]. Concentration of the $CHCl_3$ extracts under reduced pressure afforded a residue (52 mg), separation of which by chromatography on silica gel (5 g) with $CHCl_3$-EtOAc (10:1) gave the phenol (**8**) (18 mg), mp 102.0-102.5°, which was proved to be identical with the authentic sample by spectral (uv, ir, and ^1H nmr) comparison.

PTELATOSIDE-C HEXAACETATE (**7**).—A mixture of **6** (2 mg) in Ac_2O (0.5 ml) and anhydrous pyridine (0.5 ml) was stirred at room temperature for 14 h and concentrated under reduced pressure. The residue was purified by preparative tlc with $CHCl_3$-EtOAc (2:1) to afford **7** (2.2 mg) as an amorphous powder; ^1H nmr (270 MHz, C_6D_6) δ 1.61 (6H, s, 2×Ac), 1.64, 1.71, 1.72, 1.76 (3H each, s each, 4×Ac), 2.83 (1H, dd, J=12.9, 1.7 Hz, H-5″), 3.50 (2H, m, H-5′, H-6′), 3.62 (1H, dd, J=12.9, 3.0 Hz, H-5″), 3.87 (1H, brd, J=8.9 Hz, H-6′), 4.23 (1H, d, J=7.3 Hz, H-1″), 4.83 (1H, d, J=7.9 Hz, H-1′), 5.06 (2H, m, H-3″, H-4′), 5.08 (1H, dd, J=10.9, 1.0 Hz, H-8), 5.17 (1H, m, H-4″), 5.40 (1H, dd, J=9.9, 9.2 Hz, H-3′), 5.53 (1H, dd, J=9.9, 7.9 Hz, H-2′), 5.58 (1H, dd, J=9.6, 7.3 Hz, H-2″), 5.66 (1H, dd, J=17.5, 1.0 Hz, H-8),

6.65 (1H, dd, J=17.5, 10.9 Hz, H-7), 6.99 and 7.38 (4H, m, AA'BB' type, H-2, H-3, H-5, H-6).

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