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STRUCTURES OF NEW P-HYDROXYSTYRENE GLYCOSIDES, PTELATOSIDE-A AND PTELATOSIDE-B ISOLATED FROM BRACKEN FERN, PTERIDIUM AQUILINUM VAR. LATIUSCULUM, AND SYNTHESIS OF PTELATOSIDE-A

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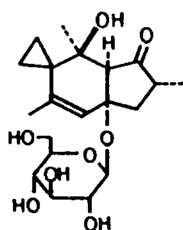
Abstract - Two new p-hydroxystyrene glycosides, ptelatoside-A (2) and ptelatoside-B (3) have been isolated from bracken fern, Pteridium aquilinum var. latiusculum, and their structures have been elucidated by chemical and spectral means. The synthesis of ptelatoside-A (2) has been carried out.

The toxic properties of bracken fern Pteridium aquilinum var. latiusculum to cattles ("cattle bracken poisoning") were first recorded at the end of the 19th century.[1] The carcinogenicity of bracken fern was found in 1960,[1,2] and clearly proved using rats in 1965.[3] Since the discovery of the carcinogenicity to various experimental animals, a number of research groups have made great efforts in search for the carcinogenic compound(s) without success.[1,4] The isolation of the carcinogen from bracken fern has been made difficult by two major factors: difficulty of applying the various short-term bioassay systems and instability of the carcinogen.[2,5]

Overcoming these difficulties, we have examined the constituents of the aqueous extract of this plant by the assay based on the carcinogenicity to rats,[6,7] isolated a new norsesquiterpene glucoside, ptaquiloside (1) from the fraction exhibiting strong carcinogenicity,[6,7] elucidated the structure,[7-9] and revealed that ptaquiloside (1) is the carcinogen of bracken fern[6,10] and also the active principle of cattle bracken poisoning.[11]

For the purpose of investigating whether or not the carcinogenic fraction from the aqueous extract of bracken fern contains another type of carcinogen(s), further scrutiny of this fraction has been made, leading to the isolation of two new p-hydroxystyrene glycosides, ptelatoside-A (2) and ptelatoside-B (3). This paper deals with the structural elucidation of these two p-hydroxystyrene glycosides (2 and 3) and the synthesis of ptelatoside-A (2) in details.[12] In addition to 2 and 3, isolation of three further p-hydroxystyrene glycosides of this type from bracken fern has been made, which was reported elsewhere.[13]



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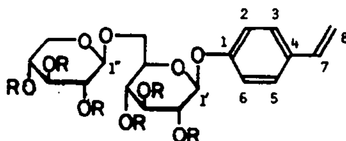
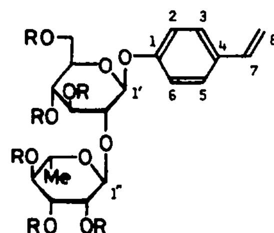


2 R = H
4 R = Ac



3 R = H
5 R = Ac

The carcinogenic fraction was prepared from the aqueous extract of dried powdered bracken fern by the use of two kinds of the resin Amberlite XAD-2 and Toyopearl HW-40(c) and by means of the repetition of the solvent partition (n-BuOH-H_2O).[14] The carcinogenic fraction (0.4%) thus obtained was chromatographed on silica gel: the fraction eluted with 5:1 chloroform-methanol gave ptaquiloside (1) and p-hydroxystyrene β-D-glucoside (6),[15,16] and the fraction eluted with 3:1 chloroform-methanol afforded a mixture of p-hydroxystyrene glycosides, separation of which by preparative HPLC provided ptelatoside-A (2) (0.0052%) and ptelatoside-B (3) (0.0048%), respectively.

Ptelatoside-A (2): $C_{19}H_{26}O_{10}$; mp 183-185 °C (H_2O-acetone); UV (MeOH) 254 (ε 19500), 288 (1700, shoulder), 299 nm (1000, shoulder); IR (KBr) 3410, 1628, 1606, 1511, 900 cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 3.17 (1H, dd, J = 11.5, 10.6 Hz), 3.26 (1H, dd, J = 9.2, 7.4 Hz), 3.36 (1H, t, J = 9.2 Hz), 3.5-3.7 (5H, m), 3.80 (1H, m), 3.90 (1H, dd, J = 11.5, 5.6 Hz), 4.14 (1H, d, J = 10.2 Hz), 4.41 (1H, d, J = 7.6 Hz, anomeric proton H-1"), 5.15 (1H, d, J = 7.3 Hz, anomeric proton H-1'), 5.24 (1H, dd, J = 11.0, 1.0 Hz), 5.76 (1H, dd, J = 17.6, 1.0 Hz), 6.76 (1H, dd, J = 17.6, 11.0 Hz), 7.13 and 7.48 (2H each, AA'BB' system); ^{13}C NMR (Table 1).

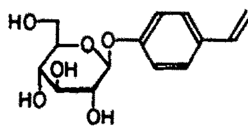
Ptelatoside-B (3): $C_{20}H_{28}O_{10}$; amorphous powder; UV (MeOH) 254 (ε 17800), 288 (1900, shoulder), 299 nm (1100, shoulder); IR (KBr) 3430, 1628, 1605, 1512, 903 cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 1.23 (3H, d, J = 6.3 Hz), 3.43 (1H, t, J = 9.8 Hz), 3.51 (1H, t, J = 8.6 Hz), 3.6-3.8 (5H, m), 3.90 (2H, m), 4.07 (1H, dd, J = 3.3, 1.7 Hz), 5.16 (1H, d, J = 1.7 Hz, anomeric proton H-1"), 5.24 (1H, d, J = 11.2 Hz), 5.31 (1H, d, J = 6.6 Hz, anomeric proton H-1'), 5.75 (1H, d, J = 17.5 Hz), 6.75 (1H, dd, J = 17.5, 11.2 Hz), 7.09 and 7.49 (2H each, AA'BB' system); ^{13}C NMR (Table 1).

The signals at δ_H 7.48, 7.13, 6.76, 5.76, and 5.24 in the ^1H NMR spectrum of 2 suggested the presence of the p-O-substituted styrene moiety in 2, which was 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 2. Methanolysis of 2 under the acidic conditions (1:200 H_2SO_4-MeOH) afforded a mixture of methyl glycosides of D-xylose and D-glucose together with a phenol 7,[17] a methanol adduct of the aglycon, p-hydroxystyrene. Acetylation of 2 gave the corresponding hexaacetate 4. In the ^{13}C NMR spectrum of 2 the signals at δ_C 69.1, 100.9, and 104.1 suggested the sugar part of 2 to be represented as β-D-xylopyranosyl-(1 →6)-β-D-glucopyranosyl (β-primeverosyl), which was further supported by the detailed analysis of the ^1H NMR spectrum of 4. Therefore ptelatoside-A was proved to be p-β-primeverosyloxystyrene (2).[18] The structure of 2 was confirmed by the unambiguous synthesis described below.

Table 1. ^{13}C NMR Spectral Data[a)] (22.5 MHz, D_2O)

C	2	3
1	157.0 (s)	156.8 (s)
2, 6	117.7 (d)	117.2 (d)
3, 5	128.4 (d)	128.5 (d)
4	133.5 (s)	133.3 (s)
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)	80.0 (d)
3'	76.4 (d)[b)]	76.8 (d)[c)]
4'	70.1 (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)	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) 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).



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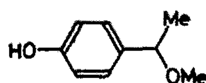

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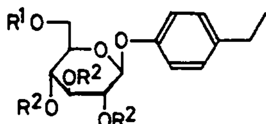
Comparison of the ^1H NMR and ^{13}C NMR spectra of **3** with those of **2** indicated the presence of the same aglycon, p-hydroxystyrene in **3** as in **2**. Acidic methanolysis of **3** (1:200 H_2SO_4-MeOH) yielded a mixture of methyl glycosides of D-glucose and L-rhamnose together with the phenol **7**.[17] Acetylation of **3** produced the corresponding hexaacetate **5**. The signals at δ_C 61.4, 80.0, 99.2, and 102.1 in the ^{13}C NMR spectrum of **3** and the signals at δ_H 3.87, 4.60, and 5.43 in the ^1H NMR spectrum of **5** 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 (**3**).



8	$R^1 = R^2 = Ac$
9	$R^1 = H$, $R^2 = Ac$
10	R^1 = 2,3,4-tri-O-acetyl-β-D-xylopyranosyl $R^2 = Ac$

In order to confirm the structure of ptelatoside-A (**2**) unambiguously and secure a large amount of **2** for examining carcinogenicity of **2**, the synthesis of **2** was carried out. p-Ethylphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**8**)[19] was converted to p-ethylphenyl 2,3,4-tri-O-acetyl-β-D-glucopyranoside (**9**) in 63% overall yield by the following sequence: (i) methanolysis (NaOMe-MeOH); (ii) tritylation (Ph_3CCl-pyridine); (iii) acetylation (Ac_2O-pyridine); (iv) detritylation ($AcOH$-H_2O). Condensation of **9** with α-acetobromo-D-xylose [$Hg(CN)_2$-$HgBr_2$, MeCN][20] provided the disaccharide **10** in 86% yield. Photobromination[16,21] of **10** (hν, Br_2-$NaHCO_3$, $CHCl_3$) and subsequent dehydrobromination[16,21] (AcONa, AcOH-Ac_2O) provided the hexaacetate **4** in 73% yield, which was identical with the hexaacetate **4** derived from natural **2** in all respects (mp, $[\alpha]_D$, UV, IR, ^1H NMR and chromatographic behaviors). Finally, methanolysis of **4** (NaOMe-MeOH) gave p-β-primeverosyloxystyrene (**2**), mp 182-184 °C in 88% yield. The spectral properties (IR, UV, ^1H and ^{13}C NMR) and physical properties (mp and $[\alpha]_D$) of synthetic **2** proved identical with those of natural ptelatoside-A (**2**).

So far, natural occurrence of p-hydroxystyrene and its glycosides is rare: while p-hydroxystyrene itself was isolated from Papaver somniferum L.[22] in 1945, isolation of its β-D-glucoside **6** from Cheilanthes kuhnii[15] and of two related glycosides from Dicranopteris dichotoma and Microlepia obtusiloba[23] was described rather recently.

Since styrene and styrene oxide are known to be the mutagens in a bacterial test system[24,25] and styrene oxide was found to be carcinogenic,[25] ptelatoside-A (**2**), ptelatoside-B (**3**), and p-hydroxystyrene β-D-glucoside (**6**) have been suspected of the carcinogens in bracken fern. Synthetic ptelatoside-A (**2**) was administered orally to rats, but no carcinogenicity was observed concerning this compound **2**.[26] The p-hydroxystyrene glycosides such as **2**, **3**, and **6** would not be responsible for the carcinogenicity of bracken fern.

Experimental

Melting points are uncorrected. UV spectra were measured on a JASCO UVIDEC-510 spectrophotometer. IR spectra were obtained with either a JASCO Model IR-S or a JASCO Model IR-810 spectrophotometer. ^1H NMR spectra were recorded on JEOL FX-90QE (90 MHz) and JEOL GX-270 (270 MHz) instruments: chemical shifts (δ) are reported in ppm downfield from internal TMS in organic solutions and from internal DSS in D_2O solutions; coupling constants are reported in Hz. ^{13}C NMR spectra were recorded on a JEOL FX-90QE (22.5 MHz) spectrometer: chemical shifts (δ) are reported in ppm downfield from internal TMS. The mass spectra were recorded on a JEOL JMS-DX303 instrument. Optical rotations were measured on a JASCO DIP-181 polarimeter. Fuji-Davison silica gel BW-820 MH was used for column chromatography. Merck precoated silica gel $60F_{254}$ plates, 0.25 mm thickness, were used for thin layer chromatography (TLC). HPLC was performed with a TRI ROTAR-II apparatus with refractive index (RI) and UV detectors. Unless otherwise stated, organic solutions were washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure by a rotary evaporator.

Ptelatoside-A (**2**) and Ptelatoside-B (**3**). The plants of P. aquilinum var. latiusculum were collected in July in the Nayoro area of Hokkaido, Japan. From the dried, finely powdered plant materials (3.0 kg)

the carcinogenic fraction (12 g) was obtained as a solid, the preparation of which is described in the preceding paper.[14] Silica gel (4 g) impregnated with the carcinogenic fraction (2 g) was placed on the top of the silica gel (40 g) column. The column was eluted successively with $CHCl_3$-MeOH [5:1 (480 ml), 3:1 (480 ml), 1:1 (120 ml)] and MeOH (120 ml). From the fractions eluted with 5:1 $CHCl_3$-MeOH there was obtained an orange powder (227 mg), column chromatography of which on Develosil ODS 30/60 (300 x 22 mm I.D.) with 4:6 MeOH-H_2O under low pressure (flow rate 9.9 ml/min) gave ptaquiloside (**1**) (68 mg, 0.014%) and p-hydroxystyrene β-D-glucoside (**6**) (9.2 mg, 0.0018%). The latter compound **6**, mp 193-194 °C (H_2O), $[\alpha]_D^{17}$ -62.3° (c 0.278, MeOH) was identified by spectral (UV, IR, and ^1H NMR) comparison with an authentic specimen synthesized by the known procedure.[16] The fractions eluted with 3:1 $CHCl_3$-MeOH were concentrated and freeze-dried to yield an orange powder (190 mg). Separation of the powder (190 mg) by HPLC [column, Develosil ODS-5 (250 x 8 mm I.D.); solvent, 25:75 EtOH-H_2O; flow rate, 1.5 ml/min] afforded the fraction of **2** (retention time 21.5 min) and that of **3** (retention time 30.0 min). Each fraction was concentrated and freeze-dried. **2** (25.9 mg, 0.0052%): the spectral data and mp are described in the text; $[\alpha]_D^{22}$ -104° (c 0.675, H_2O); FABMS m/z 437 $(M + Na)^+$, 415 $(M + H)^+$ (Found: C, 54.09; H, 6.44. $C_{19}H_{26}O_{10} \cdot 1/2\ H_2O$ requires: C, 53.90; H, 6.43%). **3** (24.0 mg, 0.0048%): the spectral data are described in the text; $[\alpha]_D^{23}$ -94.8° (c 1.00, H_2O); FABMS m/z 451 $(M + Na)^+$, 429 $(M + H)^+$ [HRFABMS. Found: 451.1578 $(M + Na)^+$. $C_{20}H_{28}O_{10}Na$ requires: 451.1581].
The other method for the isolation of **2** and **3** from bracken fern was reported.[13]

Acetylation of Ptelatoside-A (2). A mixture of **2** (10 mg), Ac_2O (0.5 ml), and pyridine (0.5 ml) was stirred at room temperature for 12 h and concentrated. The residue was chromatographed on silica gel with 3:1 $CHCl_3$-EtOAc to give **4** (15.4 mg, 96%): mp 165-166 °C (MeOH); $[\alpha]_D^{18}$ -51.7° (c 0.35, $CHCl_3$); UV (MeOH) 254 (ε 22000), 288 (2200, shoulder), 299 nm (1300, shoulder); IR ($CHCl_3$) 1760, 1630, 1606, 1510, 988, 907, 837 cm^{-1}; ^1H NMR (270 MHz, C_6D_6) δ 1.56, 1.62, 1.67, 1.71 (3H each, s), 1.73 (6H, s), 2.87 (1H, dd, J = 11.7, 9.0), 3.48 (2H, m), 3.80 (1H, m), 3.85 (1H, dd, J = 11.7, 5.1), 4.36 (1H, d, J = 6.9), 4.87 (1H, d, J = 7.6), 5.04 (2H, m), 5.09 (1H, dd, J = 10.9, 1.0), 5.24 (1H, dd, J = 8.9, 6.9), 5.32 (1H, dd, J = 8.9, 8.2), 5.41 (1H, dd, J = 9.6, 9.2), 5.54 (1H, dd, J = 9.6, 7.6), 5.68 (1H, dd, J = 17.5, 1.0), 6.67 (1H, dd, J = 17.5, 10.9), 7.00 and 7.40 (2H each, AA'BB' system) (Found: C, 55.54; H, 5.92. $C_{31}H_{38}O_{16}$ requires: C, 55.83; H, 5.75%).

Acetylation of Ptelatoside-B (3). Acetylation of ptelatoside-B (**3**) (10 mg) and subsequent purification were carried out as in the case of ptelatoside-A (**2**) to give **5** (15.7 mg, 99%): amorphous powder; $[\alpha]_D^{18}$ -32.1° (c 1.29, $CHCl_3$); UV (MeOH) 254 (ε 19000), 288 (2000, shoulder), 299 nm (1300, shoulder); IR ($CHCl_3$) 1754, 1630, 1605, 1510, 988, 907, 833 cm^{-1}; ^1H NMR (270 MHz, C_6D_6) δ 1.36 (3H, d, J = 6.3), 1.58, 1.63, 1.65, 1.70, 1.72, 2.08 (3H each, s), 3.27 (1H, ddd, J = 9.9, 5.1, 2.5), 3.87 (1H, dd, J = 9.6, 7.9), 3.98 (1H, dd, J = 12.4, 2.5), 4.24 (1H, dd, J = 12.4, 5.1), 4.45 (1H, dq, J = 9.7, 6.3), 4.60 (1H, d, J = 7.9), 5.08 (1H, dd, J = 10.9, 1.0), 5.14 (1H, dd, J = 9.9, 9.6), 5.22 (1H, d, J = 1.7), 5.43 (1H, dd, J = 3.3, 1.7), 5.43 (1H, t, J = 9.6), 5.55 (1H. dd, J = 17.5, 1.0), 5.56 (1H, dd, J = 10.2, 9.7), 5.73 (1H, dd, J = 10.2, 3.3), 6.59 (1H, dd, J = 17.5, 10.9), 7.00 and 7.23 (2H each, AA'BB' system).

Methanolysis of Ptelatoside-A (2). A solution of **2** (22.4 mg) and conc. H_2SO_4 (20 µl) in MeOH (4 ml) was refluxed for 6 h, cooled to room temperature, neutralized with a saturated $NaHCO_3$ solution, and concentrated. The residue was diluted with H_2O (3 ml) and the mixture was extracted with $CHCl_3$ (3 x 3 ml). The combined organic extracts were dried and concentrated to give an oil (9 mg), separation of which by chromatography on silica gel with 10:1 $CHCl_3$-EtOAc provided the phenol **7** (7.5 mg, 91%, mp 98.0-100.5 °C). The spectral data of **7** were in agreement with those of the literature.[17] The aqueous layer of the reaction mixture obtained after extraction with $CHCl_3$ was concentrated. A mixture of the residue in Ac_2O (2 ml) and pyridine (2 ml) was stirred at room temperature for 14 h and concentrated. The residue was extracted with $CHCl_3$ (5 ml) and the $CHCl_3$ extract was concentrated to give an oil. Separation of the oil by preparative TLC on silica gel (5:1 CCl_4-acetone) afforded methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside [8 mg, 41%, mp 63.5-64.5 °C (ether-hexane), $[\alpha]_D^{24}$ +129° (c 0.24, $CHCl_3$)] and methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside [6 mg, 38%, mp 82-83 °C (ether-hexane), $[\alpha]_D^{24}$ +122° (c 0.26, $CHCl_3$)]. Identification of these two methyl glycoside acetates was carried out by comparison of the spectral data, melting points, and specific rotations with those of the authentic samples.

Methanolysis of Ptelatoside-B (3). Methanolysis of **3** (24.6 mg) and subsequent separation of the reaction products were performed as in the case of ptelatoside-A (**2**) to give the phenol **7** (8 mg, 92%, mp 98.0-100.5 °C) and a mixture of two methyl glycoside acetates. The mixture of two methyl glycoside acetates was chromatographed on silica gel with 92:8 benzene-EtOAc to afford methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside [15 mg, 73%, mp 65-66 °C (ether-hexane), $[\alpha]_D^{21}$ +131° (c 0.77, $CHCl_3$)] and methyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside [15 mg, 87%, mp 87-88 °C (ether-hexane), $[\alpha]_D^{23}$ -55.3° (c 0.76, tetrachloroethane)]. Identification of these two methyl glycoside acetates was made by comparison of the spectral data, melting points, and specific rotations with those of the authentic samples.

p-Ethylphenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranoside (9). To a stirred suspension of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**8**)[19] (21.0 g, 46.5 mmol) in MeOH (150 ml) was added dropwise a 1.0 M solution of NaOMe in MeOH (19 ml) at room temperature under nitrogen. The mixture was stirred at room temperature for 1 h and neutralized with the ion-exchange resin Amberlite IRC-50 (H form, 28 g). The mixture was filtered and the resin was washed with MeOH. The filtrate and the washings were combined and the solution was concentrated to give colorless crystals (ca. 13 g). A solution of the crystals in EtOH (200 ml) was concentrated and the crystalline residue was kept under reduced pressure (3 mmHg) for 1 h. To a solution of the crystalline residue in pyridine (50 ml) was added trityl chloride (14.2 g, 50.9 mmol). The mixture was stirred at 100 °C for 12 h and cooled to room temperature. Acetic anhydride (40 ml) was added to the mixture. The mixture was stirred at room temperature for 3 h, diluted with 1:1 ice-water (100 g), stirred at room temperature for 5 min, and extracted with ether (3 x 150 ml). The combined ethereal extracts were washed with 4 N HCl (120

ml) and saturated NaCl solution (50 ml), dried, and concentrated to give a pale yellow oil, which was dissolved in 8:2 AcOH-H_2O (100 ml). The solution was stirred at 100 °C for 50 min, cooled to room temperature, and left overnight. The resulting crystals were filtered and washed with 75% MeOH (30 ml). The filtrate and the washings were combined and the solution was concentrated to give a residue, which was dissolved in toluene (200 ml). Concentration of the toluene solution provided crystalline **9**, which was recrystallized to give pure **9** (12.1 g, 63%): mp 138-139 °C (hexane-EtOH); $[\alpha]_D^{22}$ -13.2° (c 1.35, $CHCl_3$); IR ($CHCl_3$) 3600, 1762, 1610, 1590, 1512, 1220, 830 cm^{-1}; ^1H NMR (90 MHz, C_6D_6) δ 1.09 (3H, t, J = 7.5), 1.65 (3H, s), 1.74 (6H, s), 2.07 (1H, br t, J = 6.7, OH), 2.43 (2H, q, J = 7.5), 3.1-3.6 (3H, m), 4.92 (1H, d, J = 7.9), 5.1-5.6 (3H, m), 6.97 (4H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 15.7 (q), 20.6 (q, 3 x C), 28.1 (t), 61.4 (t), 68.8 (d), 71.5 (d), 72.9 (d), 74.5 (d), 99.3 (d, $^1J_{C-H}$ = 164), 116.8 (d, 2 x C), 128.9 (d, 2 x C), 139.2 (s), 155.0 (s), 169.3 (s), 169.8 (s), 170.2 (s); MS m/z 410 (M^+), 289, 229, 169 (Found: C, 58.44; H, 6.60. $C_{20}H_{26}O_9$ requires: C, 58.53; H, 6.38%).

p-Ethylphenyl 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**10**). To a stirred mixture of **9** (18.1 g, 44.1 mmol), $Hg(CN)_2$ (11.1 g, 44.1 mmol), and $HgBr_2$ (15.9 g, 44.1 mmol) in MeCN (180 ml) was added α-acetobromo-D-xylose (18.0 g, 53.0 mmol) at room temperature under nitrogen. The mixture was stirred at room temperature for 1 h in the dark and concentrated. Benzene (100 ml) was added to the residue and the mixture was concentrated. To the residue was added $CHCl_3$ (200 ml) and the mixture was filtered. The insoluble materials were washed with $CHCl_3$. The filtrate and the washings were combined and the solution was concentrated. The residue was dissolved in EtOH (100 ml) and the solution was concentrated to give crude crystals, recrystallization of which provided pure **10** (25.2 g, 86%): mp 84.5-87.0 °C (hexane-EtOH); $[\alpha]_D^{23}$ -46.4° (c 1.00, $CHCl_3$); IR ($CHCl_3$) 1760, 1608, 1588, 1512, 1225, 830 cm^{-1}; ^1H NMR (90 MHz, C_6D_6) δ 1.16 (3H, t, J = 7.6), 1.59, 1.65, 1.71, 1.73 (3H each, s), 1.75 (6H, s), 2.53 (2H, q, J = 7.6), 2.90 (1H, dd, J = 11.9, 9.0), 3.50 (2H, m), 3.85 (2H, m), 4.41 (1H, d, J = 6.8), 4.91 (1H, d, J = 7.7), 5.0-5.6 (6H, m), 7.02 and 7.18 (2H each, AA'BB' system); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 15.7 (q), 20.5 (q, 6 x C), 28.0 (t), 62.0 (t), 67.5 (t), 68.9 (d, 2 x C), 70.7 (d), 71.4 (d), 71.5 (d), 72.8 (d), 73.8 (d), 99.3 (d, $^1J_{C-H}$ = 163 Hz), 100.4 (d, $^1J_{C-H}$ = 164 Hz), 116.7 (d, 2 x C), 129.0 (d, 2 x C), 139.2 (s), 155.1 (s), 169.2 (s), 169.3 (s), 169.4 (s), 169.7 (s), 169.8 (s), 170.0 (s) (Found: C, 55.15; H, 6.18. $C_{31}H_{40}O_{16}$ requires: C, 55.69; H, 6.03%).

Ptelatoside-A Hexaacetate (**4**). To a stirred mixture of **10** (13.0 g, 19.5 mmol) and $NaHCO_3$ (8.2 g, 98 mmol) in $CHCl_3$ (120 ml) was added at 20 °C under nitrogen a solution of Br_2 (1.10 g, 21.4 mmol) in $CHCl_3$ (40 ml) over 50 min under irradiation with a high pressure mercury lamp (Eikosha 300 W). After addition of the bromine solution to the mixture, irradiation was continued for further 5 min. The mixture was filtered through a column of Na_2SO_4 and the column was washed with $CHCl_3$. The filtrate and the washings were combined and the solution was concentrated, until the volume of the solution became ca. 50 ml. Hexane (200 ml) was added to this solution with vigorous stirring to precipitate crystals (14.6 g). The crystals (14.6 g) were added in one portion to a mixture of NaOAc (116 g, 1.41 mol) and Ac_2O (19 ml, 0.2 mol) in AcOH (167 ml) kept under reflux. The mixture was refluxed for 40 h and the warm mixture was poured into 1:1 ice-water (700 g) with vigorous stirring. The precipitates (ca. 25 g) were collected by filtration and purified by column chromatography on silica gel with 85:15 benzene-EtOAc to give **4** (9.45 g, 73%): mp 162.5-164.0 °C (MeOH); $[\alpha]_D^{20}$ -54.4° (c 1.00, $CHCl_3$). The spectral (UV, IR, and ^1H NMR) and chromatographic properties of synthetic **4** proved identical with those of the hexaacetate **4** derived from natural ptelatoside-A (**2**).

Synthesis of Ptelatoside-A (**2**). To a stirred solution of **4** (7.42 g, 11.1 mmol) in MeOH (100 ml) was added dropwise a 1.0 M solution of NaOMe in MeOH (6.7 ml) under nitrogen. The mixture was stirred at room temperature for 1.5 h and neutralized with the ion-exchange resin Amberlite IRC-50 (H form, 10 g). The mixture was filtered and the resin was washed with MeOH. The filtrate and the washings were combined. The resulting solution was concentrated to give crude crystals, recrystallization of which provided pure **2** (4.06 g, 88%): mp 182-184 °C (H_2O-acetone); $[\alpha]_D^{24}$ -103° (c 1.00, H_2O). The spectral (UV, IR, ^1H and ^{13}C NMR) and chromatographic properties of synthetic **2** proved identical with those of natural ptelatoside-A (**2**).

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