Whisky Lactone Precursors from the Wood of *Platycarya strobilacea*

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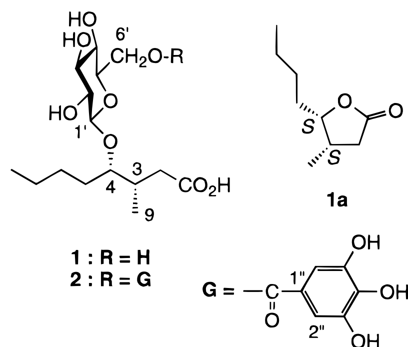
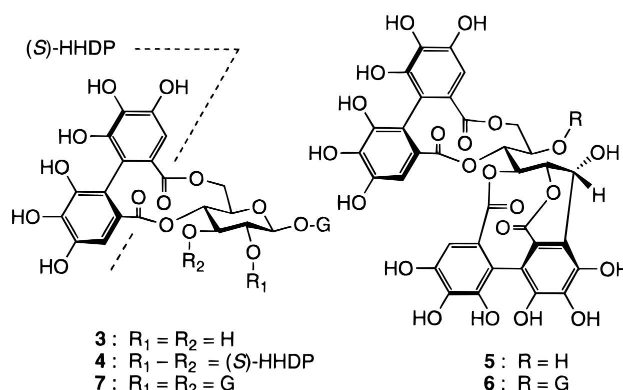
The first whisky lactone precursors, (3*S*,4*S*)-3-methyl-4-hydroxyoctanoic acid 3-*O*-β-D-glucopyranoside and its 6′-*O*-gallate, were isolated from the wood of *Platycarya strobilacea*, together with 10 known tannins and related compounds. Their structures were determined on the basis of spectroscopic and chemical evidence.

Whisky lactones (oak lactones, quercus lactones) are diastereomers of 3-methyl-4-octanolides found in whisky, wine, brandy, and extracts of oak barrels used to store and age these beverages. These lactones are important constituents of aging flavor and have so far been considered to be formed during aging from unknown precursors in wood used for barrels.[1,2] In the course of chemical studies on polyphenolic compounds, we have isolated a precursor of one of the diastereomers and its galloyl ester from the wood of *Platycarya strobilacea* Sieb. et Zucc. (Juglandaceae) together with known tannins and related compounds. This paper deals with the isolation and structure determination of these precursors. Distribution of the compounds in the wood cross sections was also examined.



The aqueous acetone extract of the wood was partitioned with H_2O and ether, and the aqueous layer was subsequently extracted with EtOAc. The final H_2O layer was separated using Sephadex LH-20, MCI-gel CHP20P, and Prep pak 500/C_{18} column chromatography (CC) (H_2O containing increasing proportions of MeOH) to yield compounds **1** and **2** together with 6-*O*-galloylglucose,[3] 2,3-(*S*)-hexahydroxydiphenoyl(HHDP)-D-glucose,[4] gallic acid 3-*O*-(6′-*O*-galloyl)-β-D-glucose,[3] strictinin (**3**),[5] pedunculagin,[4] 1-β-*O*-galloylpedunculagin (**4**),[5] casuariin (**5**), and casuarinin (**6**).[5] Compound **2** was also isolated from the AcOEt layer by similar chromatography along with 1, 2, 3, 4, 6-penta-*O*-galloyl-β-D-glucose and eugeniin (**7**).[6] Although ellagitannin composition in the wood was related to that of the bark, flavan-3-ols and proanthcyanidins that were dominantly found in the bark were absent in the wood.[7]

Compound **1** was isolated as a white amorphous powder and showed the $[M - H]^-$ peak at *m/z* 335 in the FABMS (negative ion mode). The ^{13}C-NMR spec-



trum showed 15 carbon signals including six arising from a hexosyl moiety. The *J* values ($J_{1,2}$ = 8 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9 Hz) of the sugar proton signals in the ^{1}H-NMR spectrum indicated that this sugar was β-glucopyranose. The remaining nine carbon signals were attributable to one carboxyl (C-1), one oxygenated methine (C-4), one methine (C-3), four methylenes, and two methyls (C-8 and C-9) by DEPT experiment. In the ^{1}H−^{1}H COSY spectrum, the oxygenated methine proton [δ 3.65 (m), H-4] was correlated with the methine [δ 2.29 (m), H-3] and methylene (H-5) protons. Furthermore, the methine proton (H-3) was also coupled with a doublet methyl [δ 0.96 (d), H-9] and two methylene protons [δ 2.59 (dd) and 2.14 (dd), H-2]. The chemical shifts of these methylene protons (H-2) suggested that this methylene was linked to a carboxyl group. According to these spectroscopic observations, **1** was considered to be 3-methyl-4-hydroxyoctanoic acid glucoside. Acid hydrolysis of **1** afforded **1a** along with D-glucose. The HREIMS of **1a** revealed its molecular formula of $C_9H_{16}O_2$, and the ^{1}H- and ^{13}C-NMR spectra were in agreement with those of *cis*-3-methyl-4-octanolide (whisky lactone).[8] Moreover, the $[\alpha]_D$ value (−76.8°) indicated that the absolute configurations of both C-3 and C-4 are *S*.[8] Consequently, compound **1** was characterized as (3*S*,4*S*)-3-methyl-4-hydroxyoctanoic acid 3-*O*-β-D-glucopyranoside.

Compound **2** exhibited the $[M - H]^-$ peak at *m/z* 487 in the FABMS (negative ion mode) and showed intense dark blue coloration with ethanolic $FeCl_3$. The ^{1}H-NMR spectrum of **2** was closely related to that of **1**, except for the appearance of a two-proton singlet at δ 7.16. In addition, the ^{13}C-NMR spectrum showed signals due to an aromatic ring and a conjugated ester carbon suggesting the presence of a galloyl group. This was confirmed by hydrolysis of **2** with tannase yielding gallic acid and compound **1**. The location of the galloyl group was determined to be the C-6 position of the glucose

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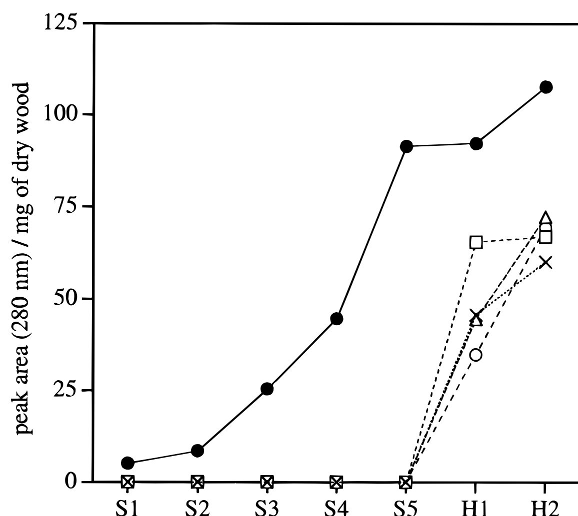

Figure 1. Comparison of HPLC peak area (280 nm) of compound **2** and tannins in wood cross sections of *Platycarya strobilacea.* The samples were extracted with aqueous acetone. Key: S1, outer sapwood; S5, inner sapwood; H1, outer heartwood; H2, inner heartwood; (●) compound **2**; (○) 1,2,3,4,6-pentagalloyl-β-D-glucose; (△) eugeniin; (□) 1-β-*O*-galloylpedunculagin; (×) casuarinin.

moiety on the basis of the large low field shifts of the glucose H-6 signals. Hence, **2** was characterized as the 6′-*O*-gallate of compound **1**.

It is well known that some of the compounds responsible for the aroma and flavor of fruits coexist with their glycosides, and these glycosides are thought to be their precursors.[9–12] Analogously, compounds **1** and **2** are probably precursors of whisky lactone, although the presence of the free lactone in the wood of *P. strobilacea* has not yet been confirmed. In this study, only one diastereomer of four possible whisky lactone precursors was isolated; other isomers were not detected. From the viewpoint of biosynthesis, it is important to clarify whether **1** or its diastereomers exist in the oak wood used for the whisky barrel. HPLC analysis of the wood demonstrated that the inner wood contained compound **2** in higher concentration compared with outer sapwood (Figure 1). The concentration of compound **1** is also higher in heartwood on the basis of TLC comparison. In addition, tannins exist only in the heartwood. Since whisky lactones have repellent activity to houseflies and mosquitoes,[1] compounds **1** and **2** and their hydrolysate may also play a role as preservatives of inner wood together with ellagitannins.[13]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Analytical HPLC was performed on a Tosoh apparatus equipped with a CCPM solvent delivery system, UV-8000 spectrometer (280 nm), and a Cosmosil 5C$_{18}$-AR (Nacalai Tesque Inc.) column (4.6 mm i.d. × 250 mm) (mobile phase, acetonitrile−50 mM H_3PO_4, gradient elution from 5 to 35% acetonitrile for 30 min; flow rate, 0.8 mL/min). CC was performed with Sephadex LH-20 (25−100 μm, Pharmacia Fine Chemical Co. Ltd.), MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries, Ltd.), Prep pak 500/C$_{18}$ (37−75 μm, Waters Associatea, Inc.), and Si gel 60 (Merck). TLC were performed on precoated Si gel 60 F$_{254}$ plates (0.2 mm thick, Merck) with benzene−ethyl formate−formic acid (1:7:1, v/v), and spots were detected by ultraviolet (UV) illumination and by spraying 2% ethanolic ferric chloride reagent or 5% H_2SO_4 followed by heating. Negative and positive FABMS were recorded on a JEOL JMX DX-303 spectrometer with glycerol as a matrix. ^{1}H and ^{13}C NMR spectra were obtained with Varian Unity plus 500, Varian Gemini 300, and Varian Gemini 200 spectrometers operating at 500, 300, and 200 MHz for ^{1}H and 125, 100 and 75 MHz for ^{13}C, respectively; chemical shifts are reported in parts per million on the δ scale from internal TMS, and coupling constants are in Hz.

Plant Material. The wood of *P. strobilacea* was collected in Sasanami, Yamaguchi prefecture, Japan. A voucher specimen is deposited in the Medical Plant Garden of Nagasaki University.

Isolation. The fresh wood (1.7 kg) was chipped into small pieces and extracted with 70% aqueous acetone. After evaporation of acetone, the insoluble precipitates were removed by filtration, and the filtrate was successively extracted with diethyl ether and EtOAc. The aqueous layer was concentrated and applied to a column of Sephadex LH-20, and the column was eluted with H_2O containing increasing proportions of MeOH. The first fraction obtained by elution with H_2O was chromatographed over MCI gel CHP20P with 50% MeOH to afford crude **1**, which was further purified by successive CC over Prep pak 500/C$_{18}$ (20% MeOH) and Si gel ($CHCl_3$−MeOH−H_2O, 70:30:5) (135 mg). The second fraction of the Sephadex LH-20 column was separated by MCI gel CHP20P CC with H_2O to give 2,3-(*S*)-HHDP-D-glucose (730 mg) and 6-*O*-galloylglucose (397 mg). The third fraction obtained by elution of the initial Sephadex column with 40% MeOH was subjected to Prep pak 500/C$_{18}$ CC (30% MeOH) to furnish **2** (843 mg). The fourth fraction containing ellagitannins was further separated by successive chromatographies on Sephadex LH-20 (60−80% MeOH), MCI-gel CHP20P (10−40% MeOH), and Prep pak 500/C$_{18}$ (10−30% MeOH) to give gallic acid 3-*O*-(6′-*O*-galloyl)-β-D-glucose (75 mg), strictinin (**3**, 208 mg), casuariin (**5**, 800 mg), pedunculagin (500 mg), 1-β-*O*-galloylpedunculagin (**4**, 760 mg) and casuarinin (**6**, 875 mg). The AcOEt layer (6 g) was similarly separated by MCI-gel CHP20P (10−40% MeOH), Sephadex LH-20 (60−80% MeOH), and Prep pak 500/C$_{18}$ (10−30% MeOH) CC to give gallic acid (235 mg), compound **2** (167 mg), 1,2,3,4,6-pentagalloyl-β-D-glucose (95 mg) and eugeniin (**7**, 30 mg).

Compound 1: white amorphous powder; $[\alpha]^{29}_D$ −24.4° (*c* 0.7, MeOH); ^{1}H-NMR (MeOH-d_4, 500 MHz) δ 4.35 (1H, d, J = 8 Hz, H-1′), 3.85 (1H, dd, J = 3, 12 Hz, H-6′), 3.70 (1H, dd, J = 5, 12 Hz, H-6′), 3.65 (1H, m, H-4), 3.38 (1H, t, J = 9 Hz, H-3′), 3.33 (1H, t, J = 9 Hz, H-4′), 3.25 (1H, ddd, J = 3, 5, 9 Hz, H-5′), 3.19 (1H, dd, J = 8, 9 Hz, H-2′), 2.59 (1H, dd, J = 6, 15 Hz, H-2), 2,29 (1H, m, H-3), 2.14 (1H, dd, J = 8, 15 Hz, H-2), 1.28−1.62 (6H, m, H-5, H-6 and H-7), 0.96 (3H, d, J = 7 Hz, H-9), 0.93 (3H, t, J = 7 Hz, H-8); ^{13}C-NMR (MeOH-d_4, 125 MHz) δ 180.3 (s, C-1), 104.5 (d, C-1′), 84.3 (d, C-4), 78.7 (d, C-3′), 78.2 (d, C-5′), 75.9 (d, C-2′), 72.2 (d, C-4′), 63.3 (t, C-6′), 39.6 (t, C-2), 35.0 (d, C-3), 32.3 (t, C-5), 29.6 (t, C-6), 24.2 (t, C-7), 15.9 (q, C-9), 14.9 (q, C-8); FABMS (negative ion mode) *m/z* 335 [M − H]$^-$. Anal. Calcd for $C_{15}H_{28}O_8\cdot1/4H_2O$: C, 52.85; H, 8.43; Found: C, 52.84; H, 8.10.

Hydrolysis of 1. **1** (40 mg) was dissolved in 1 mL of dioxane and 5 mL of 1 M HCl and heated under reflux for 2 h. The solution was extracted with ether (3 × 5 mL) and separated by Si gel chromatography (hexane−EtOAc) to yield **1a** as a colorless oil (16 mg): $[\alpha]^{20}_D$ −76.8° (*c* 1.0, MeOH); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.92 (3H, t, J = 7 Hz, H-8), 1.02 (3H, d, J = 7 Hz, H-9), 2.20 (1H, dd, J = 4, 17 Hz, H-2), 2.59 (1H, m, H-3), 2.70 (1H, dd, J = 8, 17 Hz, H-2), 4.43 (1H, m, H-4); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 13.9 and 14.0 (C-8 and C-9), 22.6 (C-7), 28.1 (C-6), 29.6 (C-5), 33.1 (C-3), 37.6 (C-2), 83.7 (C-4), 177.0 (C-1); HREIMS *m*/*z* 156.1147 ($C_9H_{16}O_2$ requires 156.1151). The acidic H_2O layer was neutralized with ion-exchange resin and examined by cellulose TLC (*n*-BuOH−pyridine−H_2O) showing the presence of glucose.

Compound 2: white amorphous powder; $[\alpha]^{29}_D$ −13.7° (*c* 0.6, MeOH); ^{1}H-NMR (acetone-d_6, 200 MHz) δ 7.16 (2H, s, H-2″ and 6″), 4.53 (1H, dd, J = 2, 12 Hz, H-6′), 4.40 (1H, d, J = 8 Hz, H-1′), 4.38 (1H, dd, J = 6, 12 Hz, H-6′), 3.60 (2H, m, H-4 and H-5′), 3.44 (2H, m, H-3′ and H-4′), 3.23 (1H, dd, J = 8, 9 Hz, H-2′), 2.62 (1H, m, H-2), 2.21 (2H, m, H-2 and H-3), 1.15 − 1.56 (6H, m, H-5, H-6 and H-7), 0.93 (3H, d, J = 7 Hz, H-9), 0.76 (3H, t, J = 7 Hz, H-8); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 175.4 (C-1), 166.9 (C-7″), 145.9 (C-3″ and C-5″). 138.6 (C-4″), 121.6 (C-1″), 109.7 (C-2″ and C-6″), 104.0 (C-1′), 83.0 (C-4), 77.7 (C-3′), 74.9 (C-5′), 74.6 (C-2′), 71.3 (C-4′), 64.6 (C-6′), 37.4 (C-2), 34.0 (C-3), 31.7 (C-5), 28.5 (C-6), 23.0 (C-7), 14.8 (C-9), 14.2 (C-8); FABMS (negative ion mode) *m/z* 467 (M − H)$^-$. Anal. Calcd for $C_{22}H_{32}O_{12}\cdot 1/2H_2O$: C, 53.11; H, 6.68; Found: C, 52.96; H, 6.24.

Hydrolysis of 2. To a solution of **2** (20 mg) in H_2O (3 mL) was added tannase (5 mg). The solution was stirred for 3 h at room temperature and directly applied to a column of MCI-gel CHP20P. The column was then eluted with H_2O containing increasing proportions of MeOH to yield gallic acid (7 mg) and **1** (15 mg).

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