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Studies on the Constituents of *Gastrodia elata* Blume.

Heihachiro Taguchi,*,[a] Itiro Yosioka,[a] Kazuo Yamasaki,[b] and Il Hyuk Kim[c]

Tsumura Laboratory,[a] 1-9-9, Izumi-Honcho, Komae-shi, Tokyo, 201, Japan, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[b] Kasumi, Hiroshima-shi, 734, Japan and College of Pharmacy, Chung-Ang University,[c] 221, Heuk-Suk Dong, Kwan-Ak Ku, Seoul, Korea

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A new glucoside, named gastrodioside, was isolated from the tubers of *Gastrodia elata* Blume. (Orchidaceae) and its structure was elucidated as bis(4-hydroxybenzyl)-ether mono-β-D-glucopyranoside (**6**). 4-Hydroxybenzaldehyde (**1**), 4-hydroxybenzyl alcohol (**2**), 4-hydroxybenzyl methyl ether (**3**, possibly an artifact), 4-(4′-hydroxybenzyl-oxy)benzyl methyl ether (**4**), bis(4-hydroxybenzyl)ether (**5**), 4-(β-D-glucopyranosyloxy)-benzyl alcohol (**7**) and tris[4-(β-D-glucopyranosyloxy)benzyl]citrate (parishin, **8**) were also isolated. This is the first time that compounds **4** and **5** have been isolated from natural sources.

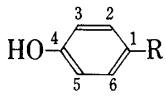
Keywords——*Gastrodia elata* Blume.; Orchidaceae; gastrodioside; bis(4-hydroxy-benzyl)ether mono-β-D-glucopyranoside; 4-hydroxybenzyl alcohol derivatives; ^{13}C NMR

Gastrodia elata Blume. (Orchidaceae) is a saprophyte growing in the woods of Japan, Korea and the central provinces of China. The steamed and dried tubers of this plant are used as a folk medicine under the name of "Tianma (天麻)".[1] This drug is considered to have very beneficial properties; it is said to aid in expelling all kinds of toxins from the body, to enhance strength and virility, and to improve the circulation and the memory. It is prescribed for rheumatism, neuralgia, paralysis, lumbago, headaches and other neuralgic and nervous affections.[1,2]

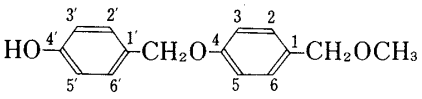
The constituents of this plant were investigated by Liu *et al.*, who reported the isolation of vanillyl alcohol.[3] Since further phytochemical studies of this plant have not been carried out, we reinvestigated the constituents of the tubers of this plant. This paper deals with the isolation of several 4-hydroxybenzyl alcoholic derivatives and glucosides, which are characteristic constituents of the Orchidaceae plants[4]: 4-hydroxybenzaldehyde (**1**, yield, 0.027%), 4-hydroxybenzyl alcohol (**2**, 0.021%), 4-hydroxybenzyl methyl ether (**3**, 0.0054%), 4-(4′-hydroxybenzyloxy)benzyl methyl ether (**4**, 0.0027%), bis (4-hydroxybenzyl) ether (**5**, 0.0039%), bis (4-hydroxybenzyl) ether mono-β-D-glucopyranoside (**6**, 0.0026%), 4-(β-D-glucopyranosyl-oxy)benzyl alcohol (**7**, 0.146%) and tris [4-(β-D-glucopyranosyloxy)benzyl]citrate(parishin, **8**, 0.041%).[5] Among them, **6**, named gastrodioside, is a new substance, and **4** and **5** were isolated for the first time from plant sources.[6]

The dried tubers of the plant (commercial crude drug) were extracted with ether and then methanol. The methanolic extract was dissolved in water and extracted with butanol. The butanolic extract, after concentration, was subjected to charcoal and then silica gel column chromatographies to furnish the compounds **2**—**7**. The ethereal extract gave compounds **1**, **3** and **5** upon silica gel column chromatography. On the other hand, another methanolic extract, prepared from the same material ,was chromatographed directly on charcoal. Elution was done first with water and then with ethanol. The ethanolic eluate was purified by silica gel column chromatography to furnish **7** and **8** (see "Experimental").

Compounds **1** and **2** were identified as 4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol, respectively, by direct comparison with authentic samples (IR and mixed mp). Compound **3** was characterized as 4-hydroxybenzyl methyl ether by elemental analysis and spectral studies.



1 : R=CHO
2 : R=CH_2OH
3 : R=$\overset{7}{C}H_2OCH_3$



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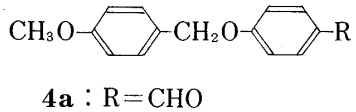
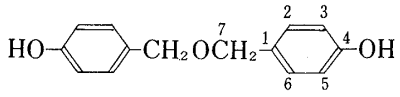
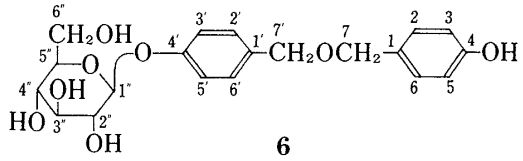


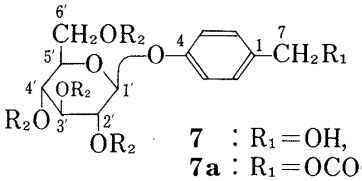
4a : R=CHO
4b : R=CH_2OH
4c : R=CH_2OCH_3



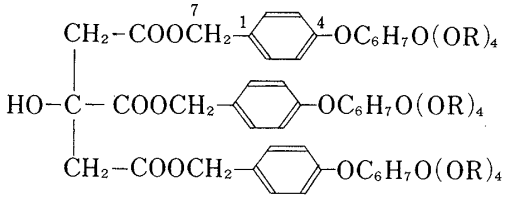
5



6



7 : R_1=OH, R_2=H
7a : R_1=$OCOCH_3$, R_2=$COCH_3$
7b : R_1=H, R_2=$COCH_3$



8 : R=H
8a : R=$COCH_3$

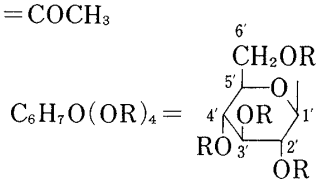


Chart 1

Compound **4** was obtained as colorless needles (from ether–hexane), $C_{15}H_{16}O_3$, mp 120—123° and gave a bluish-green color with $FeCl_3$–$K_3[Fe(CN)_6]$ in ethanol, indicating the presence of a phenolic hydroxy group. The ultraviolet (UV) spectrum of **4** was characteristic of 4-hydroxybenzyl alcoholic derivatives[5] and the infrared (IR) spectrum showed bands due to a hydroxy group and an aromatic ring, with no ester linkage. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **4** (in acetone-d_6) showed the signals of a methoxyl (δ 3.30) two benzylic methylenes bearing oxygen ($PhC\underline{H}_2O$-, δ 4.37 and 5.00, each 2H, s), two AA′BB′ type quartets [δ 6.81, 6.92, 7.25 and 7.31 (each 2H, d, J=9.5 Hz)] and a phenolic hydroxy group (δ 8.38, 1H, s, D_2O-exchangeable). The signals of a benzylic methylene at δ 4.37 and a methoxyl at δ 3.30 suggest the presence of a benzyl methyl ether moiety ($PhCH_2OCH_3$) and the other benzylic methylene signal at δ 5.00 suggests the presence of a phenoxybenzylic methylene moiety ($PhOCH_2Ph$). The mass spectrum of **4** showed strong peaks at m/z 137 ($O^+C_6H_4CH_2OCH_3$) and 107 (HO^+=C_6H_4=CH_2) as well as the molecular peak (m/z 244).

On the basis of the above spectral data, the structure of **4** was assumed to be 4-(4′-hydroxybenzyloxy)benzyl methyl ether. The structure was confirmed by the preparation of the methyl ether (**4c**). 4-Methoxybenzyl chloride and 4-hydroxybenzaldehyde were treated at 100° for 45 min in the presence of K_2CO_3 in dimethylformamide (DMF) to give 4-(4′-methoxybenzyloxy)benzaldehyde (**4a**), $C_{15}H_{14}O_3$, mp 98—99°. Reduction of **4a** with $NaBH_4$ in methanol afforded 4-(4′-methoxybenzyloxy)benzyl alcohol (**4b**), $C_{15}H_{16}O_3$, mp 114—116°. On methylation with CH_3I and Ag_2O in DMF, **4b** afforded 4-(4′-methoxybenzyloxy)benzyl methyl ether (**4c**), $C_{16}H_{18}O_3$, colorless fine plates (from MeOH–H_2O), mp 60—62.5°; this product was identical with the monomethyl ether of **4**, prepared by methylation of **4** with $(CH_3)_2SO_4$ and K_2CO_3 in acetone. The structure of **4** was thus elucidated as 4-(4′-hydroxybenzyloxy)-benzyl methyl ether.

Compound **5** was obtained as a white solid (from ether–hexane), $C_{14}H_{14}O_3$, mp 63—65° and gave a bluish-green color with $FeCl_3$–$K_3[Fe(CN)_6]$ in ethanol, indicating the presence of a phenolic hydroxy group. The UV spectrum indicated that **5** is a derivative of 4-hydroxybenzyl alcohol and the ^1H NMR spectrum (in acetone-d_6) showed signals due to a benzylic methylene bearing oxygen (δ 4.41, s, PhC$\underline{H}_2$O-), AA'BB' type aromatic protons (δ 6.80 and δ 7.20, each d, J=9 Hz) and a phenolic hydroxy group (δ 8.21, s) with a 2:4:1 ratio of integrated intensities. The carbon (^{13}C) NMR spectrum (in acetone-d_6) showed only five signals: two aromatic quaternary carbons (δ 158.3 and δ 130.2), two protonated aromatic carbons (δ 130.8 and δ 116.1) and a methylene (δ 72.6) carbon. The above spectral data indicate that **5** possesses a symmetrical structure. The mass spectrum, which showed strong peaks at m/z 123, 108 and 107 as shown in Chart 2, indicates that **5** possesses a 4-hydroxybenzyloxy moiety and that **5** is bis-(4-hydroxybenzyl)ether. The signals in the ^{13}C NMR spectrum of **5** were assigned as listed in Table I by comparison with the ^{13}C NMR spectral data for **3**. The structure of **5** was thus elucidated.

Table I. ^{13}C NMR Spectral Data for **3**, **5**, **6**, **7**, **7a**, **8a** and Trimethyl Citrate
(δ Value, ^{13}C: 20 MHz, at 25°, Solvent A=Methanol-d_4; B=$CDCl_3$)

Carbon	Solvent A				Solvent B	
	3	**5**	**6**	**7**	**7a**	**8a**
1	130.0	130.2	130.2	136.6	131.1	130.4(×3)
2, 6	130.7	130.8	130.5[a]	129.4	130.0	130.0(×3)
3, 5	116.1	116.1	116.1	117.7	117.1	117.1(×2), 116.9(×1)
4	158.3	158.3	158.2[b]	158.4	156.9	156.9(×3)
7	75.5	72.6	72.3[c]	64.8	65.7	66.3(×2), 67.4(×1)
1′	—	—	133.4	—	—	—
2′, 6′	—	—	130.8[a]	—	—	—
3′, 5′	—	—	117.7	—	—	—
4′	—	—	158.7[b]	—	—	—
7′	—	—	72.8[c]	—	—	—
OMe	57.8	—	—	—	—	—
Sugar moiety						
C-1′(1″)	—	—	102.3	102.4	99.1	99.0(×3)
C-2′(2″)	—	—	74.9	74.9	71.2	71.2(×3)
C-3′(3″)	—	—	78.1[d]	78.0[a]	72.8	72.7(×3)
C-4′(4″)	—	—	71.4	71.3	68.4	68.3(×3)
C-5′(5″)	—	—	78.0[d]	77.9[a]	72.1	72.1(×3)
C-6′(6″)	—	—	62.5	62.5	62.0	61.9(×2), 62.0(×1)
						Citric acid moiety[e]
	43.2 170.3 52.0 (×2)(×3) (×2)					43.3(×2),
	CH_2COOCH_3					$-CH_2-$
	73.3 | HO–C–COOH$_3$ 53.2					73.3(×1),
	CH_2COOCH_3					HO–C–
	Trimethyl Citrate					

a—*d*) The indicated assignments within any vertical column may be reversed.
e) Acetyl groups and carbonyl carbons of the citric acid moiety: CH_3: 20.6, CO: 169.2, 169.4, 170.2, 170.5, 173.1.

The new glucoside, named gastrodioside (**6**), was obtained as colorless needles (from MeOH–AcOEt), $C_{20}H_{24}O_8 \cdot 1/2H_2O$, mp 162—164°, $[\alpha]_D^{33}$ −42.4° (in EtOH) and gave a bluish-green color with $FeCl_3$–$K_3[Fe(CN)_6]$ in ethanol. The UV[λ_{max}^{EtOH} nm (log ε): 228 (4.33), 275 (3.43), and 278 (3.43)] and IR [ν_{max}^{KBr} cm^{-1}: 34000 (broad strong peak, OH), 1615, 1600 (sh), 1519, 1510 (aromatic)] spectra suggest that **6** might be the glucoside of a 4-hydroxybenzyl alcoholic

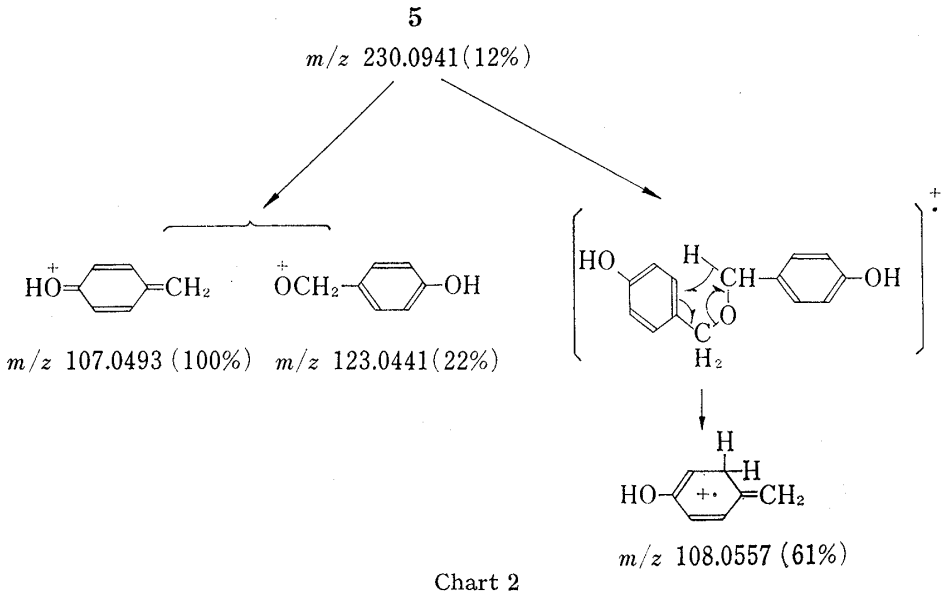


Chart 2

derivative. The ^{1}H NMR spectrum of **6** (in methanol-d_4) indicated the presence of two benzylic methylenes (δ 4.39 and δ 4.41, each 2H, s, PhC$\underline{H}_2$O–) and eight aromatic protons (δ 6.69—7.40). The ^{13}C NMR spectrum showed signals due to the β-D-glucopyranosyl moiety[7] and five pairs of signals due to the aglucone moiety: δ 158.7, 158.2 (s); 133.4, 130.2 (s); 130.8, 130.5 (d); 117.7, 116.1 (d) and 72.8, 72.3 (t). The chemical shifts of each pair are in almost the same regions as the carbons in **5**, as shown in Table I, suggesting that **6** is the monoglucoside of **5**. In fact, on hydrolysis with β-glucosidase, **6** afforded glucose and **5**, which were identified as their trimethylsilyl ethers by gas–liquid chromatography (GLC). The structure of gastrodioside was thus elucidated as bis(4-hydroxybenzyl)ether mono-β-D-glucopyranoside (**6**).

Compound **7** was obtained as colorless needles (from MeOH–AcOEt), $C_{13}H_{18}O_7 \cdot 1/2H_2O$, mp 156—157°, $[\alpha]_D^{33}$ −62.1° (in EtOH). Acetylation of **7** with Ac_2O in pyridine afforded the pentaacetate (**7a**), $C_{23}H_{28}O_{12}$, mp 120—122°, $[\alpha]_D^{33}$ −18.1° (in $CHCl_3$), and catalytic hydrogenation of **7a** with Pd–C in methanol gave *p*-cresyl-tetra-O-acetyl-β-D-glucopyranoside (**7b**), $C_{21}H_{26}O_{10}$, mp 117—118°, $[\alpha]_D^{33}$ −14.9° (in $CHCl_3$).[8] On the basis of the above reactions and spectral analysis (^{1}H and ^{13}C NMR), compound 7 was characterized as 4-(β-D-glucopyranosyloxy)benzyl alcohol.[5]

Compound **8** was obtained as an amorphous powder and purified as the acetate (**8a**), a white solid, $C_{69}H_{80}O_{37}$, mp 81—84° (from MeOH–AcOEt), $[\alpha]_D^{33}$ −17.2° (in $CHCl_3$). The IR spectrum (in $CHCl_3$) of **8a** showed a weak band at 3500 cm^{-1} due to a tertiary hydroxy group. The ^{1}H NMR spectrum (in benzene-d_6) gave a strong signal at δ 1.79 due to the acetyl groups and a broad singlet at δ 2.72, assignable to methylene adjacent to a carbonyl group (–CH_2CO). The signals at δ 4.90 (4H, s) and δ 5.00 (2H, s) can be assigned to three methylenes (3 × PhC$\underline{H}_2$O–) and AA′BB′ type signals at δ 6.90 and δ 7.13 (each 6H, d, J=9.5 Hz) to the aromatic protons. The presence of three benzylic methylene signals, twelve aromatic protons and two methylenes adjacent to a carbonyl indicates that **8a** possesses three moles of **7a** and an acid such as citric acid. A comparison of the ^{13}C NMR spectrum of **8a** with those of **7a** and trimethyl citrate, clearly supports the above assumption. The appearance of the signals at δ 66.3 (2 × C,t) and δ 67.4 (1 × C,t), assignable to benzylic methylene carbons, and those at δ 62.0 and δ 61.9 (total 3 × C,t), assignable to methylene carbons (C-6′) of the sugar moieties, indicate the presence of three moles of **7a**. The signals at δ 43.3 (2 × C,t) and δ 73.3 (1 × C,s) are assignable to methylene carbons and the quaternary carbon of citric acid, respectively. Compound **8a** was thus suggested to be the polyacetate of the triester of citric acid and **7** (*vide* elemental analysis).

Catalytic hydrogenation of **8a** with Pd–C in MeOH afforded **7b** and citric acid. On the other hand, the crude **8** showed no acetyl signal in the ^{1}H NMR spectrum. Although direct comparison was not carried out, the structure of **8** was characterized as tris[4-(β-D-glucopyranosyloxy)benzyl]citrate(parishin), which has already been isolated from *Vanda parishii* (Orchidaceae).[5]

Among the above compounds, **3** seems to be an artifact derived from **2** during the extraction procedure, since **3** could not be detected in the acetone extract of the plant by high performance liquid chromatography (HPLC), but when a methanolic solution of **2** containing SiO_2 was refluxed for 7 hr, **3** was detected in the solution. Compound **4** also could not be detected in the acetone extract,[9] but it is not yet clear whether **4** is natural or an artifact.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus (a hot stage type) and are uncorrected. The UV spectra were recorded with a Hitachi 624 digital spectrophotometer and the IR spectra with a Hitachi EPI-G2 unit. The ^{1}H NMR spectra were recorded with a Varian T-60 spectrometer and the ^{13}C NMR spectra with a Varian FT-80 spectrometer, with tetramethylsilane as an internal standard. The mass spectra were measured with Hitachi double-focusing and JEOL JMS-01-SG-2 mass spectrometers. The specific rotations were measured with a JASCO DIP-SL unit. GLC was run on a Hitachi 073 unit with a hydrogen flame ioniazation detector. Silica gel (Kieselgel 60, Merck) was used for column chromatography. Thin-layer chromatography (TLC) was carried out on Merck plates precoated with Kieselgel $60F_{254}$ and preparative layer chromatography (PLC) on plates (20×20 cm, 0.75 mm thick) coated with Kieselgel GF_{254} (Merck).

Extraction and Isolation of the Compounds——i) Isolation of **1**—**7**: The dried and pulverized tubers of the plant (1 kg, commercial crude drug) were extracted with ether (3 l×3) and then with MeOH (3 l×3) under reflux. The MeOH extracts were concentrated to give a brown mass (109 g), which was dissolved in H_2O (1 l) and extracted with BuOH (150 ml×5). The BuOH extract (20 g) was dissolved in H_2O (100 ml) and subjected to charcoal (150 g) column chromatography. Elution was carried out with H_2O (6 l), 50% MeOH (5 l), 70% MeOH (2 l) and MeOH (10 l). The 50% MeOH eluates were combined and concentrated under reduced pressure. The residue (2.76 g) was rechromatographed on silica gel (50 g) with $CHCl_3$–MeOH (19: 1, 1.4 l, each fraction 200 ml, Fr. 1—7) and $CHCl_3$–MeOH (9: 1, 4.8 l, Fr. 8—31). Fr. 1—6 were combined and concentrated, and purified by PLC with benzene–ether (1: 1) to give **4** (*Rf* 0.70, 22 mg), **3** (*Rf* 0.57, 54 mg), **5** (*Rf* 0.40, 23 mg) and **2** (*Rf* 0.25, 74 mg). Fr. 9—11 were concentrated and purified by PLC ($CHCl_3$–MeOH–H_2O, 35: 15: 3) to give **6** (26 mg). Fr. 12—27 were concentrated. Crystallization of the residue from MeOH–AcOEt gave **7** (1.16 g).

The MeOH eluates from charcoal column chromatography were concentrated and the residue was rechromatographed on silica gel (30 g) with $CHCl_3$ containing increasing amounts of MeOH. The fractions eluted with $CHCl_3$–MeOH (19: 1) were concentrated and purified by PLC [benzene–ether (2: 1), *Rf* 0.40] to give **1** (256 mg). The fractions eluted with $CHCl_3$–MeOH [(9: 1) and (17: 3)] were concentrated. The residue was crystallized from MeOH–AcOEt to give **7** (310 mg, total yield of **7**, 1.46 g).

The ethereal extract (4.3 g) was chromatographed on silica gel (100 g) with benzene containing increasing amounts of ether. The fractions eluted with benzene–ether [(9: 1) and (4: 1)] were concentrated and the residue (720 mg) was rechromatographed on silica gel with a $CHCl_3$–MeOH solvent system to give **1** (14 mg, total yield, 270 mg), **2** (130 mg, total yield, 204 mg) and **5** (16 mg, total yield, 39 mg).

ii) Isolation of **8**: Dried and pulverized tubers of the plant (2.5 kg) were extracted with MeOH and the MeOH extract, after concentration, was chromatographed on charcoal (85 g). Elution was carried out with H_2O (2 l) and then with EtOH (5 l). The H_2O eluates were concentrated to give a residue (225 g), in which the presence of glucose and fructose was demonstrated by GLC after derivatization to trimethylsilyl ethers. The EtOH eluates were concentrated and the residue (48.2 g) was rechromatographed on silica gel (180 g) with $CHCl_3$ containing increasing amounts of MeOH [$CHCl_3$ (500 ml, Fr. 1); $CHCl_3$–MeOH, 19: 1 (2 l, Fr. 2—5), 9: 1 (4 l, Fr. 6—13), 17: 3 (4 l, Fr. 14—21), 7: 3 (4 l, Fr. 22—27) and MeOH (2 l, Fr. 28—31)]. Fr. 15—24 were concentrated and the residue (6.7 g) was further purified by silica gel column chromatography (SiO_2, 65 g) with a $CHCl_3$–MeOH solvent system to give **7**. Fr. 28 was concentrated to give a residue (6.65 g). A portion (1 g) of Fr. 28 was purified by silica gel column chromatography (SiO_2, 30 g) with a $CHCl_3$–MeOH solvent system and then by PLC [$CHCl_3$–MeOH–H_2O (35: 15: 3)] to give **8** as an amorphous powder (54 mg). A portion (276 mg) of Fr. 28 was acetylated by the usual method [Ac_2O in pyridine, at room temperature]. The resulting crude acetate was purified by PLC [$CHCl_3$–MeOH (30: 1)] to give **8a** (64 mg). Fr. 29—31 were concentrated. The residue (12.6 g) was chromatographed on charcoal (20 g). Elution was carried out with H_2O and then 50% MeOH. The 50% MeOH eluates (2.82 g) were acetylated by the usual method (Ac_2O in pyridine, at room temperature). The resulting acetate was purified by PLC [$CHCl_3$–MeOH (30: 1)] to give **8a** (282 mg) [total yield of **8a**, 1.54 g; yield of **8**, 1.03 g)].

4-Hydroxybenzaldehyde (1)——Compound **1** was obtained as pale brown needles (from ether–hexane), mp 117—118°, IR ν_{max}^{KBr} cm^{-1}: 3150 (OH), 1665 (CHO), 1600, 1595, 1515 (aromatic.) ^{1}H NMR (δ in acetone-d_6): 7.01, 7.80 (each 2H, d, J=9 Hz, arom.-H), 9.86 (1H, s, CHO). This compound was identified as 4-hydroxybenzaldehyde by direct comparison with an authentic sample (IR and mixed mp).

4-Hydroxybenzyl Alcohol (2)——Compound **2** was obtained as colorless prisms (from MeOH–H_2O), mp 116—117°. UV λ_{max}^{EtOH} nm (log ε): 225 (3.93), 277 (3.19), 283 (3.12). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1595, 1515 (aromatic). ^{1}H NMR (δ in methanol-d_4): 4.46 (2H, s, PhC$\underline{H}_2$O–), 6.86, 7.16 (each 2H, d, J=9 Hz, arom.-H). This compound was identified as 4-hydroxybenzyl alcohol by direct comparison with an authentic sample (mixed mp and IR).

4-Hydroxybenzyl Methyl Ether (3)——Compound **3** was obtained as colorless prisms (from ether–pet. ether), mp 81—83°. UV λ_{max}^{EtOH} nm (log ε): 228 (3.91), 278 (3.10), 284 (3.04). IR ν_{max}^{KBr} cm^{-1}: 3280 (OH), 1615, 1595, 1515 (aromatic). ^{1}H NMR (δ in acetone-d_6): 3.28 (3H, s, OCH_3), 4.32 (2H, s, PhC$\underline{H}_2$O–), 6.79, 7.12 (each 2H, d, J=9 Hz, arom.-H), 8.23 (1H, s, OH, D_2O-exchangeable). *Anal.* Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.83; H, 7.44.

4-(4′-Hydroxybenzyloxy)benzyl Methyl Ether (4)——Compound **4** was obtained as colorless needles (from ether–hexane), mp 120—123°. $FeCl_3$–K_3[Fe(CN)$_6$] in EtOH: bluish-green. UV λ_{max}^{EtOH} nm (log ε): 231 (4.17), 277 (3.35), 280 (sh 3.32). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1610, 1595, 1580, 1515 (aromatic). ^{1}H NMR (δ in acetone-d_6): 3.30 (3H, s, OCH_3), 4.37 (2H, s, PhC$\underline{H}_2$OCH$_3$), 5.00 (2H, s, PhC$\underline{H}_2$OPh), 6.81, 6.92, 7.25, 7.31 (each 2H, d, J=9.5 Hz, 8×arom.-H), 8.38 (1H, s, OH, D_2O-exchangeable). MS m/z (%): 244 (M^+, 2.2), 137 ($\overset{+}{O}C_6H_4CH_2$-OCH$_3$, 17.5), 107 (H$\overset{+}{O}$=C$_6$H$_4$=CH$_2$, 100). High resolution MS, Calcd for $C_{15}H_{16}O_3$ (M^+), m/z: 244.1099. Found: 244.1093.

Methylation of 4——Compound **4** (4 mg) and $(CH_3)_2SO_4$ (2 drops) were dissolved in dry acetone (1 ml) and then K_2CO_3 (50 mg) was added to the solution. The mixture was stirred at room temperature for 4 hr, filtered and concentrated.

The residue was purified by PLC with benzene to give the monomethyl ether (**4c**) of **4** as colorless fine plates (from MeOH–H_2O), mp 56—58°. ^{1}H NMR (δ in acetone-d_6): 3.30 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.35 (2H, s, PhC$\underline{H}_2$OCH$_3$), 5.03 (2H, s, PhC$\underline{H}_2$OPh), 6.93, 6.95, 7.30, 7.40 (each 2H, d, J=9.5 Hz, 8×arom.-H). MS, m/z (%): 258 (M^+, 7.7), 226 (M^+—CH_3OH, 1.7), 137 ($\overset{+}{O}C_6H_4CH_2OCH_3$, 1.5), 121 ($CH_3\overset{+}{O}$=C$_6H_4$=CH$_2$, 100).

Preparation of 4-(4′-Methoxybenzyloxy)benzyl Methyl Ether (**4c**) **(Monomethyl Ether of 4)**——i) 4-Methoxybenzyl chloride (568 mg) and 4-hydroxybenzaldehyde (352 mg) were dissolved in DMF (3 ml) and then K_2CO_3 (600 mg) was added to the solution. The reaction mixture was stirred at 100° for 45 min. After cooling, the reaction mixture was diluted with H_2O (30 ml) and extracted with ether (30 ml×3). The combined ethereal solution was washed with H_2O (15 ml×4), dried over Na_2SO_4 and concentrated. The residue (754 mg) was purified by PLC [benzene–ether (1: 1)] to give 4-(4′-methoxybenzyloxy)benzaldehyde (**4a**) as colorless needles, mp 98—99°, 648 mg. IR ν_{max}^{KBr} cm^{-1}: 1700 (CHO), 1610, 1604, 1600 (sh), 1575, 1515 (aromatic). ^{1}H NMR (δ in acetone-d_6): 3.80 (3H, s, OCH_3), 5.12 (2H, s, PhC$\underline{H}_2$OPh), 6.89, 7.08, 7.38, 7.79 (each 2H, d, J=9.5 Hz, 8×arom.-H), 9.83 (1H, s, CHO). *Anal.* Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.14; H, 5.98.

ii) $NaBH_4$ (81 mg) was added to a solution of **4a** (101 mg) in MeOH (3 ml). The reaction mixture was stirred at room temperature for 1 hr, then diluted with H_2O (30 ml) and extracted with ether (20 ml×3). The combined ethereal extract was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue (87 mg) was purified by PLC [benzene–ether (3: 1)] to give 4-(4′-methoxybenzyloxy)benzyl alcohol (**4b**) as colorless prisms, mp 114—116°, 83 mg. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1610, 1586, 1518 (aromatic). ^{1}H NMR (δ in acetone-d_6): 3.79 (3H, s, OCH_3), 3.95 (1H, t, J=6 Hz, OH, D_2O-exchangeable), 4.53 (2H, d, J=6 Hz, PhC$\underline{H}_2$OH, singlet upon addition of D_2O), 5.01 (2H, s, PhC$\underline{H}_2$OPh), 6.96 (4H, d, J=9.5 Hz), 7.29 (2H, d, J=9.5 Hz), 7.36 (2H, d, J=9.5 Hz) (8×arom.-H). *Anal.* Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found: C, 73.45; H, 6.58.

iii) CH_3I (1 ml) and Ag_2O (438 mg) were added to a solution of **4b** (37.8 mg) in DMF (1 ml) and the reaction mixture was stirred at room temperature for 24 hr, diluted with $CHCl_3$ (20 ml) and filtered. The $CHCl_3$ solution was washed with H_2O (30 ml×3), dried over Na_2SO_4 and concentrated. The residue (28.7 mg) was purified by PLC [benzene–ether (10: 1)] to give 4-(4′-methoxybenzyloxy)benzyl methyl ether (**4c**) as colorless fine plates (from MeOH–H_2O), mp 60—62.5°, 16 mg. UV λ_{max}^{EtOH} nm (log ε): 230 (4.45), 275 (3.47), 282 (sh 3.39). IR ν_{max}^{KBr} cm^{-1}: 1608, 1580, 1515 (aromatic). ^{1}H NMR (δ in acetone-d_6): 3.30 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.36 (2H, s, PhC$\underline{H}_2$OCH$_3$), 5.04 (2H, s, PhC$\underline{H}_2$OPh), 6.90, 6.93, 7.29, 7.39 (each 2H, d, J=9.5 Hz, 8×arom.-H). MS, m/z (%): 258 (M^+, 1.3), 226 (M^+—CH_3OH, 2.6), 137 ($\overset{+}{O}C_6H_4CH_2OCH_3$, 2.6), 121 ($CH_3\overset{+}{O}$=C$_6H_4$=CH$_2$, 100). *Anal.* Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 74.48; H, 7.05. This compound was identical with **4c** prepared from **4** on direct comparison (^{1}H NMR, MS and mixed mp).

Bis(4-hydroxybenzyl)ether (5)——Compound **5** was obtained as a white solid (from ether–hexane), mp 63—65°. $FeCl_3$–K_3[Fe(CN)$_6$]: bluish-green. UV λ_{max}^{EtOH} nm (log ε): 230 (4.31), 278 (3.49), 285 (sh 3.39). IR ν_{max}^{KBr} cm^{-1}: 3300—3200 (OH), 1621, 1595, 1515 (aromatic). ^{1}H NMR (δ in acetone-d_6): 4.41 (4H, s, 2×

PhC$\underline{H}_2$O–), 6.80 (4H, d, J=9 Hz), 7.20 (4H, d, J=9 Hz) (8×arom.-H), 8.21 (2H, s, 2×OH, D_2O-exchangeable). High resolution MS, Calcd for $C_{14}H_{14}O_3$(M^+), m/z: 230.0943. Found: 230.0941.

Gastrodioside (6)——Compound **6** was obtained as colorless needles (from MeOH–AcOEt), mp 162—164°, $[\alpha]_D^{33}$ −42.4° (c=0.50, EtOH). UV λ_{max}^{EtOH} nm (log ε): 228 (4.33), 275 (3.43), 278 (3.43). IR ν_{max}^{KBr} cm^{-1}: 3400 (broad absorption, OH), 1615, 1600 (sh), 1519, 1510 (aromatic). ^1H NMR (δ in methanol-d_4): 4.39, 4.41 (each 2H, s, 2×PhC$\underline{H}_2$O–), 6.69—7.40 (8H, arom.-H). *Anal.* Calcd for $C_{20}H_{24}O_8 \cdot 1/2H_2O$: C, 59.84; H, 6.27. Found: C, 59.69; H, 6.30.

Enzymatic Hydrolysis of 6——Compound **6** (6 mg) was dissolved in 1/10 M acetate buffer solution (pH 5.0, 2 ml) containing 0.005% β-glucosidase [Miles Laboratories (PTY) Ltd.]. The reaction mixture was stirred at 38° for 6 hr, then diluted with H_2O (10 ml) and extracted with ether. The ethereal solution was washed with H_2O, dried over Na_2SO_4 and concentrated to give a residue. A portion of the residue was trimethylsilylated with 1,1,1,3,3,3-hexamethyldisilazane and trimethylchlorosilane in pyridine. The presence of bis(4-trimethylsilyloxybenzyl)ether was demonstrated by GLC. GLC conditions: column, 3% SE-52, Chromosorb W AW-DMCS, 80—100 mesh, 3 mm×2 m; carrier gas, N_2, 30 ml/min; oven temperature, 200°; injection temperature, 235°; t_R(min), 19.2. Authentic sample, t_R(min), 19.3. The aqueous solution was concentrated and the residue was trimethylsilylated in the same way as the ether-soluble portion. The presence of penta-O-trimethylsilylglucose was demonstrated by GLC. GLC conditions: the column and carrier gas were the same as for the aglucone; oven temperature, 180°; injection temperature, 220°; t_R(min), 7.6 and 11.2.

4-β-D-Glucopyranosyloxybenzyl Alcohol (7)[6]——Compound **7** was obtained as colorless needles (from MeOH–AcOEt), mp 156—157°, $[\alpha]_D^{33}$ −62.1° (c=0.31, EtOH). UV λ_{max}^{EtOH} nm (log ε): 223 (3.91), 273 (2.88), 278 (sh 2.78). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1615, 1590, 1575 (aromatic). ^1H NMR (δ in methanol-d_4): 3.3—3.5 (4H, m, $C_{(2')}$, $_{(3')}$, $_{(4')}$, $_{(5')}$–H), 3.6—4.0 (2H, m, $C_{(6')}$–H), 4.53 (2H, s, PhC$\underline{H}_2$O–), 7.07, 7.26 (each 2H, d, J=9.5 Hz, 4×arom.-H). *Anal.* Calcd for $C_{13}H_{18}O_7 \cdot 1/2H_2O$: C, 52.87; H, 6.49. Found: C, 52.81; H, 6.48.

Acetylation of 7——A solution of **7** (54.6 mg) in a mixture of Ac_2O (0.3 ml) and pyridine (0.7 ml) was allowed to stand at room temperature for 24 hr and then poured into ice-water. The precipitates were collected and recrystallized from MeOH–H_2O to give the pentaacetate (**7a**) as colorless needles, mp 120—122°, $[\alpha]_D^{33}$ −18.1° (c=0.61, $CHCl_3$), 74 mg. UV λ_{max}^{EtOH} nm (log ε): 221 (4.01), 269 (2.81), 276 (2.72). IR ν_{max}^{KBr} cm^{-1}: 1755, 1728 (ester), 1610, 1585, 1510 (aromatic). ^1H NMR (δ in $CDCl_3$): 2.00—2.20 (15H, s, 5×$COCH_3$), 3.90 (1H, m, $C_{(5')}$–H), 4.23 (2H, m, $C_{(6')}$–H), 5.03 (2H, s, PhC$\underline{H}_2$O–), 5.03—5.20 (4H, m, $C_{(1')}$, $_{(2')}$, $_{(3')}$, $_{(4')}$–H), 6.90, 7.26 (each 2H, d, J=9.5 Hz, 4×arom.-H). *Anal.* Calcd for $C_{23}H_{28}O_{12}$: C, 55.59; H, 5.69. Found: C, 55.67; H, 5.64.

Catalytic Hydrogenation of 7a——A solution of **7a** (96 mg) in MeOH (10 ml) was shaken with H_2 in the presence of 10% Pd-C (100 mg) as a catalyst at 25° for 45 min. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by PLC to give *p*-cresyl-tetra-O-acetyl-β-D-glucopyranoside (**7b**) as fine plates (from MeOH–H_2O), mp 117—118°, $[\alpha]_D^{33}$ −14.9° (c=0.2, $CHCl_3$), 54 mg. UV λ_{max}^{EtOH} nm (log ε): 214 (sh 3.90), 219 (3.93), 267 (sh 2.95), 273 (3.04), 280 (2.95). IR ν_{max}^{KBr} cm^{-1}: 1750, 1740 (ester), 1605, 1585, 1505 (aromatic). ^1H NMR (δ in $CDCl_3$): 2.00—2.15 (12H, s, 4×$COC\underline{H}_3$), 2.29 (3H, s, PhC$\underline{H}_3$), 3.62—4.05 (1H, m, $C_{(5')}$–H), 4.25 (2H, m, $C_{(6')}$–H), 4.82—5.42 (4H, m, $C_{(1')}$, $_{(2')}$, $_{(3')}$, $_{(4')}$–H), 6.86, 7.10 (each 2H, d, J=9.5 Hz, 4×arom.-H). *Anal.* Calcd for $C_{21}H_{26}O_{10}$: C, 57.53; H, 5.98. Found: C, 57.53; H, 5.99.

Tris[4-(tetra-O-acetyl-β-D-glucopyranosyloxy)benzyl]citrate (Parishin Acetate) (8a)——Compound **8a** was obtained as a white solid (from MeOH–AcOEt), mp 81—84°, $[\alpha]_D^{33}$ −17.2° (c=0.51, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 221 (4.47), 268 (3.33), 276 (3.27). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1750, 1740 (ester), 1605, 1505 (aromatic). ^1H NMR (δ in benzene-d_6): 1.79 (36H, $COC\underline{H}_3$), 2.72 (4H, s, 2×–CH_2CO), 4.90 (4H, s, 2×PhC$\underline{H}_2$O–), 5.00 (2H, s, PhC$\underline{H}_2$O–), 6.90, 7.13 (each 6H, d, J=9.5 Hz, 12×arom.-H). *Anal.* Calcd for $C_{69}H_{80}O_{37}$: C, 55.20; H, 5.37. Found: C, 55.23; H, 5.48; C, 55.14; H, 5.35.

Catalytic Hydrogenation of 8a——Compound **8a** (64 mg) in MeOH was shaken with H_2 in the presence of 10% Pd-C (53 mg) as a catalyst at 25° for 1 hr. The catalyst was filtered off and the filtrate was concentrated. The residue was subjected to PLC [benzene–ether (1: 1)]. The zone with *Rf* 0.55 was extracted with AcOEt and the extract was concentrated. The residue was recrystallized from a mixture of MeOH and H_2O to give colorless fine plates, mp 118—119°, 32 mg. The compound obtained here was identical with **7b** prepared from **7a** on direct comparison (IR, mixed mp and ^1H NMR). The zone near the starting line on the TLC plate was extracted with MeOH and the MeOH extract was concentrated. The residue (5 mg) was dissolved in MeOH and treated with ethereal diazomethane to give trimethyl citrate as colorless prisms (from AcOEt–ether), mp 77—78°. ^1H NMR (δ in $CDCl_3$): 2.86 (4H, br s, –CH_2CO), 3.72 (6H, s, 2×$COOCH_3$), 3.81 (3H, s, $COOCH_3$), 4.10 (1H, s, OH). This compound was identified as trimethyl citrate by direct comparison with an authentic sample (mixed mp, ^1H NMR and GLC). GLC conditions: column, 5% PNGS Chromosorb W AW-DMCS 60—80 mesh, 3 mm×2 m, carrier gas, N_2, 30 ml/min; oven temperature, 180°; injection temperature, 200°; t_R(min), 11.2).

Tris[4-β-D-glucopyranosyloxy)benzyl]citrate (Parishin) (8)——Crude **8** was obtained as an amorphous powder, which gave a single spot on TLC [$CHCl_3$–MeOH–H_2O (35: 15: 3)]. ^1H NMR (δ in methanol-d_4): 3.85 (4H, br s, 2×–CH_2CO), 4.93 (6H, s, 3×PhC$\underline{H}_2$O–), 6.86—7.40 (12H, br A_2B_2 q, arom.-H), no acetyl signal.

HPLC Investigation of the Acetone Extract of the Plant——The pulverized tubers of the plant (2 g) were extracted with acetone (2 ml) under reflux for 1 hr. The extract was concentrated to 2 ml and subjected to HPLC. HPLC conditions: apparatus, JASCO Trirotar-II; detector, UVIDEC-100-III, UV 280 nm; column, μ-Bondapak-C_{18} (Waters Associates); flow rate, 1 ml/min. Mobile phase: i) gradient conditions, mode, linear; program time, 8 min; solvent ①, CH_3CN–H_2O (1: 20), solvent ②, CH_3CN–H_2O (1: 4); t_R(min), **7**, 6.4; **2**, 10.0; **1**, 16.4; **6**, 22.0; **3**, not detected (authentic sample, 18.6). ii) solvent, CH_3CN–H_2O (3: 7), t_R(min), **5**, 11.4. iii) solvent, CH_3CN–H_2O (2: 3); t_R(min), **5**, 6.5; **4**, not detected (authentic sample, 12.8).

References and Notes

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