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Tannins and Related Compounds. XVIII.[1] A Gallotannin and Two Ellagitannins Containing a *p*-Hydroxyphenethyl Alcohol 1-*O*-*β*-D-Glucoside (Salidroside) Core from *Quercus stenophylla* MAKINO. (2)

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A gallotannin (**1**) and two ellagitannins (**2** and **3**) containing a salidroside (*p*-hydroxyphenethyl alcohol 1-*O*-*β*-D-glucoside) core have been isolated from the bark of *Quercus stenophylla* MAKINO (Fagaceae). On the basis of chemical and spectroscopic evidence, their structures have been elucidated unequivocally as 2″,3″,4″,6″-tetra-*O*-galloyl salidroside (**1**), 2″,3″-di-*O*-galloyl-4″,6″-(*S*)-hexahydroxydiphenoyl salidroside (**2**) and 3″-*O*-galloyl-4″,6″-(*S*)-hexahydroxydiphenoyl salidroside (**3**).

Keywords——*Quercus stenophylla*; Fagaceae; ellagitannin; gallotannin; *p*-hydroxyphenethyl alcohol glucoside; salidroside; tannase; ^{1}H-NMR; spin-decoupling

In a previous paper,[3] we reported the isolation of six galloyl esters of *p*-hydroxyphenethyl alcohol 1-*O*-*β*-D-glucopyranoside (salidroside) from the bark of *Quercus stenophylla* MAKINO (Fagaceae) (Japanese name: Urajirogashi). These compounds included three monogallates (6″- and 3″-*O*-gallates, and 3′-hydroxy-6″-*O*-gallate), two digallates (4′,6″- and 4″,6″-di-*O*-gallates) and one trigallate (3″,4″,6″-tri-*O*-gallate). Further chemical examination of more polar phenolic constituents occurring in this plant has resulted in the isolation of three additional salidroside derivatives, one (**1**) containing four galloyl groups and others (**2** and **3**) containing a hexahydroxydiphenoyl group in the molecule. This paper deals with the isolation and structure determination of these compounds.

Repeated chromatography (Sephadex LH-20 and Diaion HP-20) of fraction III[3] which was previously obtained from the ethyl acetate-soluble portion of the aqueous acetone extract afforded compounds **1**, **2** and **3**.

Compound **1** (**1**), an off-white amorphous powder, $[\alpha]_D$ −76.0° (MeOH), $C_{42}H_{36}O_{23}\cdot 7/2H_2O$, was strongly positive to the ferric chloride reagent (a dark blue coloration). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** revealed the presence of four galloyl groups (δ 6.93, 7.02, 7.08, 7.19, each 2H, s), one *p*-substituted aromatic ring (δ 6.60, 6.96, each 2H, d, J=8 Hz) and two methylenes which were coupled with each other (δ 2.73, 3.90, each 2H, t, J=7 Hz). The occurrence of a sugar moiety, as well as its glycosidic nature, was demonstrated by the appearance of an anomeric proton signal (δ 5.06, d, J=8 Hz) in the ^{1}H-NMR spectrum and also by characteristic carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals (δ 101.5, 73.7, 72.8, 72.5, 70.7, 63.2). On enzymatic hydrolysis with tannase in aqueous solution, **1** yielded gallic acid and the associated glycoside (**1a**). The latter was shown by comparisons of the physical data and infrared (IR) spectrum to be identical with salidroside[3,4] previously obtained by similar tannase hydrolysis of its gallates.

The locations of the galloyl groups in **1** were concluded on the basis of ^{1}H-NMR analysis to be at the C(2″)-, C(3″)-, C(4″)- and C(6″)-positions, since signals due to the sugar protons

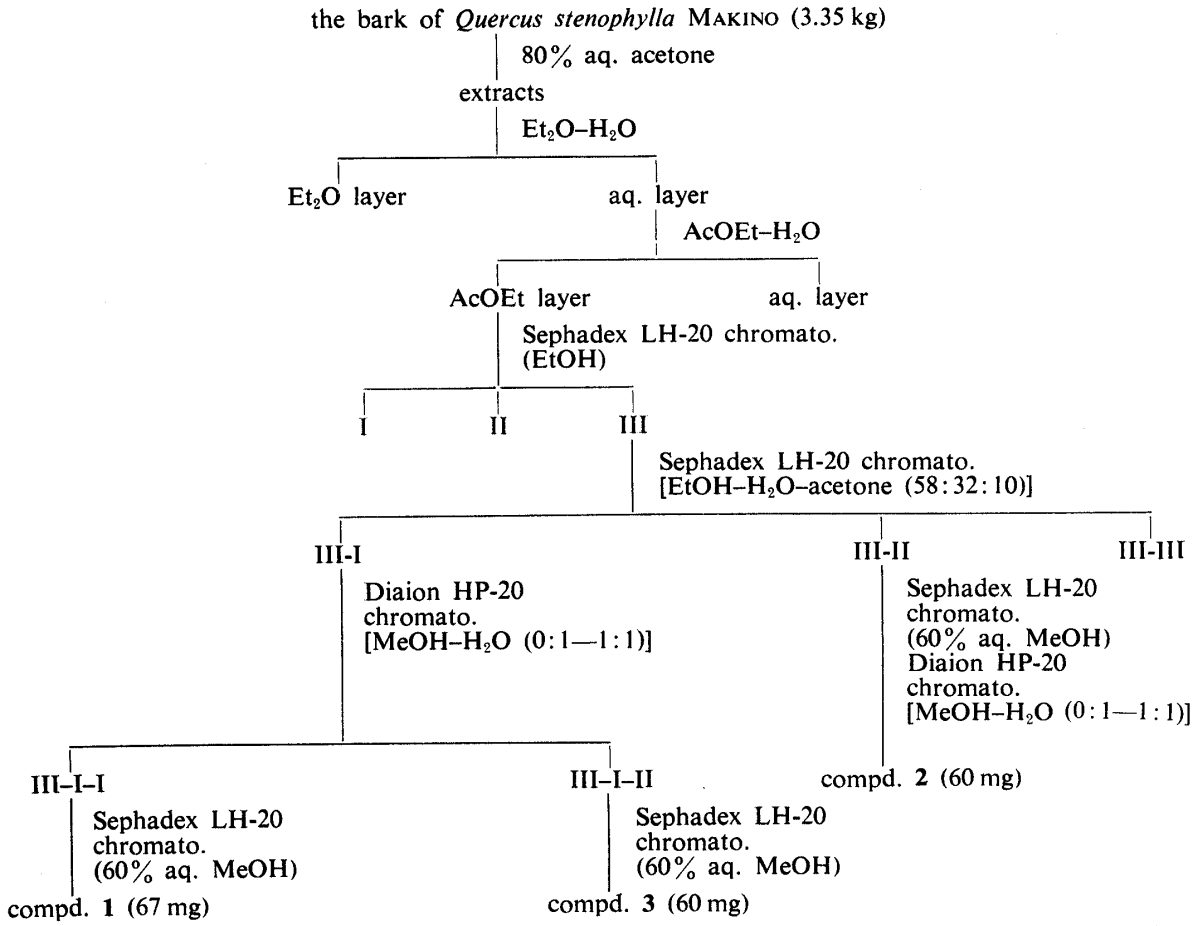
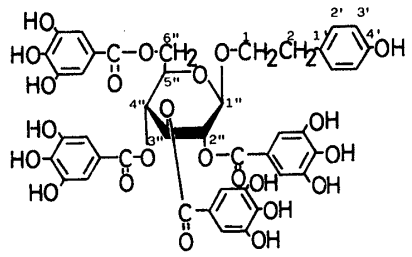
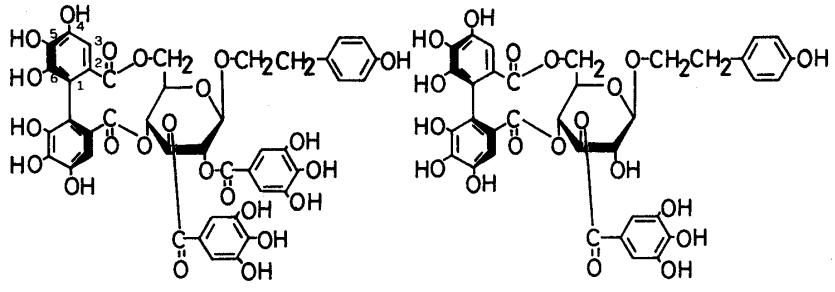


Chart 1



1

Chart 2



2 **3**

Chart 3

were all shifted downfield as compared with those of salidroside, whereas the chemical shifts for the aromatic proton signals in the aglycone moiety were almost the same. On the basis of the evidence mentioned above, the structure of **1** was characterized as 2″,3″,4″,6″-tetra-*O*-galloyl salidroside.

Compound **2** (**2**), an off-white amorphous powder, $[\alpha]_D$ +43.2° (acetone), $C_{42}H_{34}O_{23} \cdot 7/2H_2O$, emitted strong blue fluorescence under ultraviolet illumination on a thin-layer chromatography (TLC) plate. The ^{1}H-NMR spectrum exhibited aliphatic proton signals similar to those observed in **1**. An A_2B_2-type aromatic signal pattern (δ 6.62, 6.98, each 2H, d, J=8 Hz) was also observed, indicating the presence of a *p*-substituted aromatic ring. The occurrence of two galloyl groups and one hexahydroxydiphenoyl group in **2** was shown respectively by the appearance of two two-proton singlets (δ 6.94, 7.07) and a set of two aryl proton singlets (δ 6.44, 6.66). This was further supported by an examination of the ^{13}C-NMR spectrum which showed four ester carbon signals (δ 166.1, 167.0, 168.0, 168.7), together with aromatic carbon signals analogous to those reported previously.[5] Alkaline methanolysis of **2** with sodium methoxide in methanol afforded salidroside (**1a**) as the sole isolable product, while on enzymatic hydrolysis with tannase, **2** yielded gallic acid and a hydrolysate (**2a**). The ^{1}H-NMR spectrum of **2a** showed two aromatic singlets (δ 6.62, 6.75) due to the hexahydroxydiphenoyl group, along with a double doublet (δ 5.17, J=14, 7 Hz) and a triplet (δ 4.83, J=8 Hz), which were both assignable to the glucose protons attached to the carbons bearing the hexahydroxydiphenoyl group. The former glucose signal could be attributed to one of the C(6)-methylene protons owing to its large geminal coupling constant. The assignment of the latter triplet signal was achieved by means of spin-decoupling techniques as follows. Irradiation at the frequency of the anomeric proton doublet (δ 4.45, J=8 Hz) caused a change of another triplet appearing at higher field (δ 3.39, J=8 Hz) into a doublet, thus permitting the assignment of this upfield triplet to the C(2)-proton. On subsequent irradiation of this C(2)-proton signal, the triplet in question did not change, and hence this signal was assigned to the C(4)-proton. Accordingly, the structure of **2a** was characterized as 4″,6″-hexahydroxydiphenoyl salidroside.

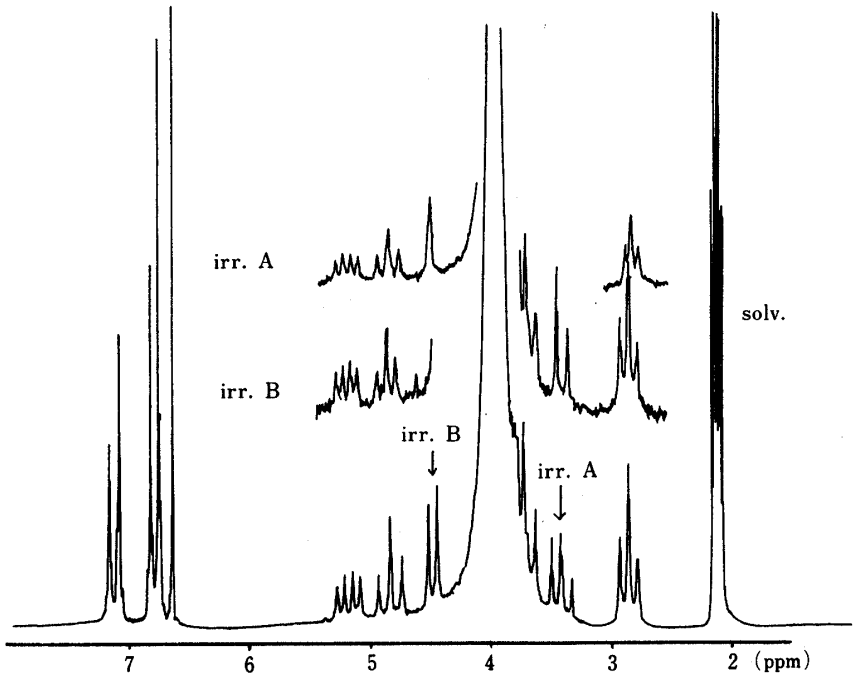
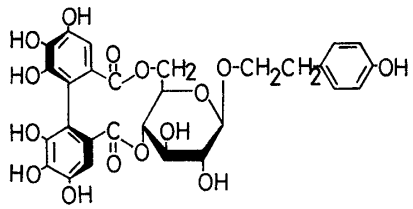


Fig. 1. ^{1}H-NMR Spectrum (Spin-decoupled) of Compound **2a** (in Acetone-d_6)
"irr." indicates irradiation.



2a

Chart 4

The locations of two galloyl groups in **2** were concluded to be at the C(2)- and C(3)-positions in the sugar moiety, since the ^{1}H-NMR spectrum of **2** exhibited two lowfield triplets (δ 5.32, $J=8$ Hz and δ 5.64, $J=8$ Hz) corresponding to these protons. The chirality of the hexahydroxydiphenoyl group was determined to be in the *S*-series on the basis of the circular dichroism (CD) properties of **2a**; the spectrum showed a negative Cotton effect at 263 nm ($\theta=-9.4\times10^4$) and a positive one at 286 nm ($\theta=+5.8\times10^4$), which were analogous to those observed in nupharin A[6] and sanguiins.[5] Based on these chemical and spectroscopic findings, compound **2** was assigned the formula (**2**).

Compound **3** (**3**), a white powder, mp 207—210 °C, $[\alpha]_D$ −9.1° (acetone), $C_{35}H_{30}O_{19}\cdot3H_2O$, gave a ^{1}H-NMR spectrum which was similar to that of **2** except for the presence of one galloyl group (δ 7.00) and an upfield shift of a triplet signal. Partial hydrolysis of **3** with tannase yielded gallic acid and **2a**, thus confirming chemically the presence of a salidroside moiety in the molecule as well as the location of an (*S*)-hexahydroxydiphenoyl group at the C(6′)- and C(4′)-positions. The galloyl group in **3** was shown to be attached to the C(3′)-position by ^{1}H-NMR spectroscopy combined with spin-decoupling. Upon irradiation at the frequency of the anomeric proton doublet (δ 4.58, $J=8$ Hz), the above-mentioned triplet changed into a doublet, indicating that this signal was due to the C(2′)-proton and that the C(2′)-position was free from the galloyl group. On the basis of these results, the structure of compound **3** was represented by the formula (**3**).

The glucopyranose ring in compounds **1**, **2** and **3** was considered from the ^{1}H-NMR *J*-values[7,8] for each sugar proton to adopt the $^4C_1$ conformation. This is the first report of the isolation of ellagitannins containing a glycoside core.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL FX-100 spectrometer (a JEOL PS-100 spectrometer for ^{1}H-NMR was also used in some cases), with tetramethylsilane as an internal standard, and chemical shifts are given on a δ (ppm) scale. IR and CD spectra were obtained with JASCO IR-G and JASCO J-20 spectrometers, respectively. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), Kieselgel 60 (70—230 mesh, Merck) and Diaion HP-20 AG (75—150 μ, Mitsubishi Chemical Industries Ltd.). TLC was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck) and cellulose F_{254} plates (0.10 mm, Merck), and spots were detected by spraying 2% methanolic ferric chloride and 10% H_2SO_4 reagents.

Isolation——Fraction III,[3] obtained previously from the ethyl acetate-soluble portion of the air-dried bark (3.35 kg) of *Quercus stenophylla* MAKINO, was subjected to Sephadex LH-20 column chromatography. Elution with the solvent system EtOH–H_2O–acetone (58 : 32 : 10)[9] afforded three fractions; fr. III-I (4.0 g), fr. III-II (6.7 g) and fr. III-III (6.1 g). Subsequent separation of fr. III-I on a Diaion HP-20 column with an increasing amount of MeOH in H_2O (0 : 1—1 : 1) gave two fractions; fr. III-I-I (210 mg) and fr. III-I-II (207 mg), the former containing compound **1** and the latter compound **3**. Purification of these compounds were achieved by chromatography over Sephadex LH-20 with 60% aqueous MeOH to yield compounds **1** (67 mg) and **3** (60 mg). Fr. III-II was repeatedly chromatographed over Sephadex LH-20 with 60% aqueous MeOH and Diaion HP-20 with a mixture of MeOH–H_2O (0 : 1—1 : 1) to give compound **2** (60 mg).

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**, **2**, **3** and **1a** (Acetone-d_6 + D_2O)

	1a	**1**	**2**	**3**
Glucose				
1	104.6	101.5	101.9	104.5
2	74.9	73.7	78.3[a]	73.1[c]
3	77.9	72.5	71.8[a]	76.0[c]
4	71.4	70.0	71.1[a]	71.1[c]
5	77.7	72.8	72.6[a]	71.9[c]
6	62.6	63.2	63.5	63.7
Aglycone				
1	71.4	71.7	71.7	71.9
2	35.7	35.7	35.6	35.9
1′	129.7	129.9	129.9	129.9
2′	130.3	130.6	130.6	130.7
3′	115.5	115.8	115.8	116.0
4′	155.9	156.2	156.1	156.5
Galloyl				
1		119.9	120.0	120.9
		120.0	120.5	
		120.5		
		120.9		
2		110.0 (4C)	110.1 (2C)	110.2
3		146.0 (4C)	145.7[b]	145.8[d]
			145.9[b]	
4		139.2	139.4 (2C)	139.1
		139.3 (2C)		
		139.6		
Hexahydroxydiphenoyl				
1			115.8 (2C)	116.0 (2C)
2			125.5	125.8
			126.1	126.2
3			107.8	107.9 (2C)
			108.1	
4, 6			144.4[b]	144.4[d]
			145.2[b]	145.3[d]
5			136.4	136.4
			136.6	136.5
–COO–		166.0	166.1	167.4
		166.2	167.0	168.0
		166.6	168.0	168.7
		167.0	168.7	

Assignments with the superscript *a*), *b*), *c*) or *d*) may be interchanged in each column.

Compound **1** (**1**): An off-white amorphous powder, $[\alpha]_D^{21}$ −76.0° (c=0.24, MeOH). *Anal.* Calcd for $C_{42}H_{36}O_{23}\cdot 7/2H_2O$: C, 51.91; H, 4.46. Found: C, 51.97; H, 4.42. ^{1}H-NMR (acetone-d_6): 2.73 [2H, t, J=7 Hz, C(2)–H], 3.70 [1H, dd, J=8, 6 Hz, C(5′′)–H], 3.90 [2H, t, J=7 Hz, C(1)–H], 4.37 [1H, dd, J=14, 6 Hz, C(6′′)–H], 4.55 [1H, dd, J=14, 2 Hz, C(6′′)–H], 5.06 (1H, d, J=8 Hz, anomeric H), 5.34 [1H, t, J=7 Hz, C(2′′)–H], 5.51 [1H, t, J=8 Hz, C(4′′)–H], 5.82 [1H, t, J=8 Hz, C(3′′)–H], 6.60, 6.96 [each 2H, d, J=8 Hz, C(2′)– and C(3′)–H], 6.93, 7.02, 7.08, 7.19 (each 2H, s, galloyl H). ^{13}C-NMR: Table I.

Compound **2** (**2**): An off-white amorphous powder, $[\alpha]_D^{20}$ +43.2° (c=0.1, acetone), *Anal.* Calcd for $C_{42}H_{34}O_{23}\cdot 7/2H_2O$: C, 52.02; H, 4.26. Found: C, 52.18; H, 4.28. ^{1}H-NMR (acetone-d_6): 2.72 [2H, t, J=7 Hz, C(2)–H], 3.6—4.1 [3H, m, C(1)– and C(6)–H], 4.26 [1H, dd, J=8, 6 Hz, C(5′′)–H], 4.94 (1H, d, J=8 Hz, anomeric H), 5.12 [1H, t, J=8 Hz, C(4′′)–H], 5.32 [1H, d, J=8 Hz, C(2′′)–H], 5.33 [1H, dd, J=14, 6 Hz, C(6′′)–H], 5.64 [1H, t, J=8 Hz, C(3′′)–H], 6.44, 6.66 (each 1H, s, HHDP[10]–H), 6.62, 6.98 [each 2H, d, J=8 Hz, C(2′)– and C(3′)–H], 6.94, 7.07 (each 2H, s, galloyl H). ^{13}C-NMR: Table I.

Compound **3** (**3**): A white powder, mp 207—210 °C, $[\alpha]_D^{21}$ −9.1° ($c=0.33$, acetone), *Anal.* Calcd for $C_{35}H_{30}O_{19}\cdot 3H_2O$: C, 51.98; H, 4.48. Found: C, 52.07; H, 4.44. ^{1}H-NMR (acetone-d_6): 2.84 [2H, t, $J=7$ Hz, C(2)-H], 3.62 [1H, t, $J=8$ Hz, C(2″)-H], 3.6—4.2 [4H, m, C(1)-, C(5″)- and C(6″)-H]], 4.58 (1H, d, $J=8$ Hz, anomeric H), 4.96 [1H, t, $J=8$ Hz, C(4″)-H], 5.34 [1H, t, $J=8$ Hz, C(3″)-H], 5.26 [1H, dd, $J=14$, 6 Hz, C(6″)-H], 6.41, 6.63 (each 1H, s, HHDP-H), 6.74, 7.10 [each 2H, d, $J=8$ Hz, C(2′)- and C(3′)-H], 7.00 (2H, s, galloyl H). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 1 with Tannase——A solution of **1** (44 mg) in H_2O was treated with tannase at room temperature for 3 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was treated with MeOH. The MeOH-soluble portion was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to furnish gallic acid (15 mg) and **1a** (10 mg), a white amorphous powder, $[\alpha]_D^{21}$ −25.4° ($c=0.8$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3280 (OH), no ester absorption. ^{1}H-NMR (acetone-d_6+D_2O): 2.32 [2H, t, $J=7$ Hz, C(2)-H], 2.1—4.1 [8H, m, C(1)-, C(2″)-, C(3″)-, C(4″)-, C(5″)- and C(6″)-H], 4.28 (1H, d, $J=8$ Hz, anomeric H), 6.70, 7.08 [each 2H, d, $J=8$ Hz, C(2′)- and C(3′)-H]. **1a** was shown to be identical with salidroside by direct comparison of their physical and spectral data.[3]

Alkaline Methanolysis of 2——**2** (15 mg) was treated with 2% NaOMe in MeOH at room temperature for 30 min. The reaction mixture, after neutralization with Dowex 50W-X8 (H^+ −form), was concentrated to dryness *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to furnish salidroside (**1a**) (4 mg).

Enzymatic Hydrolysis of 2 with Tannase——A solution of **2** (35 mg) in H_2O was incubated at 37 °C for 1 h. The reaction mixture was treated in the same way as described for **1** to give a mixture of products, which was separated by chromatography over Sephadex LH-20. Elution with EtOH furnished gallic acid (6 mg) and **2a** (10 mg), an off-white amorphous powder, $[\alpha]_D^{20}$ −49.1° ($c=0.29$, MeOH). ^{1}H-NMR (acetone-d_6+D_2O): 2.83 [2H, t, $J=7$ Hz, C(2)-H], 3.39 [1H, t, $J=8$ Hz, C(2″)-H], 3.5—4.1 [5H, C(1)-, C(3″)-, C(5″)- and C(6″)-H], 4.45 (1H, d, $J=8$ Hz, anomeric H), 4.83 [1H, t, $J=8$ Hz, C(4″)-H], 5.17 [1H, dd, $J=14$, 7 Hz, C(6″)-H], 6.62, 6.75 (each 1H, s, HHDP-H), 6.76, 7.06 [each 2H, d, $J=8$ Hz, C(2′)- and C(3′)-H]. CD ($c=2.1\times10^{-4}$, MeOH) $[\theta]^{20}$(nm): $+2.97\times10^4$ (239) (positive maximum), -0.94×10^4 (263) (negative maximum), $+0.58\times10^4$ (286) (positive maximum).

Alkaline Methanolysis of 3——A solution of **3** (3 mg) in MeOH containing 2% NaOMe was allowed to stand at room temperature for 30 min. The reaction mixture was treated in the same manner as described for **2** to give a product, which was shown to be identical with salidroside (**1a**) by TLC analysis [*Rf* 0.20; benzene–ethyl acetate–formic acid (2 : 7 : 1)].

Enzymatic Hydrolysis of 3 with Tannase——A solution of **3** (36 mg) in H_2O was incubated with tannase at 37 °C for 1 h. Work-up as before yielded the products, which were subjected to Sephadex LH-20 chromatography to furnish gallic acid (4 mg) and **2a** (12 mg).

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10) HHDP=hexahydroxydiphenoyl.