Phenolic Glycosides from the Leaves of *Alangium platanifolium* var. *platanifolium*

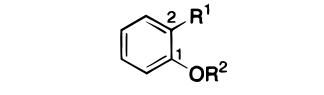
Akie Tamaki, Toshinori Ide, and Hideaki Otsuka*

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

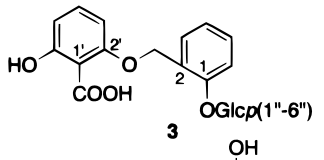
Received March 8, 2000

Chemical investigation of *Alangium platanifolium* var. *platanifolium* has resulted in the isolation of nine phenolic glycosides that were identified by means of 1D and 2D NMR experiments. Among them, catechol and salicinol *O*- and 1-*O*-*β*-D-(6-*O*-*β*-D-apiofuranosyl)glucopyranosides, respectively **1** and **2**, and two compounds characterized as adducts of 2,6-dihydroxybenzoic acid with salicin (plataplatanoside, **3**) and 4-hydroxysalicin (4-hydroxyalangifolioside, **4**) were determined structurally as new compounds.

In a previous paper, the isolation was reported of three new megastigmane diglycosides, alangionosides A−C, from the leaves of *Alangium platanifolium* (Sieb. et Zucc.) Harms var. *platanifolium* (Alangiaceae), endemic to the western area of Japan.[1] Subsequent chemical investigation has afforded five known and four new phenolic glycosides (**1**−**4**). This paper deals with the structure elucidation of these new compounds.



1 R^1=OH, R^2=Glc*p*(6'-1")Api*f*
2 R^1=CH_2OH, R^2=Glc*p*(6'-1")Api*f*



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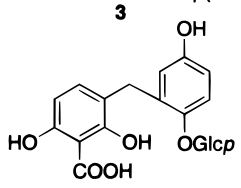

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The air-dried plant material was extracted exhaustively with MeOH. The extract was defatted, and then extracted successively with ethyl acetate and *n*-BuOH. The dried *n*-BuOH-soluble fraction was separated by means of various chromatographic methods to yield nine phenolic glycosides. In addition to the new compounds, **1**−**4**, five known compounds isolated were identified spectroscopically as salicin,[2] henryoside,[2,3] henryoside 6-*O*-*β*-D-glucopyranoside,[4] alangifolioside,[2] and demethylalangiside.[5]

Compound **1** was isolated as an amorphous powder, with a composition of $C_{17}H_{24}O_{11}$, as determined by negative-ion HRFABMS. The IR and UV spectra indicated that **1** was a phenolic glycoside. The ^{1}H NMR spectrum showed the presence of four aromatic protons (δ_C 6.79, 6.84, 6.91, and 7.18) and two characteristic anomeric protons [δ_H 4.79 (d, J = 8 Hz) and 5.00 (d, J = 2 Hz)]. The ^{13}C NMR spectrum showed five signals for a terminal *β*-apiofuranoside unit, six signals for a 6-substituted *β*-glucopyranoside unit,[6] and six signals for an aromatic ring, two of which bear hydroxyl substituents. From this evidence, the structure of **1** was elucidated as catechol *O*-*β*-D-(6′-*O*-*β*-D-apiofuranosyl)glucopyranoside.

Table 1. ^{13}C NMR Data for Compounds **1**−**4** (CD_3OD, 100 MHz)

carbon	**1**	**2**	**3**	**4**
1	148.6	157.2	156.2	150.4
2	146.9	132.4	129.0	133.5
3	117.3	129.9	129.8	117.7
4	125.0	124.0	123.7	153.6
5	121.2	130.1	129.5	114.3
6	119.4	117.6	116.6	118.6
7		61.1	67.3	29.7
1′	104.6	103.7	111.8	101.1
2′	75.0	75.1	161.1	160.6
3′	77.7	78.1	105.7	127.7
4′	71.7	71.7	132.5	138.7
5′	77.2	77.2	110.4	107.8
6′	68.8	68.9	163.5	159.9
7′			174.8	173.4
1″	111.2	111.2	103.1	104.2
2″	78.2	78.2	75.0	75.2
3″	80.6	80.5	78.2	78.3
4″	75.1	75.1	71.5	71.6
5″	65.8	65.7	78.1	78.1
6″			62.6	62.7

Compound **2** was isolated as an amorphous powder, whose elemental composition was determined to be $C_{18}H_{26}O_{11}$ in a similar manner to **1**. The ^{1}H and ^{13}C NMR spectra showed that the aglycon portion was salicinol[2] (Table 1), and the sugar moiety was the same as that of compound **1**. Therefore, the structure of **2** was determined to be the 6′-*O*-*β*-D-apiofuranoside of salicin.

Plataplatanoside (**3**) was also isolated as an amorphous powder, and its elemental composition was determined to be $C_{20}H_{22}O_{10}$. The IR spectrum indicated the presence of hydroxyl groups (3384 cm^{-1}) and aromatic ring(s) (1620, 1597, and 1462 cm^{-1}) in the molecule, and the UV absorption maxima also denoted one aromatic ring or more. The ^{13}C NMR spectrum showed the presence of six signals for one terminal *β*-glucopyranose moiety, with the remaining 14 signals representing two aromatic rings, one carboxyl group, and one hydroxymethyl group. The ^{1}H NMR spectrum showed the presence of seven aromatic protons, and the ^{1}H−^{1}H COSY spectrum confirmed the presence of two series of aromatic protons, one with three and the other

* To whom correspondence should be addressed. Tel.: +81 82 257 5335. Fax: +81 82 257 5335. E-mail: hide@pharm.hiroshima-u.ac.jp.

10.1021/np000119l CCC: $19.00 © 2000 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/12/2000

with four. From these results, together with those of the ^{13}C NMR and HSQC experiments, compound **3** was presumed to be an ether adduct of 2,6-dihydroxybenzoic acid and salicin, like henryoside and alangifolioside. The linkage position was expected to be between one of the hydroxyl groups of 2,6-dihydroxybenzoic acid and that of salicin through an ether bond, because the 2,6-dihydroxybenzoic acid moiety was substituted asymmetrically, and the carbon signals of C-2 and C-7 were shifted by −3.2 ppm and +6.2 ppm compared with those of salicin[2] (Table 1). The arrangement of the substutuents of **3** was finally confirmed by a HMBC experiment in which H_2-7a and -7b (δ_H 5.19 and 5.34) showed cross-peaks with C-2′ (δ_C 161.1), C-1 (δ_C 156.2), C-2 (δ_C 129.0), and C-3 (δ_C 129.8), and the anomeric proton (δ_H 4.95) correlated with C-1. Therefore, the structure of this isolate was elucidated as **3**.

Compound **4** was isolated as an amorphous powder with an elemental composition of $C_{20}H_{22}O_{11}$, which is one oxygen atom more than alangifolioside. The spectroscopic data indicated that **4** was similar to alangifolioside[2] (Table 1), and the ^1H NMR spectrum showed two protons [δ_H 6.36 (d, J = 8 Hz) and 7.21 (d, J = 8 Hz)] on one of the aromatic rings and three protons coupled in an ABX system on the other aromatic ring. The ^{13}C NMR spectrum showed the presence of four aromatic carbon signals with hydroxyl substituents. From this evidence, compound **4** could be assigned as alangifolioside substituted with an additional hydroxyl group on the salicin moiety. The substitution pattern was confirmed by a HMBC experiment in which H-3 [δ_H 6.51 (d, J = 3 Hz)] showed cross-peaks with C-7 (δ_C 29.7) and the anomeric proton [δ_H 4.78 (d, J = 8 Hz)] C-1 (δ_C 150.4). Therefore, the structure of **4** was concluded to be 4-hydroxyalangifolioside.

From the biosynthetic point of view, it is interesting that three adducts of 2,6-dihydroxybenzoic acid and salicin, henryoside, alangifolioside, and plataplatanoside (**3**), were isolated together in this investigation. Henryoside is an ester of both building units, while alangifolioside and plataplatanoside (**3**) are probably the electrophilic adducts of the salicin cation (or corresponding saligenol cation) at the 3-position and the phenolic hydroxyl group of 2,6-dihydroxybenzoic acid, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a Union Giken PM-101 digital polarimeter. FTIR and UV spectra were recorded on a Shimadzu FTIR-4200 and Shimadzu UV-160A spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were measured on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as internal standard. HRFABMS (negative-ion mode) were obtained with a JEOL JMS−SX-102 mass spectrometer with PEG-400 as the matrix.

Reversed-phase gravity column chromatography was performed on Cosmosil 75C_{18}−OPN (Nakalai Tesque, Kyoto, Japan) (Φ = 40 mm, L = 25 cm) with a linear gradient solvent system [H_2O−MeOH (9:1, 1 L) → (1:1, 1 L); with fractions of 10 g being collected]. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm). The ascending method was used with $CHCl_3$−MeOH−H_2O−*n*-PrOH (9:12:8:2) as solvent, and 5-g fractions were collected and numbered according to the order of elution of the mobile phase. Preparative HPLC was performed on an Inertsil column (GL Science, Tokyo, Japan) (ODS, Φ = 20 mm, L = 25 cm), with the flow rate being 6 mL/min and detection being performed by UV at 254 nm.

Extraction and Isolation. The air-dried leaves of *A. platanifolium* var. *platanifolium* (Alangiaceae)[1] (3.55 kg) were extracted with MeOH (12 L × 2). To the concentrated MeOH extract (1.5 L) was added 75 mL of H_2O, followed by washing with *n*-hexane (1.5 L). The MeOH layer was concentrated to a dark green mass and then suspended in 1.5 L of H_2O. The suspension was extracted successively with EtOAc (1.5 L) and *n*-BuOH (1.5 L) to give dried residues of 15.8 and 65.9 g of EtOAc-soluble and *n*-BuOH-soluble fractions, respectively. The *n*-BuOH-soluble fraction was subjected to passage over a column of a highly porous synthetic resin (Diaion HP-20; Mitsubishi Kagaku, Tokyo, Japan), with 20% (6 L), 40% (6 L), 60% (6 L), and 80% (6 L) MeOH in H_2O, and MeOH (6 L) as the eluents. Fractions of 2 L were collected. The residue (14.9 g) from the 40% MeOH eluate was subjected to Si gel (300 g) column chromatography by gradient elution with $CHCl_3$ and MeOH [$CHCl_3$ (1 L); $CHCl_3$−MeOH (99:1, 2 L), (49:1, 2 L), (24:1, 4 L), (93:7, 4 L), (9:1, 4 L), (17:3, 4 L), (4:1, 4 L), (3:1, 4 L), and (7:3, 4 L); fractions of 500 mL were collected]. The residue (1.52 g in fractions 31−37) from the 7% MeOH eluate was then subjected to reversed-phase column chromatography (287 mg in fractions 105−120) and then to DCCC to give 28 mg of **2** in fractions 45−55.

The residue (2.01 g in fractions 59−80) of the 10−15% MeOH eluate obtained on Si gel column chromatography was subjected to reversed-phase column chromatography, and then the residue (65 mg in fractions 41−50) was purified by DCCC to give 25 mg of **4** in fractions 3−9. The residue (158 mg in fractions 61−70) was subjected DCCC to give 37 mg of henryoside 6′-*O*-*β*-D-glucopyranoside in fractions 4−8 and 67 mg of crude **2** in fractions 16−22. The latter was finally purified by preparative HPLC [H_2O−MeOH (17:3)] to give 42 mg of **2**. The residue (59 mg in fractions 71−76) was purified by DCCC to give 30 mg of **1** in fractions 21−26.

The 60% MeOH residue (9.20 g) from the 60% MeOH eluate obtained on Diaion HP-20 column chromatography was subjected to Si gel (250 g) column chromatography by gradient elution with $CHCl_3$ and MeOH [$CHCl_3$ (1 L), $CHCl_3$−MeOH (99:1, 2 L), (49:1, 2 L), (24:1, 2 L), (47:3, 2 L), (23:2, 2 L), (9:1, 2 L), (7:1, 2 L), (17:3, 2 L), (4:1, 2 L), (3:1, 2 L), and (7:3, 2 L); fractions of 500 mL were collected]. The residue (772 mg in fractions 30−32) of the 8% MeOH eluate was successively separated by reversed-phase column chromatography (93 mg in fractions 122−127) and DCCC to give 28 mg of demethylalangiside in fractions 44−50.

The residue (10.6 g) from the 80% MeOH eluate obtained on Diaion HP-20 column chromatography was subjected to Si gel (300 g) column chromatography by gradient elution with $CHCl_3$ and MeOH [$CHCl_3$ (1 L), $CHCl_3$−MeOH (99:1, 2 L), (49:1, 2 L), (24:1, 2 L), (47:3, 2 L), (23:2, 2 L), (9:1, 2 L), (7:1, 2 L), (17:3, 2 L), (4:1, 2 L), (3:1, 2 L), and (7:3, 2 L); fractions of 500 mL were collected]. The residue (1.46 g in fractions 24−32) of the 8% MeOH eluate was purified by reversed-phase column chromatography to give 119 mg of salicin in fractions 51−62. The residue (1.69 g in fractions 42−48) of the 12.5% MeOH eluate was subjected to reversed-phase column chromatography, and the resultant subfractions [fractions 59−70 (68 mg) and fraction 105−116 (61 mg)] were then purified by two separate DCCC runs to give 42 mg of **3** in fractions 8−12 and 43 mg of alangiside in fractions 12−18, respectively.

Catechol *O*-β-D-(6′-*O*-β-D-apiofuranosyl)glucopyranoside (1): amorphous powder; $[\alpha]^{29}_D$ −85.9° (*c* 0.76, MeOH); IR (KBr) ν_{max} 3422, 2930, 1601, 1503, 1269, 1068, 754 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 204 (3.87), 274 (3.29) nm; ^1H NMR (CD_3OD) δ 3.59 (2H, s, H_2-4″), 3.65 (1H, dd, *J* = 6, 11 Hz, H-6′a), 3.76 (1H, d, *J* = 10 Hz, H-5″a), 3.92 (1H, d, *J* = 2 Hz, H-2″), 3.98 (1H, d, *J* = 10 Hz, H-5″b), 4.02 (1H, dd, *J* = 2, 11 Hz, H-6′b), 4.70 (1H, d, *J* = 8 Hz, H-1′), 5.00 (1H, d, *J* = 2 Hz, H-1″), 6.79 (1H, ddd, *J* = 2, 7, 8 Hz, H-4), 6.84 (1H, dd, *J* = 2, 8 Hz, H-6), 6.91 (1H, ddd, *J* = 2, 7, 8 Hz, H-5), 7.18 (1H, dd, *J* = 2, 8 Hz, H-3); ^{13}C NMR (CD_3OD), see Table 1; HRFABMS (negative-ion mode) *m*/*z* 403.1222 $[M - H]^-$ (calcd for $C_{17}H_{23}O_{11}$, 403.1240).

Salicin 6′-*O*-β-D-apiofuranoside (2): amorphous powder; $[\alpha]^{29}_D$ −61.6° (*c* 0.76, MeOH); IR (KBr) ν_{max} 3407, 2928, 1601, 1493, 1458, 1237, 1068, 760 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 212 (3.77), 269 (3.88) nm; ^1H NMR (CD_3OD) δ 3.45 (1H, t, *J* = 9 Hz, H-3′), 3.50 (1H, dd, *J* = 8, 9 Hz, H-2′), 3.57 (2H, s, H_2-4″), 3.63 (1H, dd, *J* = 7, 11 Hz, H-6′a), 3.74 (1H, d, *J* = 10 Hz, H-5″a), 3.90 (1H, d, *J* = 2 Hz, H-2″), 3.95 (1H, d, *J* = 10 Hz, H-5″b), 4.02 (1H, dd, *J* = 2, 11 Hz, H-6′b), 4.59 (1H, d, *J* = 13 Hz, H-7a), 4.76 (1H, d, *J* = 13 Hz, H-7b), 4.82 (1H, d, *J* = 8 Hz, H-1′), 4.98 (1H, d, *J* = 2 Hz, H-1″), 7.04 (1H, dt, *J* = 1, 8 Hz, H-4), 7.21 (1H, dd, *J* = 1, 8 Hz, H-6), 7.28 (1H, dt, *J* = 2, 8 Hz, H-5), 7.34 (1H, dd, *J* = 2, 8 Hz, H-3); HRFABMS (negative-ion mode) *m*/*z* 417.1407 $[M - H]^-$ (calcd for $C_{18}H_{25}O_{11}$, 417.1379).

Plataplatanoside (3): $[\alpha]^{29}_D$ −18.9° (*c* 0.85, MeOH); IR (KBr) ν_{max} 3384, 2944, 1620, 1597, 1462, 1381, 1221, 1084, 1042, 754 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 212 (4.36), 248 (3.67), 274 (3.20), 303 (3.48) nm; ^1H NMR (CD_3OD) δ 3.72 (1H, dd, *J* = 5, 12 Hz, H-6″a), 3.89 (1H, dd, *J* = 2, 12 Hz, H-6″b), 4.95 (1H, d, *J* = 8 Hz, H-1″), 5.19 (1H, d, *J* = 14 Hz, H-7a), 5.34 (1H, d, *J* = 14 Hz, H-7b), 6.42 (1H, dd, *J* = 1, 8 Hz, H-5′), 6.48 (1H, dd, *J* = 1, 8 Hz, H-3′), 7.01 (1H, dt, *J* = 2, 8 Hz, H-4), 7.07 (1H, t, *J* = 8 Hz, H-4′), 7.19 (1H, dd, *J* = 2, 8 Hz, H-6), 7.23 (1H, dt, *J* = 2, 8 Hz, H-5), 7.64 (1H, dd, *J* = 2, 8 Hz, H-3); HRFABMS (negative-ion mode) *m*/*z* 421.1147 $[M - H]^-$ (calcd for $C_{20}H_{21}O_{10}$, 403.1134).

4-Hydroxyalangifolioside (4): $[\alpha]^{29}_D$ −7.1° (*c* 1.40, MeOH); IR (KBr) ν_{max} 3420, 1674, 1626, 1490, 1449, 1344, 1213, 1074, 1040, 814 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 208 (4.30), 269 (3.17), 292 (3.47), 310 (3.44) nm; ^1H NMR (CD_3OD) δ 3.61 (1H, dd, *J* = 5, 12 Hz, H-6″a), 3.70 (1H, dd, *J* = 2, 12 Hz, H-6″b), 3.90 (1H, d, *J* = 16 Hz, H-7a), 3.96 (1H, d, *J* = 16 Hz, H-7b), 4.78 (1H, d, *J* = 8 Hz, H-1″), 6.36 (1H, d, *J* = 8 Hz, H-5′), 6.51 (1H, d, *J* = 3 Hz, H-3), 6.56 (1H, dd, *J* = 3, 8 Hz, H-5), 7.04 (1H, d, *J* = 8 Hz, H-6), 7.21 (1H, d, *J* = 8 Hz, H-4′); HRFABMS (negative-ion mode) *m*/*z* 437.1101 $[M - H]^-$ (calcd for $C_{20}H_{21}O_{11}$, 403.1084).

Known compounds isolated: salicin, colorless crystals, mp 201−204 °C, $[\alpha]^{31}_D$ −39.2° (*c* 0.74, MeOH); henryoside, colorless crystals, mp 133−135 °C, $[\alpha]^{31}_D$ −36.7° (*c* 0.90, pyridine); henryoside 6′-*O*-β-D-glucopyranoside, amorphous powder, $[\alpha]^{23}_D$ −36.9° (*c* 2.52, MeOH); alangifolioside, colorless needles, mp 125−128 °C, $[\alpha]^{31}_D$ −16.9° (*c* 0.83, pyridine); demethylalangiside, colorless crystals, mp 180−182 °C, $[\alpha]^{31}_D$ −72.6° (*c* 0.84, MeOH).

Acknowledgment. The authors are grateful for the access to the superconducting NMR instrument in the Analytical Center of Molecular Medicine of Hiroshima University Faculty of Medicine.

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NP000119L