JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Structural Elucidation of Some Glycosides from the Rhizomes of Smaller Galanga (*Alpinia officinarum* Hance)

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Glycosidically bound compounds were isolated from the methanol extract of fresh rhizomes of smaller galanga (*Alpinia officinarum* Hance). Nine glycosides (**1**−**9**) were finally obtained by reversed-phase HPLC and their structures were elucidated by MS and NMR analyses. They were the three known glycosides, (1*R*,3*S*,4*S*)-*trans*-3-hydroxy-1,8-cineole β-D-glucopyranoside (**1**), benzyl β-D-glucopyranoside (**3**), and 1-*O*-β-D-glucopyranosyl-4-allylbenzene (chavicol β-D-glucopyranoside, **4**); and the six novel glycosides, 3-methyl-but-2-en-1-yl β-D-glucopyranoside (**2**), 1-hydroxy-2-*O*-β-D-glucopyranosyl-4-allylbenzene (**5**), 1-*O*-β-D-glucopyranosyl-2-hydroxy-4-allylbenzene (demethyleugenol β-D-glucopyranoside, **6**), 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-2-hydroxy-4-allylbenzene (demethyleugenol β-rutinoside, **7**), 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-4-allylbenzene (chavicol β-rutinoside, **8**), and 1,2-di-*O*-β-D-glucopyranosyl-4-allylbenzene (**9**). Compounds **2**−**9** were detected for the first time as constituents of galanga rhizomes.

KEYWORDS: Glycoside; chavicol β-D-glucopyranoside; chavicol β-D-rutinoside; galanga; *Alpinia officinarum* Hance; Zingiberaceae

INTRODUCTION

Galanga (also called galangal, galingale, or galangale) is a species of the ginger family (Zingiberaceae). There are two different species of galanga, smaller galanga (*Alpinia officinarum* Hance) and greater galanga (*Alpinia galanga* Willd.) (*1*). The rhizomes of greater galanga are widely used as a spice or a ginger substitute for flavoring food throughout southeastern Asian countries. On the other hand, smaller galanga is cultivated in Vietnam and southern China, because of the use of its rhizome as a spice (fresh rhizomes) and as a traditional medicine (dried rhizomes). In recent years, it has been reported that galanga has some biological activities, including antitumor, antiulcer, antibacterial, and antifungal properties (*2*−*5*). The essential oil composition in greater galanga rhizomes has been reported by some workers (*6*−*9*). Mori et al. (*8*) reported that 1,8-cineole, linalool, geranyl acetate, and eugenol were important potent odorants. In addition, the essential oil in the rhizomes of smaller galanga has been studied (*10*, *11*). We previously reported the identification of volatile components of the essential oils in fresh and dried rhizomes of smaller galanga (*11*). The major component of the essential oil in greater galanga, 1,8-cineole, is also the major one in smaller galanga, as expected because both plants belong to the same *Alpinia* genus.

The presence of glycosidically bound volatile compounds in plants has been well established (*12*). These compounds are able to release free aroma compounds by enzymatic or acidic hydrolysis and can be considered as aroma precursors. In the ginger family, Wu et al. (*13*) confirmed the existence of glycosidically bound aroma compounds in ginger (*Zingiber officinale* Roscoe). Sekiwa et al. (*14*, *15*) have isolated some glycosides related to aroma precursors from fresh rhizomes of ginger. In rhizomes of greater galanga, three glycosides of hydroxy-1,8-cineole were isolated and identified (*16*). Therefore, such glycosides would be expected to be present in smaller galanga. We now describe the isolation and structural elucidation of some glycosides in fresh rhizomes of smaller galanga.

MATERIALS AND METHODS

Plant Material. Fresh rhizomes of smaller galanga (*Alpinia officinarum* Hance) were purchased at a local market in Hanoi, Vietnam, and botanically authenticated at the National Center for Scientific Research Technology, Vietnam. The rhizomes were then stored at −20 °C until analysis.

Apparatus. Specific rotation was determined with a SEPA-300 polarimeter (Horiba, Kyoto, Japan). Ultraviolet (UV) spectrum was measured with a Jasco Ubest-30 UV/Vis spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with an INOVA 400 FTNMR spectrometer (Varian, Palo Alto, CA) with CD_3OD as the solvent and tetramethylsilane as the internal standard. ^{1}H NMR was performed at 400.0 MHz, and the ^{1}H−^{1}H chemical shift-correlated (COSY) NMR technique was employed to assign ^{1}H shifts and couplings. ^{13}C NMR

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10.1021/jf025529p CCC: $22.00
Published on Web 07/04/2002

was at 100.6 MHz with proton decoupling. ^{1}H detected heteronuclear multiple-bond correlation (HMBC) spectroscopy was used to assign correlations between ^{1}H and ^{13}C signals. Gas chromatography−mass spectrometry (GC−MS) was performed on a JMS-GC Mate II instrument (JEOL, Tokyo, Japan) operated in the electron impact mode (70 eV). The gas chromatograph was equipped with a 25 m × 0.22 mm i.d., 0.25 μm Hicap CBP-1 capillary column (Shimadzu Co., Kyoto, Japan). For the analysis of aglycon moiety, the column temperature was held at 50 °C for 2 min, and then raised to 210 °C at 3 °C/min, whereas the temperature was programmed from 160 to 220 °C at 3 °C/min for the analysis of alditol acetates. The carrier gas (helium) flow was at 0.8 mL/min. The obtained mass spectrum (MS) was compared with an authentic one from the library of the National Institute of Standards and Technology to confirm the compound. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were obtained with a Shimadzu LCMS-QP8000α instrument equipped with ESI and APCI sources, respectively. Sample was delivered into the ion source using methanol/water (7:3, v/v) containing 0.15% acetic acid at 0.1 mL/min.

Extraction and Isolation Procedures. Fresh rhizomes of galanga (4.0 kg) were chopped and homogenized in methanol and successively extracted three times with hot methanol for 3 h. After the solvent was removed, the methanol extract (260 g) was subjected to chromatography on a 20 × 6.0 cm Amberlite XAD-2 resin column. The column was rinsed with distilled water (3 L) to eliminate sugars, acids, and other water-soluble compounds, and the retained material was eluted with methanol (3 L). The methanol eluate was concentrated to dryness in vacuo. The residue (40 g) was then dissolved and suspended in water (250 mL) and partitioned with $CHCl_3$ (each 400 mL, 3×). The water layer was lyophilized to obtain the glycosidically bound fraction (30 g).

This fraction was subjected to chromatography on an 18 × 4.0 cm ODS-A 120-S 150 silica gel column (YMC Co., Ltd., Kyoto, Japan) with stepwise elution of methanol in water (10, 20, 30, 40, 50, and 100%; each 900 mL). Each fraction was checked by thin-layer chromatography (TLC) on silica gel 60, 0.25 mm thickness (Merck, Darmstadt, Germany) developed with chloroform/methanol/water (65:25:4, v/v). Spots on the TLC plate were visualized by charring after spraying with vanillin in H_2SO_4. Two main fractions, 20% methanol eluate (1.16 g) and 30% methanol eluate (2.17 g), were further subjected to chromatography on an 18 × 3.4 cm BW-820MH, 70−200 mesh silica gel column (Fuji Silysia Chemical Ltd., Kasugai, Japan), respectively. The column was sequentially eluted by increasing the methanol concentration in mixtures of chloroform/methanol. Fractions with the same TLC patterns were pooled to give five fractions (I−V): fraction I (320 mg) was obtained from the 20% methanol eluate with elution of chloroform/methanol (90:10, v/v); whereas fractions II−V were obtained from the 30% methanol eluate: II (90.3 mg) with chloroform/methanol (95:5, v/v), III (73.5 mg) with chloroform/methanol (90:10, v/v), and IV (69 mg) and V (195 mg) with chloroform/methanol (80:20, v/v).

Fractions I−V were further purified by high-performance liquid chromatography (HPLC). Reversed-phase HPLC was done with a 250 × 10 mm Hydrosphere C18 column (YMC Co., Ltd., Kyoto, Japan) developed with acetonitrile/water (15:85, v/v; for fraction I) or acetonitrile/water (21:79, v/v; for fractions II−V) at a flow rate of 5.0 mL/min. The eluate was monitored by an absorbance at 205 nm. Compounds **1** (6.0 mg), **2** (4.3 mg), and **3** (6.5 mg) were isolated from fraction I, compound **4** (33.0 mg) was isolated from fraction II, compounds **5** (22.0 mg) and **6** (8.8 mg) were from fraction III, compounds **7** (8.9 mg) and **8** (12.0 mg) were isolated from fraction IV, and compound **9** (27.0 mg) was from fraction V.

(1*R*,3*S*,4*S*)-*trans*-3-Hydroxy-1,8-cineole β-D-glucopyranoside (1). Compound **1** is an amorphous solid, R_f 0.67. $[\alpha]^{25}_D$ −14 (*c* 0.11, methanol). UV (methanol) λ_{max} (log ϵ) 262 nm (3.05). ESIMS *m*/*z* 355.20 ($[M + Na]^+$). APCIMS *m*/*z* 355.30 ($[M + Na]^+$, 100%), 333.25 ($[M + H]^+$, 9), 315.35 ($[M - OH]^+$, 51), 163.00 ($[C_6H_{11}O_5]^+$, 2). ^{1}H NMR (CD_3OD) δ 1.03 (s, 3H, H-7), 1.21 (s, 3H, H-10), 1.29 (s, 3H, H-9), 1.54−1.61 (m, 3H, H-2a, H-6a, H-6b), 1.74 (m, 1H, H-5a), 1.81 (m, 1H, H-4), 2.08 (m, 1H, H-2b), 2.18 (m, 1H, H-5b), 3.18 (dd, *J* = 7.7, 9.2 Hz, 1H, H-2′), 3.27 (m, 1H, H-5′), 3.31 (m, 1H, H-4′), 3.33 (m, 1H, H-3′), 3.66 (m, 1H, H-6′a), 3.86 (m, 1H, H-6′b), 4.31 (d, *J* = 7.7 Hz, 1H, H-1′), and 4.51 (m, 1H, H-3).

3-Methyl-but-2-en-1-yl β-D-glucopyranoside (2). Compound **2** is a colorless wax, R_f 0.62. $[\alpha]^{25}_D$ +50 (*c* 0.086, methanol). ESIMS *m*/*z* 271.15 ($[M + Na]^+$); APCIMS *m*/*z* 271.25 ($[M + Na]^+$, 100%), 249.20 ($[M + H]^+$, 3), 231.05 ($[M - OH]^+$, 4); 163.10 ($[C_6H_{11}O_5]^+$, 7). ^{1}H NMR (CD_3OD) δ 1.70 (s, 3H, H-5), 1.75 (s, 3H, H-4), 3.16 (dd, *J* = 7.7, 9.2 Hz, 1H, H-2′), 3.23−3.27 (m, 1H, H-5′), 3.30−3.36 (m, 2H, H-3′, H-4′), 3.67 (dd, *J* = 5.5, 11.7 Hz, 1H, H-6′a), 3.86 (dd, *J* = 1.8, 11.7 Hz, 1H, H-6′b), 4.22 (dd, *J* = 7.7, 11.7 Hz, 1H, H-1b), 4.27 (d, *J* = 7.7 Hz, 1H, H-1′), 4.32 (dd, *J* = 6.2, 11.7 Hz, 1H, H-1a), and 5.37 (m, 1H, H-2).

Benzyl β-D-glucopyranoside (3). Compound **3** is a colorless wax, R_f 0.60. $[\alpha]^{25}_D$ −35 (*c* 0.13, methanol). UV (methanol) λ_{max} (log ϵ) 258 nm (2.43). ESIMS *m*/*z* 293.10 ($[M + Na]^+$). APCIMS *m*/*z* 293.20 ($[M + Na]^+$, 100%), 271.20 ($[M + H]^+$, 4), 253.20 ($[M - OH]^+$, 15), 163.10 ($[C_6H_{11}O_5]^+$, 3). ^{1}H NMR (CD_3OD) δ 3.25 (dd, *J* = 8.1, 9.2 Hz, 1H, H-2′), 3.27 (m, 1H, H-5′), 3.30−3.37 (m, 2H, H-3′, H-4′), 3.69 (dd, *J* = 5.5, 11.7 Hz, 1H, H-6′a), 3.89 (dd, *J* = 1.8, 11.7 Hz, 1H, H-6′b), 4.35 (d, *J* = 7.7 Hz, 1H, H-1′), 4.66 (d, *J* = 11.7 Hz, 1H, H-7a), 4.93 (d, *J* = 11.7 Hz, 1H, H-7b), 7.27 (d, *J* = 7.3, 1H, H-4), 7.32 (dd, *J* = 5.1, 7.3 Hz, 2H, H-3, H-5), and 7.42 (d, *J* = 8.0 Hz, 2H, H-2, H-6).

1-*O*-β-D-Glucopyranosyl-4-allylbenzene (chavicol β-D-glucopyranoside, 4). Compound **4** is an amorphous solid, R_f 0.63. $[\alpha]^{25}_D$ −53.4 (*c* 0.66, methanol). UV (methanol) λ_{max} (log ϵ) 222 (2.89) and 272 nm (2.05). ESIMS *m*/*z* 319.15 ($[M + Na]^+$). APCIMS *m*/*z* 319.20 ($[M + Na]^+$, 100%), 314.25 ($[M + H_2O]^+$, 31), 180.20 ($[C_6H_{12}O_6]^+$, 11), 163.10 ($[C_6H_{11}O_5]^+$, 7). 1H NMR (CD_3OD) δ 3.31 (d, *J* = 6.6 Hz, 2H, H-7), 3.38−3.46 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.69 (dd, *J* = 5.3, 12.1 Hz, 1H, H-6′a), 3.88 (dd, *J* = 1.8, 12.1 Hz, 1H, H-6′b), 4.86 (d, *J* = 7.7 Hz, 1H, H-1′), 5.01 (ddd, *J* = 1.5, 1.5, 9.9 Hz, 1H, H-9a), 5.02 (ddd, *J* = 1.5, 1.5, 16.8 Hz, 1H, H-9b), 5.93 (ddt, *J* = 6.6, 9.9, 16.8 Hz, 1H, H-8), 7.02 (d, *J* = 8.8 Hz, 2H, H-3, H-5, or H-2, H-6), and 7.09 (d, *J* = 8.8 Hz, 2H, H-2, H-6, or H-3, H-5). Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 59.42; H 6.58.

1-Hydroxy-2-*O*-β-D-glucopyranosyl-4-allylbenzene (5). Compound **5** is a brown wax, R_f 0.58. $[\alpha]^{25}_D$ −56.8 (*c* 0.44, methanol). UV (methanol) λ_{max} (log ϵ) 256 (2.59) and 278 nm (2.41). ESIMS *m*/*z* 335.10 ($[M + Na]^+$). APCIMS *m*/*z* 335.15 ($[M + Na]^+$, 100%), 330.25 ($[M + H_2O]^+$, 36), 180.15 ($[C_6H_{12}O_6]^+$, 9), 163.10 ($[C_6H_{11}O_5]^+$, 4). ^{1}H NMR (CD_3OD) δ 3.27 (d, *J* = 6.6 Hz, 2H, H-7), 3.41 (m, 2H, H-4′, H-5′), 3.48 (m, 2H, H-2′, H-3′), 3.72 (dd, *J* = 4.8, 12.1 Hz, 1H, H-6′a), 3.89 (dd, *J* = 1.8, 12.5 Hz, 1H, H-6′b), 4.73 (d, *J* = 7.7 Hz, 1H, H-1′), 5.00 (dd, *J* = 1.8, 10.3 Hz, 1H, H-9a), 5.04 (dd, *J* = 1.8, 17.2 Hz, 1H, H-9b), 5.93 (ddt, *J* = 7.0, 10.3, 17.2 Hz, 1H, H-8), 6.73 (dd, *J* = 1.8, 8.1 Hz, 1H, H-5), 6.75 (s, 1H, H-3), and 7.03 (d, *J* = 1.5 Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.69; H, 6.45. Found: C, 56.56; H, 6.61.

1-*O*-β-D-Glucopyranosyl-2-hydroxy-4-allylbenzene (demethyleugenol β-D-glucopyranoside, 6). Compound **6** is a brown wax, R_f 0.57. $[\alpha]^{25}_D$ −108 (*c* 0.18, methanol). UV (methanol) λ_{max} (log ϵ) 276 nm (2.89). ESIMS *m*/*z* 335.10 ($[M + Na]^+$). APCIMS *m*/*z* 335.20 ($[M + Na]^+$, 100%), 330.20 ($[M + H_2O]^+$, 29), 180.15 ($[C_6H_{12}O_6]^+$, 8), 163.10 ($[C_6H_{11}O_5]^+$, 6). ^{1}H NMR (CD_3OD) δ 3.26 (d, *J* = 6.6 Hz, 2H, H-7), 3.40 (m, or d, *J* = 5.9 Hz, 2H, H-4′, H-5′), 3.47 (m, 2H, H-2′, H-3′), 3.72 (dd, *J* = 4.8, 12.1 Hz, 1H, H-6′a), 3.87 (dd, *J* = 1.5, 12.1 Hz, 1H, H-6′b), 4.70 (d, *J* = 7.7 Hz, 1H, H-1′), 5.00 (dd, *J* = 2.1, 10.6 Hz, 1H, H-9a), 5.04 (dd, *J* = 1.8, 17.2 Hz, 1H, H-9b), 5.92 (ddt, *J* = 7.0, 10.3, 17.2 Hz, 1H, H-8), 6.60 (dd, *J* = 2.2, 8.4 Hz, 1H, H-5), 6.68 (d, *J* = 2.2 Hz, 1H, H-3), and 7.10 (d, *J* = 8.4 Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.69; H, 6.45. Found: C, 56.75; H 6.79.

1-*O*-(6-*O*-α-L-Rhamnopyranosyl-β-D-glucopyranosyl)-2-hydroxy-4-allylbenzene (demethyleugenol β-rutinoside, 7). Compound **7** is a brown wax, R_f 0.36. $[\alpha]^{25}_D$ −106 (*c* 0.18, methanol). UV (methanol) λ_{max} (log ϵ) 275 nm (2.96). ESIMS *m*/*z* 481.15 ($[M + Na]^+$). APCIMS *m*/*z* 481.20 ($[M + Na]^+$, 100%), 476.35 ($[M + H_2O]^+$, 74), 309.15 ($[C_{12}H_{21}O_9]^+$, 14), 147.10 ($[C_6H_{11}O_4]^+$, 6). ^{1}H NMR (CD_3OD) δ 1.23 (d, *J* = 6.2 Hz, 3H, H-6″), 3.26 (d, *J* = 6.6 Hz, 2H, H-7), 3.37 (m, 3H, H-3′, H-4′, H-4″), 3.46 (dd, *J* = 5.5, 7.3, 1H, H-2′), 3.52 (ddd, *J* = 1.8, 6.6, 9.5 Hz, H-5′), 3.61 (dd, *J* = 6.6, 11.0 Hz, 1H, H-6′a), 3.66

(dd, J = 3.3, 6.6 Hz, 1H, H-5″), 3.70 (dd, J = 3.7, 9.5 Hz, 1H, H-3″), 3.86 (dd, J = 1.5, 3.3 Hz, H-2″), 4.03 (dd, J = 1.5, 11.0 Hz, 1H, H-6′b), 4.67 (d, J = 7.7 Hz, 1H, H-1′), 4.73 (d, J = 1.5 Hz, 1H, H-1″), 5.03 (m, 2H, H-9), 5.92 (ddt, J = 6.6, 9.9, 16.5 Hz, H-8), 6.63 (dd, J = 2.2, 8.4 Hz, 1H, H-5), 6.68 (d, J = 2.2 Hz, 1H, H-3), and 7.07 (d, J = 8.4 Hz, 1H, H-6). Anal. Calcd for $C_{21}H_{30}O_{11}\cdot H_2O$: C, 52.94; H, 6.77. Found: C, 52.10; H, 6.57.

1-*O*-(6-*O*-α-L-Rhamnopyranosyl-β-D-glucopyranosyl)-4-allylbenzene (chavicol β-rutinoside, 8). Compound **8** is a brown wax, R_f 0.41. $[\alpha]^{25}_D$ −67.1 (*c* 0.24, methanol). UV (methanol) λ_{max} (log ϵ) 221 (3.42) and 273 nm (2.69). ESIMS *m*/*z* 465.15 ($[M + Na]^+$). APCIMS *m*/*z* 465.20 ($[M + Na]^+$, 100%), 460.25 ($[M + H_2O]^+$, 84), 309.20 ($[C_{12}H_{21}O_9]^+$, 15), 147.10 ($[C_6H_{11}O_4]^+$, 7). ^{1}H NMR (CD_3OD) δ 1.20 (d, J = 6.2, 3H, H-6″), 3.30−3.38 (m, 3H, H-3′, H-4′, H-4″), 3.31 (m, 2H, H-7), 3.44 (m, 1H, H-2′), 3.54 (ddd, J = 1.5, 6.2, 9.2 Hz, 1H, H-5′), 3.59 (dd, J = 6.6, 11.0 Hz, 1H, H-6′a), 3.65 (dd, J = 3.7, 6.2 Hz, 1H, H-5″), 3.69 (dd, J = 3.3, 9.5 Hz, 1H, H-3″), 3.84 (dd, J = 1.8, 3.3 Hz, 1H, H-2″), 4.01 (dd, J = 1.5, 11.0 Hz, 1H, H-6′b), 4.70 (d, J = 1.8 Hz, 1H, H-1″), 4.81 (d, J = 7.7 Hz, 1H, H-1′), 5.01 (ddd, J = 1.5, 1.5, 10.3 Hz, 1H, H-9a), 5.03 (ddd, J = 1.5, 1.5, 16.8 Hz, 1H, H-9b), 5.94 (ddt, J = 6.6, 10.3, 16.8 Hz, 1H, H-8), 7.01 (d, J = 8.4 Hz, 2H, H-3, H-5 or H-2, H-6), and 7.11 (d, J = 8.8 Hz, 2H, H-2, H-6 or H-3, H-5). Anal. Calcd for $C_{21}H_{30}O_{10}\cdot H_2O$: C, 54.78; H, 7.00. Found: C, 53.97; H, 6.55.

1,2-di-*O*-β-D-Glucopyranosyl-4-allylbenzene (9). Compound **9** is a yellow wax, R_f 0.22. $[\alpha]^{25}_D$ −46.8 (*c* 0.54, methanol). UV (methanol) λ_{max} (log ϵ) 272 nm (2.27). ESIMS *m*/*z* 497.15 ($[M + Na]^+$); APCIMS *m*/*z* 497.25 ($[M + Na]^+$, 100%), 492.30 ($[M + H_2O]^+$, 51), 335.15 (31), 163.00 ($[C_6H_{11}O_5]^+$, 4). ^{1}H NMR (CD_3OD) δ 3.32 (d, J = 7.7 Hz, 2H, H-7), 3.34−3.37 (m, 2H, H-5′, H-5″), 3.38−3.41 (m, 2H, H-4′, H-4″), 3.43−3.53 (m, 4H, H-2′, H-2″, H-3′, H-3″), 3.70 (dd, J = 2.2, 12.1 Hz, 1H, H-6′a), 3.71 (dd, J = 2.2, 11.7 Hz, 1H, H-6″a), 3.86 (dd, J = 1.8, 11.7 Hz, 2H, H-6″b, H-6′b), 4.82 (d, J = 7.7 Hz, 1H, H-1′), 4.84 (d, J = 7.7 Hz, 1H, H-1″), 5.03 (dd, J = 2.2, 9.9 Hz, 1H, H-9a), 5.06 (dd, J = 2.2, 16.8 Hz, 1H, H-9b), 5.94 (ddt, J = 7.0, 10.3, 17.2 Hz, 1H, H-8), 6.85 (dd, J = 2.2, 8.1 Hz, 1H, H-5), 7.09 (d, J = 2.2 Hz, 1H, H-3), and 7.17 (d, J = 8.1 Hz, 1H, H-6). Anal. Calcd for $C_{21}H_{30}O_{12}\cdot H_2O$: C, 51.22; H, 6.55. Found: C, 50.36; H, 6.67.

Analysis of Components in Glycosides. The separated compounds were hydrolyzed using 0.5 M H_2SO_4 or β-glucosidase (from almond; Oriental Yeast Co., Ltd., Tokyo, Japan) (*17*, *18*). The sugar composition was determined by gas−liquid chromatography (GLC) as alditol acetates (*19*). Compounds **1**−**6** and **9** had only glucose, whereas **7** and **8** had glucose and rhamnose as the sugar components. The aglycon moiety of **4**−**9** was analyzed by GC−MS or APCIMS. Compounds **4** and **8** showed one peak by GC−MS analysis which was identified to be 1-hydroxy-4-allylbenzene: EIMS *m*/*z* 134 (M^+, 100%), 107 ($[M - CH_2{=}CH]^+$, 50), 91 ($[C_6H_5CH_2]^+$, 23), and 77 ($[C_6H_5]^+$, 47). The aglycon moiety of **5**−**7** and **9** was identified to be 1,2-hydroxy-4-allylbenzene: negative APCIMS *m*/*z* 149 $[M - H]^-$; EIMS as the diacetate *m*/*z* 234 (M^+, 10%), 192 ($[M - CH_2CO]^+$, 40), 150 ($[C_9H_8(OH)_2]^+$, 100), 132 ($[C_9H_8O]^+$, 14), 104 (10), 91 (10), and 77 (8). The linkage of sugar moiety was analyzed by GC−MS as partially methylated alditol acetates (*18*, *20*). The GC−MS analysis of partially methylated **7** and **8** showed two peaks as the alditol acetate derivatives which were identified to be 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpentitol: EIMS *m*/*z* 175 (5%), 161 (10), 131 (30), 117 (40), 101 (70), 89 (30), and 72 (30); and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol: EIMS *m*/*z* 233 (5%), 189 (10), 161 (20), 129 (40), 117 (50), 101 (80), and 87 (40). On the other hand, **9** gave only one peak that was identified to be 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol as the alditol acetate: EIMS *m*/*z* 205 (10%), 161 (30), 145 (25), 129 (35), 117 (40), 101 (70), and 87 (25).

RESULTS AND DISCUSSION

Methanol extract from galanga rhizome was subjected to XAD-2 column chromatography followed by reversed-phase and normal-phase column chromatography. Finally, the glycosidically bound fractions were purified by preparative reversed-phase HPLC to obtain compounds **1**−**9**. The structures of compounds **1**−**9** were characterized as follows (**Figure 1**).

Compound **1** was obtained as amorphous solid (6.0 mg yield) and identified to be (1*R*,3*S*,4*S*)-*trans*-3-hydroxy-1,8-cineole β-D-glucopyranoside by comparison of its ^{1}H and ^{13}C NMR data with those of literature values (*14*, *16*). This glucoside has already been isolated from the rhizomes of ginger (*14*) and greater galanga (*16*).

Compound **2** was obtained as a colorless wax (4.3 mg yield). The APCIMS showed a significant ion at *m*/*z* 271.25 ($[M + Na]^+$), a protonated molecular ion at *m*/*z* 249.20, and glucose moiety at *m*/*z* 163.10. The ^{1}H NMR spectrum indicates the presence of two methyl proton signals at δ 1.70 and 1.75, methylene proton signals at δ 4.22 (dd, J = 7.7, 11.7 Hz) and 4.32 (dd, J = 6.2, 11.7 Hz), and one olefinic proton at δ 5.37. The anomeric proton signal at δ 4.27 (d, J = 7.7 Hz) reveals β-configuration of the glucosidic linkage. The ^{13}C NMR spectrum showed eleven carbon signals, including anomeric carbon signal at δ 102.8 (**Table 1**). The NMR data of the aglycon was in agreement with the data in the literature (*18*), in which 3-methyl-but-2-en-1-yl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside has been isolated in passion fruit. Thus, the structure of compound **2** was determined to be 3-methyl-but-2-en-1-yl β-D-glucopyranoside.

Compound **3** was obtained as a colorless wax (6.5 mg yield) and identified to be benzyl β-D-glucopyranoside by comparison of its spectroscopic data with those of literature values (*19*, *21*). Benzyl β-D-glucopyranoside has been isolated from *Cedronella canariensis* (*19*) and fresh tea leaves of *Camellia sinensis* L. var. *Yabukita* (*21*) as the flavor precursor. In addition, the disaccharide glucosides have been obtained from passion fruit (*18*), oolong tea leaves (*22*), and *Jasminum sambac* flower (*23*).

Compound **4** was obtained as an amorphous solid (33.0 mg yield), which consisted of glucose and 1-hydroxy-4-allylbenzene as the components. The structure of **4** was identified to be 1-*O*-β-D-glucopyranosyl-4-allylbenzene (chavicol β-D-glucopyranoside) by comparison of its spectroscopic data with those of literature values (*20*, *24*). Chavicol β-D-glucopyranoside has been found in *Cedronella canariensis* (*19*), in leaves of *Viburnum furcatum* (*25*), and in *Pinus contorta* needle (*26*).

Compound **5** was obtained as a brown wax (22.0 mg yield). **5** gave glucose and 1,2-dihydroxy-4-allylbenzene after hydrolysis with acid or β-glucosidase. The ^{1}H NMR spectrum indicates the presence of trisubstituted benzene protons (δ 7.03, 6.75, and 6.73), terminal olefinic protons (δ 5.00 and 5.04), and an olefinic proton (δ 5.93). The anomeric proton at δ 4.73 with its coupling constant of 7.7 Hz reveals β-configuration of the glucosidic linkage. The ^{13}C NMR data of the aglycon moiety in **Table 1** were in agreement with those in the literature (*24*). Furthermore, the connectivities of the glucose unit were established based on HMBC cross-peaks indicating long-range ^{13}C−^{1}H couplings (data not shown): the anomeric proton signal at δ 4.73 (H-1′) gave a strong cross-peak with a carbon signal at δ 146.6 (C-2); this carbon signal then gave cross-peaks with two proton signals at δ 6.75 (H-3) and 7.03 (H-6). Thus, compound **5** was determined to be 1-hydroxy-2-*O*-β-D-glucopyranosyl-4-allylbenzene.

Compound **6** was obtained as a brown wax (8.8 mg yield). Compound **6** after hydrolysis with acid or β-glucosidase gave the same aglycon, 1,2-dihydroxy-4-allylbenzene, as detected in compound **5**. The ^{1}H and ^{13}C NMR spectra of compound **6** were similar to those obtained in compound **5** (**Table 1**). The HMBC spectrum gave cross-peaks between resonances at δ 4.70 (H-1′) and 145.2 (C-1), at δ 6.60 (H-5) and 145.2 (C-1), and at

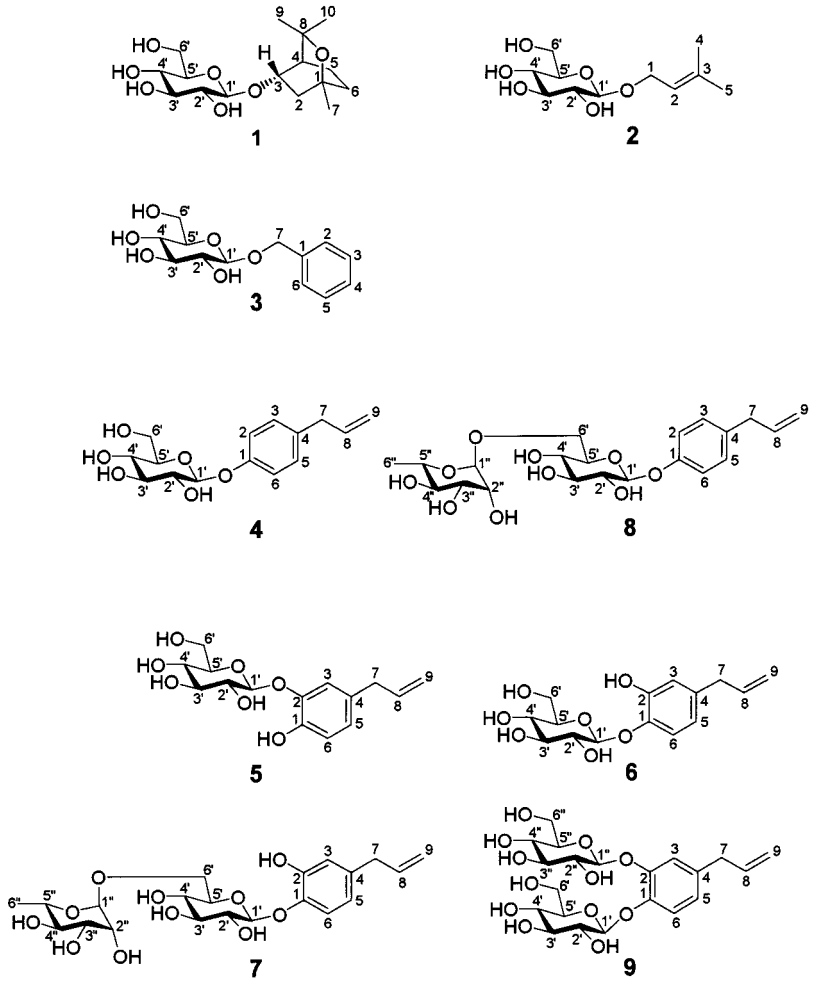

Figure 1. Structures of (1*R*,3*S*,4*S*)-*trans*-3-hydroxy-1,8-cineole β-D-glucopyranoside (**1**), 3-methyl-but-2-en-1-yl β-D-glucopyranoside (**2**), benzyl β-D-glucopyranoside (**3**), 1-*O*-β-D-glucopyranosyl-4-allylbenzene (chavicol β-D-glucopyranoside, **4**), 1-hydroxy-2-*O*-β-D-glucopyranosyl-4-allylbenzene (**5**), 1-*O*-β-D-glucopyranosyl-2-hydroxy-4-allylbenzene (demethyleugenol β-D-glucopyranoside, **6**), 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-2-hydroxy-4-allylbenzene (demethyleugenol β-rutinoside, **7**), 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-4-allylbenzene (chavicol β-rutinoside, **8**), and 1,2-di-*O*-β-D-glucopyranosyl-4-allylbenzene (**9**) isolated from fresh rhizomes of smaller galanga (*Alpinia officinarum* Hance).

δ 6.68 (H-3) and 145.2 (C-1), and at δ 7.10 (H-6) and 145.2 (C-1), respectively (data not shown). This indicates that the sugar residue is bonded to the C-1 position of 1,2-dihydroxy-4-allylbenzene. From these results, compound **6** was determined to be 1-*O*-β-D-glucopyranosyl-2-hydroxy-4-allylbenzene (demethyleugenol β-D-glucopyranoside).

Compound **7** was obtained as a brown wax (8.9 mg yield). Compound **7** after hydrolysis gave 1,2-dihydroxy-4-allylbenzene as the aglycon moiety. The sugar moiety of partially methylated compound **7** was analyzed by GC−MS as the alditol acetate derivatives. Two peaks with the area ratio of 1:1 appeared on the chromatogram, which were identified to be 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpentitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol. The results indicate that a terminal rhamnose unit is linked to the 6-position of glucose unit in the glycoside. The ^{1}H NMR spectrum shows two anomeric proton signals at δ 4.67 (d, J = 7.7 Hz) and δ 4.73 (d, J = 1.5 Hz) and a signal of methyl proton at δ 1.23, suggesting that **7** has a 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside moiety. The ^{13}C NMR spectrum in **Table 1** gave signals similar to those of glucoside moiety of compound **6**. The HMBC spectrum indicated that the anomeric proton signal of glucose at 4.67 (H-1′) gave a strong cross-peak with a carbon signal at 145.1 (C-1); this carbon signal then gave cross-peaks with proton signals at δ 6.63 (H-5), 6.68 (H-3), and 7.07 (H-6); and the anomeric proton signal of rhamnose at δ 4.73 (H-1″) gave a strong cross-peak with the glucose carbon signal at δ 62.5 (C-6′) (data not shown). Thus, compound **7** was determined to be 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-2-hydroxy-4-allylbenzene (demethyleugenol β-rutinoside).

Compound **8** was obtained as a brown wax (12.0 mg yield), which gave 1-hydroxy-4-allylbenzene as the aglycon moiety. The sugar moiety of partially methylated compound **8** gave two peaks as the alditol acetates, which were identified to be 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpentitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol. The data of ^{1}H and ^{13}C NMR spectra were similar to those of compounds **4** (for aglycon) and **7** (for sugar moiety) (**Table 1**). Thus, the structure of compound **8** was determined to be 1-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-4-allylbenzene (chavicol β-rutinoside). Another chavicol 4-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside has been isolated from *Pinus contorta* (*27*).

Compound **9** was obtained as a yellow wax (27.0 mg yield). Compound **9** after hydrolysis gave glucose and 1,2-dihydroxy-4-allylbenzene. The ^{1}H NMR spectrum shows the presence of two anomeric protons at δ 4.82 (d, J = 7.7 Hz) and δ 4.84 (d, J = 7.7 Hz), indicating β-configuration of the glucose units. The ^{13}C NMR spectrum of **9** showed two anomeric carbons at δ 103.3 and 103.5 in addition to other assignable carbon atoms (**Table 1**). From the HMBC spectrum, the anomeric proton

Table 1. ^{13}C NMR Data for the Glucosides Isolated from *Alpinia officinarum* Hance[a]

carbon	1	2	3	4	5	6	7	8	9
aglycon									
1	71.7	66.4	139.1	157.5	146.7	145.2	145.1	157.5	146.8
2	41.8	121.7	128.6	117.8	146.6	148.4	148.1	117.9	148.5
3	72.8	138.6	129.3	130.5	119.3	119.1	119.1	130.5	120.0
4	37.8	25.9	129.2	135.3	133.2	137.3	137.3	135.4	137.3
5	15.1	18.8	129.3	130.5	124.7	121.0	121.1	130.5	124.7
6	32.1		128.7	117.8	117.0	117.3	117.4	117.9	120.0
7	27.3		71.8	40.4	40.6	40.6	40.6	40.4	40.3
8	75.3			139.2	139.3	139.0	139.1	139.2	138.6
9	29.3			115.7	115.7	115.8	115.8	115.7	116.3
10	28.7								
glucose									
1′	102.3	102.8	103.3	102.5	104.5	104.7	104.6	102.6	103.3
2′	75.2	75.1	75.1	74.9	74.9	74.9	75.0	75.0	74.7
3′	78.2	78.1	78.1	78.1	78.3	78.3	77.7	78.1	77.3
4′	71.7	71.7	71.7	71.4	71.3	71.3	71.6	71.6	70.9
5′	78.0	78.0	78.1	78.0	77.6	77.7	77.1	76.9	77.8
6′	62.8	62.8	62.8	62.5	62.4	62.5	68.0	67.9	62.0
rhamnose or glucose									
1″							102.3	102.2	103.5
2″							72.4	72.4	74.7
3″							72.2	72.2	77.3
4″							74.1	74.1	70.9
5″							69.9	69.9	77.8
6″							18.0	18.0	62.0

[a] Measured in CD_3OD.

signal at δ 4.82 (H-1′) gave a strong cross-peak with a carbon signal at δ 146.8 (C-1); this carbon signal then gave cross-peaks with two proton signals at δ 6.85 (H-5), 7.09 (H-3), and 7.17 (H-6); the anomeric proton signal at δ 4.84 (H-1″) gave a strong cross-peak with a carbon signal at δ 148.5 (C-2); and this carbon signal then gave cross-peaks with two proton signals at δ 7.09 (H-3) and 7.17 (H-6) (data not shown). The sugar moiety of compound **9** after partial methylation gave only one peak, which was identified to be 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol. This indicates that glucose is linked to each hydroxyl group of 1,2-dihydroxy-4-allylbenzene. Thus, compound **9** was determined to be 1,2-di-*O*-β-D-glucopyranosyl-4-allylbenzene.

In this study, nine glycosides (**1**−**9**) were isolated from the rhizomes of smaller galanga. Their structures were determined to be (1*R*,3*S*,4*S*)-*trans*-3-hydroxy-1,8-cineole β-D-glucopyranoside (**1**), 3-methyl-but-2-en-1-yl β-D-glucopyranoside (**2**), benzyl β-D-glucopyranoside (**3**), chavicol β-glycosides (**4** and **8**), and β-glycosides of 1,2-dihydroxy-4-allylbenzene (**5**−**7** and **9**). Among the glycosides, compounds **2**−**9** were detected for the first time in the constituents of galanga rhizomes. The glycosidically bound volatile compounds have been considered as aroma precursors that release free aroma compounds by enzymatic or acidic hydrolysis (*12*, *14*). Such candidates might be compounds **1**, **2**, **3**, **4**, and **8**, although only chavicol has been detected in the essential oil in the smaller galangal (*11*). In addition, the main glycosides isolated in the present study are β-glycosides of chavicol and 1,2-dihydroxy-4-allylbenzene (**4**−**9**). These compounds have one or two phenolic hydroxyl groups in the molecule. The phenolic glycosides in ginger have been demonstrated to show strong antioxidative activities (*15*). Therefore, compounds **4**−**9** might be expected to act as antioxidants.

ACKNOWLEDGMENT

We thank Dr. M. Koketsu, Department of Chemistry, Faculty of Engineering, Gifu University, for the elemental analyses.

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Received for review March 10, 2002. Revised manuscript received June 7, 2002. Accepted June 10, 2002.

JF025529P