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FIVE MONOTERPENE GLYCOSIDES FROM ZINGIBERIS RHIZOME (SHOKYO)

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Abstract – Five monoterpene glycosides were isolated from Zingiberis rhizome (Japanese name: Shokyo). They were characterized to be (+)-angelicoidenol 2-*O*-β-D-glucopyranoside (**1**), (+)-angelicoidenol 2-*O*-β-D-glucopyranosido-5-*O*-β-D-glucopyranoside (**2**), (+)-angelicoidenol 2-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**3**), (+)-angelicoidenol 2-*O*-[6'-*O*-*S*-3-hydroxy-3-methylglutaryl]-β-D-glucopyranoside (**4**) and its methyl ester (**5**), respectively, by means of spectroscopic and chemical methods. Of them, the new glycosides (**2**, **3** and **4**) were named zingiberosides A, B and C, respectively.

INTRODUCTION

Shokyo and Kankyo (Japanese names for Zingiberis rhizome and Zingiberis siccatum rhizome, respectively) are important traditional Kampo medicines made by different processes from ginger, *Zingiber officinale* ROSCOE (Zingiberaceae) rhizome. Shokyo is a dried ginger, whereas Kankyo is prepared from ginger by steaming followed by drying. Interestingly, they have been used for different clinical purposes in Kampo medicine in spite of the same botanical origin: Shokyo has been used as a component of some Kampo formulae for its anti-emetic and anti-pyretic effects, whereas Kankyo finds applications in cough suppressants, phlegm and body warming properties.[1-4] However there is no scientific basis for discrimination between Shokyo and Kankyo. In order to make chemical basis for the discrimination to clear, we started the phytochemical survey on Shokyo.

In the previous paper,[5] we reported the isolation and characterization of six sulfonated derivatives, 4-

gingesulfonic acid, 6-gingesulfonic acid, shogasulfonic acids A, B, C and D, obtained from the 80% MeOH extract of the Zingiberis Rhizome (Shokyo). In a continuation of the phytochemical investigations on the same fractions, we have further isolated five monoterpene glycosides **1** – **5**. The isolation and characterization of these compounds is described herein.

RESULTS AND DISCUSSION

Fractions D and E obtained from the water-soluble portion of the 80% MeOH extract of Shokyo (5.0 kg) were subjected to further chromatographic separation and purification, affording glycosides (**1**) (422 mg), (**2**) (19 mg), (**3**) (15 mg), (**4**) (63 mg) and (**5**) (13 mg), in addition to the six sulfonated compounds reported previously.[5]

Glycoside (**1**), obtained as a white amorphous powder, $[\alpha]_D^{20}$ – 18.4°, was identified as (+)-angelicoidenol 2-*O*-β-D-glucopyranoside by direct comparison with the authentic sample isolated from *Glehnia littoralis* roots.[6]

Glycoside (**2**) was obtained as a white powder, $[\alpha]_D^{21}$ – 40.7°, and yielded a $[M+H]^+$ peak at *m/z* 495.2442 ($C_{22}H_{39}O_{12}$), 163 mass unit ($C_6H_{11}O_5$) larger than that of **1**, in the HR FAB-MS spectrum.

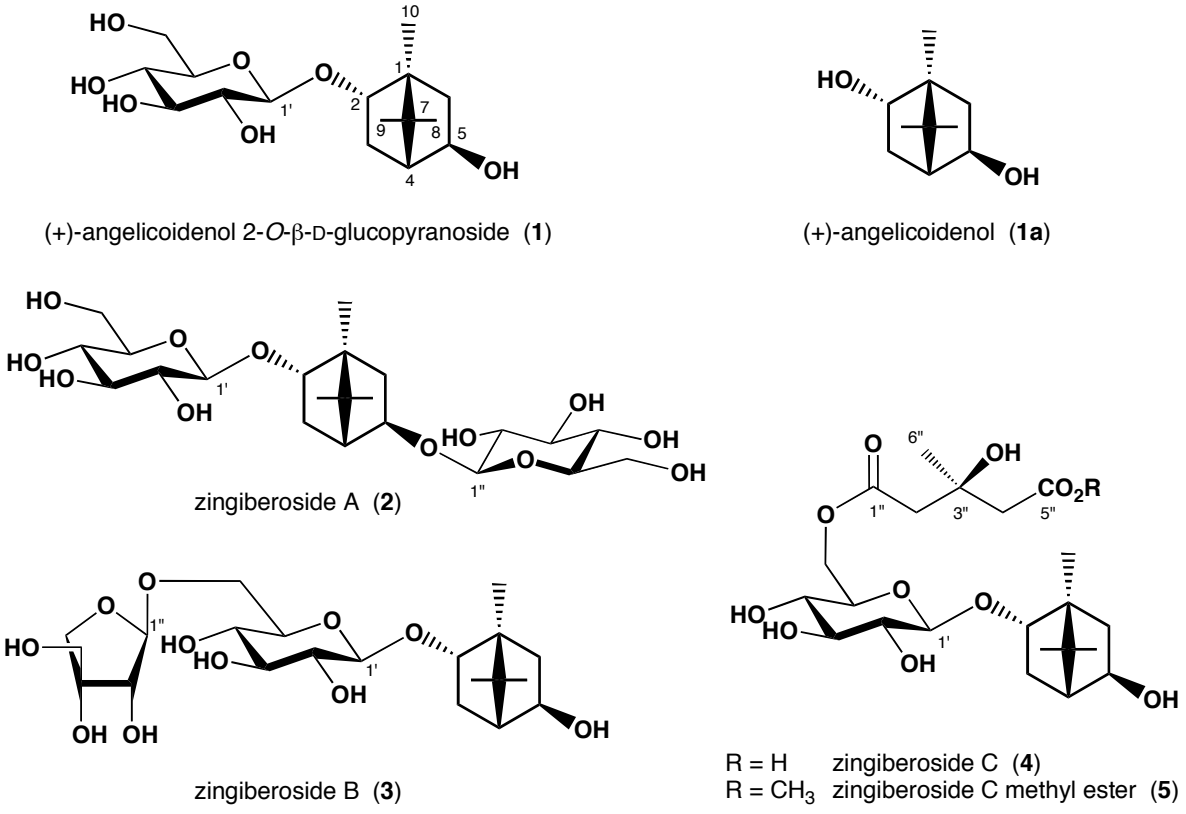


Figure 1 Structures of 1 – 5

The ^{13}C NMR spectroscopic feature was similar to those of **1**, except for the glycosylation shifts observed at the C-4, C-5 and C-6 positions by – 1.6, + 7.8 and – 2.0 ppm from those of **1**, respectively, and for the appearance of six additional carbon signals ascribable to a β-D-glucopyranosyl moiety (Table 1). On enzymatic hydrolysis, **2** furnished D-glucose and an aglycone, which was identified as (+)-angelicoidenol (**1a**) obtained from **1** by enzymatic hydrolysis. In addition, the HMBC correlations were observed between H-1' (δ_H 4.25) / C-2 (δ_C 86.1) and H-1'' (δ_H 4.25) / C-5 (δ_C 83.4). Thus, the location of the two glucopyranosyl moieties was determined to be at the 2- and 5-hydroxyl groups of **1a**, and **2** was characterized as (+)-angelicoidenol 2-*O*-β-D-glucopyranosido-5-*O*-β-D-glucopyranoside.

Glycoside (**3**) was obtained as a colorless viscous oil, $[\alpha]_D^{21}$ – 50.5°, and showed a $[M+H]^+$ peak at *m/z* 465.2333 ($C_{21}H_{37}O_{11}$), 132 mass unit ($C_5H_8O_4$) larger than that of **1**, in the HR FAB-MS spectrum. The ^{13}C NMR spectroscopic feature was similar to that of **1**, except for the appearance of five additional signals suggesting the presence of a β-D-apiofuranosyl moiety (Table 2). On mild acid hydrolysis, **3** afforded D-glucose, D-apiose, **1** and **1a**. The location of the apiosyl moiety was determined to be at the glucosyl C-6' hydroxyl group, because of the downfield shift of the glucosyl C-6' carbon signal (+ 5.6 ppm) and the HMBC correlation observed between H-1'' (δ_H 5.01) / C-6' (δ_C 68.5). Therefore, **3** was determined to be (+)-angelicoidenol 2-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

Glycoside (**4**), obtained as a colorless viscous oil, showed a $[M+H]^+$ peak at *m/z* 477.2330 ($C_{22}H_{37}O_{11}$), 145 mass unit ($C_6H_9O_4$) larger than that of **1**, in the HR FAB-MS spectrum. The IR spectrum showed a carbonyl absorption band at 1724 cm^{-1}. The ^{13}C NMR spectrum of **4** was close to that of **1**, except for the appearance of additional six carbon signals assignable to one methyl group (δ_C 27.9), two methylene groups (δ_C 47.0, 47.5), one quarternary carbon (δ_C 71.0), one ester and one carboxyl groups (δ_C 172.6, 179.2), respectively. The ^{1}H NMR spectrum exhibited a tertiary methyl group (δ_H 1.33) attached to a quaternary carbon bearing a hydroxyl group, and two methylene groups (two AB quartets at δ_H 2.43, 2.56 and 2.61, 2.66) adjacent to two quaternary carbons, suggesting the presence of a 3-hydroxy-3-methylglutaryl group (HMG) in the molecule.[7, 8] The location of the HMG group was shown to be at the glucosyl C-6' position by the cross peak between H-6' (δ_H 4.19, 4.42) / C-1'' (δ_C 172.6) observed in the HMBC spectrum.

The stereochemistry of the chiral center at the C-3'' position of the HMG moiety in **4** was determined by ^{1}H NMR spectroscopic analysis of the HMG L-alanylamide prepared from **4**.[9] The amide (**6**) derived from **4** by condensation with L-alanine methyl ester [10] was saponified with sodium methoxide in methanol to afford **1** and a HMG L-alanylamide (**7**) (Scheme 1). The characteristic feature of the *R* and *S* amides in the ^{1}H NMR spectrum was reported that the two methylene protons at the C-2'' and C-

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for **1a**, **1** and **2** (500 and 125 MHz in CD_3OD).

No.	1a		1		2	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	51.4	–	51.7	–	51.3	–
2	76.4	3.83, 1H, *ddd* (2.2, 3.4, 9.8)	86.2	3.82, 1H, *ddd* (2.2, 3.1, 9.8)	86.1	3.84, 1H, *m*
3	36.7	0.76, 1H, *dd* (3.4, 13.7)	36.2	1.09, 1H, *dd* (3.1, 14.1)	36.0	1.14, 1H, *dd* (3.0, 14.0)
		2.23, 1H, *ddd* (5.5, 9.8, 13.7)	exo	2.24, 1H, *ddd* (5.2, 9.8, 14.1)		2.26, 1H, *ddd* (5.2, 9.8, 14.0)
4	53.7	1.66, 1H, *d* (5.5)	53.8	1.65, 1H, *d* (5.2)	52.2	1.90, 1H, *d* (5.2)
5	75.9	3.79, 1H, *dd* (3.4, 8.0)	75.6	3.81, 1H, *dd* (3.1, 8.0)	83.4	3.91, 1H, *dd* (3.4, 7.9)
6	39.1	2.31, 1H, *dd* (8.0, 13.4)	39.8	2.49, 1H, *dd* (8.0, 13.4)	37.8	2.48, 1H, *dd* (7.9, 13.4)
		1.31, 1H, *br d* (13.4)	exo	1.32, 1H, *br d* (13.4)		1.50, 1H, *br d* (13.4)
7	48.6	–	48.3	–	48.3	–
8	21.7	1.07, 3H, *s*	21.4	1.06, 3H, *s*	21.3	1.05, 3H, *s*
9	20.1	0.84, 3H, *s*	20.4	0.85, 3H, *s*	20.1	0.85, 3H, *s*
10	13.2	0.86, 3H, *s*	13.8	0.95, 3H, *s*	13.6	0.96, 3H, *s*
1'			106.1	4.24, 1H, *d* (7.9)	105.8	4.25, 1H, *d* (7.9)
2'			76.1	3.16, 1H, *dd* (7.9, 8.9)	75.4	3.16, 1H, *dd* (7.9, 8.9)
3'			78.3	3.32, 1H, *dd* (8.9, 9.5)	78.1 a)	3.32, 1H, *dd* (8.9, 9.5)
4'			71.8	3.27, 1H, *dd* (9.5, 9.5)	71.7	3.26, 1H, *dd* (8.6, 9.5) a)
5'			77.9	3.21, 1H, *ddd* (2.2, 5.5, 9.5)	77.7 b)	3.21, 1H, *ddd* (2.5, 5.5, 8.6)
6'			62.9	3.65, 1H, *dd* (5.5, 11.9)	62.8	3.66, 1H, *dd* (5.5, 11.9) b)
				3.83, *1H, dd* (2.2, 11.9)		3.83, 1H, *dd* (2.5, 11.9)
1"					103.1	4.25, 1H, *d* (7.9)
2"					75	3.10, 1H, *dd* (7.9, 8.9)
3"					78.3 a)	3.32, 1H, *dd* (8.9, 9.5)
4"					71.7	3.27, 1H, *dd* (8.6, 9.5) a)
5"					77.8 b)	3.21, 1H, *ddd* (2.5, 5.5, 8.6)
6"					62.8	3.65, 1H, *dd* (5.5, 11.9) b)
						3.85, 1H, *dd* (2.5, 11.9)

a - b) The assignments may be interchangeable within the same column.
Coupling constants (*J* in Hz) are given in parentheses.

4'' positions appeared as two AB quartets in the former and two singlets in the latter.[9] The ^{1}H NMR spectrum of **7**, showing a couple of AB quartets at δ 2.43 and 2.56 (1H each, ABq, *J*=15.3 Hz), and δ 2.61 and 2.66 (1H each, ABq, *J*=14.0 Hz), respectively, was coincident with those of *S*-HMG L-alanylamide.[9] Therefore, the absolute configuration at the C-3'' position of the HMG moiety in **7** was established to be *S*, and that of the C-3'' position to be *S* as well. Thus, glycoside (**4**) was shown to be (+)-angelicoidenol 2-*O*-[6'-*O*-*S*-3-hydroxy-3-methylglutaryl]-β-D-glucopyranoside.

Glycoside (**5**), obtained as a pale yellowish syrup, showed an $[M+H]^+$ peak at *m/z* 491.2492 ($C_{23}H_{39}O_{11}$), 14 mass units (CH_2) larger than **4**, in the HR-FAB-MS spectrum. The IR spectrum exhibited ester carbonyl absorption band at 1733 cm^{-1}. The NMR spectroscopic features of **5** were similar to those of

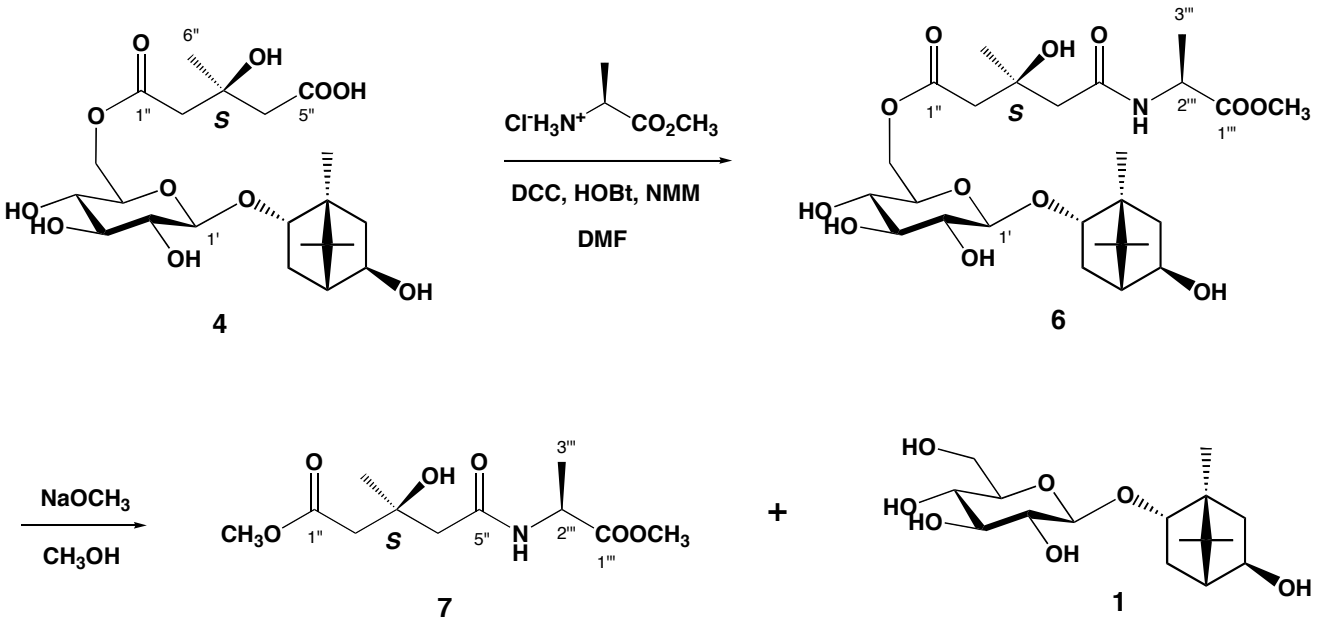
Table 2. ^{1}H and ^{13}C NMR spectroscopic data for **3**, **4** and **5** (500 and 125 MHz in CD_3OD).

No.	3		4		5	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	51.5	–	51.4	–	51.5	–
2	86.2	3.78, 1H, *brd* (9.8)	86.2	3.75, 1H, *br d* (9.8)	86.3	3.76, 1H, *br d* (9.8)
3	36.1	1.08, 1H, *dd* (3.1, 14.1)	36.0	1.07, 1H, *dd* (3.1, 14.0)	36.1	1.07, 1H, *dd* (3.0, 14.0)
		2.26, 1H, *ddd (*5.2, 9.8, 14.1)		2.18, 1H, *ddd (*5.2, 9.8, 14.0)		2.18, 1H, *ddd (*5.2, 9.8, 14.0)
4	53.7	1.65, 1H, *d* (5.2)	53.6	1.64, 1H, *d* (5.2)	53.7	1.66, 1H, *d* (5.2)
5	75.9	3.81, 1H, *dd* (3.4, 8.0)	75.8	3.79, 1H, *dd* (3.4, 7.9)	75.9	3.80, 1H, *dd* (3.4, 8.0)
6	39.7	2.48, 1H, *dd* (8.0, 13.4)	39.6	2.46, 1H, *dd* (7.9, 13.4)	39.7	2.48, 1H, *dd* (8.0, 13.5)
		1.32, 1H, *br d* (13.4)		1.32, 1H, *br d* (13.4)		1.33, 1H, *br d* (13.5)
7	48.1	–	48.0	–	48.1	–
8	21.2	1.06, 3H, *s*	21.2	1.06, 3H, *s*	21.2	1.07, 3H, *s*
9	20.3	0.85, 3H, *s*	20.3	0.84, 3H, *s*	20.3	0.86, 3H, *s*
10	13.6	0.95, 3H, s	13.6	0.94, 3H, *s*	13.5	0.95, 3H, *s*
1'	105.9	4.22, 1H, *d* (8.0)	105.8	4.24, 1H, *d* (8.0)	106.0	4.25, 1H, *d* (8.0)
2'	75.4	3.16, 1H, *dd* (8.0, 9.2)	75.2	3.19, 1H, *dd* (8.0, 8.9)	75.3	3.18, 1H, *dd* (8.0, 8.5)
3'	78.1	3.30, 1H, *dd* (9.2, 9.2)	77.8	3.31, *m*	77.9	3.32, 1H, *m*
4'	71.7	3.23, 1H, *dd* (9.2, 9.5)	71.6	3.31, *m*	71.6	3.32, 1H, *m*
5'	76.8	3.34, 1H, *ddd* (2.2, 6.1, 9.5)	75.0	3.41, 1H, *ddd* (2.1, 5.8, 8.9)	75.1	3.41, 1H, *ddd* (2.2, 6.0, 9.2)
6'	68.5	3.58, 1H, *dd* (6.1, 11.3)	64.6	4.19, 1H, *dd* (5.8, 11.9)	64.6	4.19, 1H, *dd* (6.0, 11.9)
		3.93, 1H, *dd* (2.2, 11.3)		4.42, 1H, *dd* (2.1, 11.9)		4.47, 1H, *dd* (2.2, 11.9)
1"	110.8	5.01, 1H, *d* (2.5)	172.6	–	172.3	–
2"	78.0	3.88, 1H, *d* (2.5)	47.0	2.61, 2.66, 1H each, *d* (14.0)	46.0	2.70, 2.71, 1H each, *d* (15.0)
3"	80.6	–	71.0	–	70.8	–
4"	75.0	3.75, 1H, *d* (9.5)	47.5	2.43, 2.56, 1H each, *d* (15.3)	46.5	2.67, 2.76, 1H each, *d* (14.7)
		3.94, 1H, *d* (9.5)				
5"	66.0	3.57, 2H, *s*	179.2	–	173.1	–
6"			27.9	1.33, 3H, *s*	27.9	1.38, 3H, *s*
O-CH_3					52.0	3.68, 3H, *s*

Coupling constants (*J* in Hz) are given in parentheses.

4, and only difference was the presence of an ester methyl signals (δ_H 3.68, δ_C 52.0) in **5**, suggesting **5** to be methyl ester of **4** (Table 2). The HMBC cross peaks were observed between the methoxyl signal (δ_H 3.68) / C-5" (δ_C 173.1) and H-6' (δ_H 4.19, 4.47) / C-1" (δ_C 172.3). On methylation of **4** with diazomethane, the product obtained was **1** but **5**, although **5** was detected along with **1** in the reaction mixture by TLC. On the other hand, **4** changed partly to **5** when left in the methanol solution at room temperature. Therefore, glycoside (**5**) was determined to be methyl ester of **4**, which may be an artifact formed from **4** during the isolation procedure.

The pungent constituents of ginger, Shokyo and Kankyo, 6-gingerol and 6-paradol were recently



Scheme 1

reported to reduce the viability of human leukemic cells through induction of apoptosis.[11, 12] However, in the MTT assay using U937 human leukemic cells, **1** – **5** showed no significant inhibitory effect on tumor cell growth at 40 μmol/L (data not shown).

As mentioned above, five (+)-angelicoidenol glycosides (**1** – **5**) were isolated from Shokyo (Zingiberis rhizome), and their structures identified by spectroscopic and chemical means. Of them, the bis-glucoside (**2**) and the apiosyl-glucoside (**3**) of (+)-angelicoidenol (**1a**) and the HMG ester (**4**) of **1** were novel compounds, whereas **5** is supposed to be an artifact formed during the isolation procedure. Here, we wish to propose the names zingiberosides A, B, and C for the glycosides (**2**, **3** and **4**), respectively. On the other hand, (+)-angelicoidenol 2-*O*-β-D-glucopyranoside (**1**) was isolated not only from Shokyo [13] and fresh ginger rhizomes,[14] but also from stems of *Berchemia racea (Rhamnaceae)*[15] and *Glehnia littoralis (Umbelliferae)* roots and rhizomes.[6] Of these plants, 2-*O*-β-D-glucopyranoside of the enantiomeric aglycone, (−)-angelicoidenol, was also isolated from *B. racea* and *G. littoralis*, while no glucoside of (−)-angelicoidenol has been isolated from fresh ginger rhizomes nor Shokyo so far. In addition, angelicoidenol was also isolated from aerial parts of *Piper elongatum* (Labiatae),[16] but its absolute stereostructure was not shown.

EXPERIMENTAL

General Procedure The melting points were determined on a Yanaco micro-melting point apparatus

(hot stage type) and were uncorrected. Optical rotations were obtained on a JASCO DIP 140 digital polarimeter, whereas IR spectra were measured on a JASCO FT/IR-410 spectrometer. NMR spectra were recorded on a JEOL JNM LA-500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) with chemical shifts given in ppm from TMS used as an internal standard. The signals were assigned by means of DEPT, 1D difference NOE and 2D NMR techniques (^{1}H-^{1}H COSY, HMQC and HMBC). MS spectra were obtained on a JEOL JMS SX-120A or JMS-700 spectrometer, with the matrix used for FAB-MS shown in parenthesis. HPLC was carried out on a JASCO OR-2090 Plus using a TSK-gel amide-80 column (4.6 × 250 mm). TLC was performed on precoated silica gel 60 F_{254} or RP-18W F_{254} plates (Merck) with the detection achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography (CC) was performed on silica gel 60 (< 45 μm, Merck), Sephadex LH-20 (Pharmacia), or ODS (Chromatorex DM-1020T, Fuji-Silysia Co.), whereas Sep-pack Cartridges were Plus C_{18} (Waters). β-Glucosidase (from almond) and L-alanylamide were purchased from Sigma Chemical Co.

Extraction and Isolation Commercial Shokyo (Zingiberis rhizome) powder (5.0 kg, Uchida Wakanyaku Co., Lot. No. 193012, imported from China) was percolated with MeOH – H_2O (4:1, 28 L) at rt for 6 days, with the resulting methanolic extract concentrated *in vacuo* at 40˚C. The resulting concentrate was next suspended in H_2O (1 L) and extracted with Et_2O (500 mL × 3) to afford an Et_2O extract (155 g) ultimately. The aqueous solution (546 g), after concentration to dryness, was subjected to ODS CC with a gradient mixture of H_2O and MeOH to provide the following six fractions: Frs. A (H_2O, 498.0 g), B (H_2O, 12.2 g), C (MeOH – H_2O (1:1), 1.2 g), D (MeOH – H_2O (1:1), 19.0 g), E (MeOH – H_2O (1:1), 8.2 g) and F (MeOH, 7.2 g). Fr. D was subjected to silica gel CC with $CHCl_3$-MeOH-H_2O (35:15:2) as eluant to give six fractions: Frs. D-1 (0.8 g), D-2 (2.3 g), D-3 (5.8 g), D-4 (2.9 g), D-5 (0.9 g) and D-6 (4.2 g). Fr. D-3 was further applied to a silica gel CC [$CHCl_3$-MeOH-H_2O (14:6:1)] giving four fractions: Frs. D-3-1 (0.84 g), D-3-2 (2.02 g), D-3-3 (0.70 g) and D-3-4 (1.51 g). Fr. D-3-2 was subjected to Sephadex LH-20 CC (MeOH) to give frs. D-3-2a (0.20 g), D-3-2b (1.39 g), D-3-2c (0.08 g), D-3-2d (0.15 g), D-3-2e (0.18 g) and D-3-2f (0.01 g). Fr. D-3-2b was purified by silica gel CC [$CHCl_3$-MeOH-H_2O (40:10:1)] to afford glycoside (**1**) (422 mg). Frs. D-3-2d and D-3-2e were purified by ODS CC (15% MeCN) to afford 4-gingesulfonic acid (22 mg) and shogasulfonic acid A (202 mg), respectively. Fr. D-2 subjected to silica gel CC [$CHCl_3$-MeOH-H_2O (75:25:3)] affording three compounds: glycoside (**4**) (63 mg), 4-gingesulfonic acid (31 mg) and shogasulfonic acid A (62 mg). D-4 was subjected to ODS CC with a gradient mixture of H_2O and MeOH to provide the following five fractions: Frs. D-4-1 (MeOH – H_2O (1:9), 0.53 g), D-4-2 (MeOH – H_2O (3:17), 0.21 g), D-4-3 (MeOH – H_2O (1:4), 0.16 g), D-4-4 (MeOH – H_2O (7:3), 0.82 g) and D-4-5 (MeOH, 0.98 g).

Frs. D-4-3 and D-4-4 were purified by silica gel CC [$CHCl_3$-MeOH-H_2O (35:15:2)] to afford glycoside (**2**) (19 mg), glycoside (**3**) (15 mg), shogasulfonic acids B (24 mg), C (4 mg) and D (14 mg), respectively. Fr. E was separated into three fractions by Sephadex LH-20 CC (MeOH): Frs. E-1 (0.09 g), E-2 (7.86 g) and E-3 (0.33 g). Fr. E-2 (1.0 g) was purified by silica gel CC [$CHCl_3$-MeOH-AcOEt-H_2O (2:2:4:1), lower phase] followed by Sephadex LH-20 CC (MeOH) to give glycoside (**5**) (13 mg) and 6-gingesulfonic acid (98 mg).

Glycoside (1) White amorphous powder, mp 99 – 103 ˚C (decomp), $[\alpha]_D^{20}$ – 18.4° (MeOH, *c* 1.00). Positive FAB-MS (NBA) *m/z*: 333.2064 ($[M+H]^+$, $C_{16}H_{29}O_7$: 333.1835). ^1H and ^{13}C NMR: Table 1. Glycoside **1** was identified as (+)-angelicoidenol 2-*O*-β-D-glucopyranoside by direct comparison with an authentic sample.[6]

Glycoside (2) White powder, mp 136 – 139 ˚C (decomp), $[\alpha]_D^{21}$ – 40.7° (MeOH, *c* 1.27). Positive FAB-MS (NBA) *m/z*: 495.2442 ($[M+H]^+$, $C_{22}H_{39}O_{12}$: 495.2490). IR ν_{max}^{KBr} cm^{-1}: 3403, 2926, 1456, 1372, 1262, 1162, 1077, 1025. ^1H and ^{13}C NMR: Table 1. NOE relationships: H_3-9/H_3-10, H_3-8, exo H-3, H-4, H-2; H_3-8/H_3-10, H_3-9, exo H-6, H-4; end H-6/exo H-6, H-5. Selected HMBC correlations: H-1'/C-2; H-1''/C-5.

Glycoside (3) Colorless viscous oil, $[\alpha]_D^{21}$ – 50.5° (MeOH, *c* 1.00). Positive FAB-MS (NBA) *m/z*: 465.2333 ($[M+H]^+$, $C_{21}H_{37}O_{11}$: 465.2258). IR ν_{max}^{KBr} cm^{-1}: 3398, 2951, 1456, 1391, 1366, 1289, 1232, 1168, 1049, 1032. ^1H and ^{13}C NMR: Table 2. Selected HMBC correlations: H-1'/C-2; H-1''/C-6'.

Glycoside (4) Colorless viscous oil, $[\alpha]_D^{21}$ – 1.3° (MeOH, *c* 2.27). Positive FAB-MS (NBA) *m/z*: 477.2330 ($[M+H]^+$, $C_{22}H_{37}O_{11}$: 477.2336). IR ν_{max}^{KBr} cm^{-1}: 3388, 2952, 1724, 1578, 1392, 1288, 1234, 1203, 1168, 1080, 1049, 1030. ^1H and ^{13}C NMR: Table 2. Selected HMBC correlations: H-1'/C-2; H-6'/C-1''; H-2''/C-1''; H-4''/C-5''.

Glycoside (5) Pale yellowish oily substance, $[\alpha]_D^{20}$ – 14.3° (MeOH, *c* 0.40). Positive FAB-MS (NBA) *m/z*: 491.2492 ($[M+H]^+$, $C_{23}H_{39}O_{11}$: 491.2421). IR ν_{max}^{KBr} cm^{-1}: 3421, 2929, 1733, 1653, 1456, 1375, 1204. ^1H and ^{13}C NMR: Table 2. Selected HMBC correlations: H-1'/C-2; H-6'/C-1''; H-2''/C-1''; H-4''/C-5''; O-CH_3/C-5''.

Enzymatic Hydrolysis of Glycoside (2) An aqueous solution of **2** (10 mg) containing β-glucosidase (10 mg from almond, 3.4 units/mg, Lot. 119H4029, SIGMA Co., Ltd.) was shaken in a water bath for 43 h at 39 ˚C. The reaction mixture was partitioned between EtOAc and H_2O. The EtOAc layer afforded an aglycone (**1a**) (2 mg from **2**) after removal of the solvent followed by silica gel CC. The H_2O layer was examined on its sugar component both on TLC and HPLC, and D-glucose was detected: TLC, R*f* 0.38 [$CHCl_3$-MeOH-H_2O (6:4:1)]; HPLC, t_R 10.0 min [TSK-gel amide-80 column (4.6 × 250

mm), 75% CH_3CN containing triethylamine (100 mmol/L), 1 mL/min, rt; RI (TOSOH RI-8012) and chiral (JASCO OR-2090 Plus) detectors] by comparison with authentic sugars. **Aglycone** : White amorphous powder, $[\alpha]_D^{15}$ + 15.0° (MeOH; *c* 0.33), Positive FAB-MS (NBA) *m/z*: 153.1271 ([M-H_2O+H]$^+$, $C_{10}H_{17}O$: 153.1307). ^1H and ^{13}C NMR: Table 1. Aglycone was identified with (+)-angelicoidenol (**1a**) prepared by the same manner from **1**.

Acid Hydrolysis of Glycoside (3) A solution of **3** (2 mg) in 2N H_2SO_4 (1 mL) was heated at 80 C° for 4 h, and the reaction mixture was neutralized with $BaCO_3$. After removal of the precipitates formed in the mixture by filtration, the filtrate was subjected to Sep-pack Cartridge, and eluted with H_2O and then with MeOH. From the H_2O eluent, D-Apiose and D-glucose were detected both on TLC and HPLC by comparison with authentic sugars: TLC, R*f* 0.36 (D-apiose) and 0.16 (D-glucose), CH_3Cl-MeOH-H_2O (14:6:1); HPLC, t_R 14.5 min (D-apiose) and t_R 22.0 min (D-glucose), TSK-gel amide-80 column (4.6 × 250 mm), 75% CH_3CN containing triethylamine (100 mmol/L), 0.5 mL/min, rt, RI (TOSOH RI-8012) and chiral (JASCO OR-2090 Plus) detectors. From the MeOH eluent, **1a** (R*f* 0.76) and **1** (R*f* 0.49) were detected on TLC (CH_3Cl-MeOH-H_2O, 14:6:1). The residue obtained from the MeOH eluant by removal of the solvent showed only signals due to **1a** and **1** in the ^1H NMR spectrum (the intensity ratio was nearly1:1).

Preparation of L-Alanylamide of Glycoside (4) 1-Hydroxybenzotriazole (HOBt) (7.4 mg, 48 μmol), dicyclohexylcarbodiimide (DCC) (10.0 mg, 48 μmol), and *N*-methylmorpholine (NMM) (5.3 μL, 48 μmol) were added to a dry DMF solution (1 mL) of **4** (11.5 mg, 24 μmol) and L-alanine methyl ester hydrochloride (5.1 mg, 36 μmol), and the mixture was stirred at rt for 21 h. After dilution with EtOAc, the reaction mixture was extracted with water. The aqueous extract was concentrated *in vacuo*, followed by purification with silica gel CC [EtOAc-MeOH (10:1)] to give **6** (10.4 mg, 18.5 μmol, 76 %) as colorless oil. **6**: positive FAB-MS (NBA) *m/z*: 562.2897 ([M+H]$^+$, $C_{26}H_{44}NO_{12}$: 562.2785); ^1H-NMR (CD_3OD) δ: 0.84 (3H, *s*, H_3-9), 0.94 (3H, *s*, H_3-10), 1.06 (3H, *s*, H_3-8), 1.06 (1H, *dd*, *J* = 3.0, 14.0 Hz, H-3*endo*), 1.31 (1H, *br d, J* = 13.8 Hz, H-6*endo*), 1.35 (3H, *s*, H-6''), 1.38 (3H, *d*, *J* = 7.3 Hz, H-3'''), 1.64 (1H, *d*, *J* = 5.5 Hz, H-4), 2.18 (1H, *ddd*, *J* = 5.0, 9.5, 14.0 Hz, H-3*exo*), 2.48 (1H, *dd*, *J* = 7.5, 13.0 Hz, H-6*exo*), 2.67, 2.76 (1H each, *ABq*, *J* = 14.7 Hz, H-4''a and H-4''b), 2.68, 2.72 (1H each, *ABq*, *J* = 15.0 Hz, H-2''a and H-2''b), 3.18 (1H, *dd*, *J* = 8.5, 8.5 Hz, H-2'), 3.41 (1H, *ddd*, *J* = 2.2, 6.0, 9.2 Hz H-5'), 3.32 (2H, *m*, H-3' and H-4'), 3.68 (3H, *s*, OCH_3), 3.76 (1H, *br d*, *J* = 8.6 Hz, H-2), 3.80 (1H, *dd*, *J* = 3.0, 8.5 Hz, H-5), 4.19 (1H, *dd*, *J* = 6.0, 11.9 Hz, H-6'a), 4.25 (1H, *d*, *J* = 8.0 Hz, H-1'), 4.40 (1H, *q*, *J* = 7.3 Hz, H-2'''), 4.47 (1H, *dd*, *J* = 2.2, 11.5 Hz, H-6'b); ^{13}C-NMR (CD_3OD) δ: 13.6 (C-10), 17.4 (C-3'''), 20.3 (C-9), 21.2 (C-8), 27.7 (C-6''), 36.1 (C-3), 39.7 (C-6), 46.7 (C-2''), 47.2 (C-4''),

48.1 (C-7), 49.4 (C-2'''), 51.5 (C-1), 52.8 (O-CH_3), 53.7 (C-4), 64.8 (C-6'), 71.4 (C-3''), 71.7 (C-4'), 75.1 (C-5'), 75.3 (C-2'), 75.9 (C-5), 77.9 (C-3'), 86.3 (C-2), 105.9 (C-1'), 172.6 (C-1''), 173.3 (C-5''), 174.7 (C-1'''). Selected HMBC correlations: H-1'/C-2; H-6'/C-1''; H-2''/C-1''; H-4''/C-5''; H-2'''/C-5'', C-1'''; O-CH_3/C-1'''.

Saponification of the amide (6) The amide (**6**) (10 mg) was treated with 0.1 M NaOMe in MeOH (1 mL) at rt for 30 min under stirring, and the reaction mixture was partitioned between EtOAc and water. The EtOAc and water layers were individually concentrated to dryness *in vacuo* followed by purification with silica gel CC to give an amide (**7**) as a colorless syrup and **1**, respectively. **7**: Positive FAB-MS (NBA) *m/z*: 262.1286 ($[M+H]^+$, $C_{11}H_{20}NO_6$: 262.1212); ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, *s*, H-6), 1.42 (3H, *d*, *J* = 7.2 Hz, H-3'''), 2.49, 2.55 (1H each, *ABq*, *J* = 15.0 Hz, H-2''a and H-2''b), 2.55, 2.73 (1H each, *ABq*, *J* = 15.4 Hz, H-4''a and H-4''b), 4.59 (1H, *dq*, *J* = 6.0, 7.2 Hz, H-2'''), 3.72 (3H, *s*, $COOCH_3$), 3.75 (3H, *s*, $COOCH_3$), 6.87 (1H, *br d*, *J* = 6.0 Hz, NH). The HMG L-alanylamide (**7**) was identified as 3(*S*)-form by coincidence of its ^{1}H NMR spectral data with those reported.[9]

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REFERENCES

1. M. Egawa, *Gendai Toyo Igaku (in Japanese),* 1987, **8**, 40.
2. K. Nishimoto, *Gendai Toyo Igaku (in Japanese)*, 1987, **8**, 62.
3. M. Yuta, *Gendai Toyo Igaku (in Japanese),* 1987, **8**, 45.
4. H. Itokawa*, Gendai Toyo Igaku (in Japanese),* 1993, **14**, 88.
5. Y. Hori, T. Miura, Y. Hirai, M. Fukumura, Y. Nemoto, K. Toriizuka, and Y. Ida, *Phytochemistry*, 2003, **64**, 613.
6. J. Kitajima, C. Okamura, T. Ishikawa, and Y. Tanaka, *Chem. Pharm. Bull.*, 1998, **46**, 1595.
7. M. A. Dubois, M. Wierer, and H. Wagner, *Phytochemistry*, 1990, **29**, 3369.
8. M. A. Berhow, R. D. Bennett, S. M. Poling, S. Vannier, T. Hidaka, and M. Omura, *Phytochemistry,* 1994, **36**, 1225.
9. S. Nozoe, A. Takahashi, and T. Ohta, *Chem. Pharm. Bull.*, 1993, **41**, 1738.

10. W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
11. E. Lee and J. Y. Surh, *Cancer Lett.,* 1998, **134**, 163.
12. C. C. Wang, G. L. Chen, T. L. Lee, and L. L. Yang, *In Vivo*, 2003, **17**, 641.
13. M. Yoshikawa, S. Yamaguchi, K. Kunimi, H. Matsuda, Y. Okuno, J. Yamahara, and N. Murakami, *Chem. Pharm. Bull.*, 1994, **42**, 1226.
14. Y. Sekiwa, Y. Mizuno, Y. Yamamoto, K. Kubota, A. Kobayashi, and H. Koshino, *Biosci. Biotechnol. Biochem.*, 1999, **63**. 384.
15. S. Inoshiri, M. Saiki, H. Kohda, H. Otsuka, and K. Yamasaki, *Phytochemistry*, 1988, **27**, 2869.
16. C. Masuoka, M. Ono, Y. Ito, and T. Nohara, *Food Sci. Technol. Res.*, 2003, **9**, 197.