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Terpene Glycosides and Cytotoxic Constituents from the Seeds of *Amomum xanthioides*

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Abstract

Column chromatographic isolation of the MeOH extract of the seeds of *Amomum xanthioides* afforded a new diterpene glycoside, amoxanthoside A (**1**), two new monoterpene glycosides, (1*S*,4*S*,5*S*)-5-*exo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside (**2**) and (1*R*,4*R*,5*S*)-5-*endo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside (**3**), together with four known compounds, hedychiol A (**4**), pygmol (**5**), (1*S*,4*R*,6*R*)-(+)-6-*endo*-hydroxycamphor (**6**), and dihydroyashabushiketol (**7**). The structures of the new compounds were determined through spectral analysis, including extensive 2D NMR data. The isolated compounds were tested for their cytotoxicity against four human cancer cell lines *in vitro* using a sulforhodamine B bioassay.

Key words

Amomum xanthioides · Zingiberaceae · diterpene glycoside · monoterpene glycoside · cytotoxicity

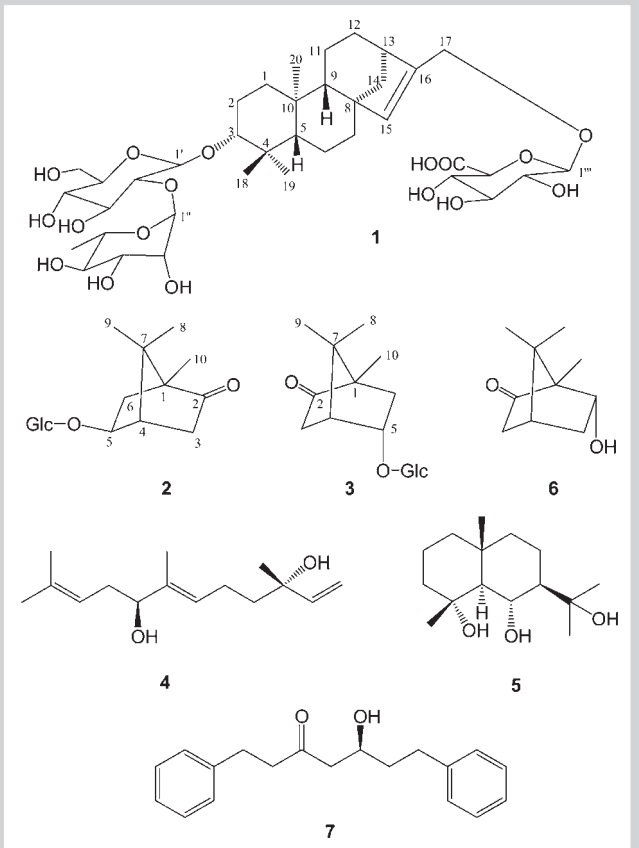

Fig. 1 Structures of compounds **1**–**7**.

The seeds of *Amomum xanthioides* (Zingiberaceae) have been used in folk medicine for the treatment of stomach and digestive disorders [1]. This herb, listed in the Japanese Pharmacopoeia as Amomum seed, contains essential oil (1–1.5%) rich in monoterpenoids [2,3]. Recently, we reported the isolation of terpene and phenolic constituents from the MeOH extract of the seeds of *A. xanthioides* [4]. In our continuing study of this source, we further isolated three new terpene glycosides (**1**–**3**), in addition to four known compounds (**4**–**7**), from the MeOH extract of this plant and tested the cytotoxicities of the isolates (**Fig. 1**).

The crude MeOH extract was analyzed by extensive chromatography, yielding the new diterpene glycoside amoxanthoside A (**1**), the two new monoterpene glycosides (1*S*,4*S*,5*S*)-5-*exo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside (**2**) and (1*R*,4*R*,5*S*)-5-*endo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside (**3**), and the four known compounds hedychiol A (**4**) [5], pygmol (**5**) [6], (1*S*,4*R*,6*R*)-(+)-6-*endo*-hydroxycamphor (**6**) [7], and dihydroyashabushiketol (**7**) [8,9]. Known compounds were identified by comparison of physicochemical and spectroscopic data with previously reported literature values.

Compound **1** was obtained as an amorphous gum. The molecular formula was established as $C_{38}H_{60}O_{17}$ from the $[M + H]^+$ peak at $m/z = 789.3925$ (calcd. for $C_{38}H_{61}O_{17}$: 789.3909) in the HR-FAB-MS. Compound **1** displayed 38 carbon signals in its ^{13}C-NMR spectrum, 20 of which could be assigned to the signals of aglycone. Extensive studies of the 1D and 2D NMR spectra (^{1}H-, ^{13}C-NMR, DEPT, HMQC, HMBC, and NOESY) led to the identification of the aglycone part of **1** as *ent*-kaur-15-en-3*β*,17-diol [10]. This was also confirmed by enzymatic hydrolysis of **1**. In the ^{13}C-NMR spectrum the chemical shifts of C-3 ($\delta_C = 88.8$) and C-17 ($\delta_C = 71.2$) indicated that three monosaccharides were present at two sites of the aglycone, one attached to C-3 and another to C-17 of *ent*-kaur-15-en-3*β*,17-diol [10]. The three anomeric protons detected at $\delta_H = 5.36$, 4.41, and 4.35 in the ^{1}H-NMR spectrum gave correlations with anomeric carbons at $\delta_C = 100.6$, 104.4, and 100.7, respectively, in the HMQC experiment. The units with anomeric protons at $\delta_H = 4.41$ (d, $J = 7.0$ Hz), 5.36 (d, $J = 1.5$ Hz), and 4.35 (d, $J = 8.0$ Hz) corresponded, respectively, to a *β*-D-glucopyranose (Glc), a terminal *α*-L-rhamnopyranose (Rha), and a terminal *β*-D-glucuronopyranosyl acid (GlcA). The deshielded value of C-2′ ($\delta_C = 78.2$) of Glc suggested the position of linkage of the terminal rhamnosyl moiety. The HMBC spectrum showed correlations between H-1′ ($\delta_H = 4.41$) of Glc and C-3 ($\delta_C = 88.8$) of aglycone, between H-1″ ($\delta_H = 5.36$) of the terminal Rha and C-2′ ($\delta_C = 78.2$) of Glc, and between H-1‴ ($\delta_H = 4.35$) of the terminal GlcA and C-17 ($\delta_C = 71.2$) of aglycone. These connectivities were also confirmed by correlations observed in the NOESY spectrum between H-3 ($\delta_H = 3.20$) and H-1′ ($\delta_H = 4.41$) of Glc, between H-2′ ($\delta_H = 3.42$) of Glc and H-1″ (δH = 5.36) of the terminal Rha, and between H-17 ($\delta_H = 4.84$) and H-1‴ ($\delta_H = 4.35$) of the terminal GlcA. The presence of D-glucose and L-rhamnose was established by GC analysis of their chiral derivatives in the acidic hydrolysate [11,12], and the D-configuration for glucuronic acid was determined by the measurement of optical rotation after separation from the crude sugar residue. Thus, **1** was elucidated as 3*β*-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-17-*O*-[*β*-D-glucuronopyranosyl]-*ent*-kaur-15-ene (amoxanthoside A). A survey of the literature revealed that diterpene glycoside from this source was reported for the first time.

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Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	> 100.0	> 100.0	> 100.0	> 100.0
2	> 100.0	> 100.0	> 100.0	> 100.0
3	> 100.0	> 100.0	> 100.0	> 100.0
4	24.29	26.72	11.08	29.20
5	22.64	27.19	24.11	16.42
6	62.64	57.19	70.11	46.42
7	12.41	17.62	11.73	14.29
Doxorubicin[b]	0.16	0.38	0.04	0.82

Table 1 Cytotoxicity of compounds **1**–**7** against four cultured human cancer cell lines using the SRB assay *in vitro*.

[a] The IC_{50} value was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*; [b] Doxorubicin was used as a positive control

Compounds **2** ($C_{16}H_{26}O_7$) and **3** ($C_{16}H_{26}O_7$) showed an $[M + H]^+$ peak at m/z = 331.1757 and 331.1774 (calcd. for $C_{16}H_{27}O_7$: 331.1757), respectively, in the HR-FAB-MS. Their NMR spectral data displayed the presence of one glucose, three *tert*-methyls, two methylenes, one methine, two quaternary carbons, one hydroxylated methine, and one carbonyl carbon, which were similar to those of 5-hydroxycamphor [13]. From the results of the HMBC experiment (HMBC between H-1′/C-5), **2** and **3** had a glucosyl moiety at C-5. Because NOE interactions between H-3_{endo}/H-5 and between H-5/H-6_{endo} were observed in NOESY spectra of **2**, the configuration of H-5 of **2** should be *endo*. The CD spectrum of **2** showed a negative Cotton effect at 295 nm [7]. Thus, **2** was identified as (1*S*,4*S*,5*S*)-5-*exo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside. In addition, NOESY correlation of **3** between H-5/H_3-8 indicated that the configuration of H-5 of **3** should be *exo*. Thus, **3** was characterized as (1*R*,4*R*,5*S*)-5-*endo*-hydroxycamphor 5-*O*-*β*-D-glucopyranoside, which was supported by a positive Cotton effect at 294 nm in the CD spectrum [7].

In this study, the cytotoxicity of the isolates (**1**-**7**) against A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cell lines was evaluated using the sulforhodamine B (SRB) assay *in vitro*. The results (**Table 1**) showed that **4, 5**, and **7** exhibited cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cells, while the other compounds showed little cytotoxicity against the tested cell lines (IC_{50} > 30 μM).

Materials and Methods

The seeds of *A. xanthioides* (2.5 kg), which were imported from China, were bought at Kyungdong Market in December 2007 and identified by one of the authors (K. R. L.). A voucher specimen (SKKU-2007–12B) of the plant was deposited at the College of Pharmacy at Sungkyunkwan University, Suwon, Korea. NMR spectra, including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. CD spectra were measured on a JASCO J-715 spectropolarimeter. FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer.

The seeds of *A. xanthioides* (2.5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (210 g), which was dissolved in water (800 mL) and partitioned with solvent to give *n*-hexane- (18 g), $CHCl_3$- (11 g), and *n*-BuOH- (23 g) soluble portions. The *n*-BuOH-soluble fraction was subjected to column chromatography (CC) over silica gel (230–400 mesh, 500 g, 6 × 90 cm), eluting with a gradient solvent system of $CHCl_3$-MeOH-H_2O (4 : 1 : 0.1, 5 : 2 : 0.1, and 5 : 2 : 0.2, 2 L of each solvent) to yield five crude fractions (F1–F5). F2 (2.4 g) was applied to CC over Sephadex LH-20 (200 g, 3 × 90 cm; Pharmacia Co.), eluting with a solvent system of MeOH-H_2O (9 : 1, 1.5 L) to give 8 subfractions (F21–F28). The subfraction F23 (500 mg) was purified further by semipreparative HPLC, using CH_2Cl_2-MeOH (13 : 1) over 30 min at a flow rate of 2.0 mL/min (Alltech Econosil Silica 5 μ column; 250 × 10 mm; Shodex refractive index detector) to yield **3** (12 mg, R_t = 15.5 min). The subfraction F25 (190 mg) was also purified by semipreparative HPLC, using $CHCl_3$-MeOH (10 : 1) to yield **2** (21 mg, R_t = 13.0 min). F3 (3.1 g) was subjected to CC over silica gel (230–400 mesh, 500 g, 9 × 60 cm) using $CHCl_3$-MeOH (4 : 1, 3 L) to yield 6 fractions (F31–F36). Compound **1** (10 mg) was isolated from F32 (100 mg) by semipreparative HPLC using $CHCl_3$-MeOH (6 : 1, flow rate of 2.0 mL/min, R_t = 14.5 min). Compounds **4**-**7** were isolated from the *n*-hexane-soluble fraction (18 g) by CC over silica gel, Sephadex LH-20, and semipreparative HPLC.

Amoxanthoside A (**1**): 10 mg; amorphous gum; $[\alpha]_D^{25}$: − 4.7 (*c* 0.2, MeOH); IR (KBr): ν_{max} = 3390, 2947, 1638, 1026 cm^{-1}; FAB-MS: m/z = 789 $[M + H]^+$; HR-FAB-MS: m/z = 789.3925 $[M + H]^+$ (calcd. for $C_{38}H_{61}O_{17}$: 789.3909). ^{1}H- and ^{13}C-NMR data: see **Table 2**.

(1S,4S,5S)-5-exo-hydroxycamphor 5-O-β-D-glucopyranoside (**2**): 21 mg; colorless gum; $[\alpha]_D^{25}$: − 20.8 (*c* 0.8, MeOH); IR (KBr): ν_{max} = 3394, 2965, 1728, 1639, 1371, 1028 cm^{-1}; CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 295 (− 2.6) nm; FAB-MS: m/z = 331 $[M + H]^+$; HR-FAB-MS: m/z = 331.1757 $[M + H]^+$ (calcd. for $C_{16}H_{27}O_7$: 331.1757). ^{1}H-NMR (500 MHz, CD_3OD): δ = 4.30 (1H, d, J = 8.0 Hz, H-1′), 4.03 (1H, dd, J = 3.5, 7.5 Hz, H-5_{endo}), 3.91 (1H, br d, J = 11.5 Hz, H-6′a), 3.72 (1H, dd, J = 3.5, 11.5 Hz, H-6′b), 3.37–3.14 (4H, m, H-2′, 3′, 4′, 5′), 2.47 (1H, dd, J = 1.0, 5.0 Hz, H-4), 2.36 (1H, dd, J = 5.0, 18.5 Hz, H-3_{exo}), 1.95 (1H, dd, J = 7.5, 15.0 Hz, H-6_{endo}), 1.78 (1H, m, H-6_{exo}), 1.76 (1H, d, J = 18.5 Hz, H-3_{endo}), 1.23 (3H, s, H-8), 0.90 (3H, s, H-10), 0.84 (3H, s, H-9); ^{13}C-NMR (125 MHz, CD_3OD): δ = 221.1 (C-2), 103.9 (C-1′), 82.7 (C-5), 78.4 (C-5′), 78.1 (C-3′), 75.2 (C-2′), 71.8 (C-4′), 62.9 (C-6′), 59.5 (C-1), 50.5 (C-4), 47.7 (C-7), 40.9 (C-6), 39.6 (C-3), 21.2 (C-9), 20.7 (C-8), 9.4 (C-10).

(1R,4R,5S)-5-endo-hydroxycamphor 5-O-β-D-glucopyranoside (**3**): 12 mg; colorless gum; $[\alpha]_D^{25}$: − 14.8 (*c* 0.4, MeOH); IR (KBr): ν_{max} = 3393, 2966, 1730, 1638, 1375, 1077 cm^{-1}; CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 294 (+ 2.1) nm; FAB-MS: m/z = 331 $[M + H]^+$; HR-FAB-MS: m/z = 331.1774 $[M + H]^+$ (calcd. for $C_{16}H_{27}O_7$: 331.1757). ^{1}H-NMR (500 MHz, CD_3OD): δ = 4.73 (1H, dddd, J = 2.0, 4.0, 4.5, 9.5 Hz, H-5_{exo}), 4.28 (1H, d, J = 7.5 Hz, H-1′), 3.87 (1H, br d, J = 11.5 Hz, H-6′a), 3.67 (1H, dd, J = 3.5, 11.5 Hz, H-6′b), 3.35–3.14 (4H, m, H-2′, 3′, 4′, 5′), 2.80 (1H, d, J = 19.0 Hz, H-3_{endo}),

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Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.61 m, 0.95 m	37.1	Glc		
2	1.65 m, 1.40 m	28.4	1′	4.41 d (7.0)	104.4
3	3.20 dd (10.0, 6.0)	88.8	2′	3.42 m	78.2
4		39.1	3′	3.36 m	78.3
5	0.95 m	54.7	4′	3.38 m	71.2
6	1.37 m, 0.78 m	20.2	5′	3.25 m	76.4
7	1.63 m, 1.35 m	39.1	6′	3.85 m, 3.65 m	61.5
8		45.4	Rha		
9	1.10 m	51.8	1′′	5.36 d (1.5)	100.6
10		35.6	2′′	4.23 m	70.2
11	1.35 m, 1.20 m	20.2	3′′	3.72 m	70.9
12	1.98 m, 1.66 m	26.2	4′′	3.38 m	72.7
13	2.15 br s	41.4	5′′	3.96 m	68.7
14	1.91 m, 1.63 m	29.3	6′′	1.21 d (6.5)	16.8
15	5.37 s	134.2	GlcA		
16		147.4	1′′′	4.35 d (8.0)	100.7
17	4.84 s	71.2	2′′′	3.26 m	75.2
18	1.06 s	27.2	3′′′	3.43 m	77.7
19	0.90 s	15.9	4′′′	3.38 m	73.2
20	0.88 s	13.5	5′′′	3.64 m	76.4
			6′′′		173.7

Table 2 ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data (δ value, J in Hz) of **1** in CD_3OD.

2.41 (1H, m, H-4), 2.22 (1H, ddd, J = 1.5, 9.5, 14.5 Hz, H-6$_{exo}$), 2.16 (1H, ddd, J = 2.0, 4.5, 19.0 Hz, H-3$_{exo}$) 1.41 (1H, dd, J = 4.0, 14.5 Hz H-6$_{endo}$), 1.05 (3H, s, H-8), 0.87 (3H, s, H-10), 0.85 (3H, s, H-9); ^{13}C-NMR (125 MHz, CD_3OD): δ = 220.2 (C-2), 101.6 (C-1′), 76.9 (C-5′), 76.8 (C-3′), 75.7 (C-2′), 73.9 (C-5), 70.5 (C-4′), 61.6 (C-6′), 58.5 (C-1), 46.9 (C-4), 46.0 (C-7), 38.9 (C-6), 34.9 (C-3), 19.3 (C-9), 18.3 (C-8), 8.3 (C-10).

Enzymatic hydrolysis of 1–3

A solution of each sample in H_2O (3 mL) was individually hydrolyzed with crude hesperidinase (30 mg, from *Aspergillus niger*; Sigma-Aldrich) at 37 °C for 72 h. Each reaction mixture was extracted with $CHCl_3$ (3 × 5 mL) to yield the individual $CHCl_3$ layer and H_2O phase after removing the solvents. The combined $CHCl_3$ layer from **1** (3 mg) was chromatographed over a silica gel Waters Sep-Pak Vac 6 cc ($CHCl_3$-MeOH, 30 : 1) to give aglycone **1a** (1.5 mg). The aglycone **1a** was identified by ^{1}H-NMR and MS data [10].

ent-Kaur-15-en-3β,17-diol (**1a**): 1.5 mg; colorless gum; $[\alpha]_D^{25}$: − 20.3 (*c* 0.08, $CHCl_3$); FAB-MS: *m/z* = 304 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.28 (1H, s, H-15), 4.15 (1H, br s, H-17), 3.24 (1H, dd, J = 6.0, 10.0 Hz, H-3), 1.01 (3H, s, H-18), 0.86 (3H, s, H-19), 0.82 (3H, s, H-20).

The combined $CHCl_3$ layers from **2** (3 mg) and **3** (3 mg) were purified over a silica gel Waters Sep-Pak Vac 6 cc ($CHCl_3$-MeOH, 100 : 1) to give **2a** (1.3 mg) [13] and **3a** (1.4 mg) [13], respectively. The sugar (glucose) from the aqueous layers of **2** and **3** was analyzed by silica gel TLC by comparison with standard sugar ($CHCl_3$-MeOH-H_2O, 8 : 5 : 1; R_f, 0.30).

Determination of sugars of 1–3

Compound **1** (2 mg) was heated with 2 N aqueous CF_3-COOH at 120 °C for 2 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ and H_2O. The sugars from the aqueous phase were identified as glucose, rhamnose, and glucuronic acid by co-TLC comparison with authentic samples. The absolute configuration of sugar residues was analyzed by GC analysis of their chiral derivatives [11, 12]. D-Glucose and L-rhamnose for **1** were detected by co-injection of hydrolysate with standard silylated samples, giving single peaks at 18.61 min and 13.12 min, respectively. In the same manner, identification of D-glucose was carried out for **2** and **3**, giving a single peak at 18.64 min. The absolute configuration of glucuronic acid for **1** was determined to be D by the measurement of optical rotation after separation from the crude sugar mixture: $[\alpha]_D^{25}$: + 10.7 (*c* 0.04, H_2O) [14].

Cytotoxicity assay

An SRB bioassay was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines [15]. The cell lines (National Cancer Institute, Bethesda, MD, USA) used were A549 (non-small-cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (purity ≥ 98%; Sigma) was used as a positive control.

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