C-Geranylated Chalcones from the Stems of *Angelica keiskei* with Superoxide-Scavenging Activity

Nobuwa Aoki, Mai Muko, Emi Ohta, and Shinji Ohta*

Nagahama Institute of Bio-Science and Technology, 1266 Tamura-cho, Nagahama, Shiga 526-0829, Japan

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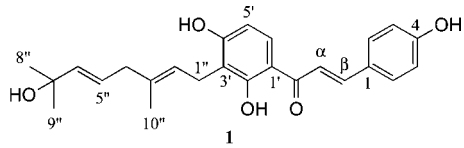
An ethyl acetate-soluble fraction of a methanol extract from the stems of *Angelica keiskei* was subjected to chromatographic separation to give three new chalcones, designated as xanthokeismins A−C (**1**−**3**), in addition to a known chalcone, xanthoangelol B (**4**). The structures of **1**−**3** were determined on the basis of the analysis of their spectroscopic data. Compounds **1**−**4** exhibited potent superoxide-scavenging activity.

The Japanese herb *Angelica keiskei* Koidzumi (Japanese name "Ashitaba") is a perennial plant belonging to the Umbelliferae and has been used in traditional medicine, food, and beverages. The herb has been reported to contain bioactive chalcones,[1−7] flavanones,[5,6] and coumarins,[2,5,6] exhibiting analeptic, antidiabetic, antimetastatic, antitumor, cancer chemopreventive, diuretic, galactagogue, and laxative effects.[5−11] In the course of our search for antioxidants from natural sources, we have found that an EtOAc-soluble fraction of the MeOH extract of *A. keiskei* stems exhibited superoxide-scavenging activity. Activity-guided fractionation of the EtOAc-soluble fraction led to the isolation of three new chalcones, designated as xanthokeismins A (**1**), B (**2**), and C (**3**), in addition to a known chalcone, xanthoangelol B (**4**). In this paper, we report the isolation, structure elucidation, and superoxide-scavenging activity of these compounds.



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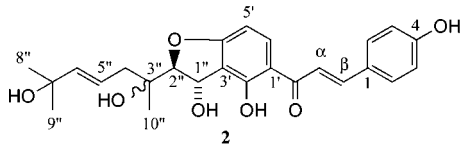

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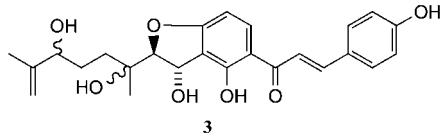

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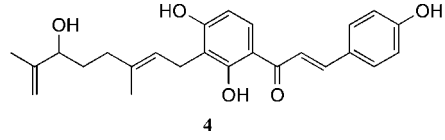

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The MeOH extract of the stems of the Japanese herb *A. keiskei* was partitioned between water and hexane. The aqueous fraction was further partitioned with EtOAc and water. Although the aqueous fraction was inactive, the EtOAc-soluble fraction exhibited potent superoxide-scavenging activity. The active EtOAc-soluble portion was chromatographed on a silica gel column by elution with 0−100% EtOAc in hexane to yield active fractions A and B. Fraction A was further subjected to column chromatography on ODS (50−100% MeOH in H_2O) and preparative HPLC on ODS employing 75% MeOH−H_2O to afford **1** and **4**. Compound **4** was identified as xanthoangelol B.[3] In turn, fraction B was subjected to preparative HPLC on ODS employing 53% MeOH−H_2O to afford **2** and **3**.

The molecular formula of xanthokeismin A (**1**) was established as $C_{25}H_{28}O_5$ on the basis of its (−)-MALDITOFMS data (*m*/*z* 407.1862 [M − H]$^-$, Δ +0.4 mmu). The IR absorption bands at 3350 (OH), 1622, 1604, 1558, and 1514 cm^{-1} (C═O, C═C, and aromatic rings) and the UV absorption at 368 nm (ε 15 400) suggested the presence of a chalcone skeleton.[3,6] Inspection of the ^{1}H and ^{13}C NMR spectra together with the DEPT and HMQC spectroscopic data revealed the presence of two aliphatic methyls, an olefinic methyl, two aliphatic methylenes, 11 olefinic methines, an aliphatic quaternary carbon, five olefinic quaternary carbons, and a carbonyl carbon. The interpretation of the ^{1}H−^{1}H COSY spectrum revealed the presence of five partial structures [C-α to C-*β*, C-5′ to C-6′, C-2 to C-3 (C-5 to C-6), C-1″ to C-2″, and C-4″ to C-6″). The connectivities among these partial structures were established from HMBC correlations (H-α/C═O and C-1; H-*β*/C═O, C-2, and C-6; H-2/C-*β* and C-4; H-5′/C-1′ and C-3′; H-6′/C═O, C-2′, and C-4′; H-1″/C-2′, C-3′, C-4′, C-2″, and C-3″; H-2″/C-4″ and C-10″; H-4″/C-2″, C-5″, C-6″, and C-10″; H-5″/C-7″; H-6″/C-4″; H-8″/C-6″, C-7″, and C-9″; H-9″/C-6″, C-7″, and C-8″; H-10″/C-2″, C-3″, and C-4″), leading to the gross structure depicted in structure **1**. The geometry of the chalcone skeleton was determined to be *E* on the basis of the vicinal ^{1}H coupling constant ($J_{\text{H-}\alpha,\text{H-}\beta}$ = 15.4 Hz). The geometry of the $\Delta^{5''}$ disubstituted olefinic bond was also determined to be *E* on the basis of the vicinal ^{1}H coupling constant ($J_{\text{H-5}'',\text{H-6}''}$ = 15.8 Hz). The observations of NOE enhancements between H-1″ and H_3-10″ indicated the *E* geometry of $\Delta^{2''}$. Thus, xanthokeismin A (**1**) was elucidated as 3-[(2*E*,5*E*)-7-hydroxy-3,7-dimethylocta-2,5-dienyl]-4,2′,4′-trihydroxychalcone.

The molecular formula of xanthokeismin B (**2**) was established as $C_{25}H_{28}O_7$ on the basis of its (−)-MALDITOFMS data (*m*/*z* 439.1749 [M − H]$^-$, Δ −0.8 mmu). The IR absorption bands at 3350 (OH), 1622, 1604, 1558, and 1514 cm^{-1} (C═O, C═C, and aromatic rings) and the UV absorption at 368 nm (ε 15 400) were similar to those of compound **1**, suggesting that compound **2** again has a *C*-geranylated chalcone skeleton. The ^{1}H and ^{13}C NMR spectra were comparable to those of compound **1**, except for the chemical shifts attributable to C-1″, C-2″, C-3″, and C-10″. The ^{1}H and ^{13}C NMR chemical shifts of C-1″ (δ_H 5.61, δ_C 68.3), C-2″ (δ_H 4.51, δ_C 95.3), and C-3″ (δ_C 71.0) together with the DEPT NMR data revealed that these carbons bear an oxygen atom. The HMBC correlations of H-2″ and H-5′ (δ_H 6.48) to C-4′ (δ_C 165.4) indicated the connectivity of C-4′ and C-2″ through an ether bond. The

* To whom correspondence should be addressed. Tel: +81-749-64-8101. Fax: +81-749-64-8140. E-mail: s_ohta@nagahama-i-bio.ac.jp.

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Table 1. ^{1}H NMR Data for **1**−**3** (400 MHz, $CDCl_3$−CD_3OD, 9:1)

	δ_H, mult. (J in Hz)		
position	**1**	**2**	**3**
α	7.44 d (15.4)	7.44 d (15.2)	7.43 d (15.4)
β	7.82 d (15.4)	7.83 d (15.2)	7.83 d (15.4)
2	7.54 d (8.8)	7.56 d (8.2)	7.55 d (8.8)
3	6.86 d (8.8)	6.88 d (8.2)	6.88 d (8.8)
5	6.86 d (8.8)	6.88 d (8.2)	6.88 d (8.8)
6	7.54 d (8.8)	7.56 d (8.2)	7.55 d (8.8)
5′	6.40 d (8.8)	6.48 d (8.7)	6.47 d (8.8)
6′	7.69 d (8.8)	7.92 d (8.7)	7.91 d (8.8)
1″	3.44 d (2H, 7.0)	5.61 d (3.5)	5.59 d (3.3)
2″	5.33 brt (7.0)	4.51 d (3.5)	4.48 d (3.3)
4″	2.74 brd (2H, 6.6)	2.36 dd (14.0, 6.6)	1.69 m (2H)
		2.43 dd (14.0, 6.6)	
5″	5.68 dt (15.8, 6.6)	5.77 dt (15.9, 6.6)	1.74 m (2H)
6″	5.66 d (15.8)	5.71 d (15.9)	4.28 brt (7.0)
8″	1.32 s (3H)	1.34 s (3H)	1.77 brs (3H)
9″	1.32 s (3H)	1.33 s (3H)	4.99 brs (2H)
10″	1.81 brs (3H)	1.17 s (3H)	1.15 s (3H)

positions of C-1″, C-3″, and C-10″ were established by HMBC correlations (H-4″/C-3″ and C-10″; H-10″/C-2″, C-3″, and C-4″; H-2″/C-1″; H-1″/C-3″ and C-4″), leading to the gross structure depicted in structure **2**. The relative stereochemistry between C-1″ and C-2″ on the dihydrobenzofuran ring was suggested to be *trans* by the absence of any NOE correlation between H-1″ and H-2″ and by the comparison of the vicinal ^{1}H coupling constant ($J_{H\text{-}1'',H\text{-}2''} = 3.5$ Hz) and the ^{1}H and ^{13}C NMR chemical shifts of C-1″ to C-3″ with those described in the literature for similar compounds.[12,13] Thus, xanthokeismin B (**2**) was elucidated as 1-[2,3-*trans*-3,4-dihydroxy-2-(1,5-dihydroxy-1,5-dimethylhex-3-enyl)-2,3-dihydrobenzofuran-5-yl]-3-(4-hydroxyphenyl)-*E*-propenone. The relative stereochemistry at C-3″ and the absolute configurations at C-1″−C-3″ of **2** remain to be determined.

The molecular formula of xanthokeismin C (**3**) was determined to be $C_{25}H_{28}O_7$ on the basis of (−)-MALDITOFMS data (*m*/*z* 439.1768 [M − H]$^-$, Δ +1.1 mmu), indicating that compound **3** is an isomer of **2**. The IR absorption bands and the UV absorption were similar to those of compound **2**, suggesting that compound **3** also has a *C*-geranylated chalcone skeleton. The ^{1}H and ^{13}C NMR spectra were similar to those of compound **2**, except for the chemical shifts attributable to C-4″−C-9″. The ^{1}H and ^{13}C NMR chemical shifts of C-4″ (δ_H 1.69, δ_C 34.1), C-5″ (δ_H 1.74, δ_C 29.4), C-6″ (δ_H 4.28, δ_C 89.0), C-7″ (δ_C 143.8), C-8″ (δ_H 1.77, δ_C 17.3), and C-9″ (δ_H 4.99, δ_C 113.5), together with the DEPT NMR data analysis, revealed that the partial structure C-4″ to C-9″ is the same as that of compound **4**, which was supported by the comparison of the ^{1}H and ^{13}C NMR chemical shifts of C-4″ to C-9″ with those of **4**. Thus, xanthokeismin C (**3**) was proposed as 1-[2,3-*trans*-3,4-dihydroxy-2-(1,4-dihydroxy-1,5-dimethylhex-5-enyl)-2,3-dihydrobenzofuran-5-yl]-3-(4-hydroxyphenyl)-*E*-propenone. The relative stereochemistry at C-3″ and C-6″ and the absolute configurations at C-1″−C-3″ and C-6″ of **3** remain to be determined.

Compounds **1**−**4** were examined for superoxide-scavenging activity. The IC_{50} values of **1**−**4** and several reference compounds are given in Table 3. Compounds **1**−**4** exhibited superoxide-scavenging activity with IC_{50} values in the range 0.51−1.1 μM, greater than that of resveratrol, used as a positive control. Among these compounds, xanthokeismin A (**1**) showed the most potent superoxide-scavenging activity. Xanthoangelol, isoliquiritigenin, farnesol, and ascorbic acid had little or no activity. These findings indicated that the specific units in the side chain of **1**−**4** play an important role in mediating superoxide-scavenging activity.

Although the herb *A. keiskei* has been reported to have various biological effects, this is the first investigation to indicate superoxide-scavenging activity of this herb. Superoxide anion radicals are precursors of hydroxyl radicals, with high reactivity against biological molecules. Consequently, it is thought that the superoxide-scavenging activity demonstrated for this herb could result in beneficial effects on ingestion.

Table 2. ^{13}C NMR Data for **1**−**3** (100 MHz, $CDCl_3$−CD_3OD, 9:1)

	δ_C, mult.		
position	**1**	**2**	**3**
C═O	192.0 s	190.7 s	192.1 s
α	117.5 d	114.9 d	116.8 d
β	144.1 d	143.4 d	144.8 d
1	126.7 s	124.7 s	126.2 s
2	130.4 d	129.0 d	130.5 d
3	115.9 d	114.3 d	115.9 d
4	159.4 s	158.3 s	159.7 s
5	115.9 d	114.3 d	115.9 d
6	130.4 d	129.0 d	130.5 d
1′	113.6 s	114.4 s	114.8 s
2′	161.7 s	160.7 s	161.9 s
3′	115.2 s	115.3 s	115.3 s
4′	163.6 s	165.4 s	166.8 s
5′	107.2 d	100.7 d	102.3 d
6′	128.9 d	132.0 d	133.5 d
1″	21.8 t	68.3 d	70.0 d
2″	123.0 d	95.3 d	97.3 d
3″	134.4 s	71.0 s	72.4 s
4″	42.6 t	40.1 t	34.1 t
5″	129.4 d	123.2 d	29.4 t
6″	134.8 d	137.1 d	89.0 d
7″	81.9 s	79.9 s	143.8 s
8″	24.3 q	22.7 q	17.3 q
9″	24.3 q	22.5 q	113.5 t
10″	16.3 q	20.1 q	21.4 q

Table 3. Superoxide-Scavenging Activity of **1**−**4**

compound	IC_{50} (μM)[a]
1	0.51 ± 0.023
2	0.69 ± 0.017
3	1.1 ± 0.12
4	0.92 ± 0.16
xanthoangelol[b]	>40
isoliquiritigenin[b]	15 ± 0.57
farnesol[b]	>40
resveratrol[b]	5.3 ± 0.59
ascorbic acid[b]	>40

[a] Each value is the mean ± SD ($n = 3$). [b] Reference compound.

Experimental Section

General Experimental Procedures. The optical rotations were measured using a Horiba SEPA-300 polarimeter. UV and IR spectra were recorded on a JASCO V-630 and a Horiba FT720 spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL AL400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25 °C. ^{1}H and ^{13}C NMR chemical shifts were referenced to an internal reference (TMS: $\delta = 0$). MALDI-TOFMS were measured on a Applied Biosystems V888630 mass spectrometer. Silica gel (Wacogel C-300) and ODS (Wacogel LP40C18) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) and RP_{18} F_{254} plates (Merck). Xanthoangelol was isolated from the roots of *A. keiskei* according to the procedure described in the literature.[8] Isoliquiritigenin and resveratrol were purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Farnesol and ascorbic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant Material. The stems of *Angelica keiskei* were purchased from Yu-ki Beverage Co. (Hachijo-jima, Japan) in June 2007 and identified by one of the authors (S.O.). Voucher samples (No. AKS0706) are stored at the Laboratory of Environmental Molecular Ecology, Nagahama Institute of Bio-Science and Technology.

Extraction and Isolation. The stems were chopped into small pieces and extracted with MeOH (10 L × 2). The combined MeOH extracts were concentrated to afford an aqueous solution, which was extracted three times with hexane. The aqueous layer was further extracted with EtOAc. The EtOAc-soluble fraction showed superoxide-scavenging

activity (30% inhibition at a concentration of 4 μg/mL). The EtOAc layer was concentrated to a small volume under reduced pressure below 20 °C. The crude extract (630 mg) was subjected to silica gel column chromatography (1.5 cm i.d. × 85 cm) using 20−80% EtOAc in hexane to afford 16 fractions. Of these fractions, fractions A and B were active in the superoxide-scavenging activity assay. Further fractionation of fraction A by column chromatography on ODS (MeOH−H_2O, 75:25) afforded a mixture of two components. The mixture was separated by ODS-HPLC (MeOH−H_2O, 9:1) to afford **1** (3 mg) and **4** (3 mg). Purification of fraction B by ODS-HPLC (MeOH−H_2O, 55:45) afforded **2** (0.5 mg) and **3** (0.5 mg).

Xanthokeismin A (1): pale yellow oil; UV (CH_3OH) λ_{max} (log ϵ) 368 (4.19) nm; IR (film) ν_{max} 3350, 1622, 1604, 1564, 1558, 1514 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 2; HRMALDITOFMS [M − H]$^-$ *m*/*z* 407.1862 ($C_{25}H_{27}O_5$, calcd [M − H]$^-$ 407.1858).

Xanthokeismin B (2): pale yellow oil; $[\alpha]_D$ +36 (*c* 0.04, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 370 (4.18) nm; IR (film) ν_{max} 3300, 1633, 1601, 1558, 1513 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 2; HRMALDITOFMS [M − H]$^-$ *m*/*z* 439.1749 ($C_{25}H_{27}O_7$, calcd [M − H]$^-$ 439.1757).

Xanthokeismin C (3): pale yellow oil; $[\alpha]_D$ +12 (*c* 0.04, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 370 (4.20) nm; IR (film) ν_{max} 3350, 1633, 1601, 1567, 1556, 1514 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD, 9:1), see Table 2; HRMALDITOFMS [M − H]$^-$ *m*/*z* 439.1768 ($C_{25}H_{27}O_7$, calcd [M − H]$^-$ 439.1757).

Superoxide-Scavenging Activity Assay. The superoxide radicals were generated in vitro by the hypoxanthine/xanthine oxidase system. The scavenging activity of compounds was measured by the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-1) method. This method was performed with the SOD assay kit WST provided by Dojindo Molecular Technologies, Inc. (Kumamoto, Japan).[14,15] Twenty microliters of sample, 200 μL of WST working solution, and 20 μL of enzyme working solution were mixed. The mixture was incubated for 20 min at 37 °C. The WST-1 formazan formed in the reaction of superoxide anion with WST-1 was measured spectrophotometrically at 450 nm using a microplate reader (Ultrospec visible plate reader II 96, Amersham Biosciences). The percent inhibition was calculated by the following equation: % inhibition = $\{[(C_{abs} - CB_{abs}) - (S_{abs} - SB_{abs})]/(C_{abs} - CB_{abs})\} \times 100$, where S_{abs}, SB_{abs}, C_{abs}, and CB_{abs} were the absorbances of the sample, the blank sample, the control, and the blank control, respectively. The IC_{50} value was determined as the concentration of sample that inhibited the formation of WST-1 formazan by 50%.

Statistical Analysis. All results are expressed as mean ± SD (n = 3). The significance of difference was calculated by the Student's t test, and values < 0.05 were considered to be significant.

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