Chalcones and Other Compounds from the Exudates of *Angelica keiskei* and Their Cancer Chemopreventive Effects

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Three new chalcones, xanthoangelol I (**1**), xanthoangelol J (**2**), and deoxydihydroxanthoangelol H (**3**), were isolated from an ethyl acetate-soluble fraction of exudates of the stems of *Angelica keiskei*, and their structures were established on the basis of spectroscopic methods. Nine aromatic compounds of known structure, **4**−**12**, and a diacetylene, **13**, were also isolated and identified from this same fraction. On evaluation of these compounds for their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, **1**, **2**, **4**, and **9**−**12** showed potent inhibitory effects on EBV-EA induction. In addition, upon evaluation of the inhibitory effects against activation of (±)-(*E*)-methyl-2[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitrogen oxide (NO) donor, six compounds, namely, **1**, **2**, **4**, **9**, **11**, and **12**, exhibited potent inhibitory effects. Further, isobavachalcone (**4**) exhibited inhibitory effects on skin tumor promotion in an in vivo two-stage mouse skin carcinogenesis test using 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator and TPA as a promoter.

Angelica keiskei Koidzumi (Japanese name "Ashitaba", Umbelliferae) is a hardy perennial herb, growing mainly along the Pacific coast of Japan, and is used traditionally as a diuretic, laxative, analeptic, and galactagogue.[1] The fresh leaves of this plant and its dry powder are used for food. Various chalcones[1−6] and coumarins[1,3,6] have been isolated and characterized from the plant. Two major chalcones of *A. keiskei*, xanthoangelol and 4-hydroxyderricin, demonstrated antitumor-promoting activity in mouse skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) plus 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[6] In the course of our search for potential antitumor-promoters (cancer chemopreventive agents) from natural sources,[7,8] we have isolated and characterized 17 compounds from an ethyl acetate (EtOAc)-soluble fraction of the exudates of *A. keiskei* stems. Their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by TPA and on activation of (±)-(*E*)-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitrogen oxide (NO) donor, were evaluated in a preliminary screen for their potential cancer chemopreventive activities.[9] Our continuing study on the constituents of the EtOAc-soluble fraction of *A. keiskei* stem exudates on a larger scale led to the isolation of 12 further compounds (**1**−**3**, **5**−**13**), including three new chalcones (**1**−**3**), along with a compound (**4**) recently reported.[9] This paper describes the isolation, characterization, and potential cancer chemopreventive effects of these constituents of *A. keiskei*.

Results and Discussion

Six chalcones, xanthoangelol I (**1**), xanthoangelol J (**2**), deoxydihydroxanthoangelol H (**3**), isobavachalcone (**4**),[10] deoxyxanthoangelol H [2′,3′-(2,2-dimethylpyrano)-4-hydroxy-4′-methoxychalcone, 2,2-dimethyl-5-methoxy-8-*p*-coumaroylchroman] (**5**),[2] and dorsmannin A (**6**);[11] three coumarins, xanthotoxin (**7**),[12] isopimpinellin (**8**),[13] and osthenol (**9**);[13] three flavanones, isobavachin (**10**),[14] munduleaflavanone B (**11**),[15] and 8-geranylnaringenin (**12**);[16] and one acetylene, (9*Z*,11*S*,16*R*)-dihydroxyoctadeca-9,17-dien-12,14-diyn-1-yl acetate (**13**),[17] were isolated from an EtOAc-soluble fraction obtained from the stem exudates of *A. keiskei*. Among these, three compounds (**1**−**3**) are new compounds, and the identification of the 10 other known compounds (**4**−**13**) was performed by MS and ^{1}H NMR comparison with corresponding and/or relevant compounds in the literature. Whereas compound **5** has been known as a semisynthetic product,[2] this is the first report of this compound being isolated from a natural source.

The molecular formula of **1** was determined to be $C_{25}H_{28}O_4$ on the basis of a HREIMS measurement ([M]$^+$, *m*/*z* 392.1988). The ^{13}C NMR spectrum (CD_3OD) showed peaks corresponding to 25 carbons (Table 1), which were classified into three CH_3, four sp^3 CH_2, nine sp^2 CH, and nine quaternary carbons by analysis of the DEPT spectrum. The ^{1}H NMR spectrum displayed 26 proton signals (Table 1), suggesting the presence of two hydroxyl groups (IR ν_{max} 3267 cm^{-1}) in the molecule. The direct connectivity of the proton and carbon atoms was determined using the HMQC spectrum. The presence of a *trans*-*p*-coumaroyl [(2*E*)-3-(4-hydroxyphenyl)-2-propenoyl] moiety was indicated by observation of ^{1}H−^{1}H COSY couplings between H-2/H-6 and H-3/H-5, and H-α and H-*β*, and by the relevant ^{13}C−^{1}H long-range couplings observed in the HMBC spectrum (Table 1). The fragment ion at *m*/*z* 147 ([M]$^+$ − $C_9H_7O_2$), formed from the bond cleavage at C═O−C-1′ in the EIMS, supported the presence of this moiety. The large coupling constant (J = 15.6 Hz) between the H-α and H-*β* ^{1}H signals suggested the *E*-geometry of the C-α/C-*β* double bond. The presence of a 1,2,3,4-tetrasubstituted benzene moiety was shown by the observation of ^{1}H−^{1}H COSY coupling (J = 8.5 Hz) between H-5′ and H-6′ and by the relevant ^{13}C−^{1}H long-range couplings observed in the HMBC spectrum (Table 1). These spectroscopic data are consistent with a chalcone structure substituted with oxygen-bearing functionalities at C-2′, C-4, and C-4′, and an alkyl group at C-3′.[2,4,5] The functional group linked to C-2′ and C-3′ was shown to be a 3″,3″-disubstituted dihydropyran ring by the observation of ^{1}H−^{1}H COSY couplings between H-1″ and H-2″ and the relevant ^{13}C−^{1}H long-range couplings in the HMBC spectrum. The ^{13}C−^{1}H long-range couplings from H-10″ to C-2″, C-3″, and C-4″ in the HMBC spectrum implied that one of the alkyl groups attached at C-3″ is a methyl group. The other alkyl group at C-3″ was indicated to be a 4-methyl-3-pentenyl group by observation of ^{1}H−^{1}H COSY couplings between H-4″ and H-5,

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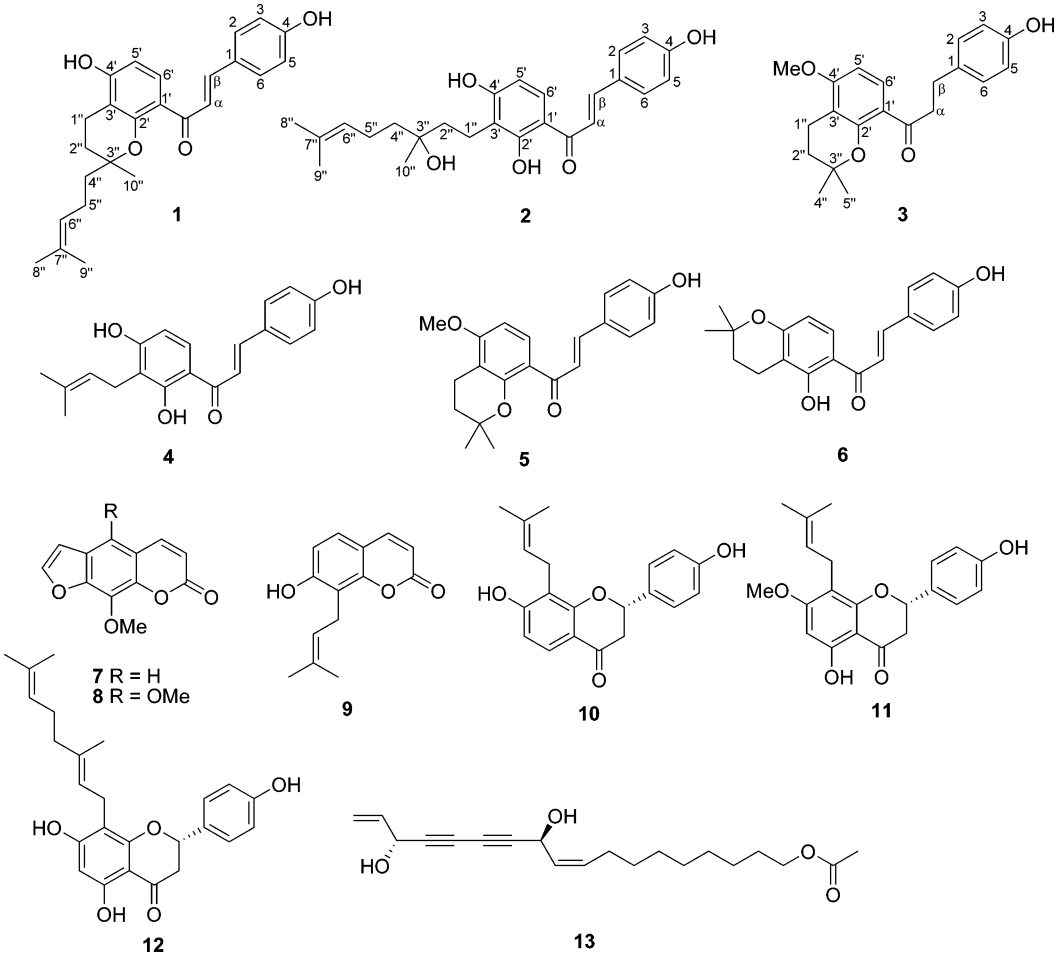
Chart 1



and H-5″ and H-6″, and by the relevant $^{13}C-^{1}H$ long-range couplings in the HMBC spectrum (Table 1). From the foregoing, it was concluded that **1** is 2′,3′-[2-methyl-2-(4-methyl-3-pentenyl)-dihydropyrano]-4,4′-dihydroxychalcone,[18] which has been named xanthoangelol I.

The HREIMS of **2** furnished a $[M]^+$ at *m*/*z* 410.2096, consistent with a molecular formula of $C_{25}H_{30}O_5$. The ^{13}C NMR spectrum [$(CD_3)_2CO$] displayed peaks corresponding to 25 carbons (Table 1), which were classified into three CH_3, four sp^3 CH_2, nine sp^2 CH, and nine quaternary carbons by analysis of the DEPT spectrum. The ^{1}H NMR spectrum displayed 27 proton signals (Table 1) including one chelated hydroxyl ^{1}H signal at δ_H 13.9 (1H, s, O*H*). The ^{13}C and ^{1}H NMR spectroscopic data were very similar to those of xanthoangelol[4] except for the signals arising from the geranyl moiety. Compound **2** possesses the molecular formula $C_{25}H_{30}O_5$, corresponding to that of hydrated xanthoangelol,[4] and the hydroxylation was shown to be at C-3″, to form a 3-hydroxy-3,7-dimethyl-6-octenyl substructure, from the prominent fragment ions at *m*/*z* 309 ($[M]^+ - C_6H_{11} - H_2O$), 283 ($[M]^+ - C_8H_{15}O$), 269 ($[M]^+ - C_9H_{17}O$), and 149 ($C_9H_{15}O_3^+$)[4] observed in the EIMS of **2**. The presence of a 3-hydroxy-3,7-dimethyl-6-octenyl group attached at C-3′ of **2** was supported by the $^{13}C-^{1}H$ long-range couplings observed in the HMBC spectrum (Table 1). Thus, it is proposed that compound **2** is 4,2′,4′-trihydroxy-3′-(3ξ-hydroxy-3,7-dimethyl-6-octenyl)chalcone, which has been named xanthoangelol J. Analysis of the $^{1}H-^{1}H$ COSY, HMQC, and HMBC spectra confirmed the proposed structure as shown.

The molecular formula of **3** was determined as $C_{21}H_{24}O_4$ from the HREIMS ($[M]^+$, *m*/*z* 340.1676). The ^{13}C NMR spectrum (CD_3-OD) showed peaks corresponding to 21 carbons (Table 1), which were classified into three CH_3 (including one CH_3-O), four sp^3 CH_2, six sp^2 CH, and eight quaternary carbons by analysis of the DEPT spectrum. The ^{1}H NMR spectrum displayed 23 proton signals (Table 1), which suggested that at least one oxygen atom is included in a hydroxyl group (IR ν_{max} 3363 cm^{-1}). The presence of a dihydro-*p*-coumaroyl [3-(4-hydroxyphenyl)propanoyl] moiety was indicated by observation of $^{1}H-^{1}H$ COSY couplings between H-2/H-6 and H-3/H-5, and H-α and H-*β*, and by the $^{13}C-^{1}H$ long-range couplings observed in the HMBC spectrum (Table 1). The fragment ion at *m*/*z* 219 ($[M]^+ - C_8H_9O$) formed from the bond cleavage at C═O−C-α in the EIMS supported the presence of this moiety. In addition, the presence of a 1,2,3,4-tetrasubstituted benzene moiety in **3**, as in the case of compound **1**, was indicated by the ^{13}C and ^{1}H NMR spectroscopic data's similarity to the corresponding signals of **1** (Table 1). Compound **3**, however, possesses a methoxyl group instead of a hydroxyl group at C-4′, as shown by a $^{13}C-^{1}H$ long-range coupling from OMe-4′ (δ_H 3.88) to C-4′ (δ_C 161.9) in the HMBC spectrum (Table 1), as well as by a diagnostic nuclear Overhauser effect (NOE) correlation between OMe-4′ and H-5′ in the NOESY spectrum. In addition, **3** was shown to possess a *gem*-dimethyl group (C-4″/C-5″) at C-3″ by the $^{13}C-^{1}H$ long-range couplings observed in the HMBC spectrum (Table 1). On the basis of these data, the structure of compound **3** was assigned as 2′,3′-(2,2-dimethyldihydropyrano)-4-hydroxy-4′-methoxydihydrochalcone[20] and has been named deoxydihydroxanthoangelol H. The $[\alpha]^{25}_D$ value of +1.43° (see Experimental Section) observed for compound **3** may be within the experimental error of the instrument since this compound does not have chiral carbon(s).

The inhibitory effects on the induction of EBV-EA induced by TPA were examined as a preliminary evaluation for antitumor-promoting activity. Table 2 shows the inhibitory effects of compounds **1**−**13** isolated from the EtOAc-soluble fraction against

Table 1. ^{13}C, ^{1}H, and HMBC NMR Spectroscopic Data (δ values) for Chalcones **1**−**3**

	1[a]				**2**[b]				**3**[a]			
position	δ_C		δ_H[c]	HMBC (H to C)	δ_C		δ_H[c]	HMBC (H to C)	δ_C		δ_H[c]	HMBC (H to C)
α	125.9	d	1, CO, *β*		119.0	d	7.70 (d, 15.3)	1, CO, *β*	46.3	t	3.23 (2H, t, 7.7)	1, CO, *β*
β	143.2	d	7.55 (d, 15.6)	1, 2, 6, CO, α	145.4	d	7.78 (d, 15.3)	1, 2, 6, CO, α	30.5	t	2.86 (2H, t, 7.7)	1, 3, 5, CO, α
CO	193.6	s			193.6	s			200.1	s		
1	128.2	s			128.1	s			133.5	s		
2, 6	131.2	d	7.47 (2H, d, 8.5)	1, 2, 4, 6, *β*	132.2	d	7.67 (2H, d, 8.4)	1, 2, 4, 6, *β*	130.0	d	7.05 (2H, d, 8.6)	2, 4, 6, *β*
3, 5	116.9	d	6.80 (2H, d, 8.5)	1, 3, 4, 5	117.3	d	6.87 (2H, d, 8.4)	1, 3, 4, 5	115.9	d	6.73 (2H, d, 8.6)	1, 3, 4, 5
4	161.1	s			161.4	s			156.4	s		
1′	121.9	s			114.8	s			122.4	s		
2′	156.7	s			165.7	s			155.3	s		
3′	110.2	s			117.9	s			110.7	s		
4′	161.4	s			163.4	s			161.9	s		
5′	107.7	d	6.40 (d, 8.5)	1′, 3′, 4′	108.8	d	6.45 (d, 8.8)	1′, 3′	102.7	d	6.57 (d, 8.9)	1′, 3′
6′	130.8	d	7.44 (d, 8.5)	2′, 4′, CO	130.6	d	7.91 (d, 8.8)	4′, CO	130.3	d	7.56 (d, 8.9)	2′, 4′, CO
1″	17.9	t	2.67 (m), 2.72 (m)	2′, 3′, 4′	18.4	t	2.71 (2H, m)	3′, 2″	17.8	t	2.66 (2H, t, 6.9)	2′, 3′, 4′, 2″, 3″
2″	31.3	t	1.86 (2H, m)	1″, 3′, 3″	41.3	t	1.65 (2H, m)	1″, 3″, 10″	32.2	t	1.84 (2H, t, 6.9)	3′, 1″, 3″, 4″, 5″
3″	78.2	s			73.0	s			75.8	s		
4″	41.4	t	1.67 (2H, m)	3″, 5″, 6″	43.0	t	1.50 (2H, m)	2″, 3″, 5″, 6″	26.9	q	1.36 (s)	2″, 3″
5″	23.3	t	2.12 (2H, br q 7.8)	3″, 4″, 6″, 7″	23.9	t	2.08 (2H, m)	4″, 6″, 7″	26.9	q	1.36 (s)	2″, 3″
6″	125.1	d	5.03 (tt, 1.5, 7.6)	5″, 8″, 9″	126.5	d	5.10 (tt, 1.5, 7.3)	5″, 8″, 9″				
7″	132.6	s			131.8	s						
8″	17.6	q	1.44 (s)	6″, 7″, 9″	18.1	q	1.58 (s)	6″, 7″, 9″				
9″	25.8	q	1.60 (s)	6″, 7″, 8″	26.3	q	1.61 (s)	6″, 7″, 8″				
10″	23.8	q	1.35 (s)	2″, 3″, 4″	27.9	q	1.19 (s)	2″, 3″, 4″				
O*H*−2′							13.9 (s)	1′, 2′, 3′				
OMe-4′									56.1	q	3.88 (s)	4′

[a] Determined in CD_3OD. [b] Determined in $(CD_3)_2CO$. [c] *J* values (Hz) determined are shown in parentheses.

Table 2. Inhibitory Effects on the Induction of Epstein−Barr Virus Early Antigen and Inhibitory Ratio (I.R.) on NOR 1 Action of Compounds 1−13 Isolated from *Angelica keiskei* and Reference Compounds

		percentage of EBV-EA induction[a]					
		concentration (mol ratio/32 pmol TPA)					
	compound	1000		500	100	10	IC_{50}[b] (mol ratio/32 pmol TPA)	I.R. of NOR 1 activation[c]
chalcone								
1	xanthoangelol I	0	(70)	20.3	64.7	87.3	273	2.4
2	xanthoangelol J	0	(70)	12.5	66.8	90.2	264	2.4
3	deoxydihydroxanthoangelol H	27.4	(60)	56.7	81.6	100	563	1.6
4	isobavachalcone[d]	0	(60)	26.3	63.0	98.4	320	2.0
5	deoxyxanthoangelol H	32.1	(60)	60.5	85.2	100	578	1.5
6	dorsmannin A	34.2	(60)	61.7	86	100	583	1.9
coumarin								
7	xanthotoxin	14.2	(60)	55.2	83.1	100	490	1.3
8	isopimpinellin	12.1	(60)	57.4	81.5	100	486	1.4
9	osthenol	0	(70)	30.1	69.8	90.6	290	2.0
flavanone								
10	isobavachin	0	(70)	23.5	68.0	88.9	225	1.2
11	munduleaflavanone B	0	(70)	24.9	66.7	90.3	230	2.3
12	8-geranylnaringenin	0	(70)	18.5	56.4	89.3	215	2.1
diacetylene								
13	(9*Z*,11*S*,16*R*)-dihydroxyoctadeca-9,17-dien-12,14-diyn-1-yl acetate	18.7	(60)	53.1	81.4	100	511	1.2
	reference compounds							
	retinoic acid	15.3	(60)	49.3	76.3	100	482	
	glyzyrrhizin	27.4	(>80)	63.3	83.3	100	572	2.2
	carboxy-PTIO							8.0

[a] Values represent percentages relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. [b] IC_{50} represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA. [c] Determined at the concentration of 350 nmol. Inhibitory ratio of NOR 1 (positive control; 350 nmol) was taken as 1.0. [d] Bioassay data were taken from the literature.[9]

TPA (32 pmol)-induced EBV-EA activation in Raji cells. All of the compounds tested caused high viability (60−70%) of Raji cells even at 32 nmol (mol ratio of compound to TPA = 1000:1), indicating their very low cytotoxicity at this high concentration. Of the substances tested, seven compounds, **1**, **2**, **4**, and **9**−**12**, showed potent inhibitory effects with IC_{50} values (concentration of 50% inhibition with respect to positive control) of 215−320 mol ratio/32 pmol TPA and were found to be more potent than the

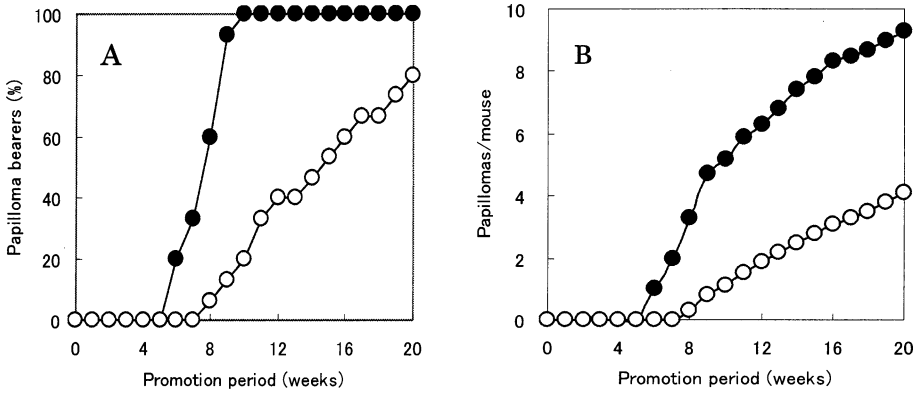


Figure 1. Inhibition of TPA-induced tumor promotion by multiple applications of isobavachalcone (**4**). All mice (n = 15 for each of groups I and II) were initiated with DMBA (390 nm) and promoted with 1.7 nmol of TPA given twice weekly starting 1 week after initiation. (A) Percentage of mice bearing papillomas. (B) Average number of papillomas per mouse. (●) Control TPA alone (group I); (○) TPA + 85 nmol of compound **4** (group II). At 20 weeks of promotion, the number of papillomas per mouse differed significantly by Student's t-test ($P < 0.05$) between groups I and II. The number of papillomas per mouse for each group was 9.3 (SD = ±1.6) and 4.1 (SD = ±1.4) for groups I and II, respectively.

reference compound, retinoic acid (IC_{50} value, 482 mol ratio/32 pmol TPA), one of the retinoids that has been studied as a cancer chemoprevention strategy for various organ site cancers.[21] Since the inhibitory effects against EBV-EA induction have been demonstrated to correlate with those against tumor promotion in vivo,[22] these seven compounds may be potential antitumor-promoters. All of these compounds possess a prenyl or a geranyl group in the molecule. This suggests that prenylation or geranylation of the parent skeletal structure gives rise to more potent activity in this bioassay for compounds of chalcone, coumarin, and flavanone types.

Using an in vitro screening model for NO scavenging,[23] the inhibitory effects of compounds **1**−**13** were evaluated for their scavenging activity against NO generation by NOR 1 in a cultured cell system. Table 2 shows the inhibitory ratios (I.R.) of the 13 compounds and two reference compounds, the natural product, glycyrrhizin, and the synthetic NO scavenger, carboxy-PTIO, on NOR 1 action. Among the isolates tested, six compounds, **1**, **2**, **4**, **9**, **11**, and **12**, exhibited potent inhibitory effects (I.R. 2.0−2.4) that were almost equivalent to the value for glyzyrrhizin (I.R. 2.2). It is noteworthy that all of these six compounds exhibited potent inhibitory effects also on EBV-EA induction, as mentioned above. NO is known to be involved in several potential toxic mechanisms and is a mutagen and can cause mutations in both microorganisms and mammalian cells.[24,25]

On the basis of the results of the in vitro assays described above, we determined subsequently the inhibitory effects of compound **4** in a two-stage carcinogenesis test in mouse skin using DMBA as an inhibitor and TPA as a promoter. The incidence (%) of papilloma-bearing mice and the average numbers of papillomas per mouse are presented in Figures 1A and 1B, respectively. The incidence of papillomas in group I (untreated) was highly significant at 100% of mice at 10 weeks of promotion. Further, more than five and nine papillomas were formed per mouse at 10 and 20 weeks of promotion, respectively. The formation of papillomas in mouse skin was delayed and the mean numbers of papillomas per mouse were reduced by treatment with **4**. Thus, in group II (treated), the percentage ratios of papilloma-bearing mice were 20 and 80% at 10 and 20 weeks, respectively, and the mean papillomas per mouse were 1.1 and 4.1, respectively, compared with the control group at the same time points.

From the results of in vitro EBV-EA induction, in vitro NOR 1 inhibition, and in vivo two-stage carcinogenesis tests, it may be suggested that chalcones, coumarins, and flavanones from the exudates of *A. keiskei* stems may be useful as agents that inhibit chemical carcinogenesis.

Experimental Section

General Experimental Procedures. Crystallizations were performed in MeOH, and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter in $CHCl_3$ at 25 °C. UV spectra, using a Shimadzu UV-2200 spectrometer, and IR spectra, using a JASCO FTIR-300E spectrometer, were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a JEOL LA-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer in CD_3OD or in $(CD_3)_2$-CO with tetramethylsilane as an internal standard. EIMS (70 eV) and HREIMS were recorded on a JEOL JMS-BU20 spectrometer using a direct inlet system, while HRESIMS were recorded on an Agilent G1969A spectrometer. Silica gel (silica gel 60, 220−400 mesh, Merck) and octadecyl silica gel (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Reversed-phase preparative HPLC was carried out on a 25 cm × 10 mm i.d. C_{18} silica column at 25 °C using either a Hypersil ODS 5 μm column (Senshu Scientific Co., Ltd., Tokyo, Japan) with MeOH−H_2O−acetic acid (AcOH) (75:25:1) as mobile phase at 2.0 mL/min (HPLC I), a Pegasil ODS II column (Senshu Scientific Co., Ltd.) with MeOH−H_2O−AcOH (80:20:1; flow rate 2.0 mL/min; HPLC II), or an ERC-ODS-2532 5 μm column (ERC Co., Tokyo, Japan) with MeOH−H_2O−AcOH (65:35:1; flow rate 2.0 mL/min; HPLC III). A refractive index detector was used for reversed-phase HPLC.

Chemicals and Materials. Yellow-colored stem exudates were obtained from *Angelica keiskei* (Hachijo-type) cultivated at a farm at Lombok Island (Indonesia) and harvested in May 2000.[9] The plant was identified by Mr. Kazuya Ogasawara (Japan Bio Science Laboratory Co., Ltd., Oita, Japan), and a voucher specimen (No. IAPB0926) has been deposited in the Research Laboratory, Japan Bio Science Laboratory Co., Ltd. (Oita, Japan).[9] The following chemicals were purchased: TPA from ChemSyn Laboratories (Lenexa, KS), DMBA, (all-*trans*-)retinoic acid, and glyzyrrhizin from Sigma Chemical Co. (St. Louis, MO), the EBV cell culture reagents and *n*-butyric acid from Nacalai Tesque, Inc. (Kyoto, Japan), and NOR 1 and carboxy-PTIO from Dojindo Laboratories (Kumamoto, Japan).

Extraction and Isolation. Evaporation under reduced pressure of the stem exudates of *A. keiskei* (300 g) yielded the dried exudates (22.3 g), which were partitioned with *n*-hexane−MeOH−H_2O (19:19:2), giving *n*-hexane- (0.5 g) and MeOH−H_2O-soluble fractions. The latter was further partitioned with EtOAc−H_2O (1:1) to yield EtOAc (15.3 g) and H_2O fractions. The yellow-colored EtOAc fraction (15.3 g) was chromatographed on a silica gel (500 g) column, which was eluted successively with solvents of increasing polarity [*n*-hexane−EtOAc, 19:1 (1.5 L), 4:1 (2.0 L), 1:1 (4.0 L), 0:1 (5.0 L); EtOAc−MeOH, 4:1 (2.0 L), 1:1 (3.5 L), 0:1 (0.5 L)] to afford 12 fractions, A−L. Fraction D (7817 mg) was subjected to further chromatography on silica gel and yielded 10 fractions, D1−D10. Fraction D5 (138 mg), upon preparative HPLC (HPLC I), afforded **11** (5 mg, t_R 15.6 min). Fraction

D8 (755 mg) was chromatographed on an ODS column to give four fractions, D8a−D8d. On preparative HPLC (HPLC II), fraction D8d (88 mg) yielded **3** (3 mg, t_R 17.0 min) and **5** (17 mg, t_R 15.9 min). Fraction D9 (679 mg), after chromatography over an ODS column afforded four fractions, D9a−D9d. From fraction D9b (393 mg) were isolated **1** (3 mg, t_R 18.5 min), **2** (2 mg, t_R 20.5 min), and **12** (5 mg, t_R 24.4 min) by preparative HPLC (HPLC II). Fraction D10 (1663 mg) was chromatographed on an ODS column and yielded five fractions, D10a−D10e. Upon preparative HPLC (HPLC II), fraction D10b (142 mg) gave **13** (58 mg, t_R 7.2 min). Fraction E (3577 mg) afforded four fractions, E1−E4, upon chromatography on an ODS column. Fraction E1 (1592 mg) was subjected to preparative HPLC (HPLC III) to give **7** (3 mg, t_R 7.8 min) and **9** (6 mg, t_R 14.6 min). Preparative HPLC (HPLC III) of fraction E2 (126 mg) yielded **8** (2 mg, t_R 10.9 min). Fraction F (529 mg), upon chromatography using an ODS column, afforded four fractions, F1−F4. Preparative HPLC (HPLC II) of fractions F2 (54 mg) and F4 (32 mg) yielded **4** (30 mg, t_R 13.7 min) and **6** (3 mg, t_R 31.7 min), respectively.

2′,3′-[2-Methyl-2-(4-methyl-3-pentenyl)dihydropyrano]-4,4′-dihydroxychalcone (1; xanthoangelol I): yellow needles; mp 106−109 °C; $[\alpha]^{25}_D$ +8.0 (*c* 0.1, MeOH); UV λ_{max} (log ϵ) 206 (4.24), 240 (sh, 3.81), 350 (4.05) nm; IR ν_{max} 3267 (OH), 2971, 2930 (Ph-O), 1639 (C═O), 1588, 1512 (aromatic C═C), 1441, 1334, 1233, 1167, 1072, 980, 832, 811 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 392 ($[M]^+$, 70), 349 (6), 323 (9), 309 (13), 295 (15), 281 (12), 269 (85), 203 (17), 149 (100) 147 (22), 123 (16); HREIMS *m*/*z* 392.1988 (calcd for $C_{25}H_{28}O_4$ $[M]^+$, 392.1987).

4,2′,4′-Trihydroxy-3′-(3-hydroxy-3,7-dimethyl-6-octenyl)chalcone (2; xanthoangelol J): yellow needles; mp 120−123 °C; $[\alpha]^{25}_D$ +6.0 (*c* 0.1, MeOH); UV λ_{max} (log ϵ) 206 (4.24), 368 (4.21) nm; IR ν_{max} 3369 (OH), 2969, 2928 (Ph-O), 1615 (C═O), 1605, 1562, 1512 (aromatic C═C), 1442, 1370, 1236, 1168, 1107, 980, 832, 799 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 410 ($[M]^+$, 11), 392 ($[M]^+ - H_2O$, 42), 377 (4), 349 (7), 323 (14), 309 (14), 307 (20), 283 (9), 269 (50), 203 (36), 149 (100), 123 (25), 120 (34); HREIMS *m*/*z* 410.2096 (calcd for $C_{25}H_{30}O_5$ $[M]^+$, 410.2093).

2′,3′-(2,2-Dimethyldihydropyrano)-4-hydroxy-4′-methoxydihydrochalcone (3; deoxydihydroxanthoangelol H): colorless needles; mp 120−123 °C; $[\alpha]^{25}_D$ +1.43 (*c* 0.14, MeOH); UV λ_{max} (log ϵ) 223 (4.53), 275 (4.28), 304 (4.01) nm; IR ν_{max} 3363 (OH), 2926 (Ph-O), 2852, 1637 (C═O), 1584, 1515 (aromatic C═C), 1442, 1380, 1316, 1271, 1240, 1160, 1097, 883, 829, 796 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 340 ($[M]^+$, 87), 309 (4), 297 (6), 285 (36), 219 (98), 192 (80), 179 (27), 164 (73), 163 (100), 136 (27), 132 (25); HREIMS *m*/*z* 340.1676 (calcd for $C_{21}H_{24}O_4$ $[M]^+$, 340.1674).

In Vitro EBV-EA Activation Experiment. The EBV genome-carrying lymphoblastoid cells, Raji cells, derived from Burkitt's lymphoma, were cultured in RPMI-1640 medium. The Raji cells were incubated for 48 h at 37 °C in a medium containing *n*-butyric acid (4 mM), TPA (32 pM), and various amounts of each test compound. Smears were made from the cell suspension, and the EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. Details of this in vitro assay on EBV-EA induction have been reported previously.[22]

In Vitro NOR 1 Inhibition Experiment.[23] Chang liver cells (normal human hepatic cells; 5×10^5/mL), derived from human liver in an MEM Eagle medium, were cultured 3 days before treatment. NOR 1 was added into the culture dish and incubated for 1 h under a CO_2 incubator as a control. For the screening assay, test samples were added to the culture dish 1 min before NOR 1 treatment. Transformed cells were observed under light microscopy (×100). All observed cells counted were more than 250. The inhibitory ratio was then calculated, as follows:

$$\text{inhibitory ratio (I.R.)} = \frac{\text{transformed cell \% (NOR 1 alone)}}{\text{transformed cell \% (NOR 1 + test sample)}}$$

In Vivo Two-Stage Carcinogenesis Assay on Mouse Skin Papillomas. Each group was composed of 15 mice housed five per cage and given water ad libitum. The back of each mouse was shaved with surgical clippers, and the mice were treated topically with DMBA (100 μg, 390 nM) in acetone (0.1 mL) for the initiation treatment. One week after the initiation, papilloma formation was promoted by the application of TPA (1 μg, 17 nM) in acetone (0.1 mL) on the skin twice a week for 20 weeks. Group I received the TPA treatment alone, and group II received a topical application of test sample (85 nM) in acetone (0.1 mL) 1 h before each TPA treatment. The incidence and numbers of papillomas were observed and reported weekly for 20 weeks; only typical papillomas larger than about 1 mm in diameter were counted. Details of this in vivo two-stage carcinogenesis test have been reported previously.[26]

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