Chemical Constituents of *Millettia taiwaniana*: Structure Elucidation of Five New Isoflavonoids and Their Cancer Chemopreventive Activity[1]

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We describe the isolation and identification of five new isoflavonoids, millewanins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), together with six known isoflavonoids and three rotenoids, from the stems of *Millettia taiwaniana* collected in Japan. The major component, auriculasin (**6**), exhibited significant inhibitory effect on mouse skin tumor promotion in an in vivo two-stage carcinogenesis test. The results of the present investigation indicate that **6** might be a valuable antitumor promoter.

Continuing our search for cancer chemopreventive compounds from plant sources, we examined constituents of stems of *Millettia taiwaniana* Hayata (Leguminosae) collected in Japan. The chemical constituents of many *Millettia* species have been studied, and isoflavonoids,[2] rotenoids,[2] chalcones,[2] isoflavans,[3] isocoumarins,[4] pterocarpans,[5] flavanones,[5] and flavones[6] have been identified. Some isoflavonoids have been found to show biological activities against several kinds of cancer cells.[7] In a previous paper, we reported the antitumor-promoting effects of 15 natural isoflavonoids, including millewanin B,[8] millewanin D,[8] auriculasin, and millepurone, isolated from *Millettia* plants on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells (in vitro).[9] Among these isoflavonoids, millepurone, which has a structure corresponding to that of an oxidized and additionally prenylated and hydroxylated analogue of 5,7,4′-trihydroxy-6,8-diprenylisoflavone, was found to have a significant inhibitory effect on EBV-EA activation and in two-stage mouse skin carcinogenesis.[9]

In this paper, we describe the isolation and characterization of five new isoflavonoids, millewanins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), from the stems of *M. taiwaniana* collected in Japan, and the major known compound auriculasin (**6**)[9,10] was found to exhibit a marked inhibitory effect on mouse skin tumor promotion in a two-stage carcinogenesis test.

Results and Discussion

The acetone extract of the stems of *M. taiwaniana* was fractionated by silica gel column chromatography and preparative TLC to obtain five new isoflavones along with six known isoflavones and three known rotenoids.

Millewanin A (**1**) was obtained as a colorless powder having the molecular formula $C_{26}H_{28}O_6$. The IR spectrum exhibited bands at ν_{max} 3533, 3240, and 1655 cm^{-1} due to hydroxy and carbonyl groups, respectively. The signals at δ_H 7.67 and δ_C 153.9 assignable to H-2 and C-2 in ^1H and ^{13}C NMR spectra (Table 1), respectively, were suggestive of an isoflavone type skeleton. The UV spectrum was similar to that of 6, 8-di-γ,γ-dimethylallylorobol,[11] which we isolated from the leaves of this plant, indicating the presence of the 5,7,3′,4′-tetraoxygenated isoflavone skeleton. The ^1H NMR spectrum further revealed the presence of a methoxy group at δ 3.81, an isolated proton at δ 6.70, two broad singlets at δ 6.28 and 6.35 assignable to H-6 and H-8, respectively, and a broad singlet at δ 5.77 due to 4′-OH, in addition to a hydrogen-bonded hydroxy proton at δ 12.83. The appearance of the remaining signals at δ 5.31 (1H, m), 4.98 (1H, m), 3.33 (2H, d, J = 7.3 Hz), 3.28 (2H, br s), 1.73, 1.71, 1.53, and 1.44 (each 3H, s) in the ^1H NMR spectrum, a characteristic MS fragment ion at *m*/*z* 366 arising from loss of [·C_5H_9] followed by a hydrogen transfer from the molecular ion, suggested the presence of two prenyl moieties [$-CH_2CH{=}C(CH_3)_2$] in the molecule. Observation of MS fragment ion peaks at *m*/*z* 284 and 153 derived from a retro-Diels−Alder type cleavage followed by a hydrogen transfer suggested the location of a methoxy group, a hydroxy group, and two prenyl moieties on the B ring. In the NOE experiments, irradiation of the singlet at δ 7.67 (H-2) resulted in 2% enhancement of the singlet at δ 6.70 (H-6′). Irradiation of the methoxy signal at δ 3.81 caused a 2% area increase of the signal at δ 3.28 (H-1″) on the prenyl moiety. Irradiation of the signal at δ 3.28 (H-1″) caused a 5% area increase of the methoxy signal at δ 3.81. Irradiation of the signal of another prenyl moiety at δ 3.33 (H-1‴) gave 9% enhancement of the singlet at δ 6.70 (H-6′). Furthermore, in the HMBC spectrum (Figure 2), correlation from C-3 to H-6′, from C-2′ to H-6′, from C-3′ to 4′-OH and 3′-OCH_3, and from C-4′ to H-6′ and H-1‴ confirmed the location of two prenyl moieties at C-2′, C-5′, a methoxy group at C-3′, and a hydroxy group at C-4′ on the B ring. From these spectroscopic data and further HMBC results (Table 1 and Figure 2), we proposed structure **1** for millewanin A.

Millewanin B (**2**) was isolated as a pale yellow amorphous solid and had the molecular formula $C_{31}H_{36}O_6$ on the basis of the HREIMS. The ^1H NMR spectrum (Table 1) showed a signal pattern similar to that of **1**, except for signals due to the $C_{10}H_{17}$ substituent, instead of the prenyl moiety. The appearance of ^1H NMR signals at δ 5.33 (1H, m), 5.08 (1H, m), 3.35 (2H, d, J = 7.3 Hz), 2.08 (2H, m), 2.03 (2H, m), 1.70, 1.62, and 1.57 (each 3H, s) and NOE (Figure 3) between the H-1‴ (δ 3.35) and H-4‴ (δ 1.70)

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Millewanins (**1**−**5**)[a]

	millewanin A (**1**)		millewanin B (**2**)		millewanin C (**3**)		millewanin D (**4**)[b]		millewanin E (**5**)[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	7.67 (s)	153.9	7.66 (s)	153.8	7.71 (s)	153.7	8.09 (s)	154.2	8.27 (s)	154.4
3		124.3		124.4		124.5		124.5		123.8
4		181.1		181.1		181.1		181.7		182.1
4a		106.1		106.1		106.1		106.2		106.4
5		162.8		162.9		162.9		163.9		156.2
5-OH	12.83 (s)		12.85 (s)		12.85 (s)		13.06 (s)		13.43 (s)	
6	6.28 (br s)	99.4	6.29 (d, 2.2)	99.4	6.30 (br s)	99.4	6.26 (d, 1.8)	99.8		105.6
7		162.2		161.9		162.1		164.9		157.9
7-OH			5.84 (br s)							
8	6.35 (br s)	93.9	6.36 (d, 2.2)	93.9	6.37 (br s)	93.9	6.39 (d, 1.8)	94.4		104.7
8a		158.2		158.1		158.2		159.0		156.1
1″		122.1		122.1		*d*		123.4		122.9
2″		132.2		132.2		132.7	7.19 (d, 2.2)	128.9	7.47 (d, 8.8)	131.1
3″		145.3		145.3		142.7		128.7[e]	6.90 (d, 8.8)	116.0
3″-OCH_3	3.81 (3H, s)	61.4	3.82 (3H, s)	61.4						
4″		147.5		147.5		143.0		153.4		158.5
4″-OH	5.77 (br s)		5.76 (br s)		5.64 (br s)				8.55 (s)	
5″		126.2		126.2		125.2		128.6[e]	6.90 (d, 8.8)	116.0
6″	6.70 (s)	127.7	6.71 (s)	127.6	6.53 (s)	123.9	7.20 (d, 2.2)	128.9	7.47 (d, 8.8)	131.1
1″	3.28 (2H, br s)	26.8	3.28 (2H, br s)	26.8	3.24 (2H, br s)	27.6	3.39 (2H, d, 6.6)	29.5[e]	6.69 (d, 9.9)	116.1
2″	4.98 (m)	123.2	4.98 (m)	123.3	5.23 (m)	122.3	5.35 (m)	123.4	5.77 (d, 9.9)	129.1
3″		131.6		131.4[e]		134.2		133.0		79.0
4″	1.44 (3H, s)	17.5	1.45 (3H, s)	17.5	1.66 (3H, s)	17.7	1.72 (3H, s)	17.9	1.50 (3H, s)	28.5
5″	1.53 (3H, s)	25.4	1.53 (3H, s)	25.4	1.69 (3H, s)	25.4	1.71 (3H, s)	25.8	1.52 (3H, s)	28.5
1‴	3.33 (2H, d, 7.3)	28.1	3.35 (2H, d, 7.3)	28.0	3.35 (2H, d, 7.3)	29.0	3.40 (2H, d, 6.6)	29.2[e]	2.86[c]	25.2
									3.01 (dd, 7.0, 7.3)	
2‴	5.31 (m)	121.8	5.33 (m)	121.6	5.34 (m)	121.7	5.38 (m)	123.2	4.60 (dd, 7.3, 13.4)	88.7
3‴		133.1		136.8		137.0		137.1		145.7
4‴	1.71 (3H, s)	17.8	1.70 (3H, s)	16.1	1.74 (3H, s)	16.1	1.724 (3H, s)	16.2	1.84 (3H, s)	17.3
5‴	1.73 (3H, s)	25.8	2.03 (2H, m)	39.7	2.07 (2H, m)	39.7	2.06 (2H, m)	40.5	4.82 (br s)	113.3
									4.79 (br s)	
6‴			2.08 (2H, m)	26.6	2.10 (2H, m)	26.4	2.10 (2H, m)	27.3		
7‴			5.08 (m)	124.2	5.07 (m)	123.3	5.10 (m)	125.0		
8‴				131.5[e]		131.8		131.8		
9‴			1.57 (3H, s)	17.7	1.58 (3H, s)	17.7	1.56 (3H, s)	17.7		
10‴			1.62 (3H, s)	25.6	1.65 (3H, s)	25.6	1.60 (3H, s)	25.9		
other					5.63 (br s, 3′-OH)				10.59 (s, OOH)	

[a] Values in (δ_H and δ_C) ppm. All signals correspond to 1H, unless otherwise stated. Figures in parentheses are coupling constants (*J*) in Hz. [b] Spectra were recorded in acetone-d_6. [c] Overlapped with H_2O. [d] Because of the small quantity obtained, these quaternary carbon signals could not be assigned. [e] Assignments may be reversed.

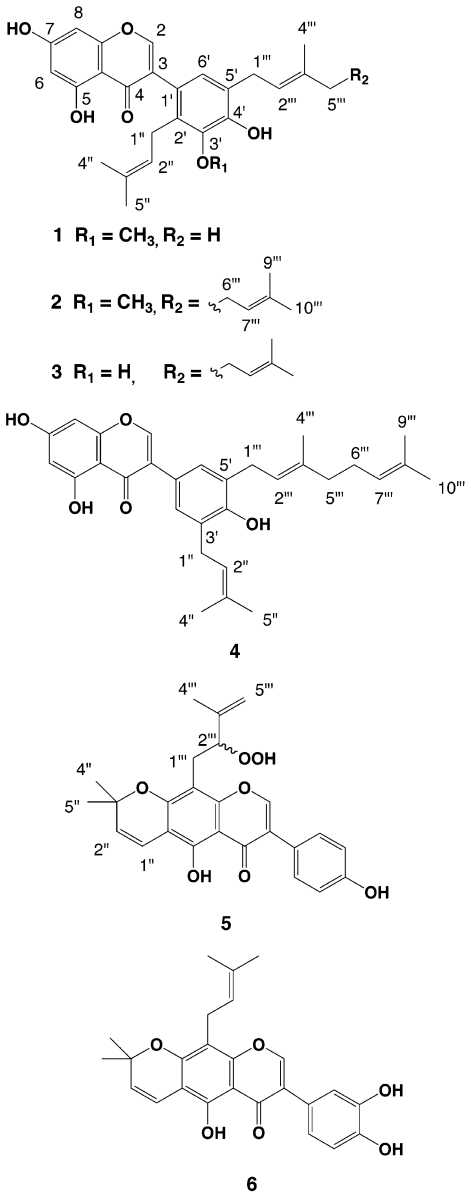

Figure 1. Structures of isoflavonoids from *Millettia taiwaniana.*

proton signals suggested the presence of a geranyl moiety [$-CH_2CH{=}C(CH_3)-CH_2CH_2-CH{=}C(CH_3)_2$] in **2**. The location of the geranyl moiety was confirmed by the HMBC spectrum (Figure 2). C−H long-range correlations from C-6′ to H-1‴ and from C-4′ to H-1‴ deduced the location of a geranyl moiety at C-5′ on the B ring. On the basis of these results, coupled with other observed long-range correlations (Figure 2), the structure of millewanin B was proposed as **2**.

The molecular formula ($C_{30}H_{34}O_6$) of millewanin C (**3**) was found to differ by CH_2 compared with **2** by HREIMS. The signal pattern of the ^{1}H NMR spectrum was similar to that of **2**, except for the hydroxy singlet at δ 5.63, instead of the methoxy singlet at δ 3.82. The HMBC data (Figure 2) were in agreement with structure **3** for millewanin C.

Millewanin D was obtained as a pale yellow oil with a molecular formula of $C_{30}H_{34}O_5$. The UV spectrum was similar to that of 5,7,4′-trihydroxy-3′,5′-di-γ,γ-dimethylallylisoflavone,[12] which was also isolated from this plant. The signals at δ_H 8.09 assignable to H-2 and δ_C 154.2 (C-2) in the ^{1}H and ^{13}C NMR spectra (acetone-d_6, Table 1), respectively, coupled with IR bands at ν_{max} 3589, 3435, 3246, and 1655 cm^{-1} due to hydroxy and carbonyl groups, and MS fragment ion peaks at *m*/*z* 321 and 153 derived from a retro-Diels−Alder type cleavage followed by a hydrogen transfer suggested the presence of a 5,7,4′-trioxygenated isoflavone skeleton. The ^{1}H NMR spectrum revealed the presence of two pairs of doublets at δ 6.26 and 6.39 assignable to H-6 and H-8, δ 7.20 and 7.19 assignable to H-6′ and H-2′, respectively, and a hydrogen-bonded hydroxy proton at δ 13.06. The appearance of the remaining signals (Table 1) suggested the presence of a geranyl and a prenyl moiety in the molecule. The locations of these moieties were confirmed by the HMBC spectrum (Figure 2). C−H long-range correlations between C-2′ and H-1″, C-4′ and H-1″, C-4′ and H-1‴, and C-6′ and H-1‴ suggested the locations of the prenyl moiety at C-3′ and the geranyl moiety at C-5′ on the B ring. On the basis of these results, together with other HMBC correlations, the structure of millewanin D was proposed as **4**.

Millewanin E was obtained as a colorless oil, $[\alpha]_D^{24}$ ±0° (MeOH), and determined to have the molecular formula $C_{25}H_{24}O_7$ by HRFABMS. The UV spectrum was similar to that of warangalone,[13] which was isolated from this plant. In the ^{1}H NMR spectrum (acetone-d_6), a downfield signal at δ 13.43 was confirmed for a hydroxy group at C-5. The signal observed at δ 8.27 was assigned to H-2 of an isoflavonoid skeleton. The ^{1}H NMR spectrum also indicated the typical dimethylpyran ring signals at δ 6.69 (1H, d, J = 9.9 Hz), 5.77 (1H, d, J = 9.9 Hz), and 1.52, 1.50 (each 3H, s) and 1,4-disubstituted aromatic ring signals at δ 7.47 and 6.90 (each 2H, d, J = 8.8 Hz). In millewanin E, the occurrence of a typical mass fragment at *m*/*z* 420 (M^+ − O), a hydrogen-bonded 1H singlet (δ_H 10.59), and oxygen-linked methine-carbon and -proton signals (δ_C 88.7, δ_H 4.60), which were deshielded relative to that of a secondary alcohol, suggested the presence of a hydroperoxy substitutent in the molecule. Furthermore, the proton signals [δ 3.01 (1H, dd, J = 7.0, 7.3 Hz, H-1‴) and 2.86 (1H, overlapped with H_2O, H-1‴), δ 4.60 (1H, dd, J = 7.3, 13.4 Hz, H-2‴)] assignable to a methylene linked to a methine bearing a hydroperoxy group, a vinylmethyl, and an exo-methylene proton signal were observed. These spectroscopic data, coupled with the observation of HMBC correlations (Figure 2) from C-2‴ to the exo-methylene (H-5‴) and vinylmethyl protons, and an MS fragment base peak at *m*/*z* 349 arising from cleavage at the benzylic position indicated the structure of $-CH_2-CH(OOH)-C(CH_3){=}CH_2$ for the side chain. The arrangement of these substituents on the isoflavonoid nucleus was revealed by HMBC analysis. C−H long-range correlations from C-6 to 5-OH and H-2″ indicated a [5,6-*g*] orientation of the pyran ring. Further, HMBC correlations from C-8a to H-2 and H-1‴ indicated the location of the side chain at C-8. On the basis of these data, the structure of millewanin E was concluded to be **5**.

Six known isoflavones, auriculasin (**6**),[10] 5,7,4′-trihydroxy-3′,5′-di-γ,γ-dimethylallylisoflavone,[12] warangalone,[13] 8-γ,γ-dimethylallylwighteone,[11] alpinumisoflavone,[14] and barbigerone,[15] and three known rotenoids, deguelin,[16] α-toxicarol,[16] and tephrosin,[16] were isolated and identified by comparison of spectroscopic data published.

Two-Stage Mouse Skin Carcinogenesis. The inhibitory effect of auriculasin (**6**), isolated as a major component of this plant, was investigated in a two-stage carcinogenesis test focusing on mouse skin papillomas induced by dimethylbenz[*a*]anthracene (DMBA) as an initiator and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as a promoter. The activity evaluated in terms of both the rate (%) of

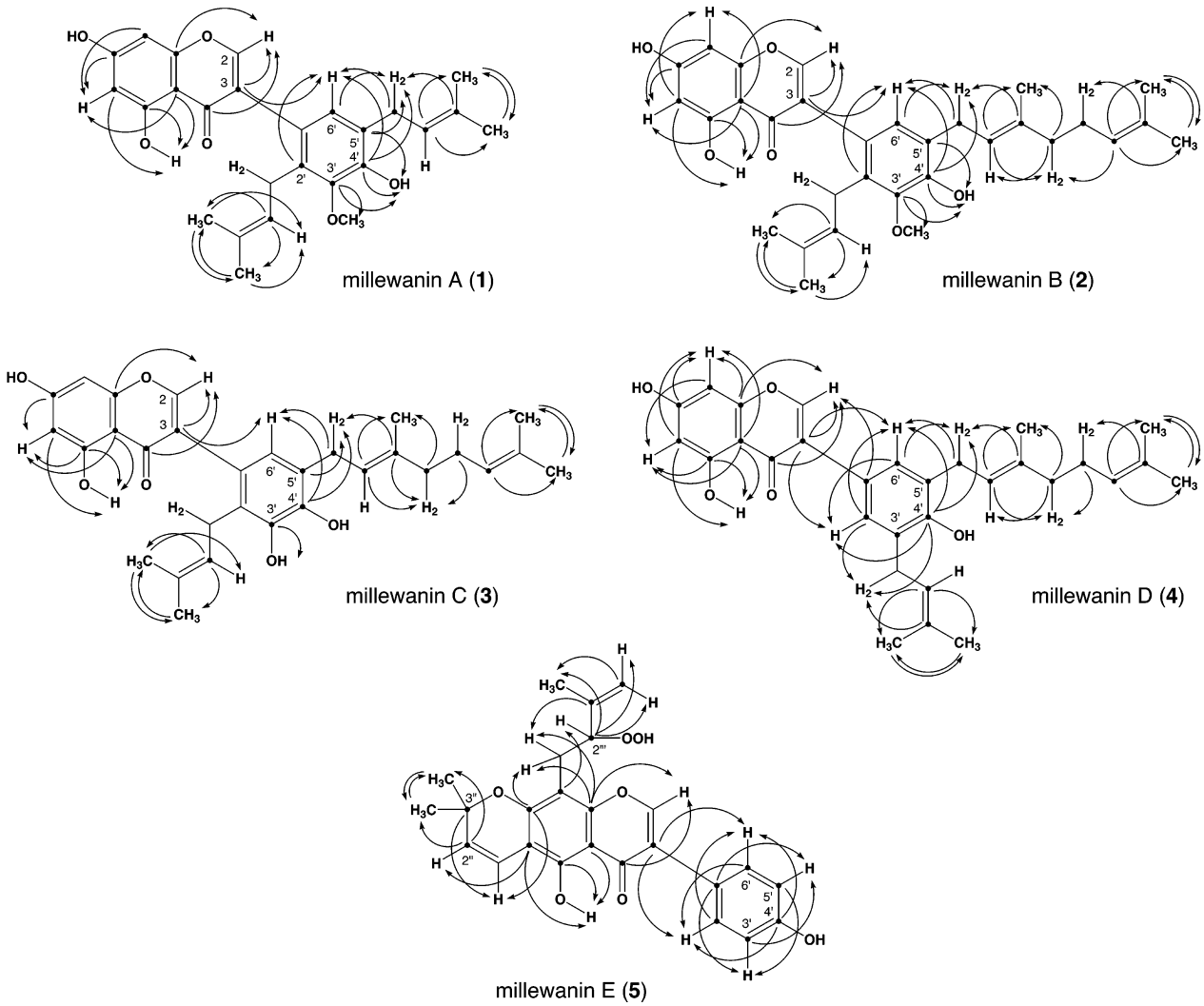

Figure 2. C−H long-range correlations in the HMBC spectra of millewanins A−E (**1**−**5**).

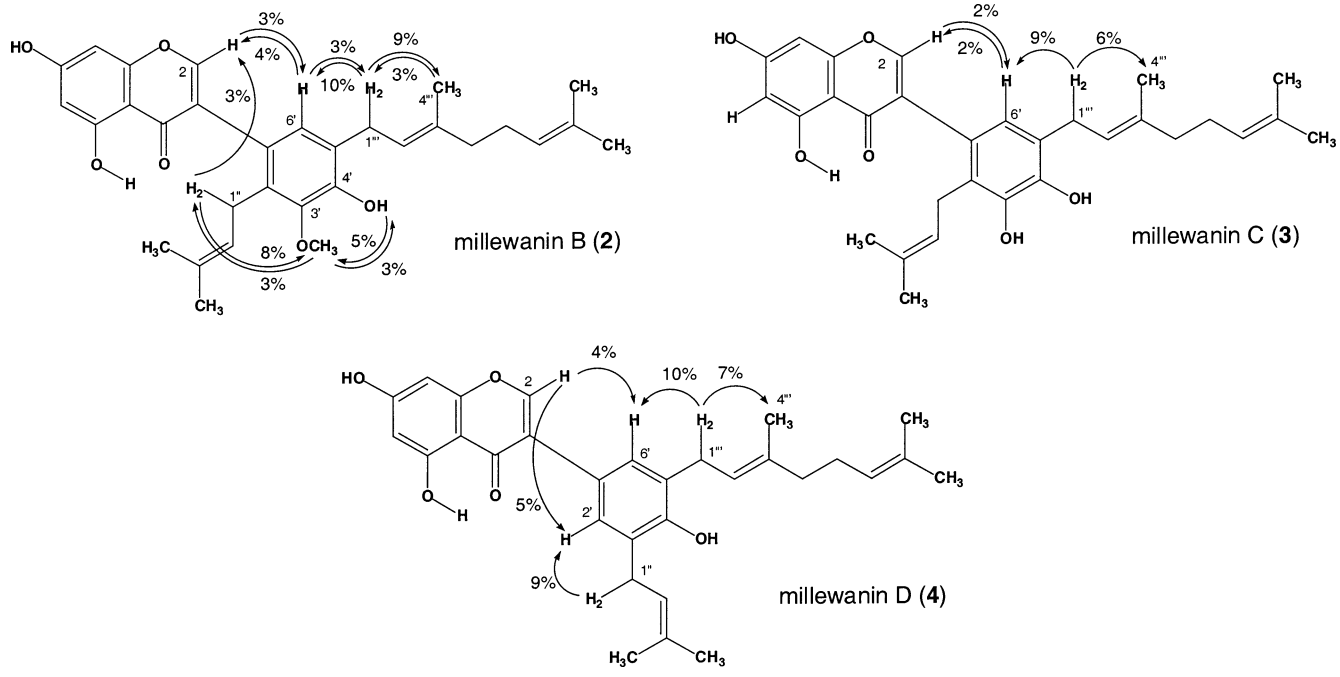

Figure 3. NOE spectra of millewanins B (**2**), C (**3**), and D (**4**).

papilloma-bearing mice (Figure 4A) and the average number of papillomas per mouse (Figure 4B) was compared with that of a positive control. In the case of the positive control, 80 and 100% of the mice bore papillomas after 8

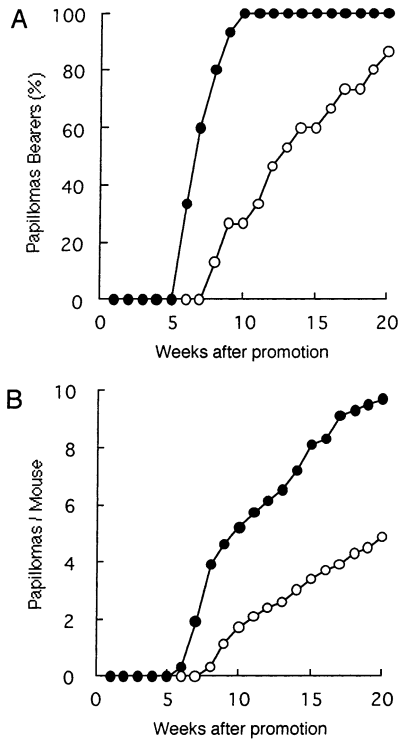

Figure 4. Inhibitory effects of auriculasin (**6**) on DMBA-TPA mouse skin carcinogenesis. Tumors were initiated in all mice with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice weekly starting 1 week after initiation. (A) Percentage of mice with papillomas. (B) Average number of papillomas per mouse. ●, control TPA alone; ○, TPA + 85 nmol of auriculasin (**6**). After 20 weeks of tumor promotion, a significant difference in the number of papillomas per mouse between the groups treated with auriculasin (**6**) and the control group was evident ($p < 0.05$).

and 10 weeks of promotion, respectively, and more than 9.7 papillomas were formed per mouse after 20 weeks, as shown in Figure 4. When auriculasin (**6**) was applied before TPA treatment, it delayed the formation of papillomas as follows. In the group treated with auriculasin (**6**), only about 13 and 27% of mice bore papillomas after 8 and 10 weeks of promotion, respectively, and 87% of the mice bore papillomas, after 20 weeks. Auriculasin (**6**) reduced the number of papillomas per mouse, as follows. Less than 3 and 4 papillomas were formed per mouse after 14 and 17 weeks of promotion, respectively, and only about 4.9 papillomas were formed per mouse after 20 weeks, as shown in Figure 4B. In previous studies, we reported that auriculasin (**6**) and millepurone showed significant inhibitory activity in a short-term in vitro assay of TPA-induced EBV activation in Raji cells.[9] In the present study, auriculasin (**6**) showed about the same inhibitory activity as millepurone[9] in the in vivo two-stage mouse skin carcinogenesis test. The results of the present investigation indicate that auriculasin (**6**) might be a potentially valuable cancer chemopreventive agent (antitumor promoter).

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC (J = 8 Hz), and NOE were measured on JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers. Chemical shifts are shown in δ (ppm), with tetramethylsilane (TMS) as an internal reference. All mass spectra were recorded under EI conditions, unless otherwise stated, using HX-110 (JEOL) and/or JMS-700 (JEOL) spectrometers having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH, and IR spectra on an IR-230 (JASCO) in $CHCl_3$. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Plant Materials. The plant materials used in this study, *Millettia taiwaniana* Hayata cultivated at Higashiyama Zoo & Botanical Garden (Nagoya) in Japan, were collected in October 1997. A voucher specimen was deposited in Meijo University under number MUY0005.

Isolation of Millewanins A (1), B (2), C (3), D (4), and E (5) from *M. taiwaniana*. The dried stems (863 g) of *M. taiwaniana* were extracted with acetone at room temperature, and the solvent was evaporated under reduced pressure to give the acetone extract (6.6 g). The acetone extract was subjected to silica gel column chromatography eluted with hexane−acetone (19:1, 9:1, 4:1, 3:1, 3:2, 1:4), CH_2Cl_2−MeOH (3:1), and MeOH, successively, to give nine fractions. Successive column (silica gel) chromatography and preparative TLC of each fraction using appropriate combinations of solvents (hexane, EtOAc, $CHCl_3$, CH_2Cl_2, acetone, iPr$_2$O, benzene, and MeOH) as eluting or developing solvents gave the following compounds. Fraction 4 (hexane−acetone, 4:1) gave millewanin E (**5**, 1.5 mg), warangalone (95.0 mg), and α-toxicarol (2.5 mg), and fraction 5 (hexane−acetone, 3:1) gave millewanins A (**1**, 2.1 mg), B (**2**, 5.9 mg), C (**3**, 1.0 mg), and D (**4**, 24.8 mg), auriculasin (**6**, 511.1 mg), 5,7,4′-trihydroxy-3′,5′-di-γ,γ-dimethylallylisoflavone (2.1 mg), 8-γ,γ-dimethylallylwighteone (4.1 mg), alpinumisoflavone (16.3 mg), barbigerone (3.5 mg), deguelin (97.7 mg), and tephrosin (19.5 mg).

Millewanin A (1) (3-[4-hydroxy-3-methoxy-2,5-bis(3-methyl-2-butenyl)phenyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one): colorless powder; UV (MeOH) λ_{max} 208, 260, 289 nm; IR ($CHCl_3$) ν_{max} 3533, 3240, 1655, 1624 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; EIMS m/z 436 (M^+, 40), 366 (8%, $M^+ - \cdot C_5H_9 - \cdot H$), 321 (5), 284 (13), 228 (5), 153 (base peak); HREIMS m/z 436.1884 (calcd for $C_{26}H_{28}O_6$, 436.1879).

Millewanin B (2) (3-[5-(3,7-dimethyl-2,6-octadienyl)-4-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one): pale yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 207 (4.71), 260 (4.41), 285 (4.00) nm; IR ($CHCl_3$) ν_{max} 3581, 3533, 3239 br, 1651, 1624 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; NOE, see Figure 3; EIMS m/z 504 (M^+, 24), 419 (5), 380 (9), 351 (4), 313 (5), 153 (100); HREIMS m/z 504.2509 (calcd for $C_{31}H_{36}O_6$, 504.2497).

Millewanin C (3) (3-[5-(3,7-dimethyl-2,6-octadienyl)-3,4-dihydroxy-2-(3-methyl-2-butenyl)phenyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one): colorless oil; UV (MeOH) λ_{max} 207, 261, 286 nm; IR ($CHCl_3$) ν_{max} 3545, 3228, 1653, 1624 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; NOE, see Figure 3; EIMS m/z 490 (M^+, base peak), 421 (13, $M^+ - \cdot C_5H_9$), 405 (57), 389 (18), 366 (34), 353 (17, $M^+ - \cdot C_{10}H_{17}$), 349 (29), 337 (28), 311 (18), 295 (15), 236 (17); HREIMS m/z 490.2355 (calcd for $C_{30}H_{34}O_6$, 490.2361).

Millewanin D (4) (3-[3-(3,7-dimethyl-2,6-octadienyl)-4-hydroxy-5-(3-methyl-2-butenyl)phenyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 2.04 (4.66), 263 (4.51) 289 (4.07) nm; IR ($CHCl_3$) ν_{max} 3589, 3435 br, 3246 br, 1655, 1624 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; NOE, see Figure 3; EIMS m/z 474 (M^+, 41), 457 (9, $M^+ - \cdot OH$), 405 (9, $M^+ - \cdot C_5H_9$), 389 (19), 349 (41), 337 (12, $M^+ - \cdot C_{10}H_{17}$), 295 (21), 153 (100); HREIMS m/z 474.2403 (calcd for $C_{30}H_{34}O_5$, 474.2369).

Millewanin E (5) (5-hydroxy-10-(2-hydroperoxy-3-methyl-3-butenyl)-7-(4-hydroxyphenyl)-2,2-dimethyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one): colorless oil; $[\alpha]_D^{24}$ ±0° (c 0.18, acetone); UV (MeOH) λ_{max} (log ϵ) 202 (4.39), 226 (4.19), 286 (4.36) nm; IR ($CHCl_3$) ν_{max} 3587, 3315 br, 1653, 1616 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HMBC, see Figure 2; EIMS m/z 420 (9, $M^+ - \cdot O$), 405 (5), 368 (12), 349 (base peak, $M^+ - \cdot C_4H_7O_2$), 335 (33, $M^+ - \cdot C_5H_9O_2$), 313 (6), 255 (6), 236 (11); FABMS m/z 437 ($M + H$)$^+$; HR-FABMS m/z 437.1601 (calcd for $C_{25}H_{25}O_7$, 437.1624).

In Vivo Two-Stage Mouse Skin Carcinogenesis Test. Female ICR mice were obtained at 5−6 weeks of age from SLC

Co. Ltd. (Shizuoka, Japan). Groups of animals (15 animals per group) were housed in bunches of five in polycarbonate cages and given food and water ad libitum throughout the experiment. The back of each mouse was shaved with surgical clippers before the first day of initiation. Tumors on the back of the mice were initiated with DMBA (390 nmol) in acetone (0.1 mL). One week after initiation, they were promoted twice a week by the application of TPA (1.7 nmol) in acetone (0.1 mL). For the animals in the test compound treated groups the mice were treated with the test compounds (85 nmol) in acetone (0.1 mL) 1 h before each TPA treatment. The incidence of papillomas was observed weekly for 20 weeks.

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References and Notes

(1) Part of this paper was presented at the 119th Annual Meeting of the Pharmaceutical Society of Japan (Tokushima, Japan), 1999.
(2) Yenesew, A.; Midiwo, J. O.; Waterman, P. G. *Phytochemistry* **1998**, *47*, 295−300.
(3) Kumar, R. J.; Krupadanam, G. L. D.; Srimannarayana, G. *Phytochemistry* **1989**, *28*, 913−916.
(4) Khalid, S. A.; Waterman, P. G. *Phytochemistry* **1983**, *22*, 1001−1003.
(5) Baruah, P.; Barua, N. C.; Sharma, R. P.; Baruah, J. N.; Kulanthaivel, P.; Herz, W. *Phytochemistry* **1984**, *23*, 443−447.
(6) Sritularak, B.; Likhitwitayawuid, K.; Conrad, J.; Vogler, B.; Reeb, S.; Klaiber, I.; Kraus, W. *J. Nat. Prod*. **2002**, *65*, 589−591.
(7) Li, L.; Wang, H. K.; Chang, J. J.; McPhail, A. T.; McPhail, D. R.; Terada, H.; Konoshima, T.; Kokumai, M.; Kozuka, M.; Estes, J. R.; Lee, K.-H. *J. Nat. Prod*. **1993**, *56*, 690−698.
(8) The structures of millewanin B [286462-31-5] and millewanin D [86462-31-5] have been reported previously to explain their biological activity (Ito, C.; et al. *Cancer Lett.* **2000**, *152*, 187−192), and their isolation, structure elucidation, and spectroscopic data have been published here for the first time.
(9) Ito, C.; Itoigawa, M.; Tan, H. T. W.; Tokuda, H.; Mou, X. Y.; Mukainaka, T.; Ishikawa, T.; Nishino, H.; Furukawa, H. *Cancer Lett.* **2000**, *152*, 187−192.
(10) Minhaj, N.; Khan, H.; Kapoor, S. K.; Zaman, A. *Tetrahedron* **1976**, *32*, 749−751.
(11) Singhal, A. K.; Sharma, R. P.; Thyagarajan, G.; Herz, W.; Govindan, S. V. *Phytochemistry* **1980**, *19*, 929−934.
(12) Labbiento, L.; Menichini, F.; Monache, F. D. *Phytochemistry* **1986**, *25*, 1505−1506.
(13) Falshaw, C. P.; Harmer, R. A.; Ollis, W. D.; Wheeler, R. E.; Lalitha, V. R.; Rao, N. V. S. *J. Chem. Soc.* (*C*) **1969**, 374−382.
(14) Tahara, S.; Narita, E.; Ingham, J. L.; Mizutani, J. *Z. Naturforsch*. **1990**, *45c*, 154−160.
(15) Vilain, C. *Phytochemistry* **1980**, *19*, 988−989.
(16) Carlson, D. G.; Weisleder, D.; Tallent, W. H. *Tetrahedron* **1973**, *29*, 2731−2741.

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