New Bisabolane Sesquiterpenes from the Rhizomes of *Curcuma xanthorrhiza* ROXB. and Their Inhibitory Effects on UVB-Induced MMP-1 Expression in Human Keratinocytes

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Two new bisabolane sesquiterpenoids, **1** and **2**, along with five known ones, 13-hydroxyxanthorrhizol (**3**), 12,13-epoxyxanthorrhizol (**4**), xanthorrhizol (**5**), β-curcumene (**6**), and β-bisabolol (**7**), were isolated from the rhizomes of *Curcuma xanthorrhiza* ROXB. The chemical structures of the new compounds were determined to be (7*R*,10*R*)-10,11-dihydro-10,11-dihydroxyxanthorrhizol 3-*O*-β-D-glucopyranoside (**1**) and (−)-curcuhydroquinone 2,5-di-*O*-β-D-glucopyranoside (**2**) on the basis of 1D- and 2D-NMR spectroscopic analyses and optical-rotation characteristics. Compounds **2** and **3** decreased MMP-1 expression in UVB-treated human keratinocytes by *ca.* 8.9- and 7.6-fold at the mRNA level, and by *ca.* 9.2- and 6.6-fold at the protein level, respectively. The results indicate that the isolated compounds may have anti-aging effects through inhibition of MMP-1 expression in skin cells.

Introduction. – The rhizomes of *Curcuma xanthorrhiza* (turmeric spices, *temu lawaq*) of the Zingeberaceae have been used for medicinal purposes mainly in Southeast Asia. In Indonesia, the rhizome is widely used as a tonic and cholagogue [1], and in Europe it is also employed in choleretic drug preparations [2]. Curcuminoids (diarylheptanoids) and essential oils are major bioactive ingredients exhibiting stimulant, aspirant, carminative, cordeal, emenagogue, astringent, detergent, anti-cancer, antinociceptive, diuretic, and martirnet bioactivities [3–6]. Xanthorrhizol is a phenolic bisabolane sesquiterpene obtained as the main constituent of rhizome oil from *C. xanthorrhiza* [7]. Previously, more than 50 bisabolane sesquiterpenes have been identified from the turmeric rhizomes of the genus *Curcuma* [8–20]. The current study was initiated to search for other active compounds from the rhizomes of *C. xanthorrhiza* and led to the isolation of seven bisabolane sesquiterpenoids, among which two were new compounds and two were compounds isolated for the first time from this source. 13-Hydroxyxanthorrhizol (**3**) and 12,13-epoxyxanthorrhizol (**4**) were previously isolated from the rhizomes of *C. longa*, and xanthorrhizol (**5**), β-curcumene (**6**), and β-bisabolol (**7**) were previously isolated from the rhizomes of *C. xanthorrhiza*. The two new compounds were identified as (7*R*,10*R*)-10,11-dihydro-10,11-dihydroxy-xanthorrhizol 3-*O*-β-D-glucopyranoside (**1**) and (−)-curcuhydroquinone 2,5-di-*O*-β-D-glucopyranoside (**2**; *Fig. 1*).

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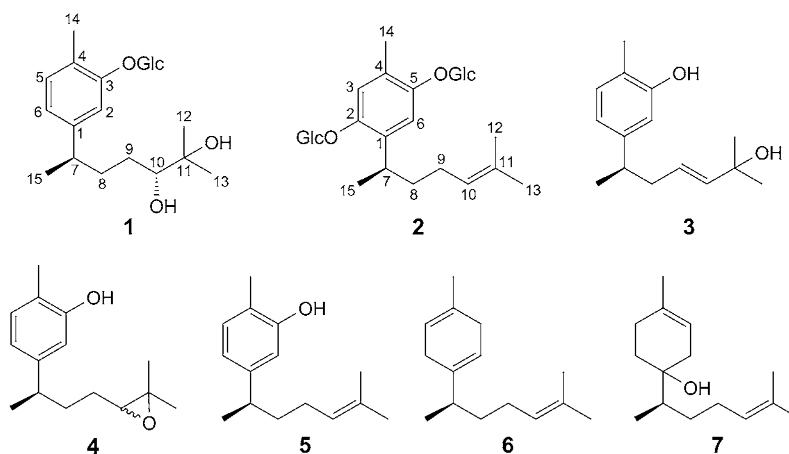

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Curcuma xanthorrhiza ROXB., *and compounds* **3**–**7**

Recently, a variety of herbs and plants have been widely used in applications for the treatment of collagen synthesis and collagenase inhibition [21]. UV Irradiation causes various adverse effects on human skin such as skin cancer and premature skin aging (photoaging) [22]. Type 1 collagen accounts for 70–90% of all collagen, which is synthesized by fibroblasts as a soluble precursor, type 1 procollagen, which is secreted and processed to form insoluble collagen fibers in the dermis. The production of collagen in the skin is reduced by UV irradiation, whereas gene expression of matrix metalloproteinases (MMPs) is increased. MMP-1 is the protein most responsible for the degradation of type 1 collagen in the skin [23]. It has been reported that natural compounds from plants are potential sources of MMP inhibitors, which may protect from UV-induced skin aging [24]. Herein, we describe the isolation of seven bisabolane sesquiterpenoids, **1**–**7**, and the structure determination of the two new ones, **1** and **2**. We also investigated the anti-aging protective effects of these compounds against UV irradiation.

Results and Discussion. – Compound **1** was isolated as a yellow amorphous powder and showed IR absorbance bands of OH (3374 cm^{-1}) and aromatic (1578 cm^{-1}) groups. The molecular weight was determined to be 414 from the molecular-ion peak at *m/z* 415 ($[M+H]^+$) in the FAB mass spectrum (positive-ion mode), and a molecular formula of $C_{21}H_{34}O_8$ was deduced from the molecular-ion peak (*m/z* 415.2307 ($[M+H]^+$; $C_{21}H_{35}O_8$; calc. 415.2333) in the HR-FAB-MS. The ^{1}H-NMR spectrum (*Table*) exhibited three H–C (sp^2) signals of a 1,2,4-trisubstituted benzene ring at δ(H) 7.01 (*d*, $J = 8.0$, H–C(5)), 6.98 (*d*, $J = 1.2$, H–C(2)), and 6.75 (*dd*, $J = 8.0, 1.2$, H–C(6)). An O-bearing CH signal at δ(H) 3.18 (*dd*, $J = 10.8, 2.0$, H–C(10)) was also observed. In the high magnetic field region, a CH signal at δ(H) 2.67–2.61 (*m*, H–C(7)), two CH_2 signals at δ(H) 1.84–1.81, 1.60–1.58 (CH_2(8)) and 1.48–1.45, 1.28–1.25 (CH_2(9)), an allyl-Me signal at δ(H) 2.22 (*s*, Me(14)), a Me signal at δ(H) 1.22 (*d*, $J = 6.8$, Me(15)), and two Me signals at δ(H) 1.09 (*s*, Me(12)) and 1.05 (*s*, Me(13)) were observed. In

Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; CD_3OD) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1		148.0		136.9
2	6.98 (*d*, *J* = 1.2)	115.0		151.7
3		157.2	6.97 (*s*)	120.0
4		126.2		127.3
5	7.01 (*d*, *J* = 8.0)	131.4		152.9
6	6.75 (*dd*, *J* = 8.0, 1.2)	121.9	6.99 (*s*)	115.6
7	2.67 – 2.61 (*m*)	41.3	3.50 – 3.34 (*m*)	32.4
8	1.84 – 1.81 (*m*), 1.60 – 1.58 (*m*)	36.9	1.63 – 1.59 (*m*), 1.49 – 1.43 (*m*)	38.6
9	1.48 – 1.45 (*m*), 1.28 – 1.25 (*m*)	30.6	1.94 – 1.87 (*m*), 1.84 – 1.78 (*m*)	27.3
10	3.18 (*dd*, *J* = 10.8, 2.0)	79.8	5.12 (*t*-like, *J* = 6.4)	126.1
11		73.8		131.7
12	1.09 (*s*)	25.6	1.51 (*s*)	17.8
13	1.05 (*s*)	24.9	1.63 (*s*)	25.9
14	2.22 (*s*)	16.1	2.23 (*s*)	16.3
15	1.22 (*d*, *J* = 6.8)	22.8	1.15 (*d*, *J* = 6.8)	22.1
1′	4.85 (*d*, *J* = 7.6)	102.8	4.72 (*d*, *J* = 7.6)	103.9
2′	3.47[a])	75.0	3.46[a])	75.0
3′	3.40[a])	78.3	3.42[a])	78.2
4′	3.34[a])	71.5	3.32[a])	71.4
5′	3.38[a])	78.1	3.36[a])	78.0
6′	3.87 (*dd*, *J* = 12.0, 2.0), 3.69 (*d*, *J* = 12.0, 6.4)	62.6	3.87[a]), 3.72[a])	62.5
1″			4.73 (*d*, *J* = 7.2)	104.1
2″			3.45[a])	75.1
3″			3.41[a])	78.8
4″			3.33[a])	71.4
5″			3.35[a])	78.1
6″			3.86[a])	62.6
			3.72[a])	

[a]) Overlapped signals, reported without multiplicity.

addition, signals of a hemiacetal H-atom (δ(H) 4.85 (*d*, *J* = 7.6, H–C(1′))), O-bearing CH_2 H-atoms (δ(H) 3.87 (*dd*, *J* = 12.0, 2.0, H_a–C(6′)) and 3.69 (*dd*, *J* = 12.0, 6.4, H_b–C(6′)), and four O-bearing CH H-atoms (δ(H) 3.48 – 3.46 (*m*, H–C(2′)), 3.40 – 3.36 (overlapped, H–C(3′) and H–C(5′)), and 3.31 – 3.30 (*m*, H–C(4′)) were detected due to a hexose moiety.

The ^{13}C-NMR spectrum (*Table*) showed signals corresponding to 21 C-atoms: an O-bearing olefin quaternary, two olefin quaternary, three olefinic CH C-atoms, an O-bearing quaternary and an O-bearing CH C-atom, a CH, two CH_2, and four Me group, and hexose signals, indicating that compound **1** was a sesquiterpene monoglycoside. The sugar was identified as a β-glucopyranose from the chemical shifts observed, including a hemiacetal signal at δ(C) 102.8 (C(1′)), four O-bearing CH C-atom signals at δ(C) 78.3 (C(3′)), 78.1 (C(5′)), 75.0 (C(2′)), and 71.5 (C(4′)), and of an O-bearing CH_2 C-atom at δ(C) 62.6 (C(6′)). The olefinic region displayed six aromatic signals,

including an O-bearing olefin quaternary signal (δ(C) 157.2 (C(3)), two olefin quaternary signals (δ(C) 148.0 (C(1)) and 126.2 (C(4))), and three olefin CH signals (δ(C) 131.4 (C(5)), 121.9 (C(6)), and 115.0 (C(2))). Furthermore, the oxygenated region showed an O-bearing quaternary signal (δ(C) 73.8 (C(11))) and an O-bearing CH signal (δ(C) 79.8 (C(10))). The Me signals at δ(C) 25.6, 24.9, 22.8, and 16.1 were assigned to C(12), C(13), C(15), and C(14), respectively. The unsaturation degree was five as deduced from the molecular formula $C_{21}H_{34}O_8$, indicating that aglycone was composed of one benzene ring moiety with an aliphatic side chain. Consequently, compound **1** was identified as a bisabolane sesquiterpene glucopyranoside. The relative configuration of the anomeric C-atom was confirmed to be β from the coupling constant of the hemiacetal H-atom signal ($J = 7.6$ Hz) in the ^{1}H-NMR spectrum. To determine the locations of the glucose moiety, aliphatic chain, and OH and Me groups in compound **1**, 2D-NMR experiments such as gHSQC and gHMBC were conducted. In the gHMBC spectrum, two olefinic CH H-atom signals at δ(H) 6.98 (H–C(2)) and 6.75 (H–C(6)) showed cross-peaks with CH C-atom signals at δ(C) 41.3 (C(7)) through ^3J correlation. Also, the anomeric H-atom signal at δ(H) 4.85 (H–C(1′)) showed a correlation with the O-bearing olefin quaternary signal at δ(C) 157.2 (C(3)) through ^3J correlation. Based on these results, the aliphatic chain and the β-D-glucose moiety were determined to be located at C(1) and C(3), respectively. The O-bearing CH signal at δ(H) 3.18 (H–C(10)) of the aliphatic chain showed correlations with the O-bearing quaternary signal at δ(C) 73.8 (C(11)) and CH_2 signal at δ(C) 36.9 (C(9)), and the O-bearing quaternary signal at δ(C) 73.8 (C(11)) showed cross-peaks with the Me signals at δ(H) 1.09 (Me(12)) and 1.05 (Me(13)). From these results, two OH groups were determined to be located at the terminal end of the aliphatic chain. As a result, the new compound **1** was determined as a 10,11-dihydro-10,11-dihydroxyxanthorrhizol 3-*O*-β-D-glucopyranoside. The configurations of both of C(7) and C(10) were determined as (*R*) by the comparison of the specific rotation value of the aglycone **1a** with that reported in the literature [7]. The specific rotation value ($[\alpha]^{20}_{D}$) of the aglycone of compound **1**, which was obtained through acid hydrolysis of compound **1**, was as -34. Hence, compound **1** was (7*R*,10*R*)-10,11-dihydro-10,11-dihydroxyxanthorrhizol 3-*O*-β-D-glucopyranoside, a new compound.

Compound **2** was isolated as a brown amorphous powder and showed IR absorbance bands of OH (3374 cm^{-1}) and aromatic (1595 cm^{-1}) groups. The molecular weight was determined as 558 from the molecular-ion peak at *m*/*z* 559 ($[M+H]^+$) in the FAB-MS (positive-ion mode), and a molecular formula of $C_{27}H_{42}O_{12}$ was deduced from the molecular-ion peak (*m*/*z* 559.2730 ($[M+H]^+$, $C_{27}H_{43}O_{12}$; calc. 559.2755) in the HR-FAB-MS. In the ^{1}H-NMR (400 MHz, CD_3OD) spectrum of compound **2**, most signals were similar to those of compound **1**, with the exception of the signals of an additional sugar moiety, an olefinic H-atom (δ(H) 5.12 (*t*-like, $J = 6.4$)), and two aromatic H-atom *singlet*s (δ(H) 6.99 and 6.97) due to a 1,2,4,6-tetrasubstituted benzene ring. The ^{13}C-NMR (100 MHz, CD_3OD) spectrum of **2** exhibited signals corresponding to 27 C-atoms, including those of two hexoses and a benzene moiety, two O-bearing olefinic quaternary C-atom signals (δ(C) 152.9 (C(5)) and 151.7 (C(2))), two olefinic quaternary C-atom signals (δ(C) 136.9 (C(1)) and 127.3 (C(4))), two olefinic CH signals (δ(C) 120.0 (C(3)) and 115.6 (C(6))), and two olefinic signals (δ(C) 131.7 (C(11)) and 126.1 (C(10))). The two sugar moieties were identified as β-glucopyranoses

from their chemical shifts. The 2D-NMR experiment confirmed the location of two glucopyranoses and other key functional groups in **2**. In the gHMBC spectrum, the correlations of the olefin CH signal at δ(H) 6.99 (H–C(6)) showed a cross-peak with the C-signal at δ(C) 32.4 (C(7)). The signals of two anomeric H-atoms at δ(H) 4.73 (H–C(1″)) and 4.72 (H–C(1′)) showed correlations with the O-bearing olefinic quaternary C-atom signals at δ(C) 151.7 (C(2)) and 152.9 (C(5)). Based on these results, two β-D-glucose moieties were determined to be located at C(2) and C(5). As a result, compound **2** was determined to be a curcuhydroquinone 2,5-di-*O*-β-D-glucopyranoside. The configuration of C(7) was determined as (*R*) by the comparison of the specific rotation value of the aglycone with that in the literature [25]. The aglycone of compound **2** was determined to be (7*R*)-configured from the specific rotation value ($[\alpha]_D^{20} = -28$) of the aglycone obtained through acid hydrolysis of compound **2**. As a result, compound **2** was determined to be a new compound (−)-curcuhydroquinone 2,5-di-*O*-β-D-glucopyranoside.

The known compounds were identified as 13-hydroxyxanthorrhizol (**3**) [26], 12,13-epoxyxanthorrhizol (**4**) [7], xanthorrhizol (**5**) [27], β-curcumene (**6**) [28], and β-bisabolol (**7**) [29] by comparing their spectroscopic data with those reported in the literature.

The compounds isolated from *C. xanthorrhiza* ROXB. were evaluated for their effects on viability of HaCaT keratinocyte cells. None of the phytochemicals exhibited inhibition of cell viability up to a 20 μM concentration (*Fig. 2,a*), indicating that they are not toxic to cells in the range of concentrations used in this study. Each compound was also evaluated for its effects on the expression of MMP-1 in UVB-treated HaCaT

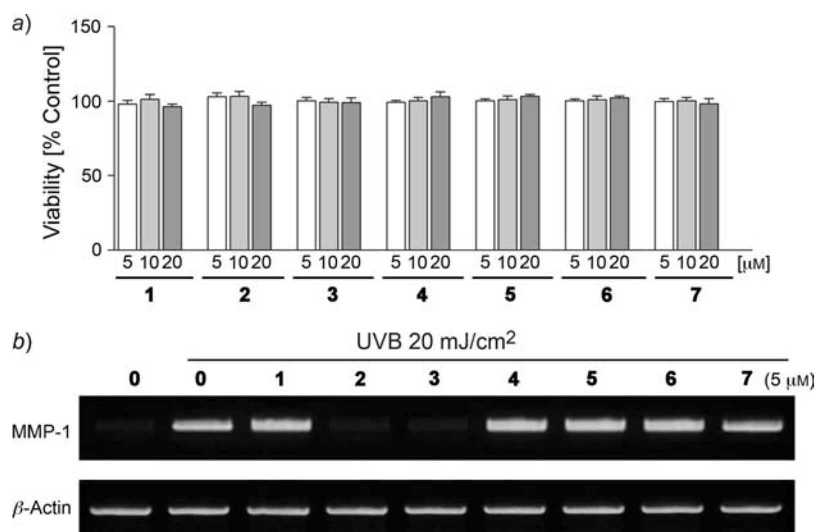

Fig. 2. *Effects of the isolated compounds on cell viability and MMP-1 mRNA expression levels: a) cells grown in the culture medium in the presence of various concentrations of isolated compounds for 48 h*. Cell viability was measured by MTT assays. All data are given as means ± SD of at least three independent experiments with triplicate samples. *b) Levels of MMP-1 mRNA determined by RT-PCR analysis.* β-Actin mRNA was used as an internal control.

cells. The keratinocyte cells were treated with each of the compounds at 5 μM concentrations for 24 h, exposed to UVB for 15 s (20 mJ/cm^2) and incubated for 24 h. Reverse transcription polymerase chain reaction (RT-PCR) analysis showed that the new compound, (−)-curcuhydroquinone 2,5-di-*O*-*β*-D-glucopyranoside (**2**) and 13-hydroxyxanthorrhizol (**3**) strongly suppressed MMP-1 mRNA expression in UVB-treated HaCaT cells. The inhibitory effects of the two compounds on MMP-1 expression were further determined at the protein level, as well as at mRNA levels in UVB-irradiated cells in the presence of various concentrations of the compounds. RT-PCR analysis revealed that treatment of cells with **2** or **3** resulted in inhibition of the elevated MMP-1 mRNA expression in UVB-stimulated HaCaT cells in a concentration-dependent manner, without affecting the mRNA levels of *β*-actin (*Fig. 3*,*a* and *b*). Compounds **2** and **3** reduced the MMP-1 mRNA expression in keratinocytes by *ca.* 8.9- and 7.6-fold, respectively. The protein levels of MMP-1 were analyzed by *Western* blot analysis, which evidenced that the protein levels of MMP-1 were decreased in HaCaT cells treated with **2** or **3** by *ca.* 9.2- and 6.6-fold, respectively (*Fig. 3*,*c* and *d*). Taken together, our results demonstrated that **2** and **3** from *C. xanthorrhiza* ROXB. inhibit production of MMP-1 in UV-irradiated HaCaT cells. These findings suggest that *C. xanthorrhiza* ROXB. could be utilized as an effective agent to prevent the UVB-mediated cutaneous alterations and photoaging.

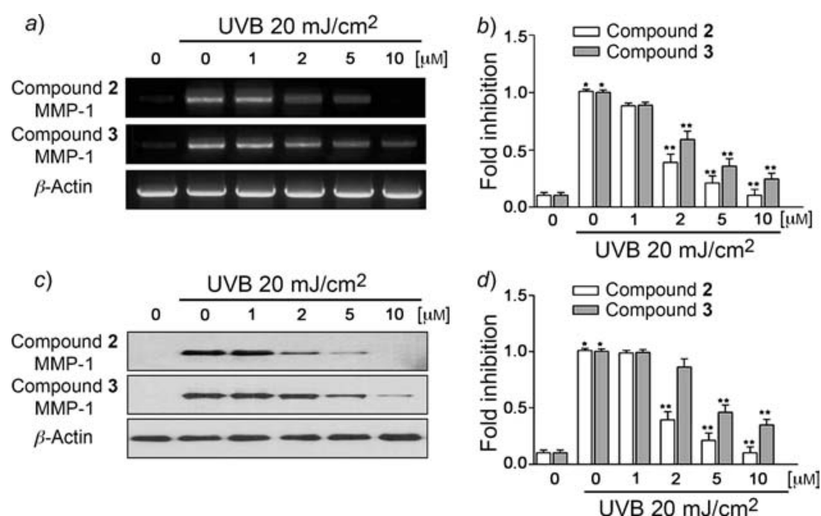

Fig. 3. *Effect of (−)-curcuhydroquinone 2,5-di-*O*-β-D-glucopyranoside* (**2**) *and 13-hydroxyxanthorrhizol* (**3**) *on expression of matrix metalloproteinase-1* (*MMP-1*). HaCaT Cells were pretreated with various concentrations of **2** or **3** for 24 h and exposed to UVB (20 mJ/cm^2). The UV-treated cells were then cultured in serum-free medium for 24 h. *a*) and *b*) Levels of MMP-1 mRNA determined by RT-PCR analysis; quantification by densitometry. *β*-Actin mRNA was used as an internal control. *c*) Levels of MMP-1 protein measured by *Western* blot analysis using a monoclonal antibody against human MMP-1. The blot underwent rehybridization with antibody against *β*-actin to verify equal loading of proteins in each lane. *d*) *Western* blot data quantified by densitometry. Significance compared with the control, *: $P < 0.05$. Significance compared with UVB treatment alone, **: $P < 0.05$. The data are expressed as the means ± SD of three individual experiments.

Experimental Part

General. FAB-MS: *JEOL JMSAX-700* (Tokyo, Japan). TLC: *Kieselgel 60 F_{254}* and *RP-18 F_{254S}* (*Merck*) plates; visualization with UV lamp *Spectroline* model *ENF-240 C/F* (*Spectronics Corporation*, New York, NY, USA) and a 10% H_2SO_4 soln. SiO_2 (*Kieselgel 60*, *Merck*, DE-Darmstadt) and *ODS* (*LiChroprep RP-18*, *Merck*) resins. Column chromatography (CC): Optical rotations *JASCO P-1010* digital polarimeter (*Jasco*, Tokyo, Japan). IR Spectra: *PerkinElmer Spectrum One FT-IR* spectrometer (GB-Buckinghamshire). ^{1}H- and ^{13}C-NMR (400 and 100 MHz, resp.) spectra: *Varian Unity Inova AS-400 FT-NMR* spectrometer (Palo Alto, CA, USA). Antibody against human MMP-1 was obtained from *R&D Systems* (Minneapolis, MN, USA). HRP-Conjugated anti-mouse IgG antibody was obtained from *Sigma* (St. Louis, MO, USA). Other chemicals were obtained commercially from *Sigma–Aldrich* (St. Louis, MO, USA).

Plant Material. C. xanthorrhiza ROXB. was supplied from the Korea Food Research Institute, Sungnam, Korea, in January 2012, and was identified by *Dae-Keun Kim*, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU12-0111) has been deposited with the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Extraction and Isolation. The dried and powdered rhizomes of *C. xanthorrhiza* were extracted at r.t. using 80% aq. MeOH (3 × 36 l) for 24 h, followed by filtration. The filtrates were evaporated in vacuum, and the obtained MeOH extracts were suspended in H_2O (3 l) and then extracted successively with AcOEt (2 × 3 l) and BuOH (2 × 22.8 l). The org. and aq. layers were concentrated to give the residues of the AcOEt fraction (*CXE*; 901 g), the BuOH fraction (*CXB*; 40 g), and the H_2O fraction (*CXW;* 1445 g). The *CXE* (178 g) was subjected to CC (SiO_2 CC (ϕ 12 × 15 cm); hexane/AcOEt 1 : 1; 27 l). The eluting solns. were monitored using TLC analysis and ultimately produced 16 fractions, *CXE-1* to *CXE-16*. *Fr. CXE-2* (68 g, elution volume/total volume (V_e/V_t) 0.05 – 0.08) was subjected to CC (SiO_2 (ϕ 8.5 × 15 cm); hexane/AcOEt 20 : 1; 17.5 l) to yield 15 fractions, *CXE-2-1 – CXE-2-15*. *Fr. CXE-2-2* (3.3 g; V_e/V_t 0.03 – 0.07) was subjected to CC (*ODS* (ϕ 4 × 11 cm); MeOH/H_2O 5 : 1, 5.4 l) to ultimately furnish 19 fractions along with a purified compound **6** (*CXE-2-2-14*; 133 mg; V_e/V_t 0.61 – 0.74; TLC (*ODS F_{254S}*; acetone/H_2O 6 : 1): R_f 0.40). *Fr. CXE-2-5* (3.2 g, V_e/V_t 0.17 – 0.23) was subjected to CC (*ODS* CC (ϕ 6 × 10 cm); MeOH/H_2O 2 : 1; 6.7 l) to yield 13 fractions, *CXE-2-5-1 – CXE-2-5-13*. *Fr. CXE-2-5-12* (648 mg; V_e/V_t 0.93 – 1.00) was purified by CC (*ODS* (ϕ 4.5 × 7 cm); MeOH/H_2O 3 : 1; 600 ml) to furnish nine fractions along with a purified compound **5** (*CXE-2-5-12-4*; 206 mg, V_e/V_t 0.34 – 0.44; TLC (*ODS F_{254S}*; MeOH/H_2O 8 : 1): R_f 0.49) and compound **7** (*CXE-2-5-12-8*; 26 mg; V_e/V_t 0.66 – 1.00; TLC (*ODS F_{254S}*; MeOH/H_2O 8 : 1): R_f 0.41). *Fr. CXE-2-15* (805 mg; V_e/V_t 0.98 – 1.00) was subjected to CC (*ODS* (ϕ 4 × 10 cm); MeOH/H_2O 3 : 2; 3 l) to furnish 19 fractions, *CXE-2-15-1 – CXE-2-15-19*. *Fr. CXE-2-15-12* (25 mg; V_e/V_t 0.42 – 0.45) was purified by CC (SiO_2 (ϕ 3 × 10 cm); hexane/AcOEt 2 : 1; 215 ml) to produce six fractions along with a purified compound **4** (*CXE-2-15-12-2*; 4.6 mg; V_e/V_t 0.31 – 0.42; TLC (*ODS F_{254S}*; MeOH/H_2O 6 : 1): R_f 0.61). *Fr. CXE-6* (1.6 g; V_e/V_t 0.19 – 0.23) was subjected to CC (ODS (ϕ 4.5 × 8 cm); MeOH/H_2O (2 : 1 → 5 : 1, 2 l of each) to yield 23 fractions, *CXE-6-1 – CXE-6-23*. *Fr. CXE-6-7* (260 mg; V_e/V_t 0.04 – 0.06) was purified by CC (SiO_2 (ϕ 3 × 12 cm); $CHCl_3$/AcOEt 15 : 1; 4.9 l) to afford 18 fractions along with a purified compound **3** (*CXE-6-7-15*; 34 mg; V_e/V_t 0.36 – 0.42; TLC (SiO_2 *F_{254}*; $CHCl_3$/MeOH 15 : 1): R_f 0.58). The BuOH extract (*CXB*; 34 g) was submitted to CC (SiO_2 (ϕ 9 × 15 cm); $CHCl_3$/MeOH 12 : 1 → 10 : 1 → 8 : 1; 8 l of each; and $CHCl_3$/MeOH/H_2O 8 : 3 : 1; 14 l). The eluting solns. were monitored using TLC analysis and ultimately 22 fractions, *CXB-1 – CXB-22* were obtained. *Fr. CXB-21* (1.2 g; V_e/V_t 0.68 – 0.71) was subjected to CC (*ODS* (4 × 11 cm); MeOH/H_2O 1 : 2; 3 l) to yield nine fractions *CXB-21-1 – CXB-21-9*. *Fr. CXB-21-8* (103 mg; V_e/V_t 0.80 – 1.00) was purified by CC (*ODS* (3 × 5 cm); MeOH/H_2O 1 : 1; 320 ml) to furnish nine fractions, *CXB-21-8-1 – CXB-21-8-9*, along with the purified compound **1** (*CXB-21-8-5*; 58 mg; V_e/V_t 0.41 – 0.43; TLC (*ODS F_{254S}*; MeOH/H_2O 3 : 2): R_f 0.50). *Fr. CXB-22* (830 mg; V_e/V_t 0.72 – 0.74) was subjected to the CC (*ODS* (4 × 6 cm); MeOH/H_2O 1 : 2; 1.8 l) to give 14 fractions, *CXB-22-1 – CXB-22-14*, and also yielded a purified compound **2** (*CXB-22-9*; 24 mg; V_e/V_t 0.14 – 0.33; TLC (*ODS F_{254S}*; MeOH/H_2O 1 : 1): R_f 0.40).

*(7*R*,10*R*)-10,11-Dihydro-10,11-dihydroxyxanthorrhizol 3-*O*-β-*D*-Glucopyranoside* (*=5-[(1*R*,4*R*)-4,5-Dihydroxy-1,5-dimethylhexyl]-2-methylphenyl β-*D*-Glucopyranoside*; **1**). Amorphous powder.

$[\alpha]_D^{20} = -33.8$ ($c = 1.0$, MeOH). IR (CaF_2): 3374, 2923, 1578, 1451, 1246. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-FAB-MS: 415.2307 ($[M+H]^+$, $C_{21}H_{35}O_8^+$; calc. 415.2333).

*(−)-Curcuhydroquinone 2,5-Di-*O*-β-*D*-glucopyranoside* (*=2-[(1*R*)-1,5-Dimethylhex-4-en-1-yl]-4-(β-*D*-glucopyranosyloxy)-5-methylphenyl β-*D*-Glucopyranoside*; **2**). Amorphous powder. $[\alpha]_D^{20} = -40.8$ ($c = 0.5$, MeOH). IR (CaF_2): 3374, 2919, 1595, 1500, 1448, 1190. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-FAB-MS: 559.2730 ($[M+H]^+$, $C_{27}H_{43}O_{12}^+$; calc. 559.2755).

Acid Hydrolysis of **1** *and* **2**, *and Isolation of the Aglycones.* A soln. of **1** (6 mg) in 1M HCl (3 ml) was heated at 90° in a heating block for 3 h. The mixture was added to 2 ml of MeOH and extracted with hexane (5 ml). The hexane layer was concentrated and then purified by a *Supelclean LC-Si SPE* tube (hexane/AcOEt 3 :1) to furnish the aglycone (**1a**; 0.6 mg).

A soln. of compound **2** (8.6 mg) in 1M HCl (3 ml) was treated as described for **1** to yield the aglycone (**2a**; 0.8 mg).

*(7*R*,10*R*)-10,11-Dihydro-10,11-dihydroxyxanthorrhizol* (*=(3*R*,6*R*)-6-(3-Hydroxy-4-methylphenyl)-2-methylheptane-2,3-diol*; **1a**). $[\alpha]_D^{20} = -34$ ($c = 0.04$, $CHCl_3$). ^{1}H-NMR (400 MHz)[1]): 7.00 (d, $J=7.6$, H–C(5)); 6.65 (dd, $J=7.6$, 1.6, H–C(6)); 6.60 (d, $J=1.6$, H–C(2)); 3.26 (dd, $J=10.8$, 2.0, H–C(10)); 2.61–2.57 (m, H–C(7)); 2.19 (s, Me(14)); 1.79–1.83 (m, CH_2(8)); 1.32–1.36 (m, CH_2(9)); 1.20 (s, Me(15)); 1.17 (s, Me(13)); 1.09 (s, Me(12)).

(−)-Curcuhydroquinone (*=2-Methyl-5-[(2*R*)-6-methylhept-5-en-2-yl]benzene-1,4-diol*; **2a**). $[\alpha]_D^{20} = -28$ ($c = 0.01$, $CHCl_3$). ^{1}H-NMR (400 MHz)[1]): 6.52 (s, H–C(6)); 6.42 (s, H–C(3)); 5.07 (dd, $J=7.2$, 3.6, H–C(10)); 2.94–2.97 (m, H–C(7)); 2.21 (s, Me(14)); 1.87–1.90 (m, CH_2(9)); 1.66 (s, Me(13)); 1.49–1.52 (m, CH_2(8)); 1.49 (s, Me(12)); 1.12 (d, $J=7.2$, Me(15)).

Cell Culture. Human keratinocyte HaCaT cells were grown in *Dulbecco*'s modified *Eagle*'s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 unit/ml penicillin and 100 μg/ml streptomycin) at 37° in a humidified incubator containing 5% CO_2 and 95% air. DMEM, FBS, antibiotics, and trypsin EDTA were obtained from *Invitrogen* (Carlsbad, CA, USA).

Cell Viability Assay. HaCaT Cells were seeded in 96-well plates at a density of 5×10^3 cells/well and allowed to attach for 24 h. After discarding the growth medium, cells were treated with various concentrations of phytochemicals isolated from *C. xanthorrhiza* ROXB. in serum-free medium for 48 h. After incubation, cells were treated with 100 μg/ml of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) for 1 h. The formazan precipitate was dissolved in 200 μl of DMSO and the absorbance at 560 nm was determined spectrophotometrically. Analyses were repeated three times, and the results are expressed as the means of three independent experiments.

UV Irradiation. In brief, HaCaT cells were pretreated with or without phytochemicals in serum-free media for 24 h and then rinsed twice with phosphate-buffered saline (PBS). The cells were then irradiated with UVB (20 mJ/cm^2) at 312 nm for 15 s using a UVB lamp (*Bio-Sun Lamps*, *Vilber Lourmat*, F-Marine), which provided uniform irradiation at a distance of 15 cm, and radiation intensities were monitored with a *JIC 119* amplifier (*BEC*, Brookline, MA, USA.). All irradiations were performed under a thin layer of PBS. After irradiation, cells were treated with or without phytochemicals in serum-free media for an additional 24 h. Mock-irradiated controls followed the same schedule of medium changes without UVB irradiation.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Total RNAs were prepared from cells using a *TRIzol Reagent* kit (*Invitrogen*, Carlsbad, CA, USA). Total RNA (2 μg) was reverse-transcribed using M-MuLV reverse transcriptase (*Fermentas Life Science*, Glen Burnie, MD, USA). The primers used for PCR amplification in this study were as follows: MMP-1, forward 5′- AGCGTGTGACAGTAAGCTAA-3′ and reverse 5′-GTTTTCCTCAGAAAGAGCAGCAT-3′; β-actin mRNA levels were used as internal controls.

Western *Blot Analysis.* Cells were lysed in radio immunoprecipitation assay (RIPA) buffer (150 mM NaCl, 1% *Triton X-100*, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM *Tris* · HCl, pH 7.5, 2 mM ethylenediaminetetraacetic acid (EDTA)-containing proteinase inhibitor cocktail (*Roche*, Indianapolis, IN, USA)). Protein concentrations were quantified with a protein assay kit (*Bio-Rad Laboratories*, Philadelphia, PA, USA). Proteins (30 μg/lane) were resolved with 8–12% SDS-

[1]) Atom numbering as indicated in *Fig. 1*.

polyacrylamide gel electrophoresis. Peroxidase-conjugated antibody was used as a secondary antibody. The membranes were developed with an enhanced chemiluminescence system from *GE Healthcare* (UK-Buckinghamshire) and exposed to X-ray film (*Fuji Photo Film Co. Ltd.*, Japan).

Statistical Analysis. All experiments were performed with triplicate samples and repeated at least three times. The data are presented as means ± SD, and statistical comparisons between groups were performed using one-way ANOVA, followed by *Student*'s *t*-test.

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