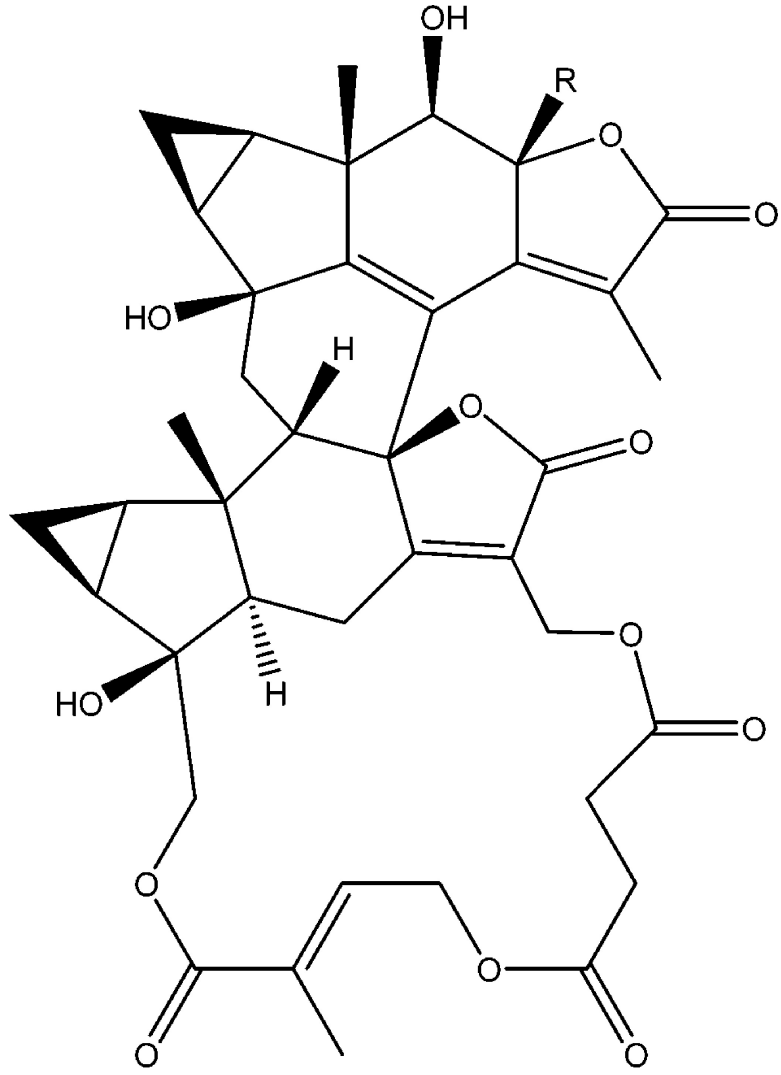

1 R=OH
2 R=OCH_3

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Complex Sesquiterpenoids with Tyrosinase Inhibitory Activity from the Leaves of *Chloranthus tianmushanensis*

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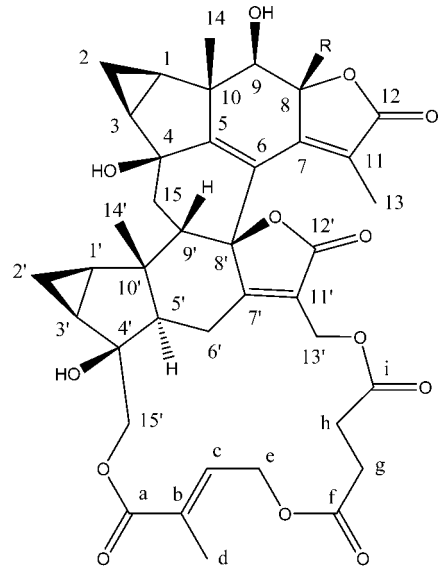
Investigation of the leaves of *Chloranthus tianmushanensis* resulted in the isolation and characterization of two new sesquiterpene dimers with a rare 18-membered triester ring, tianmushanol (**1**) and 8-*O*-methyltianmushanol (**2**), and four known sesquiterpenes. Their structures were established by spectroscopic means. The inhibitory activities against tyrosinase of all isolates were also evaluated.

The use of Traditional Chinese Medicine, particularly to cure multiple skin diseases and for cosmetics, is a common and widespread practice in most areas of China. Herbal cosmetic products can be used for enhancement of skin color, tattoos, hair care, and coloring of nails, palms, and teeth, and to cure different skin disease.[1] Tyrosinase is a multifunctional copper-containing enzyme widely distributed in plants and animals, which catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals.[2,3] Tyrosinase inhibitors, therefore, can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They also find uses in cosmetics for whitening and depigmentation after sunburn. Several tyrosinase inhibitors that suppress melanogenesis have been studied for the treatment of hyperpigmentation.[4]

In the previous paper we described the isolation of two tyrosinase inhibitory sesquiterpenes from *Chloranthus henryi*.[5] In continuation of our search for bioactive natural products that can be used for the treatment of dermatological disorders associated with melanin hyperpigmentation, we investigated the MeOH extract of *Chloranthus tianmushanensis* K.F. Wu.

Species of the genus *Chloranthus* are known to be rich in sesquiterpenes of the lindenane, germacrane, and eudesmane-type[6–15] including sesquiterpenoid dimers and trimers.[16–21] Recently, we found a new sesquiterpene skeleton named chloranthane from this genus.[22] Other constituents like flavonoids and amides have also been reported.[12,23] However, no phytochemical investigation on *C. tianmushanensis* has been reported. Chemical study of this plant led to the isolation of two new sesquiterpene dimers with a macrocyclic triester ring, tianmushanol (**1**) and 8-*O*-methyltianmushanol (**2**), and four known sesquiterpenes, atractylenolide III, isofuranodiene, glechomanolide, and chloranthalactone A. The two new compounds showed inhibitory activity against tyrosinase.

Tianmushanol (**1**) was obtained as colorless needles from MeOH. The HRFTICRMS exhibited an ion peak at *m/z* 757.2469 [M + Na]$^+$ (calcd 754.2467), indicating that the molecular formula was $C_{39}H_{42}O_{14}$ with 19 degrees of unsaturation, and **1** was a sesquiterpene dimer like the known compound chloramultilide A isolated from the same genus.[18] The IR spectrum revealed the presence of hydroxy, α,β-unsaturated butyrolactone, and ester groups characterized by absorptions at ν_{max} 3455, 1760, and 1735 cm^{-1}. The NMR data of **1** were similar to those of chloramultilide A except that the Me-13 signal in **1** was shifted upfield at δ_C 10.8 (q), characteristic of Me-13 in the series of lindenane sesquiterpenes lactones,[24,25] and there was a dioxygenated C-8 in **1** instead of a carbonyl in chloramultilide A. These differences suggested a five-membered lactone ring between C-8 and C-12 in **1**, which was further confirmed by the observation of HMBC correlation from the proton signal at δ_H 3.79 (s, H-9) to the dioxygenated quaternary carbon signal at δ_C 105.0 (s, C-8). The ^{1}H and ^{13}C NMR assignments for **1** were made by comparing its NMR data with those of chloramultilide A and detailed analysis of 2D NMR spectra.[18] The macrocyclic ring unit in **1** was proved to be constructed via hydroxytiglyl and succinyl moieties, the same as in chloramultilide A and shizukaol B, by comparison of NMR data.[18,21,26] The stereochemical relationship between the rings and the two lindenane sesquiterpene units was determined by a 2D NOESY experiment. The correlation between H-9 and H-5′ characteristic of lindenane sesquiterpene dimers connected at C-15−C-9′ and C-6−C-8′ suggested that the molecule is folded back at the six-membered ring between two sesquiterpene units.[16,18] The relative configuration of C-8 is derived from that of atractylenolide III isolated from the same plant, on the assumption that the two are biogenetically related. The identity of atractylenolide III was confirmed by comparison of its NMR data with those published,[27] and the similar chemical shift of C-8 of atractylenolide III supported this assumption. The complete ^{1}H and ^{13}C NMR assignments are listed in Table 1.



1 R=OH
2 R=OCH$_3$

Compound **2** was obtained as colorless needles from MeOH and was found to be unstable on storage. After 2 weeks at room

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Table 1. NMR Data (500 MHz) for Compounds **1** and **2**

	1 (CD_3OD)		**1** (DMSO-d_6)	
position	δ_C[a,b]	δ_H[c] mult. (J in Hz)	δ_C[a,b]	δ_H[c] mult. (J in Hz)
1	29.9 (d)	1.86 (m)	28.4 (d)	1.68 (m)
2α	9.7 (t)	0.86 (m)	8.81 (t)	0.67 (ddd, J = 8.5, 8.5, 3.0)
2β		1.07 (m)		1.01 (m)
3	31.5 (d)	1.84 (m)	30.7 (d)	1.73 (m)
4	78.2 (s)		76.4 (s)	
5	164.6 (s)		164.3 (s)	
6	124.2 (s)		123.5 (s)	
7	154.4 (s)		152.5 (s)	
8	105.0 (s)		105.6 (s)	
9	79.9 (d)	3.79 (s)	74.2 (d)	3.76 (d, J = 7.0)
10	51.0 (s)		49.8 (s)	
11	125.4 (s)		122.2 (s)	
12	174.5 (s)		172.4 (s)	
13	10.8 (q)	1.64 (s)	10.3 (q)	1.51 (s)
14	14.4 (q)	0.87 (s)	14.3 (q)	0.73 (s)
15α	41.5 (t)	1.84 (m)	40.1 (t)	1.68 (m)
15β		2.74 (m)		2.58 (m)
1′	27.8 (d)	1.67 (m)	26.4 (d)	1.54 (m)
2′α	10.8 (t)	0.62 (m)	10.6 (t)	0.51 (ddd, J = 8.5, 8.5, 4.5)
2′β		1.24 (m)		1.09 (m)
3′	30.3 (d)	1.49 (m)	29.1 (d)	1.46 (m)
4′	78.2 (s)		76.0 (s)	
5′	56.2 (d)	2.38 (dd, J = 12.5, 7.5)	54.9 (d)	2.11 (dd, J = 12.0, 7.5)
6′α	25.0 (t)	2.74 (m)	23.0 (t)	2.50 (m)
6′β		2.99 (dd, J = 18.0, 12.0)		2.80 (dd, J = 18.0, 12.0)
7′	177.4 (s)		175.9 (s)	
8′	87.4 (s)		85.6 (s)	
9′	51.9 (d)	2.68 (m)	50.5 (d)	2.53 (m)
10′	46.6 (s)		44.8 (s)	
11′	123.9 (s)		121.2 (s)	
12′	173.4 (s)		171.4 (s)	
13′a	50.0 (t)	4.54 (d, J = 12.0)	54.2 (t)	4.48 (d, J = 12.0)
13′b		5.18 (d, J = 12.0)		4.80 (d, J = 12.0)
14′	24.4 (q)	0.95 (s)	23.9 (q)	0.81 (s)
15′a	74.8 (t)	3.89 (d, J = 11.5)	73.1 (t)	3.76 (d, J = 11.0)
15′b		4.60 (d, J = 11.5)		4.62 (d, J = 11.0)
a	168.9 (s)		166.7 (s)	
b	130.3 (s)		128.4 (s)	
c	137.7 (t)	6.73 (t, J = 5.0)	136.8 (t)	6.47 (t, J = 5.5)
d	12.9 (q)	1.87 (s)	12.8 (q)	1.79 (s)
ea	62.8 (t)	4.67 (dd, J = 14.5, 5.0)	61.2 (t)	4.56 (dd, J = 15.0, 6.5)
eb		4.85 (overlap)		4.97 (dd, J = 15.0, 5.5)
f	174.2 (s)		171.8 (s)	
ga	29.8 (t)	2.55 (m)	28.7 (t)	2.43 (m)
gb		2.67 (m)		2.55 (m)
ha	28.9 (t)	2.62 (m)	29.1 (t)	2.55 (m)
hb		2.67 (m)		2.57 (m)
i	173.8 (s)		171.6 (s)	
OCH_3			51.6 (q)	3.22 (s)

[a] Recorded at 125 MHz. [b] Multiplicities inferred from DEPT and HMQC experiments. [c] Recorded at 500 MHz.

temperature, it had undergone ca.50% decomposition to yield a mixture of products. The original compound could be repurified; however, no other compounds were characterized. The HRFTICRMS exhibited an ion peak at m/z 771.2624 $[M + Na]^+$ (calcd 771.2623), corresponding to the molecular formula $C_{40}H_{44}O_{14}$. The UV and IR spectra of **2** exhibited similar general patterns to those of **1**. The ^1H and ^{13}C NMR spectra of **2** (Table 1) showed similar chemical shifts and the same multiplicities of most carbon atoms as in **1**, except for a methoxy group for **2**, indicating also a lindenane sesquiterpene dimer backbone with three hydroxy groups, a methoxy group, and an 18-membered triester ring. Analysis of 2D NMR data (COSY, HMQC, and HMBC) led to assignment of the NMR signals, locating the hydroxy groups at C-4, C-4′, and C-9 and the ester ring at C-15′ and C-13′. The methoxy group was inferred to be located at C-8 from the HMBC correlation between the methoxy protons at δ_H 3.22 (s, H-OCH_3) and the dioxygenated

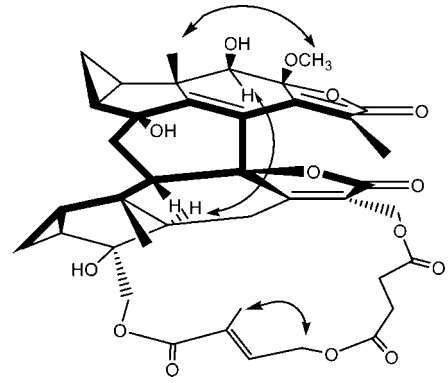


Figure 1. Key NOESY correlations of compound **2**.

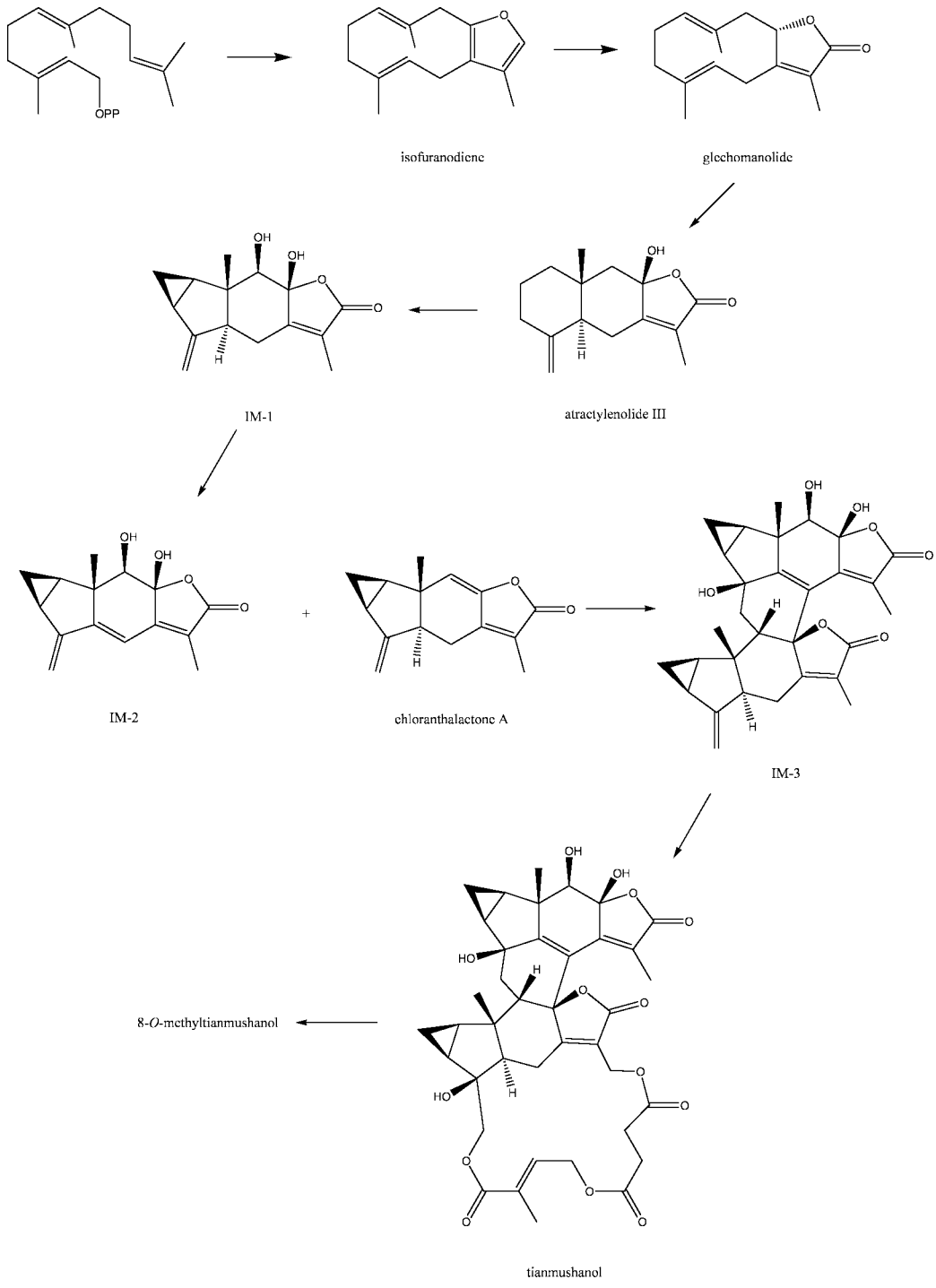
quaternary carbon at δ_C 105.6 (s, C-8). The relative configuration of **2** was deduced to be the same as in **1** from the analysis of the NOESY data. The configuration of C-8 was further confirmed by the NOESY correlation from the methoxy protons to Me-14 (Figure 1). Thus, compound **2** was identified as 8-*O*-methyltianmushanol.

Furthermore, a known eudesmane-type sesquiterpene, atractylenolide III, two known germacrane-type sesquiterpenes, isofuranodiene and glechomanolide, and a known lindenane-type sesquiterpene, chloranthalactone A, were identified by comparison of their spectroscopic data with published data.[8,24,27,28] Since some sesquiterpene dimers were reported to be artificial, for example the photodimer of shizukanolide B (chloranthalactone A) from *C. japonicus*,[29] an LC/MS investigation on the extract of fresh roots was made to determine whether compounds **1** and **2** were natural or not. Results showed that both compounds were detectable in the EtOAc extract of the fresh roots. Therefore, it could be concluded that the sesquiterpene dimers **1** and **2** are indeed true natural products.

Since two new dimers and key intermediates, atractylenolide III, isofuranodiene, glechomanolide, and chloranthalactone A, were found in the same plant, we could tentatively outline a plausible biogenetic relationship of the isolates (Scheme 1). Furanogermacrane-type sesquiterpenes are considered to be key intermediates of other furanosesquiterpenes, which are regarded as precursors to sesquiterpene lactone.[8] Thus, glechomanolide may be derived from isofuranodiene. Atractylenolide III, isolated as the major component of this plant, is derived from cycloaddition between the two olefin bonds of glechomanolide. Atractylenolide III affords intermediate IM-1, which is then selectively dehydrogenated to yield intermediate IM-2. Lindenane sesquiterpene dimers are possibly formed via an enzyme-catalyzed intermolecular Diels-Alder cycloaddition of two lindenane sesquiterpenes.[16,17] Thus, IM-2 reacts with chloranthalactone A to yield the intermediate IM-3, followed by rearrangements and esterifications to afford tianmushanol (**1**), which is methylated to give 8-*O*-methyltianmushanol (**2**).

All the compounds were tested for tyrosinase inhibitory activities.[30] Compounds **1** and **2** showed IC_{50} values of 358 ± 3 and 312 ± 3 μM, when the active compounds were compared to the standard tyrosinase inhibitor kojic acid (IC_{50} = 243 ± 4 μM). However, atractylenolide III, isofuranodiene, glechomanolide, and chloranthalactone A exhibited virtually no inhibition against tyrosinase. Sesquiterpenes like 6-oxo-8-hydroxygermacra-1(10),*E*,4*Z*,7(11)-trien-12,8-olide, 6-oxo-8-methoxygermacra-1(10),*E*,4*Z*,7(11)-trien-12,8-olide, molephantin, molephantinin, phantomolins, tomenphantin A, tomenphantin B, tomenphantopin A, tomenphantopin B, deoxyelephantopin, isodeoxyelephantopin, isoelephantopin, and 15-ethoxy-4α*H*-isocentratherin were indeed reported to possess tyrosinase inhibitory activity or used as skin-lightening agents.,[31] This is the first time that tyrosinase inhibitory activity of lindenane sesquiterpene dimers is reported.

Scheme 1. Plausible Biogenetic Relationships of Isolates (IM-1, intermediate 1; IM-2, intermediate 2; IM-3, intermediate 3)



Experimental Section

General Experimental Procedures. The melting points (uncorrected) were obtained on a Reichert apparatus. Optical rotations were recorded on a Perkin-Elmer-341 polarimeter. The IR spectra ($CHCl_3$) were run on a NicoletAvatar-360FT-IR spectrometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a Bruker AVANCE DMX 500 NMR spectrometer with TMS as internal standard (at 25 °C). HRFTICRMS were recorded on a Bruker Apex III spectrometer. ESIMS were recorded on a Bruker Esquire-3000^{plus} spectrometer. TLC was performed using Merck precoated plates (Si gel 60 F254) of 0.25 mm thickness. A Waters 600 preparative HPLC, with a Shim-pack PREP-ODS (250 × 20 mm) column, was used for preparative HPLC. Sephadex LH-20 (Amersham) was used for column chromatography.

Plant Material. The leaves of *C. tianmushanensis* K.F. Wu were collected in Linan County, Zhejiang Province, People's Republic of China, in September 2003 and identified by Prof. Changxi Zhang (Jinhua Medical College, Jinhua, People's Republic of China.). A voucher specimen (zju6945) is maintained at the College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, People's Republic of China.

Extraction and Isolation. The shade-dried, powdered leaves (3 kg) of *C. tianmushanensis* were extracted at room temperature with MeOH (3 × 5 L). The extracts were evaporated in vacuo to afford a gummy residue (308 g). This residue was partitioned in H_2O (3 L) and extracted with petroleum ether (4 × 3 L) and EtOAc (4 × 3 L), successively. The EtOAc extract (40 g) was adsorbed onto Si gel (80 g) and subjected to chromatography on Si gel (9 × 100 cm, 2000 g, 200–300 mesh), eluting with *n*-hexane−EtOAc gradient mixtures. Eleven main fractions were obtained by checking with TLC and combined. Small samples of the fractions were submitted to testing of tyrosinase inhibitory activity, with oil obtained from the fourth and seventh fractions showing bioactivities. The fourth fraction was subjected to preparative HPLC (flow rate 8 mL/min,

UV detector 210 nm), using MeOH−H_2O (45:55) as eluent, to afford atractylenolide III (79.8 mg, t_R 35 min), chloranthalactone A (12.7 mg, t_R 53 min), isofuranodiene (10.2 mg, t_R 65 min), and glechomanolide (15.6 mg, t_R 69 min). The seventh fraction was applied to a Sephadex LH-20 column (4 × 150 cm, 300 g, Amersham) and eluted with MeOH. Fractions collected were purified by preparative HPLC using MeOH−H_2O (20:80) as eluent, to yield compounds **1** (8.5 mg) and **2** (14.8 mg).

Tianmushanol (1): colorless needles from MeOH; mp 162–163 °C; $[\alpha]^{24}_D$ −45 (*c* 0.001, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (4.02) nm; IR ν_{max} 3455, 1760, 1735, 1493, 1135, 892 cm^{-1};^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 1491.1 $[2M + Na]^+$, 733.1 $[M - H]^-$; HRFTICRMS *m/z* 757.2469 $[M + Na]^+$ (calcd for $C_{39}H_{42}O_{14}Na$, 757.2467).

8-*O*-Methyltianmushanol (2): colorless needles from MeOH; mp 151–153 °C; $[\alpha]^{24}_D$ −75 (*c* 0.001, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.38) nm; IR ν_{max} 3456, 1765, 1735, 1375, 1280, 1135, 1120, 955, 894 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 1519.1 $[2M + Na]^+$; HRFTICRMS *m/z* 771.2624 $[M + Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$, 771.2623).

Tyrosinase Inhibition Assay. The tyrosinase inhibition assays were performed in 96-well microplate format using a SpectraMax 340 microplate reader.[29] Compounds were initially screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All active compounds from the preliminary screening were subjected to IC_{50} studies. Compounds were dissolved in MeOH to a concentration of 2.5%. Mushroom tyrosinase (28 nM) was preincubated with the compounds in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was added to the reaction mixture, and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37 °C) due to the formation of the DOPAchrome for 10 min. The percent inhibition of the enzyme was calculated as follows:

$$\text{Percent inhibition } (\%) = [B - S/B] \times 100$$

B and *S* are the absorbance for the blank and samples, respectively. After screening of the compounds, median inhibitory concentration (IC_{50}) was calculated. All the studies have been carried out in triplicate, and the results represent the mean SEM (standard error of the mean). Kojic acid was used as standard inhibitor for the tyrosinase. All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

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Supporting Information Available: ^{13}C and ^{1}H NMR and DEPT spectra of tianmushanol (**1**) and 8-*O*-methyltianmushanol (**2**), and the LC/MC spectra of EtOAc extract are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Ding, H. *Chin. J. Trad. Chin. Med. Pharm.* **2006**, *21*, 185–187.
(2) Shiino, M.; Watanabe, Y.; Umezawa, K. *Bioorg. Med. Chem.* **2001**, *9*, 1233–1240.
(3) Shiino, M.; Watanabe, Y.; Umezawa, K. *Bioorg. Chem.* **2003**, *31*, 129–135.
(4) Curto, E. V.; Kwong, C.; Hermersdörfer, H.; Glatt, H.; Santis, C.; Virador, V.; Hearing, V. J., Jr.; Dooley, T. P. *Biochem. Pharmacol.* **1999**, *57*, 663–672.
(5) Wu, B.; He, S.; Wu, X.; Pan, Y. *Chem. Biodiversity*Accepted for publication.
(6) Wu, B.; He, S.; Wu, X.; Wu, D.; Pan, Y. *Helv. Chim. Acta* **2007**, *90*, 1586–1592.
(7) Wu, B.; He, S.; Pan, Y. *Planta Med.* **2006**, *72*, 1334–1338.
(8) Kawabata, J.; Tahara, S.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *46*, 1447–1453.
(9) Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1989**, *53*, 203–207.
(10) Li, Y.; Zhang, D.-M.; Li, J.-B.; Yu, S.-S.; Li, Y.; Luo, Y.-M. *J. Nat. Prod.* **2006**, *69*, 616–620.
(11) Tsui, W.-Y.; Brown, G. D. *Phytochemistry* **1996**, *43*, 819–821.
(12) Takeda, Y.; Yamashita, H.; Matsumoto, T.; Terao, H. *Phytochemistry* **1993**, *33*, 713–715.
(13) Kusano, G.; Abe, M.; Koike, Y.; Uchida, M.; Nozoe, S.; Taira, Z. *Yakugaku Zasshi* **1991**, *111*, 756–764.
(14) Tahara, S.; Fukushi, Y.; Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *45*, 1511–1512.
(15) Takemoto, T.; Uchida, M.; Kusano, G. *Chem. Pharm. Bull.* **1976**, *24*, 531–533.
(16) Kawabata, J.; Fukushi, Y.; Tahara, S.; Mizutani, J. *Phytochemistry* **1990**, *29*, 2332–2334.
(17) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121–125.
(18) Yang, S.-P.; Yue, J.-M. *Tetrahedron Lett.* **2006**, *47*, 1129–1132.
(19) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1993**, *32*, 1347–1349.
(20) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1998**, *47*, 231–235.
(21) Kawabata, J.; Mizutani, J. *Phytochemistry* **1992**, *31*, 1293–1296.
(22) Wu, B.; He, S.; Pan, Y. *Tetrahedron Lett.* **2007**, *48*, 453–456.
(23) Takemoto, T.; Uchida, M.; Koike, K. *Chem. Pharm. Bull.* **1975**, *23*, 1161–1163.
(24) Uchida, M.; Koike, Y.; Kusano, G.; Kondo, Y. *Chem. Pharm. Bull.* **1980**, *28*, 92–102.
(25) Okamura, H.; Nakashima, N.; Iwagawa, T.; Nakayama, N. *Chem. Lett.* **1994**, *11*, 1541–1542.
(26) Fukushi, E.; Kawabata, J. *Magn. Reson. Chem.* **1995**, *33*, 909–912.
(27) Endo, K.; Taguchi, T.; Taguchi, F.; Hikino, H.; Yamahara, J.; Fujimura, H. *Chem. Pharm. Bull.* **1979**, *27*, 2954–2958.
(28) Rücker, G.; de Assis Brasil e Silva, G. A.; Bauer, L *Phytochemistry* **1971**, *10*, 221–224.
(29) Okamura, H.; Iwagawa, T.; Nakatani, M. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3465–3467.
(30) Hearing, V. J. J. *Methods Enzymol.* **1987**, *142*, 154–165.
(31) Hasegawa, K.; Yokokawa, Y.; Umishio, K.; Maeda, N. Japan Patent JP 200500 2050, 2005.

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