Constituents of the Stigmas of *Crocus sativus* and Their Tyrosinase Inhibitory Activity

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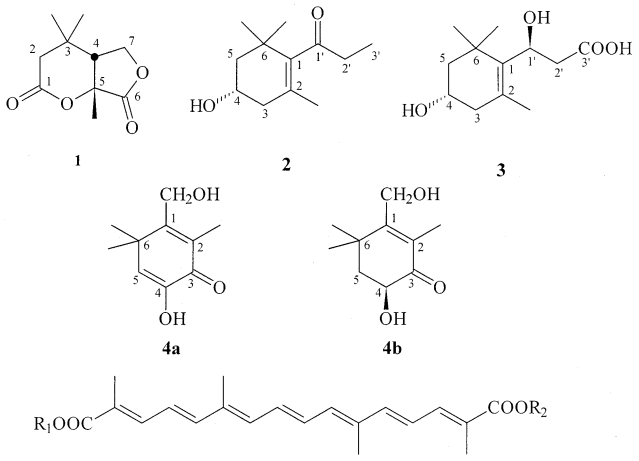
Four new compounds, crocusatins F (**1**), G (**2**), H (**3**), and I (**4a**), together with 21 known compounds, were isolated from an aqueous extract of the stigmas of *Crocus sativus* (saffron). The structures of **1**−**4** were established by spectral methods. The tyrosinase inhibitory activities of all 25 compounds isolated were evaluated in vitro using mushroom tyrosinase. Among them, crocusatin H (**3**), crocin-1 (**5**), and crocin-3 (**6**) showed significant tyrosinase inhibitory activity.

The stigmas of *Crocus sativus* L. (Iridaceae), commonly known as saffron, have a very high commercial value and are cultivated widely in Spain, France, Italy, Greece, Turkey, Iran, India, and mainland China.[1−3] Saffron is employed mainly to provide color and flavor to foods. In addition, the stigmas of *C. sativus* are used as a well-known traditional medicine to stimulate the blood in the treatment of amenorrhea, chest and abdominal pains, and painful swellings due to blood stasis, hepatomegaly, and splenomegaly.[1] Saffron is utilized also as an antispasmodic, eupeptic, nerve sedative, stomachic, and aphrodisiac.[1] Earlier investigators have reported the isolation of carotenoids, monoterpenoids, flavonoids, and anthocyanins from the stigmas, petals, pollen, and leaves of this plant.[4−11] Recently, several bioactive constituents have been reported from this plant with antitumor,[12−14] hypolipidemic,[15] and tissue oxygenation enhancement[16−20] activities.

We have reinvestigated the constituents of the dried stigmas of *Crocus sativus*, since the H_2O extract of this plant showed significant antityrosinase activity. Tyrosinase is known as a key enzyme implicated in the metabolism of melanin in melanocytes.[21] In the present report, we describe the isolation and structural elucidation of four new compounds, crocusatins F−I (**1**−**3**, **4a**). These compounds and 21 known compounds were evaluated for their mushroom tyrosinase inhibitory activity in vitro.

Results and Discussion

Crocusatin F (**1**) was isolated as an optically active colorless oil. The molecular formula was determined as $C_{10}H_{14}O_4$ by HREIMS. The IR absorptions of **1** at 1774 and 1740 cm^{-1} indicated the presence of γ-lactone and δ-lactone units in the molecule, respectively. The ^{13}C NMR and HMQC spectra of **1** revealed 10 carbon signals, namely, three methyls, two methylenes, one methine, two quaternary carbons, and two lactone carbonyl carbons. The ^1H NMR spectrum of **1** displayed a methylene proton at δ 2.43 and 2.26 (each 1H, d, J = 16.4 Hz), ABX type protons at δ 4.37 (1H, dd, J = 10.0, 6.0 Hz), 4.32 (1H, dd, J = 10.0, 2.0 Hz), and 2.29 (1H, dd, J = 6.0, 2.0 Hz), and three methyl signals at δ 1.09, 0.98, and 1.67 (each 3H, s). The correlations between the carbonyl carbon resonance at δ_C 173.6 (C-6) and signals at δ_H 4.32 (1H), 2.29 (1H), and 1.67 (3H) and between the signals at δ_C 79.9 (C-5) and δ_H 2.29 (H-4β) in the HMBC experiment (Figure 1), respectively, indicated the presence of a γ-lactone ring and a methyl group on the α-carbon of the γ-lactone ring in the molecule of **1**. The presence of the partial structure −O(C=O)-$CH_2C(CH_3)_2CH(CH_2)$− in **1** was established by the HMBC correlations between the methine carbon signal at δ_C 48.9 and the methyl protons at δ_H 1.09 and 0.98, and the methylene protons at δ_H 2.43 and 2.26. Also, HMBC correlations between the signal at δ_C 167.3 and δ_H 2.43 and 2.26 as well as the signal at δ_C 32.1 with δ_H 4.37 and 4.32 were observed. Analysis of the available spectral data suggested that the δ-lactone and γ-lactone rings are fused at C-4 and C-5. Therefore, structure **1** was assigned to crocusatin F (**1**), which may be rationalized as a product of oxidative cleavage and recyclization of safranal.[4] The stereochemistry at the junction of the lactone rings was assigned as *cis*, on the basis of a correlation observed between the methyl resonance (δ_H 1.67, 3H, s, Me-5) and the methine proton (δ_H 2.29, 1H, dd, J = 6.0, 2.0 Hz) in a NOESY NMR experiment (Figure 2).



5 $R_1 = R_2 =$ β-D-gentiobiose
6 $R_1 =$ H, $R_2 =$ β-D-gentiobiose

Crocusatin G (**2**) was isolated as a colorless oil and in its HREIMS exhibited a $[M]^+$ ion at m/z 196.1461, corresponding to a molecular formula of $C_{12}H_{20}O_2$. The IR absorptions at 1684 (carbonyl) and 3356 cm^{-1} (hydroxyl) and the UV absorption band at 235 nm, coupled with signals observed at δ_C 210.6 (s), 143.2 (s), 126.7 (s), and 63.6 (d) in the ^{13}C NMR spectrum, indicated the presence of a conjugated ketone system and an oxygenated carbon in the molecule. The ^1H and ^{13}C NMR spectra of **2** displayed

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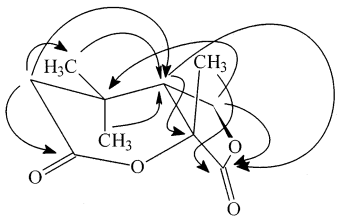

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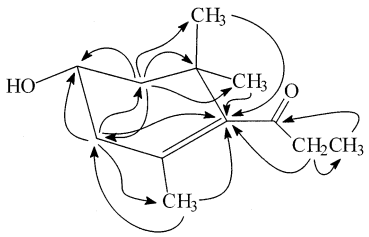

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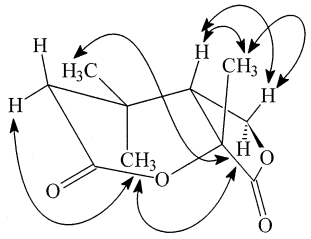
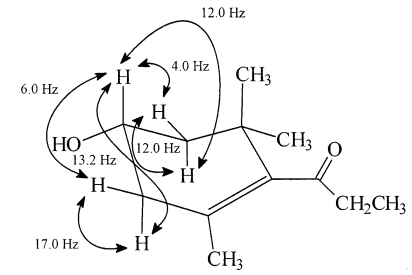
Figure 1. Selected HMBC correlations of **1** and **2**.



1

Figure 2. Selected NOE correlations of **1**.



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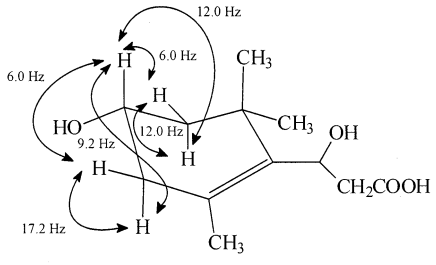

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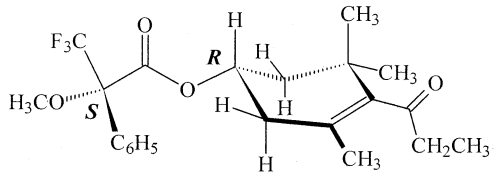
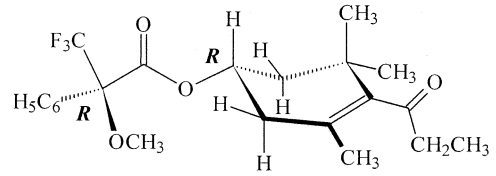
Figure 3. Selected $^1H-^1H$ COSY correlations of **2** and **3**.

signals of four methyl groups (including one vinyl methyl and two methyls geminal to a quaternary carbon), three methylenes, a methine-bearing oxygen, and four quaternary carbons (of which two were olefinic and one a carbonyl). The COSY (Figure 3) and HMQC NMR spectra were used to establish the presence of the partial structures $-CH_2CH(OH)CH_2-$ and $-COCH_2CH_3$ in the molecule of **2**. The skeleton of **2** was constructed on the basis of an HMBC experiment (Figure 1). The orientation at the chiral



2a



2b

Figure 4. Conformations of the (*S*)- and (*R*)-MTPA derivatives of **2**.

Table 1. ^1H NMR Data of Mosher Esters of **2** in Acetone-d_6

compound	H-3_{ax}	H-3_{eq}	Me-2	H-5_{ax}
(*S*)-MTPA derivative of **2** (**2a**)	1.92	2.08	1.54	1.67
(*R*)-MTPA derivative of **2** (**2b**)	2.04	2.23	1.58	1.56
Δ ($\delta_{\mathbf{2a}} - \delta_{\mathbf{2b}}$)	−0.12	−0.15	−0.04	+0.11

center at C-4 was determined by the coupling constants of the H-4 resonance at δ_H 3.92 (dddd, J = 13.2, 12.0, 6.0, 4.0 Hz) with the two pairs of methylene protons, which inferred an equatorial orientation of the hydroxyl group at C-4. The absolute stereochemistry of C-4 was achieved by Mosher ester derivatization.[22] Both the (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenyl acetate (MTPA) esters of **2** were prepared (Figure 4) and subjected to ^1H NMR analysis (Table 1). In the ^1H NMR spectrum, the H-3_{ax}, H-3_{eq}, and Me-2 signals of **2a** shifted upfield due to the phenyl group, whereas in **2b**, the H-5_{ax} signal experienced shielding. Therefore, the absolute configuration of **2** at C-4 was determined as *R*. If C-4 had been in the *S* configuration, the opposite shielding results would have been obtained. On the basis of the above results, the structure of crocusatin G was assigned as **2**.

Crocusatin H (**3**) was obtained as colorless needles, and HRMS established its molecular formula as $C_{12}H_{20}O_4$. The ^{13}C NMR spectrum showed signals for three methyls, three methylenes, two methines, and four quaternary carbons. The IR absorption at 3400 and 1681 cm^{-1} indicated the presence of a hydroxyl and a carbonyl group in the molecule. The ^1H NMR spectrum of **3** showed the occurrence of a *gem*-dimethyl group at δ_H 1.07 and 1.20 and a vinyl methyl group at δ_H 1.70. The ^1H NMR spectrum in combination with the $^1H-^1H$ COSY NMR spectrum (Figure 3) indicated the presence of the partial structure $-CH_2-CH(OH)CH_2-$ from the multiplet signal at δ_H 3.94 (ddt, J = 12.0, 9.2, 6.0 Hz), which coupled with two pairs of methylene protons at δ_H 2.31 (dd, J = 17.2, 6.0 Hz), 1.94 (dd, J = 17.2, 9.2 Hz), 1.71 (dd, J = 12.0, 6.0 Hz), and 1.36 (t, J = 12.0 Hz). Another partial structure, $-CH(OH)CH_2-COOH$, was accounted for by the observation of the signals at δ_C 73.3, 64.4, and 171.4 in the ^{13}C NMR spectrum and the signals at δ_H 3.64 (dd, J = 6.0, 5.0 Hz), 3.57 (dd, J = 10.8, 5.0 Hz), and 3.50 (dd, J = 10.8, 6.0 Hz) in the ^1H NMR spectrum. The spectral data of **3** were similar to those of crocusatin G (**2**), except for the signals of H-1′, C-1′, and C-3′ of **3** at δ_H 3.64 and δ_C 73.3 (d) and 171.4 (s), respectively, which appeared instead of the signals of C-1′, C-3′, and H-3′ at δ_C 210.6 (s), 7.0 (q), and δ_H 1.01 in **2**. According to the coupling constants of H-4 with the C-2′ methylene protons (ddt, J = 12.0, 9.2, 6.0 Hz), the orienta-

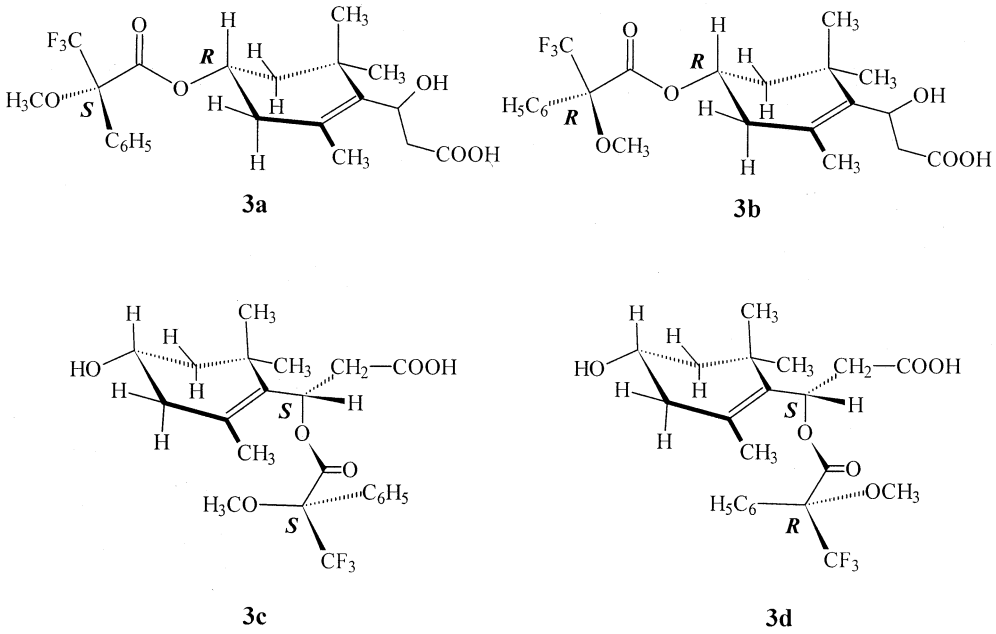


Figure 5. Conformations of the (*S*)- and (*R*)-MTPA derivatives of **3**.

Table 2. ^{1}H NMR Data of Mosher Esters of **3** in Acetone-d_6

compound	H-3$_{ax}$	H-3$_{eq}$	H-5$_{ax}$	Me-2	H-2′
(*S*)-MTPA derivative of **3** (**3a**)	1.69	2.11	1.21	1.68	
(*R*)-MTPA derivative of **3** (**3b**)	1.84	2.20	1.14	1.73	
Δ ($\delta_{3a} - \delta_{3b}$)	−0.15	−0.09	+0.07	−0.05	
(*S*)-MTPA derivative of **3** (**3c**)				1.81	3.32
					3.38
(*R*)-MTPA derivative of **3** (**3d**)				1.75	3.37
					3.54
Δ ($\delta_{3c} - \delta_{3d}$)				+0.06	−0.05
					−0.16

tion of the hydroxyl group at C-4 was determined to be equatorial. The absolute configurations at C-4 and C-1′ were determined by Mosher's method.[22] The (*S*)- and (*R*)-MTPA esters of **3** were prepared (Figure 5), and an analysis of the ^{1}H NMR spectrum of these derivatives is summarized in Table 2. The shielding effects of H-3$_{ax}$, H-3$_{eq}$, and Me-2 in **3a** and H-5$_{ax}$ in **3b** due to the phenyl group were similar to those of **2a** and **2b**, respectively, so that the absolute configuration of C-4 was assigned as *R*. The stereochemistry of the side chain alcohol was resolved by the ^{1}H NMR analysis of **3c** and **3d** (Table 2). The signal of Me-2 of **3c** appeared more downfield than that of **3d**, and the H-2′ protons of **3c** were shielded by a phenyl group and shifted more upfield than that of **3d**. These observations indicated that the configuration at C-1′ was *S*. On the basis of the above data, the structure of crocusatin H was assigned as **3**.

Crocusatin I (**4a**) was isolated as a colorless oil, and its molecular formula, $C_{10}H_{14}O_3$ (M^+ at *m*/*z* 198.0897), indicated four degrees of unsaturation. The presence of the α-hydroxyenone system of **4a** was implied by the observation of IR bands at 1668 and 3400 cm^{-1} and the UV absorptions at 254 and 287 nm.[4] The ^{1}H NMR spectrum of **4a** showed the presence of a *gem*-dimethyl group at δ 1.31 (6H, s) and a vinyl methyl group at δ 2.07 (3H, s), a hydroxymethyl group at δ 4.44 (2H), an olefinic proton at δ 6.03 (1H, s), and a hydroxyl group at δ 6.28 (1H, brs, D_2O exchangeable). The foregoing spectral studies suggested that **4a** has a 2,6,6-trimethylcyclohexenone monoterpene skeleton. These spectral data were similar to those of crocusatin D (**4b**),[23] but differed from **4b** by having a double bond between C-4 and C-5 (δ 6.03). On the basis of the above observations, the structure of crocusatin I was elucidated as **4a**. Straubinger et al. isolated 6-hydroxy-3-(hydroxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one 6-*O*-β-D-glucopyranoside and subjected this glycoside to hydrolytic cleavage to give the aglycon **4a** and identified the structure by GC/MS.[24] However, this is the first time **4a** has been obtained as a natural product.

The known compounds 2-formyl-5-methoxyfuran,[25] crocusatin C,[23] 4-hydroxy-3,5,5-trimethylcyclohex-2-enone,[4] crocusatin B,[23] picrocrocin,[8] 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde,[4] 2-hydroxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione,[4] 4-hydroxymethyl-3,5,5-trimethylcyclohex-3-enol,[26] 3-hydroxy-β-ionone,[27] methylparaben,[28] 5-methyluracil,[29] uracil,[30] adenosine,[31] pyridin-3-ylmethanol,[32] α-crocetin,[5] crocetin monomethyl ester,[33] crocetin mono(β-D-glucosyl) ester,[5] crocin-3 (**6**),[5] crocin-4,[5] crocin-2,[5] and crocin-1 (**5**)[5] were also isolated and identified from the dried stigma of *C. sativus*. The structures of these known compounds were identified by comparison of their spectroscopic data (UV, IR, and mass spectroscopy) with literature values.

The isolated compounds were subjected to an evaluation of their antityrosinase activity. Among them, crocusatin H (**3**) and crocin-3 (**6**) inhibited mushroom tyrosinase, and their activities were more potent (IC$_{50}$ values of 0.87 and 0.96 mM, respectively) than those of arbutin (IC$_{50}$ 24 mM)[34] and hydroquinone (IC$_{50}$ 4.5 mM).[35] In turn, crocin-1 (**5**) exhibited more potent inhibitory activity (IC$_{50}$ 140 μM) than that of kojic acid (IC$_{50}$ 235 μM).[36] The known compounds arbutin, hydroquinone, and kojic acid are usually present in commercial whitening cosmetics, with tyrosinase inhibitors having become increasingly important for cosmetic products related to hyperpigmentation. The results of the present biological evaluation suggest that the stigmas of *C. sativus* might be able to be used as an additive in whitening cosmetics.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were obtained with a JASCO DIP-370 polarimeter. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH

solution. The IR spectra were measured on a Shimadzu FTIR-8501 spectrophotometer as KBr disks. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian-400 Unity Plus 400 spectrometer. Chemical shifts are recorded in δ values with tetramethylsilane as an internal reference. The mass spectra were performed in the EI mode on a VG70-250S mass spectrometer.

Plant Material. The stigmas of *Crocus sativus* L. were purchased in Shanghai, People's Republic of China, in July 1995, and authenticated by Prof. C. S. Kuoh. A voucher specimen (NCKU-WU-950701) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried stigmas of *C. sativus* (2.0 kg) were extracted with H_2O under reflux (8 L × 7), then the residue was refluxed with MeOH three times. The combined extracts were concentrated to give a dark brown syrup (1.1 kg). The crude extract was partitioned between H_2O (1.5 L) and $CHCl_3$ (1.5 L × 15). The $CHCl_3$-soluble layer (160 g) was directly chromatographed over silica gel by eluting with a gradient of $CHCl_3$−MeOH (100% to 0%) to afford 10 fractions. Fraction 1 was chromatographed over silica gel with a gradient of *n*-C_6H_{14}−Me_2CO (19:1 to 1:1) to yield crocusatin I (**4a**) (0.6 mg), crocusatin C (1.7 mg), 4-hydroxy-3,5,5-trimethylcyclohex-2-enone (3.6 mg), crocusatin B (4.1 mg), 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde (446 mg), and 2-hydroxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione (7.8 mg), in order of polarity. Fraction 2 was chromatographed on a silica gel column eluting with $CHCl_3$−MeOH (49:1) to give crocusatin F (**1**) (4.5 mg) and 2-formyl-5-methoxyfuran (5.3 mg). Fraction 4 was subjected to column chromatography over silica gel with a gradient of $CHCl_3$−MeOH (9:1 to 1:1) to afford picrocrocin (6.7 g). Fraction 5 was treated in the same manner to give crocusatin H (**3**) (6.9 mg), crocusatin B (2.3 mg), methylparaben (4.3 mg), α-crocetin (45 mg), crocetin monomethyl ester (16 mg), and crocin-4 (43 mg), respectively. Fraction 6 was chromatographed over silica gel eluting with a gradient of $CHCl_3$−Me_2CO (4:1 to 1:1) to yield crocusatin G (**2**) (6.2 mg), crocusatin I (**4a**) (0.5 mg), crocetin monomethyl ester (12 mg), and crocin-3 (**6**) (688 mg), successively. Fraction 10 was chromatographed on silica gel eluting with $CHCl_3$−EtOAc−MeOH (3:1:1) to yield crocin-3 (**6**) (1.4 g).

The H_2O-soluble layer (900 g) was chromatographed on Diaion HP-20 eluting with a gradient of H_2O and MeOH (100% to 0%) to give 13 fractions. Fraction 5 was further purified over Sephadex G-10 using 5% MeOH as eluent to give 5-methyluracil (68 mg) and uracil (39 mg). Fraction 7 on column chromatography over silica gel with $CHCl_3$−MeOH (3:1) afforded picrocrocin (21 g), 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde (3.2 mg), 4-hydroxymethyl-3,5,5-trimethylcyclohex-3-enol (2.1 mg), and adenosine (6.5 mg). Fraction 8 was treated in a manner similar to fraction 7 to afford pyridin-3-ylmethanol (1.3 mg). Fraction 10 was chromatographed on Sephadex LH-20 eluting with a gradient of H_2O and MeOH (100% to 50%) to give 4-hydroxy-3,5,5-trimethylcyclohex-2-enone (0.8 mg), 3-hydroxy-β-ionone (2.6 mg), α-crocetin (51 mg), and crocetin mono(β-D-glucosyl) ester (34 mg), in turn. Fractions 11 and 12 were combined and recrystallized from MeOH−H_2O to yield crocin-1 (**5**) (104 g). The mother liquor was rechromatographed on Diaion HP-20 eluting with a gradient of H_2O and MeOH (70% to 30%) to give crocin-3 (**6**) (1.4 g), crocin-4 (5.6 g), and crocin-1 (**5**) (26 g). Fraction 13 was recrystallized from MeOH−H_2O to afford crocin-2 (29 g).

Crocusatin F (1): colorless oil; $[\alpha]^{25}_D$ −58° (*c* 0.012, MeOH); IR (KBr) ν_{max} 1774, 1740, 1218, 1101, 764 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.37 (1H, dd, J = 10.0, 6.0 Hz, H-7β), 4.32 (1H, dd, J = 10.0, 2.0 Hz, H-7α), 2.43 (1H, d, J = 16.4 Hz, H-2β), 2.29 (1H, dd, J = 6.0, 2.0 Hz, H-4β), 2.26 (1H, d, J = 16.4 Hz, H-2α), 1.67 (3H, s, Me-5), 1.09 (3H, s, βMe-3), 0.98 (3H, s, αMe-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6 (s, C-6), 167.3 (s, C-1), 79.9 (s, C-5), 65.4 (t, C-7), 48.9 (d, C-4), 42.6 (t, C-2), 32.1 (s, C-3), 28.3 (q, βMe-3) 25.3 (q, Me-5), 21.9 (q, αMe-3); EIMS *m*/*z* 198 [M]$^+$ (16), 185 (21), 154 (36), 110 (24), 83 (100); HREIMS *m*/*z* 198.0892 (calcd for $C_{10}H_{14}O_4$, 198.0893).

Crocusatin G (2): colorless oil; $[\alpha]^{25}_D$ +54° (*c* 0.05, C_5H_5N); $[\alpha]^{25}_D$ +72° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (3.15) nm; IR (KBr) ν_{max} 3356, 2948, 1684, 1543 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.92 (1H, dddd, J = 13.2, 12.0, 6.0, 4.0 Hz, H-4), 3.70 (1H, brs, OH), 2.59 (1H, dq, J = 18.8, 7.2 Hz, H-2′), 2.49 (1H, dq, J = 18.8, 7.2 Hz, H-2′), 2.26 (1H, dd, J = 17.0, 6.0 Hz, H-3$_{eq}$), 1.93 (1H, dd, J = 17.0, 13.2 Hz, H-3$_{ax}$), 1.68 (1H, dd, J = 12.0, 4.0 Hz, H-5$_{eq}$), 1.54 (3H, s, Me-2), 1.38 (1H, t, J = 12.0 Hz, H-5$_{ax}$), 1.12 (3H, s, Me-6), 1.01 (3H, t, J = 7.2 Hz, H-3′), 0.98 (3H, s, Me-6); ^{13}C NMR (acetone-d_6, 100 MHz) δ 210.6 (s, C-1′), 143.2 (s, C-2), 126.7 (s, C-1), 63.6 (d, C-4), 48.1 (t, C-5), 41.1 (t, C-3), 38.5 (t, C-2′), 35.8 (s, C-6), 29.3 (q, Me-6), 28.9 (q, Me-6), 20.1 (q, Me-2), 7.0 (q, C-3′); EIMS *m*/*z* 196 [M]$^+$ (41), 183 (37), 178 (26), 167 (22), 163 (100), 139 (35); HREIMS *m*/*z* 196.1461 (calcd for $C_{12}H_{20}O_2$, 196.1463).

Crocusatin H (3): colorless needles; $[\alpha]^{25}_D$ +43° (*c* 0.02, MeOH); IR (KBr) ν_{max} 3400, 1681, 1317, 1076 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.94 (1H, ddt, J = 12.0, 9.2, 6.0 Hz, H-4), 3.83 (1H, brs, OH), 3.64 (1H, dd, J = 6.0, 5.0 Hz, H-1′), 3.57 (1H, dd, J = 10.8, 5.0 Hz, H-2′), 3.50 (1H, dd, J = 10.8, 6.0 Hz, H-2′), 2.31 (1H, dd, J = 17.2, 6.0 Hz, H-3$_{eq}$), 1.94 (1H, dd, J = 17.2, 9.2 Hz, H-3$_{ax}$), 1.71 (1H, dd, J = 12.0, 6.0 Hz, H-5$_{eq}$), 1.71 (3H, s, Me-2), 1.36 (1H, t, J = 12.0 Hz, H-5$_{ax}$), 1.20 (3H, s, Me-6), 1.07 (3H, s, Me-6); ^{13}C NMR (acetone-d_6, 100 MHz) δ 171.4 (s, C-3′), 135.7 (s, C-1), 131.7 (s, C-2), 73.3 (d, C-1′), 64.4 (t, C-2′), 64.1 (d, C-4), 48.4 (t, C-5), 41.7 (t, C-3), 36.1 (s, C-6), 29.4 (q, Me-6), 28.9 (q, Me-6), 21.3 (q, Me-2); EIMS *m*/*z* 228 [M]$^+$ (25), 213 (54), 210 (34), 195 (75), 184 (62), 183 (56), 121 (100); HREIMS *m*/*z* 228.1365 (calcd for $C_{12}H_{20}O_4$, 228.1362).

Crocusatin I (4a): colorless oil; UV (MeOH) λ_{max} (log ϵ) 254 (3.85), 287 (3.77) nm; IR (KBr) ν_{max} 3400, 2979, 1668, 1305, 1249, 1113 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.28 (1H, brs, OH), 6.03 (1H, s, H-5), 4.44 (2H, s, CH_2OH), 2.07 (3H, s, Me-2), 1.31 (6H, s, Me-6 × 2); EIMS *m*/*z* 182 [M]$^+$ (7), 164 (46), 151 (23), 138 (25), 123 (100), 93 (60); HREIMS *m*/*z* 182.0954 (calcd for $C_{10}H_{14}O_3$, 182.0941).

Preparation of MTPA Esters. Aliquots (5 μL) of the enantiomerically pure α-methoxy-α-trifluoromethylphenylacetyl chloride [(*S*)- or (*R*)-MTPA chloride] were added to a mixture of 0.5 mg of **2**, 0.5 mL of anhydrous $CHCl_3$, and 0.5 mL of anhydrous pyridine in a sealed vial. Each reaction mixture was allowed to stand at 25 °C for 16 h. Then, H_2O and $CHCl_3$ (each 5 mL) were added, and the $CHCl_3$ layer was washed several times with H_2O and then evaporated to dryness. The residue was subjected to silica gel preparative TLC with $CHCl_3$−MeOH (19:1) to yield **2a** or **2b**. The MTPA esters of **3** were prepared by a similar procedure. Compound **3** (1.5 mg) and 10 μL of the enantiomerically pure α-methoxy-α-trifluoromethylphenylacetyl chloride were dissolved in a mixture of 0.5 mL of anhydrous $CHCl_3$ and 0.5 mL of anhydrous pyridine, and the reaction mixture was allowed to stand at 25 °C for 16 h. Then, H_2O and $CHCl_3$ were added, and the $CHCl_3$ layer was washed several times with H_2O and then dried by rotary evaporation. The residue was subjected to silica gel preparative TLC with *n*-C_6H_{14}−Me_2CO (3:1) to yield **3a** and **3c** or **3b** and **3d**, successively.

Tyrosinase Inhibitory Activity. The mushroom tyrosinase and L-dopa used for the bioassay were purchased from Sigma Chemical Co. Antityrosinase activity was measured by spectrophotometry according to the method of Mason and Peterson,[37] with minor modifications. The test substance was dissolved in 0.1 mL of 10% DMSO in aqueous solution and incubated with 0.1 mL of mushroom tyrosinase (135 U/mL, PBS pH 6.8) at 25 °C for 10 min, and then 0.1 mL of 0.5 mM L-dopa in phosphate buffer solution (PBS, pH 6.8) was added. The reaction mixture was incubated for 5 min. The amount of dopachrome in the mixture was determined by the optical density (OD) at 475 nm using a μ Quant universal microplate spectrophotometer. Kojic acid, arbutin, and hydroquinone (Sigma Chemical Co.) were used as standard agents. The inhibitory percentage of tyrosinase was calculated as follows: % inhibition = $\{[(A - B) - (C - D)] - (A - B)\} \times 100$ (*A*, OD

at 475 nm without test substance; *B*, OD at 475 nm without test substance and tyrosinase; *C*, OD at 475 nm with test substance; *D*, OD at 475 nm with test substance, but without tyrosinase.)

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References and Notes

(1) Jiangsu New Medical College. *Encyclopedia of Chinese Materia Medica*; Shanghai Science and Technology Press: Shanghai, 1977; Vol. 2, pp 2671−2672.
(2) Rios, J. L.; Recio, M. C.; Giner, R. M.; Manez, S. *Phytother. Res.* **1996**, *10*, 189−193.
(3) Sujata, V.; Ravishankar, G. A.; Venkataraman, L. V. *J. Chromatogr.* **1992**, *624*, 497−502.
(4) Zarghami, N. S.; Heinz, D. E. *Phytochemistry* **1971**, *10*, 2755−2761.
(5) Dhingra, V. K.; Seshadri, T. R.; Mukerjee, S. K. *Indian J. Chem.* **1975**, *13*, 339−341.
(6) Pfander, H.; Rychener, M. *J. Chromatogr.* **1982**, *234*, 443−447.
(7) Ghosal, S.; Singh, S. K.; Battacharrya, S. K. *J. Chem. Res.* (*S*) **1989**, 70−71.
(8) Castellar, M. R.; Montijano, H.; Manjon, A.; Iborra, J. L. *J. Chromatogr.* **1993**, *648*, 187−190.
(9) Tarantilis, P. A.; Tsoupras, G.; Polissiou, M. *J. Chromatogr. A* **1995**, *699*, 107−118.
(10) Song, C. Q. *Zhongcaoyao* **1990**, *21*, 439−441.
(11) Song, C. Q.; Xu, R. S. *Acta Chim. Sin.* **1991**, *49*, 917−920.
(12) Nair, S. C.; Pannikar, B.; Panikkar, K. R. *Cancer Lett.* **1991**, *57*, 109−114.
(13) Salomi, M. J.; Nair, S. C.; Panikkar, K. R. *Nutr. Cancer* **1991**, *16*, 67−72.
(14) Mathews, M. M. *Oncology* **1982**, *39*, 362−364.
(15) Abdullaev, F. I. *Toxicol. Lett.* **1994**, *70*, 243−251.
(16) Gainer, J. L.; Chisolm, G. M., III. *Atherosclerosis* **1974**, *19*, 135−138.
(17) Gainer, J. V.; Nugent, G. R. *J. Neurosurg.* **1976**, *45*, 535−538.
(18) Gainer, J. V. *J. Neurosurg.* **1977**, *46*, 358−360.
(19) Seyde, W. C.; Mckernan, D. J.; Laudeman, J.; Gainer, J. L.; Longnecker, D. E. *J. Cereb. Blood Flow Metab.* **1986**, *6*, 703−707.
(20) Holloway, G. M.; Gainer, J. L. *J. Appl. Physiol.* **1988**, *65*, 683−686.
(21) Naish-Byfield, S.; Riley, P. A. *Biochem. J.* **1992**, *288*, 63−67.
(22) Erickson, K. L.; Beutler, J. A.; Cardellina, J. H.; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8188−8192.
(23) Li, C. Y.; Wu, T. S. *Chem. Pharm. Bull.* **2002**, in press.
(24) Straubinger, M.; Bau, B.; Ekstein, S.; Fink, M.; Winterhalter, P. *J. Agric. Food Chem.* **1998**, *48*, 3238−3243.
(25) Schwartz, D. A.; Yates, P. *Can. J. Chem.* **1983**, *61*, 1126−1131.
(26) Ruettimann, A.; Mayer, H. *Helv. Chim. Acta* **1980**, *63*, 1456−1462.
(27) Machida, K.; Kikuchi, M. *Phytochemistry* **1996**, *41*, 1333−1336.
(28) Carter, M. J.; Fleming, I.; Percival, A. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2415−2434.
(29) Claydon, N.; Grove, J. F.; Pople, M. *Phytochemistry* **1985**, *24*, 937−943.
(30) Schneider, H. J.; Blatter, T.; Palm, B.; Pfingstag, U.; Ruediger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704−7708.
(31) Karlik, S. J.; Elgavish, G. A.; Eichhorn, G. L. *J. Am. Chem. Soc.* **1983**, *105*, 602−609.
(32) Ito, A.; Kasai, R.; Yamasaki, K.; Sugimoto, H. *Phytochemistry* **1993**, *33*, 1133−1137.
(33) Corradi, C.; Micheli, G. *Boll. Chim. Farm.* **1979**, *118*, 537−552.
(34) Isao, K.; Ikuyo, K. H. *Planta Med.* **1999**, *65*, 19−22.
(35) Kazuhisa, M.; Minoru, F. *J. Soc. Cosmet. Chem.* **1991**, *42*, 361−368.
(36) Ryuju, H.; Senji, T.; Kunikatsu, H.; Tadasu, M.; Akira, N. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1725−1726.
(37) Mason, H. S.; Peterson, E. W. *Biochim. Biophys. Acta* **1965**, *111*, 134−139.

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