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Isolation of a Natural Antioxidant, Dehydrozingerone from *Zingiber officinale* and Synthesis of Its Analogues for Recognition of Effective Antioxidant and Antityrosinase Agents

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In the present study, the antioxidative and inhibitory activity of *Zingiber officinale* Rosc. rhizomes-derived materials (on mushroom tyrosinase) were evaluated. The bioactive components of *Z. officinale* rhizomes were characterized by spectroscopic analysis as zingerone and dehydrozingerone, which exhibited potent antioxidant and tyrosinase inhibition activities. A series of substituted dehydrozingerones [(*E*)-4-phenyl-3-buten-2-ones] were prepared in admirable yields by the reaction of appropriate benzaldehydes with acetone and the products were evaluated in terms of variation in the dehydrozingerone structure. The synthetic analogues were examined for their antioxidant and antityrosinase activities to probe the most potent analogue. Compound **26** inhibited Fe^{2+}-induced lipid peroxidation in rat brain homogenate with an IC_{50} = 6.3±0.4 μM. In the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical quencher assay, compounds **2**, **7**, **17**, **26**, **28**, and **29** showed radical scavenging activity equal to or higher than those of the standard antioxidants, like α-tocopherol and ascorbic acid. Compound **27** displayed superior inhibition of tyrosinase activity relative to other examined analogues. Compounds **2**, **17**, and **26** exhibited non-competitive inhibition against oxidation of 3,4-dihydroxyphenylalanine (L-DOPA). From the present study, it was observed that both number and position of hydroxyl groups on aromatic ring and a double bond between C-3 and C-4 played a critical role in exerting the antioxidant and antityrosinase activity.

Key words: Dehydrozingerone, Zingerone, Fe^{2+}-Induced lipid peroxidation, Antioxidation, Antityrosinase, (*E*)-4-Phenyl-3-buten-2-one

INTRODUCTION

Augmentation of reactive superoxide radicals has been known to induce lipid peroxidation and to damage membranes in biological systems, thus resulting in the initiation and/or progression of a number of diseases such as cancer, senescene, and inflammation (Fridovich, 1978). Antioxidant activity is a fundamental property important for life. Moreover, in food, autooxidation damages many constituents, mainly lipids, proteins, colorants, and flavor compounds. Frequently this process leads to negative nutritive and sensoric value of many food products. To diminish oxidative damage antioxidants are added, primarily to inhibit the autooxidation of highly unsaturated lipids or flavor compounds (Halliwell *et al.*, 1995). In particular, the autooxidation of unsaturated lipids generates typical flavors and frequently off-flavors. In addition, oxidized lipids can be absorbed by the normal pathways in mammals and can be incorporated into the lipoproteins, such as chylomicrons and LDL (Staprans *et al.*, 1993). Oxidized lipids are cytotoxic and can negatively influence

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lipid metabolism. Hence it is imperative to probe for antioxidants, which can protect membranes against oxidative damage and can diminish autooxidation of lipids by inhibiting or quenching free radicals and reactive species.

Tyrosinase is a multifunctional enzyme that catalyzes both the hydroxylation of monophenols, such as tyrosine to *o*-diphenols by monophenol monooxygenase, and the oxidation of *o*-diphenols to *o*-quinones by catechol oxidase (Raper, 1928). Tyrosinase is one of the most important key enzymes in the insect molting process, and its inhibitors might ultimately provide clues to the control of insect pests (Shirota *et al.*, 1994; Anderson, 1985). In the food industry, tyrosinase, which is also known as polyphenol oxidase (PPO), is responsible for enzymatic browning reactions in damaged fruits during post-harvest handling and processing (CoSeteng and Lee, 1987; Lee *et al.*, 1990; Sanchez-Ferrer *et al.*, 1995). Moreover, tyrosinase inhibitors are becoming important constituents of cosmetic products that are related to hyperpigmentation (Maeda and Fukuda, 1991). Tyrosinase inhibitors may prevent the production of dermal melanin pigmentation since tyrosinase plays an important role in the process of melanin biosynthesis (Pomerantz, 1963; Mason and Peterson, 1965). Thus, melanin synthesis inhibitors are used topically for treatment of localized hyperpigmentation in humans such as lentigo, nevus, ephelis, post-inflammatory state, and melanoma of pregnancy. These observations offer a crucial insight to look effective tyrosinase inhibitors.

On the basis of an earlier report (Huang *et al.*, 2001), the active components of *Z. officinale* rhizomes were isolated and characterized by spectroscopic analyses. A series of analogues of dehydrozingerone, a more active compound were synthesized and their antioxidative potential to develop new antioxidants for cosmetic or food uses were evaluated. Additionally, the antityrosinase activities of isolates and synthetic analogues were also examined due to their structural similarity with L-DOPA and in relation to the antioxidant results obtained.

MATERIALS AND METHODS

General intrumentation

All chemicals were purchased from Merck Co., U.S.A. otherwise mentioned specifically. Melting points were measured on Yanaco MP-S3 micro-melting point apparatus and were uncorrected. UV-Vis spectra were recorded on a Hitachi UV-3210 spectrophotometer. IR spectra were measured on a Shimadzu FT-IR 8501 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AC-200 and Avance 300 spectrometers, respectively. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. EI and HREIMS spectra were measured on a VG70-250S spectrometer by a direct inlet system. Elemental analyses were performed on a Heraeus CHN-*O*-Rapid analyzer and were within 0.4 % of the theoretical values.

Isolation and identification

The shade dried and powdered rhizomes (100 g) of *Zingiber officinale* Rosc. were extracted with hot methanol (2L×3) and filtered. The extracts were combined and concentrated *in vacuo* at room temperature. The concentrated extract (10 g) was suspended in water (100 mL) and then partitioned with chloroform (100 mL×10) to obtain chloroform solubles (2 g). The aqueous layer was concentrated to give brown syrup (8 g). The residue of the chloroform extract was chromatographed on a silica gel (230-400 mesh, 60 g) column (3×20 cm), and chloroform-methanol (1:0, 19:1, 9:1, 5:1, 3:1, 1:1, 0:1, each 200 mL) gradient system was employed as the mobile phase, with a subsequent yield of 8 fractions. Fraction 3 was directly subjected to silica gel column chromatography (230-400 mesh, 30 g, 2×15 cm) by mixing *n*-hexane and ethyl acetate (3:1) as eluent, to obtain 3 subfractions. Recrystallization of subfraction 2 with acetone resulted in the formation of zingerone (**1**). Similarly, fraction 5 chromatographed on a silica gel column (230-400 mesh, 30 g, 2×15 cm) with *n*-hexane and acetone (2:1) as mobile phase, followed by the recrystallization of second subfraction (obtained with ethyl acetate as an eluent) resulted in the formation of dehydrozingerone (**2**).

The characteristics of the compounds are as followed; Zingerone (**1**): colorless solid, m.p.: 125-127°C; UV (MeOH) λ_{max}: 289 (sh), 281, 227; IR (KBr) ν_{max}: 3300, 1715 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.13 (3H, s, CH_3-1), 2.73 (2H, t, *J* = 6.9 Hz, H-3), 2.82 (2H, t, *J* = 6.9 Hz, H-4), 3.87 (3H, s, OCH_3-3'), 5.54 (1H, br s, OH-4'), 6.66 (1H, dd, *J* = 8.0, 1.2 Hz, H-6'), 6.69 (1H, d, *J* = 1.2 Hz, H-2'), 6.82 (1H, d, *J* = 8.0 Hz, H-5'); EI-MS (*rel. int.*): *m/z* 148 ([M]$^{+}$, 5), 133 (100).

Dehydrozingerone [4-(4'-Hydroxy-3'-methoxyphenyl)-(*E*)-3-buten-2-one] (2)

Yellow solid, m.p.: 124-125°C; UV (MeOH) λ_{max}: 337 (sh), 303, 242, 225; IR (KBr) ν_{max}: 3535, 3020, 2964, 2943, 1665, 1514, 1429 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.37 (3H, s, CH_3-1), 3.93 (3H, s, OCH_3-3'), 6.14 (1H, br s,

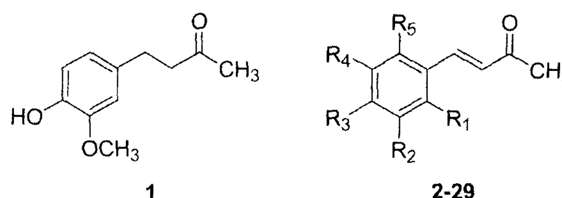

Fig. 1. Structures of compounds **1-29**

OH), 6.59 (1H, d, *J* = 16.4 Hz, H-3), 6.93 (1H, d, *J* = 8.2 Hz, H-5'), 7.06 (1H, d, *J* = 1.8 Hz, H-2'), 7.09 (1H, dd, *J* = 8.2, 1.8 Hz, H-6'), 7.46 (1H, d, *J* = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 10), 162 (28), 147 (100), 145 (25), 103 (26).

General synthetic procedures for (*E*)-4-phenyl-3-buten-2-ones (2-17) (Denniff *et al.*, 1976)

To the solution of appropriate benzaldehyde (0.275 g) and acetone (1 mL) in ethanol (2.5 mL), 5% aqueous sodium hydroxide (NaOH) (5 mL) was added. The reaction mixture was stirred at room temperature for 1.5 h then diluted with water (20 ml) and cooled to 0°C. The diluted solution was neutralized with 2 N hydrochloric acid (HCl). Excessive alcohol was evaporated in a vacuum and the products were extracted with ethyl acetate (EtOAc) (50 mL×3). The combined organic layer was washed with brine and dried over anhydrous sodium sulfate (Na_2SO_4). Thereafter, the drying agent was removed by filtration and the solvent was evaporated under reduced pressure to give crude product. The product was purified by silica gel column chromatography.

4-(3'-Hydroxy-4'-methoxyphenyl)-(*E*)-3-buten-2-one (3)
Yellow solid, m.p.: 85-86°C; IR (KBr) ν_{max}: 3545, 3020, 2845, 1665, 1607, 1512 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.37 (3H, s, CH_3-1), 3.94 (3H, s, OCH_3-3'), 5.78 (1H, br s, OH), 6.59 (1H, d, *J* = 16.2 Hz, H-3), 6.86 (1H, d, *J* = 8.2 Hz, H-5'), 7.06 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 7.16 (1H, d, *J* = 2.0 Hz, H-2'), 7.44 (1H, d, *J* = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 78), 177 (100), 149 (19), 134 (14), 117 (17), 91 (23).

4-(4'-Hydroxy-2'-methoxyphenyl)-(*E*)-3-buten-2-one (4)
Yellow solid, m.p.: 170-172°C; IR (KBr) ν_{max}: 3329, 1635, 1592, 1508, 1467 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.37 (3H, s, CH_3-1), 3.87 (3H, s, OCH_3-2'), 6.42~6.48 (2H, m, H-3' & -5'), 6.67 (1H, d, *J* = 16.4 Hz, H-3), 7.43 (1H, d, *J* = 9.1 Hz, H-6'), 7.82 (1H, d, *J* = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 62), 177 (100), 161 (79), 150 (20), 134 (51).

4-(2'-Hydroxy-3'-methoxyphenyl)-(*E*)-3-buten-2-one (5)
Yellow solid, m.p.: 78-80°C; IR (KBr) ν_{max}: 3460, 2970, 1618, 1480 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.40 (3H, s, CH_3-1), 3.93 (3H, s, OCH_3-3'), 6.14 (1H, br s, OH-2'), 6.81 (1H, d, *J* = 16.5 Hz, H-3), 6.85~6.91 (2H, m, H-4' & -6'), 7.11~7.15 (1H, m, H-5'), 7.84 (1H, d, *J* = 16.5 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 53), 177 (100), 162 (28), 150 (21), 134 (55), 121 (43).

4-(2'-Hydroxy-4'-methoxyphenyl)-(*E*)-3-buten-2-one (6)
Yellow solid, m.p.: 128-129°C; IR (KBr) ν_{max}: 3178, 1605, 1510, 1433 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3-1), 3.82 (3H, s, OCH_3-4'), 6.44 (1H, d, *J* = 2.4 Hz, H-3'), 6.51 (1H, dd, *J* = 8.6, 2.4 Hz, H-5'), 6.86 (1H, d, *J* = 16.5 Hz, H-3), 7.41 (1H, d, *J* = 8.6 Hz, H-6'), 7.77 (1H, d, *J* = 16.5 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 42), 177 (100), 149 (19), 133 (14), 121 (34).

4-(2'-Hydroxy-5'-methoxyphenyl)-(*E*)-3-buten-2-one (7)
Yellow solid, m.p.: 125-126°C; IR (KBr) ν_{max}: 3083, 1635, 1594, 1509, 1423 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.42 (3H, s, CH_3-1), 3.78 (3H, s, OCH_3-5'), 6.84~6.85 (2H, m, H-3' & -6'), 6.89 (1H, d, *J* = 16.4 Hz, H-3), 6.98~7.00 (1H, m, H-4'), 7.86 (1H, d, *J* = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 85), 177 (100), 175 (39), 150 (48), 135 (22), 121 (55).

4-(2'-Hydroxy-6'-methoxyphenyl)-(*E*)-3-buten-2-one (8)
Yellow solid, m.p.: 148-150°C; IR (KBr) ν_{max}: 3099, 3062, 2960, 2839, 1684, 1623, 1593, 1472, 1436 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.43 (3H, s, CH_3-1), 3.88 (3H, s, OCH_3-6'), 6.46 (1H, d, *J* = 8.3 Hz, H-3'), 6.61 (1H, d, *J* = 8.3 Hz, H-5'), 7.18 (1H, t, *J* = 8.3 Hz, H-4'), 7.42 (1H, d, *J* = 16.6 Hz, H-3), 8.03 (1H, d, *J* = 16.6 Hz, H-4); EI-MS (*rel. int.*): *m/z* 192 ($[M]^+$, 24), 177 (100), 175 (33), 161 (34), 149 (15), 134 (23).

4-(2'-Hydroxyphenyl)-(*E*)-3-buten-2-one (9)
Yellowish solid, m.p.: 136-137°C; IR (KBr) ν_{max}: 3273, 3020, 2949, 1666, 1601, 1456 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.44 (3H, s, CH_3-1), 6.98 (2H, m, H-3' & -5'), 7.07 (1H, d, *J* = 16.4 Hz, H-3), 7.26 (1H, ddd, *J* = 8.2, 7.2, 1.6 Hz, H-4'), 7.47 (1H, dd, *J* = 7.6, 1.6 Hz, H-6'), 7.89 (1H, d, *J* = 16.4 Hz, H-4), 7.99 (1H, br s, OH); EI-MS (*rel. int.*): *m/z* 162 ($[M]^+$, 36), 147 (100), 145 (33), 103 (36), 91 (53), 65 (17).

4-(3'-Hydroxyphenyl)-(*E*)-3-buten-2-one (10)
Yellow solid, m.p.: 91-92°C; IR (KBr) ν_{max}: 3304, 3020, 2829, 1666, 1606, 1580, 1454 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.40 (3H, s, CH_3-1), 6.69 (1H, d, *J* = 16.4 Hz, H-3), 6.96 (1H, ddd, *J* = 8.2, 2.6, 1.2 Hz, H-6'), 7.07 (1H, dd, *J* = 7.6, 1.2 Hz, H-4'), 7.09 (1H, d, *J* = 2.6 Hz, H-2'), 7.26 (1H, dd, *J* = 8.2, 7.6 Hz, H-5'), 7.50 (1H, d, *J* = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 162 ($[M]^+$, 55), 147 (100), 145 (29), 103 (20), 91 (52), 65 (24).

4-(4'-Hydroxyphenyl)-(*E*)-3-buten-2-one (11)
Yellow solid, m.p.: 102-103°C; IR (KBr) ν_{max}: 3277, 3022, 2818, 1665, 1593, 1514, 1441 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.40 (3H, s, CH_3-1), 6.61 (1H, d, *J* = 16.2 Hz, H-3), 6.93 (2H, d, *J* = 8.6 Hz, H-3' & -5'), 7.45 (2H, d, *J* = 8.6 Hz, H-2' & -6'), 7.53 (1H, d, *J* = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 162 ($[M]^+$, 38), 147 (100), 145 (32), 144 (24), 115 (21).

4-(2'-Methoxyphenyl)-(*E*)-3-buten-2-one (12)
Yellow solid, m.p.: 48-49°C; IR (KBr) ν_{max}: 1665, 1597, 1465 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3-1), 3.90 (3H, s, OCH_3-2'), 6.75 (1H, d, *J* = 16.5 Hz, H-3), 6.93 (1H, d, *J* = 8.3 Hz, H-3'), 6.95~7.01 (1H, m, H-5'), 7.33~7.42 (1H, m, H-4'), 7.55 (1H, dd, *J* = 7.7, 1.7 Hz, H-6'), 7.89 (1H, d, *J* = 16.5 Hz, H-4); EI-MS (*rel. int.*): *m/z* 176 ($[M]^+$, 19), 161 (46), 145 (100), 118 (20), 105 (23).

4-(3'-Methoxyphenyl)-(*E*)-3-buten-2-one (13)
Yellow syrup; IR (KBr) ν_{max}: 1670, 1605, 1579, 1496, 1456, 1433 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3-1), 3.84 (3H, s, OCH_3-3'), 6.70 (1H, d, *J* = 16.2 Hz, H-3), 6.92~6.98 (1H, m, H-4'), 7.05~7.08 (1H, m, H-2'), 7.14 (1H, d, *J* = 7.8 Hz, H-6'), 7.32 (1H, t, *J* = 7.8 Hz, H-5'), 7.48 (1H, d, *J* = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 176 ($[M]^+$, 85), 161 (100), 145 (66), 133 (40), 118 (37).

4-(4'-Methoxyphenyl)-(*E*)-3-buten-2-one (14)
Yellow solid, m.p.: 67-68°C; IR (KBr) ν_{max}: 3005, 2963, 2841, 1663, 1599, 1512 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.36 (3H, s, CH_3-1), 3.84 (3H, s, OCH_3-4'), 6.61 (1H, d, *J* = 16.4 Hz, H-3), 6.81 (2H, d, *J* = 9.0 Hz, H-3' & -5'), 7.48 (1H, d, *J* = 16.4 Hz, H-4), 7.50 (2H, d, *J* = 9.0 Hz, H-2' & -6'); EI-MS (*rel. int.*): *m/z* 176 ($[M]^+$, 5), 162(28), 161(15), 147(100), 145(22), 103(31).

4-Phenyl-(*E*)-3-buten-2-one (15)
Light yellowish solid, m.p.: 40-42°C; IR (KBr) ν_{max}: 1699, 1634, 1606, 1575, 1498, 1450 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.38 (3H, s, CH_3-1), 6.72 (1H, d, *J* = 16.3 Hz, H-3), 7.52 (1H, d, *J* = 16.3 Hz, H-4), 7.38~7.57 (1H, m, H-2', -3', -4', -5', & -6'); EI-MS (*rel. int.*): *m/z* 146 ($[M]^+$, 10), 131 (12), 102 (48).

4-(3', 4'-Methylenedioxyphenyl)-(*E*)-3-buten-2-one (16)
Yellow solid, m.p.: 103-104°C; IR (KBr) ν_{max}: 3020, 2901, 1665, 1599, 1497, 1448 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.36 (3H, s, CH_3-1), 6.02 (2H, s, $O\underline{CH_2}O$), 6.55 (1H, d, *J* = 16.2 Hz, H-3), 6.82 (1H, d, *J* = 7.6 Hz, H-5'), 7.03 (1H, dd, *J* = 7.6, 1.8 Hz, H-6'), 7.05 (1H, d, *J* = 1.8 Hz, H-2'), 7.43 (1H, d, *J* = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 190 ($[M]^+$,100), 175 (92), 145 (51), 117 (27), 89 (40), 63 (21).

4-(4'-Hydroxy-3', 5'-dimethoxyphenyl)-(*E*)-3-buten-2-one (17)
Yellow solid, m.p.: 134-135°C; IR (KBr) ν_{max}: 3518, 1666, 1645, 1597, 1515, 1466, 1425 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.36 (3H, s, CH_3-1), 3.92 (6H, s, OCH_3-3' & -5'), 5.89 (1H, br s, OH-4'), 6.59 (1H, d, *J* = 16.1 Hz, H-3), 6.79 (2H, d, *J* = 1.8 Hz, H-2' & -6'), 7.42 (1H, d, *J* = 16.1 Hz, H-4); EI-MS (*rel. int.*): *m/z* 222 ($[M]^+$, 100), 207(60), 191(29), 175(23), 147(33), 121(23).

General synthetic procedures for isopropylated (*E*)-4-phenyl-3-buten-2-ones (18-25)

To the solution of 2, 3-dihydroxybenzaldehyde (0.350 g) in anhydrous *N,N*-dimethylformamide (DMF; 20 mL), potassium carbonate (K_2CO_3; 0.350 g) was added. The reaction mixture was stirred at 65°C under argon (Ar) atmosphere. After the mixture was stirred for 10 min, 2-bromopropane (0.95 mL) was added and the reaction mixture was stirred at 65°C for 5 h. After cooling to 0°C, aqueous HCl (5%) solution was added and the resulting solid was filtered. The filtrate was extracted with EtOAc (50 mL×3), and the combined organic layer was washed by brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to yield isopropyl protected benzaldehydes, which were used to prepare **18-25** by similar procedure as described above.

4-(3'-Hydroxy-2'-isopropyloxyphenyl)-(*E*)-3-buten-2-one (18)
Yellow solid, m.p.: 92-93°C; IR (KBr) ν_{max}: 3531, 3020, 2980, 2932, 1668, 1607, 1462 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.37 (6H, d, *J* = 6.0 Hz, $OCH(\underline{CH_3})_2$), 2.40 (3H, s, CH_3-1), 4.19 (1H, septet, *J* = 6.0 Hz, $O\underline{CH}(CH_3)_2$), 5.87 (1H, br s, OH), 6.70 (1H, d, *J* = 16.6 Hz, H-3), 6.98~7.07 (2H, m, H-4' & -6'), 7.10~7.15 (1H, m, H-5'), 7.75 (1H, d, *J* = 16.6 Hz, H-4); EI-MS (*rel. int.*): *m/z* 220 ($[M]^+$, 19), 178 (44), 163 (100), 161 (40), 136 (26).

4-(2'-Hydroxy-4'-isopropyloxyphenyl)-(*E*)-3-buten-2-one (19)
Yellow solid, m.p.: 110-111°C; IR (KBr) ν_{max}: 3267, 3020, 2986, 2936, 1666, 1605, 1512, 1435 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.31 (6H, d, *J* = 6.0 Hz, $OCH(\underline{CH_3})_2$), 2.41 (3H, s, CH_3-1), 4.55 (1H, septet, *J* = 6.0 Hz, $O\underline{CH}(CH_3)_2$),

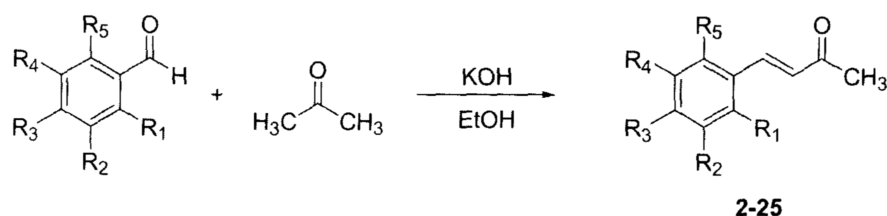

Fig. 2. General equation for the formation of compounds **2-25**

6.44 (1H, dd, J = 8.6, 2.6 Hz, H-5'), 6.51 (1H, d, J = 2.6 Hz, H-3'), 7.00 (1H, d, J = 16.4 Hz, H-3), 7.36 (1H, d, J = 8.6 Hz, H-6'), 7.81 (1H, d, J = 16.4 Hz, H-4), 8.60 (1H, br s, OH); EI-MS (*rel. int.*): *m/z* 220 ($[M]^+$, 22), 202 (22), 163 (100), 161 (37), 160 (75).

4-(5'-Hydroxy-2'-isopropyloxyphenyl)-(*E*)-3-buten-2-one (20)
Yellow solid, m.p.: 145-146°C; IR (KBr) ν_{max}: 3331, 3016, 2984, 2934, 1663, 1605, 1493, 1450 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.35 (6H, d, J = 6.2 Hz, $OCH(\underline{CH_3})_2$), 2.40 (3H, s, CH_3-1), 4.46 (1H, septet, J = 6.2 Hz, $O\underline{CH}(CH_3)_2$), 6.67 (1H, d, J = 16.6 Hz, H-3), 6.83 (1H, d, J = 8.8 Hz, H-3'), 6.89 (1H, dd, J = 8.8, 2.6 Hz, H-4'), 7.07 (1H, d, J = 2.6 Hz, H-6'), 7.89 (1H, d, J = 16.6 Hz, H-4); EI-MS (*rel. int.*): *m/z* 220 ($[M]^+$, 31), 178 (27), 163 (100), 161 (47), 136 (37), 135 (21).

4-(3'-Hydroxy-4'-isopropyloxyphenyl)-(*E*)-3-buten-2-one (21)
Yellow solid, m.p.: 81-82°C; IR (KBr) ν_{max}: 3537, 3014, 2986, 2936, 1665, 1605, 1506 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.39 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$), 2.36 (3H, s, CH_3-1), 4.66 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$), 5.83 (1H, br s, OH), 6.58 (1H, d, J = 16.2 Hz, H-3), 6.85 (1H, d, J = 8.4 Hz, H-5'), 7.03 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 7.16 (1H, d, J = 2.0 Hz, H-2'), 7.43 (1H, d, J = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 220 ($[M]^+$, 39), 177 (38), 163 (100), 161 (17), 136 (32).

4-(2', 3'-Diisopropyloxyphenyl)-(*E*)-3-buten-2-one (22)
Yellow syrup; IR (KBr) ν_{max}: 2980, 2932, 2874, 1666, 1607, 1574, 1462, 1377, 1261 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 6.2 Hz, $OCH(\underline{CH_3})_2$-3'), 1.36 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$-2'), 2.39 (3H, s, CH_3-1), 4.52 (1H, septet, J = 6.2 Hz, $O\underline{CH}(CH_3)_2$-3'), 4.56 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$-2'), 6.65 (1H, d, J = 16.6 Hz, H-3), 6.94 (1H, dd, J = 8.2, 1.8 Hz, H-4'), 7.02 (1H, dd, J = 8.2, 7.4 Hz, H-5'), 7.18 (1H, dd, J = 7.4, 1.8 Hz, H-6'), 7.97 (1H, d, J = 16.6 Hz, H-4); EI-MS (*rel. int.*): *m/z* 262 ($[M]^+$, 39), 220 (40), 178 (51), 177 (43), 163 (83), 161 (38), 136 (22), 86 (47), 84 (100).

4-(2', 4'-Diisopropyloxyphenyl)-(*E*)-3-buten-2-one (23)
Yellow syrup; IR (KBr) ν_{max}: 3022, 2982, 2934, 1659, 1597, 1497 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.35 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$-4'), 1.39 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$-2'), 2.36 (3H, s, CH_3-1), 4.57 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$-4'), 4.58 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$-2'), 6.43 (1H, dd, J = 2.4 Hz, H-3'), 6.48 (1H, dd, J = 8.6, 2.4 Hz, H-5'), 6.67 (1H, d, J = 16.4 Hz, H-3), 7.47 (1H, d, J = 8.6 Hz, H-6'), 7.82 (1H, d, J = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 262 ($[M]^+$, 30), 205 (12), 163 (100), 161 (23), 135 (10).

4-(2', 5'-Diisopropyloxyphenyl)-(*E*)-3-buten-2-one (24)
Yellow syrup; IR (KBr) ν_{max}: 3020, 2980, 2934, 1663, 1603, 1489 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 6.2 Hz, $OCH(\underline{CH_3})_2$-5'), 1.36 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$-2'), 2.38 (3H, s, CH_3-1), 4.45 (1H, septet, J = 6.2 Hz, $O\underline{CH}(CH_3)_2$-5'), 4.48 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$-2'), 6.69 (1H, d, J = 16.4 Hz, H-3), 6.88 (1H, d, J = 8.8 Hz, H-3'), 6.90 (1H, dd, J = 8.8, 2.4 Hz, H-4'), 7.08 (1H, d, J = 2.4 Hz, H-6'), 7.88 (1H, d, J = 16.4 Hz, H-4); EI-MS (*rel. int.*): *m/z* 262 ($[M]^+$, 57), 220 (34), 178 (21), 163 (100), 161 (34), 136 (22).

4-(3', 4'-Diisopropyloxyphenyl)-(*E*)-3-buten-2-one (25)
Yellow solid, m.p.: 49-50°C; IR (KBr) ν_{max}: 3022, 2982, 2934, 1665, 1599, 1504 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 1.35 (6H, d, J = 6.2 Hz, $OCH(\underline{CH_3})_2$-3'), 1.37 (6H, d, J = 6.0 Hz, $OCH(\underline{CH_3})_2$-4'), 2.37 (3H, s, CH_3-1), 4.48 (1H, septet, J = 6.2 Hz, $O\underline{CH}(CH_3)_2$-3'), 4.56 (1H, septet, J = 6.0 Hz, $O\underline{CH}(CH_3)_2$-4'), 6.58 (1H, d, J = 16.2 Hz, H-3), 6.90 (1H, d, J = 9.0 Hz, H-5'), 7.13 (1H, dd, J = 9.0, 2.0 Hz, H-6'), 7.13 (1H, d, J = 2.0 Hz, H-2'), 7.44 (1H, d, J = 16.2 Hz, H-4); EI-MS (*rel. int.*): *m/z* 262 ($[M]^+$, 42), 220 (14), 178 (76), 177 (32), 163 (100), 161 (22), 138 (90), 137 (48), 120 (7), 92 (8).

General synthetic procedures for dihydroxy-(*E*)-4-phenyl-3-buten-2-ones (26-29) (Sala and Sargent, 1979)

To the solution of monoisopropyloxy-(*E*)-4-phenyl-3-buten-2-one **18-21** (0.100 g) in anhydrous dichloromethane (CH_2Cl_2; 15 mL), BCl_3/*n*-hexane (1.0 M; 4.6 mL) was added at –10°C. The reaction was continued for 3 h under Ar atmosphere at –10°C, and was terminated by adding ice/water mixture slowly. The reaction mixture was extracted by EtOAc (50 mL×3), and the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure to yield crude product, which was then purified by silica gel column chromatography.

4-(2', 3'-Dihydroxyphenyl)-(*E*)-3-buten-2-one (26)
Yellow solid, m.p.: 170-172°C; IR (KBr) ν_{max}: 3264, 1661, 1599, 1583, 1474 cm^{-1}; ^1H-NMR (200 MHz, $CDCl_3$): δ 2.31 (3H, s, CH_3-1), 6.73 (1H, t, J = 7.9 Hz, H-5'), 6.82 (1H, d, J = 16.5 Hz, H-3), 6.91 (1H, dd, J = 7.9, 1.6 Hz, H-4'), 7.13 (1H, dd, J = 7.9, 1.6 Hz, H-6'), 7.90 (1H, d, J = 16.5 Hz, H-4), 8.38 (2H, br s, OH); EI-MS (*rel. int.*): *m/z* 178 ($[M]^+$, 10), 176 (47), 161 (100), 145 (36), 133 (21), 118 (27).

4-(2', 4'-Dihydroxyphenyl)-(*E*)-3-buten-2-one (27)
Yellow solid, m.p.: 183°C (dec.); IR (KBr) ν_{max}: 3398,

3221, 1612, 1461, 1418 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.26 (3H, s, CH_3-1), 6.43 (1H, dd, J = 8.5, 2.3 Hz, H-5'), 6.48 (1H, d, J = 2.3 Hz, H-3'), 6.69 (1H, d, J = 16.3 Hz, H-3), 7.48 (1H, d, J = 8.5 Hz, H-6'), 7.83 (1H, d, J = 16.3 Hz, H-4), 8.96 (2H, br s, OH); EI-MS (*rel. int.*): *m/z* 178 ($[M]^+$, 20), 163 (100), 161 (62), 160 (53), 147 (29), 131 (25).

4-(2', 5'-Dihydroxyphenyl)-(*E*)-3-buten-2-one (28)
Yellow solid, m.p.: 148-150°C; IR (KBr) ν_{max}: 3328, 3280, 1620, 1602 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.30 (3H, s, CH_3-1), 6.73 (1H, d, J = 16.5 Hz, H-3), 6.77~6.80 (2H, m, H-3' & -4'), 7.06 (1H, d, J = 2.3 Hz, H-6'), 7.85 (1H, d, J = 16.5 Hz, H-4), 7.93 (1H, br s, OH), 8.49 (1H, br s, OH); EI-MS (*rel. int.*): *m/z* 178 ($[M]^+$, 46), 163 (100), 161 (47), 136 (40), 107 (86).

4-(3', 4'-Dihydroxyphenyl)-(*E*)-3-buten-2-one (29)
Yellow solid, m.p.: 172-173°C; IR (KBr) ν_{max}: 3466, 1602, 1558 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.27 (3H, s, CH_3-1), 6.54 (1H, d, J = 16.2 Hz, H-3), 6.87 (1H, d, J = 8.1 Hz, H-5'), 7.06 (1H, dd, J = 8.1, 2.1 Hz, H-6'), 7.17 (1H, d, J = 2.1 Hz, H-2'), 7.48 (1H, d, J = 16.2 Hz, H-4), 8.19 (1H, br s, OH), 8.48 (1H, br s, OH); EI-MS (*rel. int.*): *m/z* 178 ($[M]^+$, 13), 163 (24), 161 (10), 85 (38).

Bioassays

Rat brain homogenate lipid peroxidation assay

The rat brain homogenate lipid peroxidation assay was based on reported methods (Ko *et al.*, 1995). Whole rat brain homogenates were freshly prepared in Krebs buffer containing 15 mM Hepes (pH 7.4), 10 mM glucose, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4 and 0.7 mM $MgCl_2$. Male Wistar rats (250-300 g) were decapitated, and the brains (minus cerebellum) were rapidly removed and homogenized in 10 vol. of ice-cold Krebs buffer using a glass Dounce homogenizer, and centrifuged at 1000×g for 10 min. Supernatant (1 mg protein/mL, 500 μL) was incubated with test agents or vehicle at 37°C for 10 min, then Fe^{2+} (200 μM) was added and incubated for another 30 min. The reaction was terminated by adding 10 μL thiobarbituric acid (TBA, 5 mg/mL in 50% w/v acetic acid), and boiled for 15 min. The sample was extracted with *n*-butanol (1 mL) and centrifuged at 10000×g for 10 min. The absorbance of the butanol phase was determined at 532 nm in a spectrophotometer (Hitachi, Model U-3200), and the amount of thiobarbituric acid reactive substance (TBARS) present was determined by linear regression analysis of a standard curve using tetramethoxypropane as a standard.

Diphenyl-picryl-hydrazyl (DPPH) assay

The DPPH assay was based on reported methods (Ko *et al.*, 1995; Mellors and Tappel, 1966). An ethanolic solution of 100 μM of the chemically stable free radical 1,1-diphenyl-2-picryl-hydrazyl was incubated with antioxidant for 30 min. Radical scavenging activities of drugs tested were measured and expressed in terms of $IC_{0.200}$, the final concentration of the drug in a reaction cuvette was supposed to decrease by 0.200 in absorption of DPPH at 517 nm.

Iron chelation study

Iron chelation was determined by adding 200 μM of $FeSO_4$ to the solutions containing 200 μM of test compounds. The UV and visible spectra (190–900 nm) were recorded 6.5 min after the addition of iron salt to the incubation mixture. Iron chelation was determined by measuring the absorbance change and/or spectral shift after subtracting the absorbance from blank (Braughler *et al.*, 1988).

Tyrosinase inhibitory activity

Antityrosinase activity was measured according to the reported method (Mason and Peterson, 1965). The test substance was dissolved in 0.5 mL 1/15 M phosphate buffer (PBS, pH 6.8) and was incubated at 25°C for 10 min. After incubation, 0.5 mL mushroom tyrosinase (135 U/mL, Sigma Chemical Co., U.S.A.) and 0.5 mL 0.03% 3,4-dihydroxyphenylalanine (L-dopa) (Sigma Chemical Co., U.S.A.) were added. The reaction mixture was incubated for 5 min. The amount of dopachrome formed was determined by measuring the optical density (OD) at 475 nm by a μ Quant universal microplate spectrophotometer, and kojic acid was used as standard agent. The percentage of inhibition of tyrosinase was calculated as follows: the activities of test substances were expressed as the 50% inhibitory concentration (IC_{50}; μM) by the method of Litchifield-Wilcoxon:

$$\% \text{ inhibition} = [(A - B) - (C - D)]/(A - B) \times 100$$

A: OD at 475 nm without test substance
B: OD at 475 nm without test substance and enzyme
C: OD at 475 nm with test substance
D: OD at 475 nm with test substance, but without enzyme

RESULTS AND DISCUSSION

In our routine screening using DPPH radical, we observed that methanol extracts of *Z. officinale* rhizomes showed significant scavenging of DPPH radical. In fractionation guided by DPPH radical scavenging activity, chloroform fraction of methanol extracts exhibited strong scavenging activity against DPPH radical. Little or no activity was present in the water fraction. However, two active compounds were isolated. Structural determination of the isolates was made by spectroscopic analyses and by direct comparison

Table I. Structures, yields and melting points (MP) of synthetic products **2-29**

Product	R_1	R_2	R_3	R_4	R_5	Yield (%)	MP (Lit. MP)
2	H	OCH_3	OH	H	H	89	124–125 (129)
3	H	OH	OCH_3	H	H	81'	85–86 (89–91)
4	OCH_3	H	OH	H	H	75	170–172 (165–167)
5	OH	OCH_3	H	H	H	45	78–80 (83)
6	OH	H	OCH_3	H	H	46	128–129 (131)
7	OH	H	H	OCH_3	H	48	125–126 (124)
8	OH	H	H	H	OCH_3	58	148–150 (145–147)
9	OH	H	H	H	H	93	136–137 (137–138)
10	H	OH	H	H	H	73	91–92 (97–98)
11	H	H	OH	H	H	65	102–103 (111–112)
12	OCH_3	H	H	H	H	84	48–49 (44–46)
13	H	OCH_3	H	H	H	79	–
14	H	H	OCH_3	H	H	95	67–68 (72–74)
15	H	H	H	H	H	43	38–40 (40–42)
16	H	OCH_2O		H	H	88	103–104 (107–108)
17	H	OCH_3	OH	OCH_3	H	62	134 (137–138)
18	O^iPr	OH	H	H	H	84	92–93
19	OH	H	O^iPr	H	H	72	110–111
20	O^iPr	H	H	OH	H	91	145–146
21	H	OH	O^iPr	H	H	91	81–82
22	O^iPr	O^iPr	H	H	H	90	–
23	O^iPr	H	O^iPr	H	H	87	–
24	O^iPr	H	H	O^iPr	H	82	–
25	H	O^iPr	O^iPr	H	H	91	49–50
26	OH	OH	H	H	H	52	170–172
27	OH	H	OH	H	H	72	183(decomp.)
28	OH	H	H	OH	H	77	148–150 (151)
29	H	OH	OH	H	H	88	172–173 (176)

with the authentic reference compounds.

Compound **1** was obtained as colorless powder and the UV maxima at 289 (sh), 281, and 227 nm was typical of a benzenoid derivative (Scott, 1964). The IR absorption bands at 1715 cm^{-1} showed the presence of carbonyl group. The ^1H-NMR spectra of **1** displayed a set of ABX signals at δ 6.82 (1H, d, J = 8.0 Hz, H-5'), 6.69 (1H, d, J = 1.2 Hz, H-2') and 6.66 (1H, dd, J = 8.0, 1.2 Hz, H-6') indicating the presence of trisubstituted benzene ring. Triplets at δ 2.82 (2H, t, J = 6.9 Hz, H-4) and 2.73 (2H, t, J = 6.9 Hz, H-3) corresponded to two mutually coupled methylene groups, and a methyl at δ 2.13 and a methoxy signal at δ 3.87 were also observed. Comparison of these data with that of reported (Connell and Sutherland, 1969) values permitted the identification of **1** as zingerone. Dehydrozingerone (**2**), obtained as colorless powder, was determined to be $C_{11}H_{12}O_3$ *from* its elemental analysis. The UV absorption maxima at 337, 303 (sh), 242, and 225 nm were typical of a benzenoid derivative (Scott, 1964). The IR absorption band at 1665 cm^{-1} was consistent with the presence of a conjugated carbonyl group. The ^1H-NMR of **2** exhibited similar signals with those of **1**, except for the presence of signals for *trans* coupled double bond at δ 7.46 and 6.59 (each 1H, J = 16.4 Hz) instead of two mutually coupled triplets. These data allowed the identification of **2** as dehydrozingerone (De Bernardi *et al.*, 1976).

Investigation of potent antioxidant activity with dehydrozingerone in our DPPH and rat-brain homogenate lipid peroxidation inhibition assays led us to synthesize the structurally similar (*E*)-4-phenyl-3-buten-2-ones by aldol condensation. Besides, the dihydroxy-(*E*)-4-phenyl-3-buten-2-ones (**26-29**), all the aldol products were one-step yielded from the corresponding benzaldehydes as per the Whiting's method. Because of the high reactivity of dihydroxybenzaldehydes, compounds **26-29** were obtained

Table II. Antioxidant activity of compounds **2-29**

compounds	DPPH assay		Rat brain assay	
	$IC_{0.200}$ (μM)	ΔA_{517} of 100 μM	IC_{50} (μM)	Inhibition % of 100 μM
2	12.8			31.7±2.1
3		0.04±0.001		20.9±1.9
4		0.00±0.020		19.1±0.8
5		0.13±0.001		35.3±3.3
6		0.01±0.001		29.2±3.8
7	8.7		60.2±5.8	
8		0.00±0.001		16.9±3.1
9		0.03±0.003		12.2±1.7
10		0.02±0.003		5.7±3.2
11		0.02±0.002		13.7±2.6
12		0.02±0.002		0.9±2.0
13		0.02±0.001		4.2±2.5
14		0.02±0.004		4.6±2.5
15		0.02±0.001		1.8±1.2
16		0.02±0.001		4.3±2.9
17	12.4		58.5±3.2	
18		0.04±0.003		21.2±2.0
19		0.03±0.001		45.1±3.2
20		0.08±0.020	70.2±3.1	
21		0.02±0.020		29.3±1.9
22		0.02±0.010		14.1±3.2
23		0.02±0.002		21.2±2.0
24		0.00±0.001		13.3±3.5
25		0.00±0.003		13.8±2.9
26	3.2		6.3±0.4	
27		0.07±0.004		29.1±1.2
28	4.9		41.8±6.3	
29	7.9		12.0±0.8	
Vitamine C	23.7			
BHT (30μM)		0.12±0.002	1.3±0.6	
Vitamine E	8.3		2.5	

after protection and deprotection of the hydroxyl groups with isopropyl groups. All the products were purified prior to analysis and evaluated by chromatographic methods. All these purified compounds were identified by ^1H- and ^{13}C-NMR, IR, elemental and mass spectral analyses. All the signals in ^1H- and ^{13}C-NMR spectra corresponded to the proposed structures. In all the cases, the double bond geometry of the phenyl-butene-2-one moieties was *E*, because the coupling constants (^3J) between α and β protons were 15-16 Hz. For all the new compounds, satisfactory HRMS or elemental analysis was measured. Structures of these synthetic products are listed in Table I. To determine the radical scavenging and antioxidant action of the synthetic analogues **2-29**, we used two *in vitro* assay systems, including rat brain homogenate lipid peroxidation inhibition and α,α-diphenyl-β-picrylhydrazyl free radical (DPPH) scavenging assays and the obtained results are summarized in Table II. Brain homogenate is useful for investigation of lipid peroxidation and has also been employed to elucidate antioxidant activity (Braughler *et al.*, 1987). Lipid peroxidation in rat brain homogenate was stimulated by the addition of Fe^{2+} through various mechanisms, e.g., the decomposition of lipid peroxides, the generation of hydroxyl radical or by forming perferryl or ferryl species (Gutteridge *et al.*, 1979; Braughler *et al.*, 1987). Compound **26** showed inhibitory activity (IC_{50} = 6.3±0.4 μM) comparable to the standard antioxidant, α-tocopherol (IC_{50} = 2.5±0.1 μM). Compounds **7**, **17**, **28**, and **29** showed only moderate activity in the lipid peroxide inhibition assay. The tested compounds **2**, **7**, **17**, **26**, **28**, and **29** did not form complex with ferrous ion in the iron chelation study since addition of ferrous ion did not cause any spectral shift or absorbance change. Thus, the test compounds may exert their effects on lipid peroxidation primarily by scavenging free radicals rather than functioning as iron chelators. This notation was further supported by the DPPH test that provided information regarding the reactivities of the tested compounds with a stable free radical. In this test, free radical scavenging activity was expressed by $IC_{0.200}$. Compounds **26** ($IC_{0.200}$ = 3.2 μM) and **28** ($IC_{0.200}$ = 4.9 μM) were about two and five times more reactive than α-tocopherol ($IC_{0.200}$ = 8.3 μM) and ascorbic acid ($IC_{0.200}$ = 23.7 μM), respectively in scavenging DPPH radical. Activity of compounds **2**, **7**, **17**, and **29** was comparable to that of α-tocopherol but was about two times more active than ascorbic acid. It is plausible that the presence of *o*-dihydroxy and/or *p*-dihydroxy groups in the test compounds could be the putative radical target sites (Kitagawa *et al.*, 1992; Terao *et al.*, 1993; Laranjinha *et al.*, 1994). The antioxidant effect of these compounds may also be due to their α and β unsaturated side chain and the vanillyl based structures which inherits from the antioxidant potency (Huang *et al.*, 2001).

Due to the structural similarity between the synthetic dehydrozingerone analogues and L-DOPA, and their antioxidant potentials, the antityrosinase activity of all the synthetic compounds was tested. In addition, a commercial skin whitening agent, kojic acid, was also examined for the antityrosinase activity for the sake of comparison. Compound **27** exhibited profound inhibitory activity (IC_{50} = 27 μM) than kojic acid (IC_{50} = 154 μM). The activity of tyrosinase was analyzed in the presence of compounds **2**, **7**, **17**, **26**, **27**, **28**, and **29** without L-DOPA to determine the mode of inhibition. The curves obtained for **2**, **17**, and **26** resembled with those obtained for resveratrol (Bernard and Berthon, 2000). This indicates that compounds **2**, **17**,

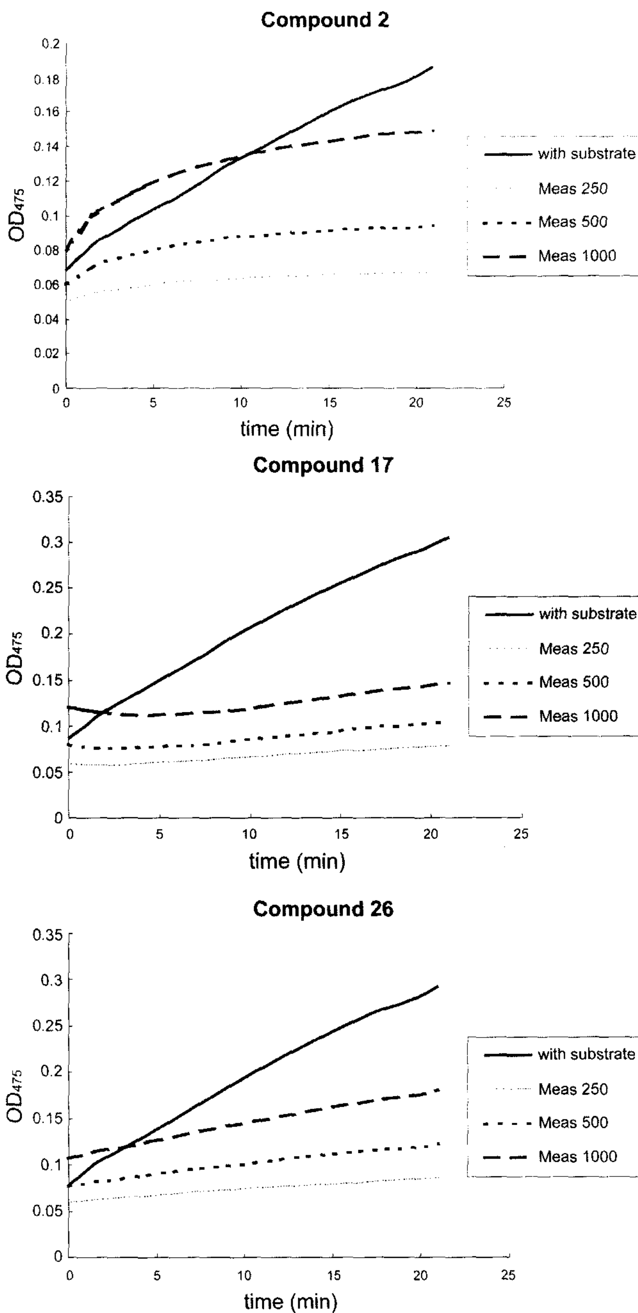

Fig. 3. Tyrosinase activity of **2**, **17**, and **26** (without preincubation time and L-dopa) represented by OD_{475} versus time. The solid line corresponds to tyrosinase activity with 250 μM tested compounds and L-dopa, whereas Meas 250, 500, and 1000 are without substrates (L-dopa) and correspond to concentrations of tested compounds at 250, 500, and 1000 μM, respectively.

and **26** may be metabolized by tyrosinase like resveratrol, as the spectrophotometric tests record the formation of quinone derivatives. In Fig. 3, increase in OD indicates that quinone derivatives were formed from **2**, **17**, and **26** and thus they probably act as substrates for tyrosinase. Furthermore, the inhibition kinetics of active tyrosinase

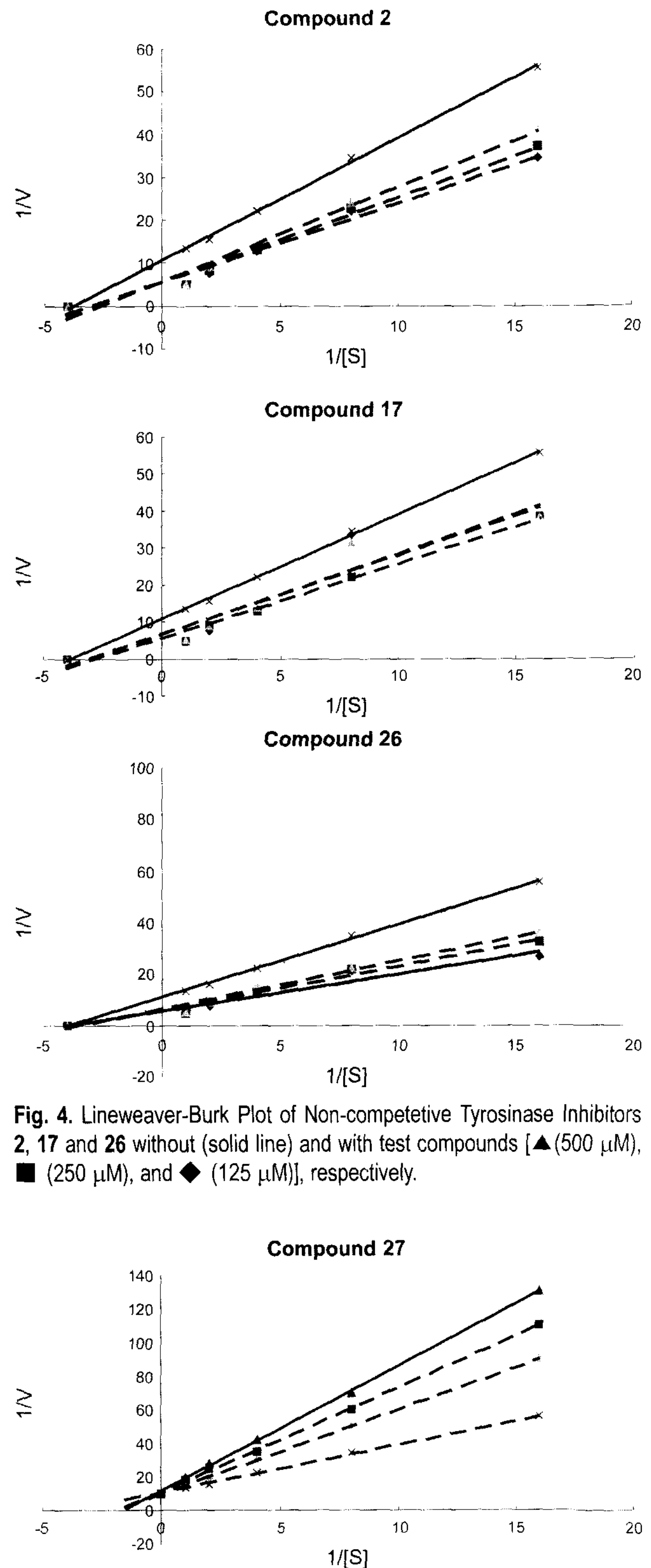

Fig. 4. Lineweaver-Burk Plot of Non-competetive Tyrosinase Inhibitors **2**, **17** and **26** without (solid line) and with test compounds [▲(500 μM), ■ (250 μM), and ◆ (125 μM)], respectively.

Fig. 5. Lineweaver-Burk Plot of Competetive Tyrosinase Inhibitor **27** without (solid line) and with tested compound [▲(500 μM), ■ (250 μM), and ◆ (125 μM)], respectively.

inhibitors was analyzed by Lineweaver-Burk plots. The slopes, obtained from the uninhibited enzyme and from the three different concentrations of compound **27** intercepted on the original axis. It was evident that compound **27** was a direct inhibitor of mushroom tyrosinase, since on pre-incubation with the enzyme in the absence of the substrate, it siginificantly decreased the enzyme activity. The results indicated that compound **27** was a characteristic competitive inhibitor. However, for compounds **2**, **17**, and **26**, the four lines obtained from the uninhibited enzyme and from the three different concentrations of 250, 500, and 1000 μM, intersected on the horizontal axis. This result indicates that these compounds exhibited a noncompetitive inhibition for L-DOPA oxidation by mushroom tyrosinase. On the other hand, compounds **7**, **28**, and **29** were neither competitive nor noncompetitive inhibitors.

Antioxidant activity assays of synthetic analogues with diverse substituents inferred that the presence of hydroxyl substituents on the phenyl nucleus enhanced activity, whereas substitution by alkoxyl groups like methoxyl and isopropyloxyl groups decreased antioxidant activity remarkably. Substitution patterns on the phenyl ring especially affected antioxidant potency of the 4-phenyl-3-buten-2-ones. The dihydroxyl substitution at 2' and 3' of the phenyl ring was particularly important to the radical scavenging activity than at 2' and 5', and 3' and 4'. This study also revealed that a double bond at the C-3 and C-4 position would be essential for antioxidant activity. Experimental results indicated that compound **26** may be a promising antioxidant in light of fact that antioxidant therapy apparently offers protection against a wide range of free radical-induced diseases. However, further investigation of its *in vivo* antioxidant activity is warranted. The remarkable capability of tyrosinase inhibition by compound **27** is another potentially important finding for skincare product research.

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