1 Introduction

As a consequence of air pollution, the ozone layer is being destroyed progressively, and the intensity of ultraviolet radiation to the surface of the earth is increasing accordingly (1). Due to this increase in intensity of ultraviolet radiation, skin pigmentation such as stains and freckles and cutaneous disorders such as wrinkles and laxity are now common in the population.

To cope with these troubles, cosmetic manufacturers have recently developed new effective compounds for use in skin-whitening cosmetics, which are now available in the market. Such compounds include arbutin, ellagic acid, kojic acid and 4-n-butyl resorcinol which are extracted from plants, and thus are gaining popularity with consumers, who show an increasing fondness for cosmetics containing substances derived from natural sources (2).

To cope with these troubles, cosmetic manufacturers have recently developed new effective compounds for use in skin-whitening cosmetics, which are now available in the market. Such compounds include arbutin, ellagic acid, kojic acid and 4-n-butyl resorcinol which are extracted from plants, and thus are gaining popularity with consumers, who show an increasing fondness for cosmetics containing substances derived from natural sources (2). We have also derived numerous chemicals having the skin whitening activity from estragole and trans-anethole. These chemicals are essential oils extracted from *Foeniculum vulgare Miller*, an umbelliferous plants that is used in Chinese medicine (3). We previously reported interesting results indicating that trans-anethole condensed with monoterpenoids have strong skin whitening effects.

The present study is a continuation of our previous investigation (4). We used estoragole (1), an essential oil of *Foeniculum vulgare Miller*, as a starting substance to synthesize new cyclic acetals by various simple reactions. We then examined the ability of these new derivatives to inhibit tyrosinase activity in vitro.

We report that some of the new derivatives inhibit the activity are of tyrosinase more potently than do arbutin, ellagic acid and kojic acid, skin whitening agents that are currently available on the market.

2 Experimental

2.1 Materials

The starting material, estragole (4-allyl anisole) (1) was obtained from Kanto Chemical Co., Inc. (1): bp.215 – 216°C, d_20^0 = 0.965, n_20^0 = 1.520

The tyrosinase using physiological activity tests was obtained from Sigma Chemical Co. (EC 1.14.18.1). The

---

* Correspondence to: Masato NOMURA, 1, Umenobe, Takaya, Higashihiroshima, Hiroshima 739-2116, JAPAN
E-mail: nomura@hiro.kindai.ac.jp
tyrosine and L-DOPA (L-3-(3,4-dihydroxyphenyl)-α-alanine) were obtained from Kanto Chemical Co., Inc.
Arubutin, which was used as a comparative material was obtained from Lancaster synthesis.

2.2 Analysis
$^1$H-NMR spectra were obtained with a JEOL JNM-EX 400 instrument in CDCl$_3$ with TMS as an internal standard. MS spectra were obtained with a JEOL JMS-HX 100, and eluates from an OV-1 (1%) column were measured by the EI (Electron Impact ionization) method in the range of temperature $80 \sim 250^\circ C$ (10°C/ min).

2.3 Synthesis
2.3.1 Synthesis of alcohol with hydroboration (5)
Into a 50 ml three-neck flask fitted with a magnetic stirrer and reflux condenser were placed 0.13 g (3.4 x 10$^{-3}$ mol) of NaBH$_4$ and a solution containing 0.50 g (3.4 x 10$^{-3}$ mol) of BF$_3$-Et$_2$O was added in slowly, and the solution was stirred and refluxed for 2 h at 50°C, and the solution was stirred and refluxed for 2 h at 50°C on an oil bath. The mixture was allowed to cool to room temperature. Added 2.5 ml of water, 2.5 ml of 3M NaOH (aq.) (1 M = 1 mol dm$^{-3}$), and 2.5 ml of H$_2$O$_2$ (about 30%) were added slowly and the mixture was stirred and refluxed for 2 h at 50°C on an oil bath. The mixture was extracted with ether, washed with saturated brine, and dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent gave a crude product that was purified by silica gel column chromatography (n-hexane : ethyl acetate = 9 : 1) to yield 0.30 g (53.6%) of the 3-(4-Methoxyphenyl)-1-propanol (2).

2.3.2 Synthesis of aldehyde with PDC (pyridinium dichromate) oxidation (6)
Into a 500 ml four-neck flask fitted with a magnetic stirrer and air condenser are placed 13.6 g (3.6 x 10$^{-3}$ mol) of PDC (pyridinium dichromate), and added 350 ml of CH$_2$Cl$_2$ and 4.0 g (2.4 x 10$^{-2}$ mol) of (2). The mixture was then stirred at 0°C on an ice bath. After further stirring for 24 h at room temperature, the CH$_2$Cl$_2$ solution was evaporated and the oil was extracted with n-hexane. The organic layer was washed with water and dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent gives of crude product that was purified by silica gel column chromatography (n-hexane : ethyl acetate = 9 : 1) to yield 2.90g (73.4%) of the 3-(4-Methoxyphenyl) propanal (3).

2.3.3 Synthesis of cyclic acetal (7)
(The synthesis of (2-[2-(4-Methoxyphenylethyl)] dioxorane (3a) is described as an example.)
Into a 50 ml flask fitted with a magnetic stirrer and Dean-Sark extractor were placed 0.5 g (3.0 x 10$^{-3}$ mol) of (3), 0.4 g (6.4 x 10$^{-3}$ mol) of ethyleneglycol, 0.6 g (3.2 x 10$^{-3}$ mol) of p-TsOH, and 30 ml of dry benzene. The mixture was stirred and refluxed for 24 h at 120 ~ 130°C on oil bath. The benzene solution was evaporated, and the oil was extracted with ether. The organic layer was washed with saturated brine and dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent gave a crude product that is purified by silica gel column chromatography (n-hexane : ethyl acetate = 9 : 1) to yield 0.46 g (72.5 %) of the (2-[2-(4-Methoxyphenylethyl)] dioxorane (3a). The above synthesis was also carried out using propyleneglycol, ethylenedithiol and propylenedithiol in the initial condensation step to yield (3b) (58.6 % yield), (3c) (64.4 % yield) and (3d) (62.6 % yield), Respectively.

(3a) $^1$H-NMR δ CDCl$_3$ (ppm); 1.92 (2H, m, -CH$_2$CH$_2$CH$_2$-), 2.67 (2H, m, -CH$_2$CH$_2$-), 3.70 (3H, s, -OCH$_3$), 3.85 (each 2H, m, -OCH$_2$), 6.92 (4H, m, φ).

(3b) $^1$H-NMR δ CDCl$_3$ (ppm); 1.76 ~ 1.82 (2H, m, -CH$_2$CH$_2$CH$_2$-), 1.87 ~ 2.02 (2H, -OCH$_2$CH$_2$CH$_2$O-), 2.61 (2H, t, J = 8.0 Hz, -CH$_2$CH$_2$CH$_2$-), 3.63 ~ 3.66, 3.97 ~ 4.02 (each 2H, m, -OCH$_2$CH$_2$CH$_2$O-), 3.71 (3H, s, -OCH$_3$), 4.46 (1H, t, J = 5.6 Hz, -CH$_2$CH$_2$-), 6.79 ~ 7.05 (4H, m, φ).

(3c) $^1$H-NMR δ CDCl$_3$ (ppm); 2.04 (2H, q, J = 7.2 Hz, -CH$_2$CH$_2$CH$_2$-), 2.67 (2H, m, -CH$_2$CH$_2$CH$_2$-), 3.13, 3.19 (each 2H, m, -SCH$_2$CH$_2$S-), 3.70 (3H, s, -OCH$_3$), 4.39 (1H, t, J = 7.2 Hz, -CH$_2$CH$_2$-), 6.95
Skin Whitening Effects of Estragole Derivatives

(4H, m, ϕ).

MS m/z (rel.int%): 240 (M⁺, 100), 147 (62), 121 (84), 105 (68), 91 (11), 77 (10).

(3d) ¹H - NMR δ CDCl₃ (ppm): 1.97 (2H, m, -CH₂CH₂-), 2.04 (2H, m, -SCH₂CH₂CH₂S-), 1.97 (2H, m, -CH₂CH₂CH₂CH₃), 2.74 (2H, J = 8.0 Hz, -CH₂CH₂), 2.78, 2.83 (each 2H, m, -SCH₂CH₂CH₂S-), 3.72 (3H, s, -OCH₃), 4.00 (1H, t, J = 7.2 Hz, -CH₂CH₂), 6.97 (4H, m, ϕ).

MS m/z (rel.int%): 254 (M⁺, 100), 147 (85), 121 (80), 91 (8), 45 (5).

3 Physiological Activity Tests

3.1 Tyrosinase Activity Inhibition Test

Using Tyrosine as a Substrate

A solution of individual compounds that was used at a concentration of 0.08 mmol and tested for their ability to inhibit tyrosinase activity as described previously (8) using tyrosinase (Sigma Chemical Co., EC 1.14.18.1) as a substrate. Readings were taken at 475 nm with a spectrophotometer.

3.2 Tyrosinase Activity Inhibition Test

Using DOPA as a Substrate

A solution of 3,4-dihydroxy-L-phenylalanine (DOPA, Wako Pure Chemical Industries Ltd.) was prepared at the concentration determined previously (1.66 mmol), and the synthesized compounds were tested for their ability to inhibit tyrosinase activity as described previously ((8), section 2.4.1) using L-DOPA as a substrate.

3.3 Reactive Oxygen-Species Scavenging Effect Test

A solution of individual compounds were prepared at the concentration determined previously (0.714 mmol) and tested for their reactive oxygen-species scavenging ability as described previously (8) using the superoxide dismutase (SOD) test and Wako reagent (Wako Pure Chemical Industries Ltd.). The concentration of diformazan generated by the compounds was measured at 560 nm with a spectrophotometer.

4 Results and Discussion

Chemicals such as arbutin, ellagic acid, kojic acid and/or 4-n-butylresorcinol are contained in cosmetics used for skin whitening effect (9, 10). In addition, many chemicals containing sulfur atoms, such as cystine and glutathione, are sold on the market as skin whitening agents (11). Therefore, we have used these chemicals (4) as a substrate compound to synthesize derivatives containing sulfur atoms by the method shown in Fig. 1, because compounds containing sulfur atoms were expected to have higher inhibitory activity against tyrosinase. The cyclic acetals obtained were tested individually for their skin whitening properties. When tyrosine was used as a substrate in the tyrosinase activity inhibition test, the substrate compound (1) itself had a relatively high inhibitory activity at 0.1 mM as shown in Table 1. Except for compound (3d) derivatives produced by introducing a cyclic acetal radical had lower activity than the substrate compound (1), as shown in Table 1. However, they were more potent than arbutin.

Fig. 1 Synthetic Pathway of (3a) ~ (3d).
Table 1  Inhibition of Tyrosinase and Superoxide Dismutase for (1) ~ (3d).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tyrosinase</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyrosine</td>
<td>L - DOPA</td>
</tr>
<tr>
<td>(1)</td>
<td>34.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.6</td>
</tr>
<tr>
<td>(3)</td>
<td>5.3</td>
<td>14.2</td>
</tr>
<tr>
<td>(3a)</td>
<td>7.7</td>
<td>1.0</td>
</tr>
<tr>
<td>(3b)</td>
<td>8.6</td>
<td>22.9</td>
</tr>
<tr>
<td>(3c)</td>
<td>16.2</td>
<td>23.8</td>
</tr>
<tr>
<td>(3d)</td>
<td>34.7</td>
<td>83.0</td>
</tr>
<tr>
<td>Arbutin</td>
<td>7.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Ellagic Acid</td>
<td>-13.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>91.2</td>
<td>82.3</td>
</tr>
</tbody>
</table>

* a) Concentration; 0.1mM  
* b) Inhibitory rate (%)  
* c) Superoxide Dismutase

and ellagic acid, which were tested as control substances. Notably, the inhibitory activity of compound (3d) was four times higher than that of arbutin. When L-DOPA was used as a substrate in the tyrosinase activity inhibition test, compounds (3b), (3c) and (3d) were found to have high tyrosinase inhibitory activity. Notably, compound (3d) was more than five times more active than arbutin. Kojic acid was the most active of all, followed by compound (3d) and then by arbutin.

The above results indicate that compound (3c) is more active than compound (3a) and that compound (3d) is more active than compound (3b), suggesting that sulfur atoms contribute to an increase in the potency of the compounds to inhibit tyrosinase activity. With regard to the action of sulfur atoms, Prota et al. (12, 13) found that SH compounds do not inhibit the activity of tyrosinase by acting at its catalytic site, but rather they react with dopaquinone produced by melanocytes to form complexes such as cystinylldopa (when, for example, the SH compound is cystine), thereby producing pheomelanin. It is conceivable that the compounds synthesized in this study, conceivably exert their inhibitory action by a similar mechanism. Alternatively, the split of the C-S bond under acidic conditions may result in the production of SH compounds, which subsequently form complexes with dopaquinone and thereby lead to the production of pheomelanin.

References