NOTE

Cosmetic Substance Synthesis from Estragole Derivatives for Skin Depigmentation

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Edited by Y. Hirose, Yamanashi Univ., and accepted September 3, 2001 (received for review January 19, 2001)

Abstract: Natural material extracts are presently used as cosmetic ingredients. Their physiological effects were examined using estragole isolated from the essential oil of *Foeniculum vulgare* Miller as starting materials. Thirty derivatives were synthesized by condensation using ten thiols. The derivatives were examined for potential use as whitening agents possessing tyrosinase activity and superoxide dismutase. Derivative compounds were found good inhibitors of tyrosinase.

J. Oleo Sci., **51**, 57-62 (2002).

Key words: *Foeniculum vulgare* Miller, estragole, whitening effect, tyrosinase, primary irritation test

1 Introduction

In recent years, it is feared that the stratospheric ozone layer depletion by chlorofluorocarbons released into the atmosphere. They are only released into the atmosphere might adversely affect animals, humans and plants. It is said that a 1% depletion of the ozone layer causes about a 2% increase in the ultraviolet rays of 280 to 320 nm in wavelength (*i.e.*, so-called UV-B rays harmful to living organisms). Since many biological substances such as genes and plant hormones have absorption peaks in the UV region, effects of the increase in UV-B rays on ecological systems have attracted public attention as a serious problem (1). As one of these effects, tyrosinase in the skin is activated and thus causes melanogenesis, thereby inducing pigmentation. It has been found that this phenomenon can be prevented by plant components such as arbutin, ellagic acid and resorcinol (2). On the other hand, we paid

our attention to the pharmacological activities of anethole and estragole, which are essential oil components of *Foeniculum vulgare* Miller, a Chinese herbal drug, and found out that derivatives of these substances obtained via various reactions show remarkable effects of inhibiting tyrosinase activity (3). These results have been already reported. In this study, which relates to the above findings, we attempted to synthesize a compound having a high whitening effect by preparing estragole derivatives having an S atom in the side chain. The compound thus obtained was subjected to a tyrosinase activity inhibition test and those showing high inhibitory activities were further subjected to a skin primary irritation test. As a result, a favorable compound could be obtained, as will be reported hereinafter. For comparison, *p*-anisic acid and 4-methoxyphenylacetic acid, each having a similar mother nuclear structure, were subjected to the same reactions as in the case of estragole. The compounds thus obtained were examined for whitening effects and thus the difference in the effects was clarified.

2 Experimental

2.1 Materials

The starting material, estragole (**1**) was obtained from KANTO CHEMICAL CO., INC..

(**1**): bp 215–216°C, $d_4^{20} = 0.9658$.

2.2 Analysis

The structures of the compounds were determined by IR spectra (JASCO IR-810), ^1H -NMR spectra (JEOL JNM-EX400) and GC-MS data (JEOL JMS-HX100).

2.3 Synthesis

2.3.1 3-(4-Methoxyphenyl)-1-propanal (**2**) (**4**)

Added 0.13 g (3.4×10^{-3} mol) of NaBH_4 and 5 mL of THF to a 50 mL three-neck flask and refluxed. A solution 0.50 g (3.4×10^{-3} mol) of (**1**) dissolved in 2 mL of distilled THF was added, $\text{BF}_3 \cdot \text{Et}_2\text{O}$ 0.68 g (4.8×10^{-3} mol) was dropped slowly and stirred for 2 h at 50°C on an oil bath. Added 2.5 mL water, 2.5 mL 3M- $\text{NaOH}(\text{aq.})$ ($1\text{M} = 1\text{mol dm}^{-3}$), and 2.5 mL H_2O_2 slowly and stirred for 12 h. The mixture was extracted with ether, washed with saturated $\text{NaCl}(\text{aq.})$, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 6 : 4) to give (**2**) (0.30 g, 53.6% yield).

(**2**): IR ν max (cm^{-1}); 3400(-OH), 2950(- OCH_3), 1510, 1610(ϕ). ^1H -NMR δ CDCl_3 (ppm); 1.80 (2H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 2.59 (2H, t ($J=7.2$ Hz), $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 3.58 (2H, t ($J=7.2$ Hz), $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 3.70 (3H, s, $-\text{OCH}_3$), 6.77~7.07 (4H, m, ϕ).

2.3.2 3-(4-Methoxyphenyl) propanal (**3**)

Added pyridinium dichromate 13.6g (3.6×10^{-2} mol) to a 500 mL four-neck flask and added CH_2Cl_2 and 4.0 g (2.4×10^{-2} mol) of (**2**) then stirred for 1 h at 0°C on an ice bath. Stirred for 24 h at room temperature. Removed chrome by silica gel column chromatography (ethyl acetate only) and evaporated. The residue was acidified by 10% $\text{HCl}(\text{aq.})$, washed with saturated $\text{NaCl}(\text{aq.})$, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography using silica gel (hexane : ethyl acetate = 9 : 1) to give (**3**) (2.9g, 72.5% yield).

(**3**): IR ν max (cm^{-1}); 2950(- OCH_3), 1730(-CHO), 1510, 1610(ϕ). ^1H -NMR δ CDCl_3 (ppm); 2.84 (2H, t ($J=7.2$ Hz), $-\text{CH}_2\text{CH}_2\text{CHO}$), 3.01 (2H, t ($J=7.2$ Hz), $-\text{CH}_2\text{CH}_2\text{CHO}$), 3.88 (3H, s, $-\text{OCH}_3$), 6.95~7.24 (4H, m, ϕ), 9.89 (1H, s, -CHO).

2.3.3 3-(4-Methoxyphenyl) propionirile (**4**)

Added (**3**) (1.8×10^{-2} mol), *N,N*-dimethylhydrazine 1.6g (2.7×10^{-2} mol) and dry benzene into a 300 mL four-neck flask and refluxed for 3 h at 75~85°C. Then azeotroped water and benzene at 120°C and stirred for 3 h at 0°C. Added CH_3I (4.4 g, 3.1×10^{-2} mol) was added then stirred for 1 h at the same temperature and refluxed for 4 h at 70~80°C. Filtrated hydrazinium salt with ether was added to 1M- KOH - $\text{MeOH}(\text{aq.})$, then refluxed for 10 h at 60~70°C. The mixture was extracted with ether, washed with saturated $\text{NaCl}(\text{aq.})$, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 9 : 1) to give (**4**) (1.5g, 50.0% yield).

(**4**): IR ν max (cm^{-1}); 2225(-CN), 2950(- OCH_3), 1510, 1610(ϕ). ^1H -NMR δ CDCl_3 (ppm); 2.54 (2H, t ($J=7.3$ Hz), $-\text{CH}_2\text{CH}_2\text{CN}$), 2.86 (2H, t ($J=7.3$ Hz), $-\text{CH}_2\text{CH}_2\text{CN}$), 3.76 (3H, s, $-\text{OCH}_3$), 6.83~7.14 (4H, m, ϕ).

2.3.4 3-(4-Methoxyphenyl) propanoic acid (**5C**)

Added (**4**) (9.3×10^{-3} mol) and 1M- KOH - $\text{MeOH}(\text{aq.})$ to a 300 mL four-neck flask and refluxed for 48 h at 60~70°C. Evaporated MeOH and the residue were extracted with saturated $\text{NaCl}(\text{aq.})$, dried over anhydrous Na_2SO_4 , and evaporated. The residue was chromatographed on silica gel (hexane-ethyl acetate = 3 : 7) to give (**5C**) (1.1g, 68.8% yield).

(**5C**): IR ν max (cm^{-1}); 2850(- OCH_3), 1700(-COOH), 1420, 1620 (ϕ). ^1H -NMR δ CD_3OD (ppm); 2.06 (2H, t ($J=7.6$ Hz), $-\text{CH}_2\text{CH}_2\text{COOH}$), 2.57 (2H, t ($J=7.2$ Hz), $-\text{CH}_2\text{CH}_2\text{COOH}$), 3.75 (3H, s, $-\text{OCH}_3$), 6.74~7.09 (4H, m, ϕ).

2.3.5 Condensation reaction (**5A-a**) ~ (**5C-j**)

Dissolved (**5C**) (1.0 g, 6.4×10^{-3} mol) in ethanethiol 0.4g (6.4×10^{-3} mol), diethyl cyanophosphonate (0.9 g, 5.5×10^{-3} mol) and Et_3N (0.6 g, 5.9×10^{-3} mol) under a nitrogen atmosphere at 0°C, and then stirred for 24 h at room temperature. The residue was extracted with ether, washed with saturated $\text{NaCl}(\text{aq.})$, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (hexane-ether = 9 : 1), then deodorized by activated carbon to give (**5C-a**) (0.9g, 72.0% yield). By using the same method, condensed (**5A-a**) ~ (**5C-j**) (0.4~1.4 g, 40.6~96.2% yield). Physical constants and yields are shown in **Table 1**.

(**5C-a**): ^1H -NMR δ CDCl_3 (ppm); 1.19 (3H, t ($J=7.6$ Hz), $-\text{CH}_2\text{CH}_3$), 2.76 (2H, m, ϕ - CH_2CH_2 -), 2.82 (2H, q ($J=7.6$ Hz), $-\text{CH}_2\text{CH}_3$), 2.87 (2H,

m, ϕ -CH₂CH₂-), 3.69 (3H, s, -OCH₃), 6.76~7.06 (4H, m, ϕ).

MS m/z (rel.int %); 224 (M⁺, 40), 134 (45), 121 (100), 105 (4), 91 (8), 77 (6).

(5C-b): ¹H-NMR δ CDCl₃ (ppm); 0.89 (3H, t (J=7.2 Hz), -CH₂CH₃), 1.52 (2H, sex (J=7.2 Hz), -CH₂CH₃), 2.74 (2H, m, ϕ -CH₂CH₂-), 2.78 (2H, t (J=7.2 Hz), -CH₂CH₂CH₃), 2.85 (2H, m, ϕ -CH₂CH₂-), 3.65 (3H, s, -OCH₃), 6.73~7.04 (4H, m, ϕ).

MS m/z (rel.int %); 238 (M⁺, 35), 134 (51), 121 (100), 105 (3), 91 (7), 77 (5).

(5C-c): ¹H-NMR δ CDCl₃ (ppm); 0.88 (3H, t (J=7.2 Hz), -CH₂CH₃), 1.32 (2H, sex (J=7.2 Hz), -CH₂CH₃), 1.49 (2H, qui (J=7.2 Hz), -SCH₂CH₂-), 2.76 (2H, m, ϕ -CH₂CH₂-), 2.83 (2H, t (J=7.2 Hz), -SCH₂-), 2.87 (2H, m, ϕ -CH₂CH₂-), 3.68 (3H, s, -OCH₃), 6.75~7.06 (4H, m, ϕ).

MS m/z (rel.int%); 252 (M⁺, 32), 134 (57), 121 (100), 105 (3), 91 (6), 77 (5).

(5C-d): ¹H-NMR δ CDCl₃ (ppm); 2.73 (2H, m, ϕ -CH₂CH₂-), 2.87 (2H, m, ϕ -CH₂CH₂-), 2.96 (1H, sep (J=7.2 Hz), -SCH-), 3.21 (3H $\times$ 2, d (J=7.2 Hz), gem-CH₃), 3.69 (3H, s, -OCH₃), 6.76~7.06 (4H, m, ϕ).

MS m/z (rel.int %); 238 (M⁺, 25), 134 (58), 121 (100), 105 (3), 91 (6), 77 (5).

2.4 Assay

2.4.1 in vitro (5)

Inhibition of tyrosinase activity. Tyrosinase activity was determined by the dopachrome method with L-tyrosine or L-DOPA as substrate.

a) With the L-tyrosine substrate: The reaction mixture (2 mL) which contained 1.66 mM L-tyrosine, 10.0 mM phosphate buffer (pH 6.8), 30 units of mushroom tyrosinase [Sigma Chemical Co. (EC 1.14.18.1)] and the sample (a 1.66 mM DMSO solution; 1.0 mL) was incubated at 37°C for 10 min. Absorbance was then measured at 475 nm. A control reaction was carried out without the sample.

Percent inhibition of tyrosinase was calculated as follows:

Tyrosinase inhibition (%) = $\{(T_2 - (T_1 - T_3))/T_2 \times 100\}$
T₁ and T₂ represent the absorbance of the test solution and simultaneous control, respectively; T₃ represents

absorbance of the sample itself.

b) With the L-DOPA substrate: The reaction mixture (3 mL) which contained 1.66 mM L-DOPA, 19.0 mM phosphate buffer (pH 6.8), 30 units mushroom tyrosinase and the sample (a 1.66 mM DMSO solution; 1.0 mL) was incubated at 25°C for 1.75-2.75 min. The control reaction was carried out without the test sample. Absorbance was measured at 475 nm before and after incubation. Percent inhibition of tyrosinase was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = (T_1 - T_2) / T_1 \times 100$$

T₁ represents the difference in absorbance of the control before and after incubation, and T₂ is the difference in absorbance of the test solution before and after incubation.

Superoxide dismutase. The reaction mixture (30 mL) which contained the sample (a 0.3 mM DMSO solution; 0.1 mL), xantin, hydroxylamine hydrochloride, N-1-naftylethylenediamine dihydrochloride and xantinoxidase, was incubated at 37°C for 20 min. Sodium dodecyl sulfate was then added to the mixture, and the absorbance was measured at 560 nm.

$$\text{Superoxide Dismutase (\%)} = (T_1 - T_2) / T_1 \times 100$$

T₁ represents the absorbance of the control after incubation, and T₂, absorbance of the test solution after incubation.

2.4.2 in vivo

Primary irritation test using a guinea pig. The hair was removed from both abdomens of the guinea pig and the test body was smeared with a prepared fixed concentration (0.1% sample + squalane, 1.0% sample + squalane, 5.0% sample + squalane and squalane only). Patch bonds were fixed on one side of each of two test bodies, a part in right front in turn. Affixing was carried out by the circuit method and fixed by tape. After 24 h, patch bonds were removed, and erythema and edema of the part of fixing test body were estimated by removing patch bonds after 1 h, 24 h 48 h and 72 h. The results are shown in Table 3 (Draize method) (6). The Stimulus index was calculated as follows:

Stimulus index = (each erythema score + each edema score) / the number of animals

Absolute estimation score: Adopted higher score of stimulus index after 1 h and 24 h. Stimulus estimation data are as follows:

Absolute estimation score	estimation
0.00 ~ 0.20	non - stimulus
0.21 ~ 0.50	weak - stimulus
0.51 ~ 1.00	middle - stimulus
1.01 ~ 1.50	middle - strong - stimulus
1.50 ~	strong - stimulus

The absolute estimation score was less than 1.00, which is suitable, and more than 1.51, which is not suitable as a cosmetic ingredient.

3 Results and Discussion

Thirty products were examined for tyrosinase inhibi-

Fig. 1 Synthesis Procedure from (1) ~ (5C).

Fig. 2 Synthesis Procedure from (5A-a) ~ (5C-l).

Table 1 Physical Constants and Yields of (5A-a) ~ (5C-d).

Compound	n_D^{20} ^{a)}	d_4^{20} ^{b)}	Yield (%)
(5A - a)	1.5820	1.346	65.1
(5A - b)	1.5708	1.297	74.6
(5A - c)	1.5639	1.260	63.3
(5A - d)	1.5683	1.292	40.6
(5B - a)	1.5491	1.256	61.4
(5B - b)	1.5411	1.218	60.4
(5B - c)	1.5365	1.190	51.0
(5B - d)	1.5400	1.216	54.8
(5C - a)	1.5376	1.211	72.0
(5C - b)	1.5325	1.183	84.9
(5C - c)	1.5241	1.151	87.1
(5C - d)	1.5310	1.180	73.5

a) refractive index

b) density

Table 2 Inhibition of Tyrosinase and Superoxide Dismutase for (5A-a) ~ (5C-d).

Compound ^{a)}	Tyrosinase		SOD ^{c)}
	Tyrosine	L - Dopa	
(5A - a)	10.8	5.0	-7.5
(5A - b)	10.4	2.0	-3.2
(5A - c)	8.3	-1.5	1.2
(5A - d)	-1.8	2.0	0.1
(5B - a)	12.5	10.0	-2.5
(5B - b)	19.0	20.1	-1.7
(5B - c)	27.4	16.8	41.6
(5B - d)	12.9	18.6	-17.8
(5C - a)	42.9	35.3	2.1
(5C - b)	74.2	57.2	13.2
(5C - c)	86.2	46.0	11.1
(5C - d)	27.6	30.7	9.4
Arbutin	7.5	14.9	0.5
Ellagic Acid	-13.6	2.0	-3.7
Kojic Acid	91.2	82.3	25.4

a) Concentration; 0.1 mM

b) Inhibitory rate (%)

c) Superoxide Dismutase

Table 3 The Effects of Primary Skin Irritation Test of (5C-b).

Test body	1 h	24 h	48 h	72 h	Absolute estimation	Irritation level
5.0%sample+squalane	0.00	0.20	0.20	0.00	0.20	non-stimulus
1.0%sample+squalane	0.00	0.20	0.00	0.00	0.20	non-stimulus
0.1%sample+squalane	0.00	0.40	0.20	0.00	0.40	weak-stimulus
Squalane only	0.00	0.00	0.00	0.00	0.00	non-stimulus

tion activity and superoxide dismutase to develop whitening agents for cosmetics. (5A-a) ~ (5C-j) were tested for a whitening effect *in vitro* (5) and *in vivo* (6). The effects of the assay are shown in Tables 2 and 3. The effects of *in vitro* are shown in Table 2.

We paid special attention to the (5C) series. (5C-a)~(5C-j) inhibited more strongly than arbutin or ellagic acid. (5C-b) and (5C-c) indicated good inhibition at 74.2% and 86.2% of tyrosinase activity with the tyrosine substrate. As inhibitors these two compounds are as strong as kojic acid with tyrosine substrate, and stronger than arbutin or ellagic acid with the L-DOPA substrate. We synthesized (5C-k) and (5C-l) each possessing an estragole derivatives as analogous compounds of (5C-b) and (5C-c). The difference of (5C-k), (5C-l) and (5C-b), (5C-c) is in their structure regarding

the presence of estragole or thioestragole derivatives. (5C-b) and (5C-c) which include a thioestragole derivatives have a higher inhibition of tyrosinase activity than (5C-k) and (5C-l), which includes an estragole derivatives. In the S atom the influence with the inhibition of tyrosinase activity was determined. We considered the physical property of the C-S and C-O bonds. The bond dissociation energy of C-S is weaker than C-O (7). When the C-S bond dissociates, SH compounds are formed under acidic condition. It is clear that SH compounds do not inhibit the tyrosinase activity but form colorless melanin which is known as pheomelanin by reaction of dopaquinone (8, 9). Human skin is a weak acid (10) and cosmetics are desirable weak acids (11). SH compounds formed by dissociation of C-S bonds under weak acid react with dopaquinone, then switch to

the pathway of pheomelanin.

By increasing the number of methylene groups from the aromatic ring to the thioesteratic site, inhibition of tyrosinase activity increases. The length of the side chain, which includes the thioesteratic site, may thus be a factor of inhibition of tyrosinase activity. In the primary irritation test, a concentration of 0.1% was a weak stimulus and as for other concentrations, the response toward the skin was small (**Table 3**). So, we judged that there is no problem with the primary irritation test.

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