近畿大学工学部研究報告 №40, 2006年, pp.1-7
Research Reports of the School of Engineering,
Kinki University №40, 2006, pp.1-7

Skin Protection Effect of Heterocyclic Compounds Synthesized from *Trans-Anethole* Derivatives

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Abstract

The phenylpropanoids (1-(2-methoxyphenyl) -2-propanone (**1**), 1-(3-methoxyphenyl)-2-propanone (**2**), 1-(4-methoxyphenyl)-2-propanone (**3**), 2-hydroxypropiophenone (**4**) and 4-hydroxypropiophenone(**5**)) were condensed with diols and dithiols, by which we obtained 25 acetals (**1a**) to (**5a**), (**4d**), (**5d**) thioacetals (**1b**) to (**5b**), (**1e**) to (**5e**), and (**1c**) to (**5c**). These compounds were examined for their potential to use as whitening agents by measuring their anti-tyrosinase activity, superoxide scavenging effects and anti-hyaluronidase activity. The results showed that compounds (**3b**), (**3e**), (**4e**) and (**5c**) inhibited tyrosinase activity towards both tyrosine and *L*-DOPA oxidation by more than 80%. Compounds (**3c**), (**3e**) and (**5c**) had stronger superoxide scavenging activity than kojic acid. Overall we found that compound (**3e**) has greater activity as a cosmetic whitening agent than arbutin and kojic acid used, which are currently used in commercial cosmetics.

Key words : phenylpropanoids, whitening agents, acetals, cosmetics.

1. Introduction

We synthesized the phenylpropanoid compounds of trans-anethole and estragole which are major components of the essential oil of fennel (*Foeniculum vulgare miller*), a member of the parsley plant family. Fennel is used in Chinese herbal medicines [1,2] such as aromatic stomachic, carminative and expectorant, and its monoterpenyl radical or heterocyclic C_2-C_3 compounds are used as cosmetic raw materials [3-5]. We are interested in new uses of these compounds in skin whitening cosmetic and have developed various evaluation tests such as *in vitro* tyrosynase inhibitory activity, reactive oxygen scavenging ability and hyaluronidase inhibitory activity. As a result, we previously reported the test results of compounds with greater inhibitory effects than those of the commercially used arbutin. In this study, as part of our ongoing research, we have undertaken a condensation reaction between 1-(2-methoxyphenyl)-2-propanone (**1**) derived from either trans-anethole or phenylpropanoids, such as the related compounds (**1**) to (**5**), and diol, dithiol (C_2-C_3) or 2-mercaptoethanol. We then, investigated how the different orientations of functional radical existing in 25 kinds of heterocyclic compounds (**1a**) to (**5e**) influence the tyrosinase inhibitory activity, reactive oxygen scavenging ability and hyaluronidase inhibitory activity.

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2. Experimental

2.1 Materials

The starting materials, 1-(2-methoxyphenyl)-2-propanone (**1**), 1-(3-methoxyphenyl)-2-propanone (**2**), 1-(4-methoxyphenyl)-2-propanone (**3**), 2-hydroxypropiophenone (**4**) and 4-hydroxypropiophenone (**5**) was obtained from Lancaster synthesis Ltd..

The tyrosinase using physiological activity tests was obtained from Sigama Chemical Co. (EC 1.14. 18.1). The 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 Ltd..

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 80～250℃ (10℃/min).

2.3 Synthesis

2.3.1 Synthesis of cyclic acetals [6]

The synthesis of 2-(4-methoxybenzyl)-2-methyl-[1,3]- dioxolane (**3a**) is described as an example. Into a 50 mL flask fitted with a magnetic stirrer and a Dean-Sark extractor were placed 0.5 g (3.0 mmol) of (**3**), 0.4 g (6.5 mmol) of ethylenglycol (**a**), 0.6 g (3.2 mmol) of *p*-TsOH, and 40 mL of dry benzene. The mixture was stirred and refluxed for 24 h at 120～130℃ on an 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_2SO_4. Evaporation of solvent gave a crude product that was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 9 : 1) to yield 0.40g (63%) of the 2-(4-methoxybenzyl)-2-methyl-[1,3]-dioxolane (**3a**). The above synthesis was also carried out using ethylenedithiol (**b**), 2-mercaptoethanol (**c**), propyleneglycol (**d**) and propylenedithiol (**e**) in the initial condensation step to yield (**3b**) (69%), (**3c**) (44%), (**3d**) (44%) and (**3e**) (70%), respectively.

2.3.2 Synthesis of (**1a**),(**2a**),(**3a**),(**4a**) and (**5a**) [7-10]

The phenyl compound derivatives (**1a**) ～ (**3a**) to(**4a**) and (**5a**) were prepared according to reported procures.

2-(2-Methoxybenzyl)-2-methyl-[1,3]dithiolane(**1b**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.70(3H, s, -$CH_2CC\underline{H}_3$),3.32 (2H, s, -$C\underline{H}_2CCH_3$), 3.33(4H, m, -SCH_2-×2), 3.80

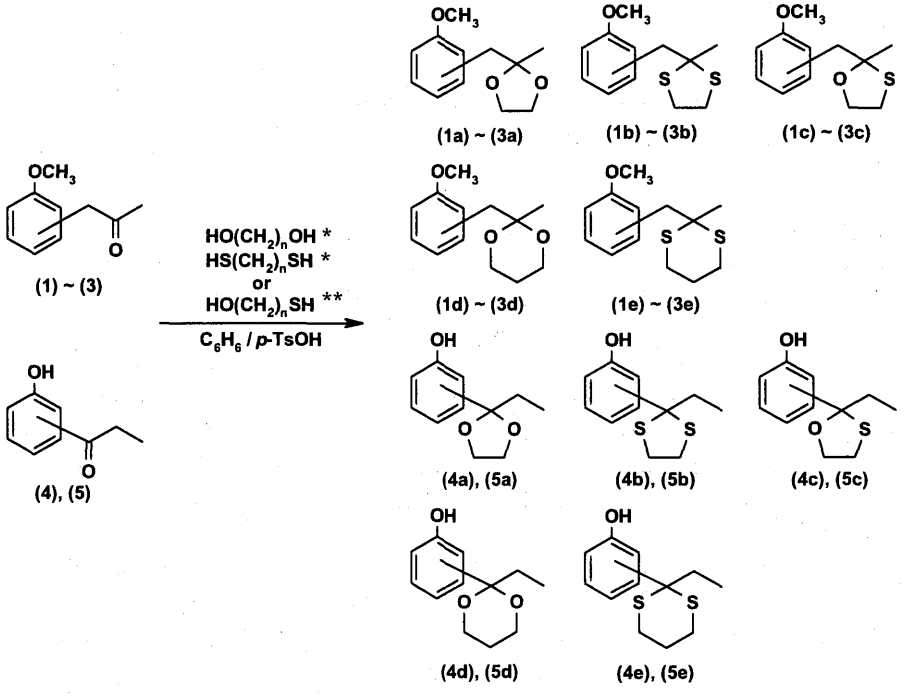

Fig.1 Synthetic Pathway of (1a)～(5e)

Table 1 Physical constants and Yield of (1a)~(5e)

Compound	mp(℃)	d_4^{27}	n_D^{20}	Yield(%)*
(1a)	—	1.1158	1.5201	47
(1b)	—	1.1702	1.5909	41
(1c)	—	1.1480	1.5589	73
(1d)	—	1.1116	1.5259	59
(1e)	105.2 ~ 106.3	—	—	52
(2a)	—	1.1119	1.1579	63
(2b)	—	1.1832	1.5990	41
(2c)	—	1.1467	1.5581	59
(2d)	—	1.1079	1.5238	59
(2e)	—	1.1468	1.5819	52
(3a)	—	1.1119	1.5179	63
(3b)	—	1.1854	1.6004	69
(3c)	—	1.1463	1.5579	44
(3d)	—	1.1119	1.5261	44
(3e)	—	1.2690	1.6602	70
(4a)	—	1.1357	1.5202	62
(4b)	—	1.2185	1.6131	40
(4c)	—	1.1860	1.5720	29
(4d)	—	1.1303	1.5268	29
(4e)	44.0 ~ 45.3	—	—	25
(5a)	70.0 ~ 71.0	—	—	62
(5b)	50.0 ~ 52.1	—	—	66
(5c)	—	1.1942	1.5768	57
(5d)	112.4 ~ 114.0	—	—	58
(5e)	68.0 ~ 69.0	—	—	38

* Isolated yield.

(3H, s, -OCH_3), 7.13(4H, m, ϕ).
MSm/z(rel.int %) ; 240(M^+,7), 119(100), 91(12), 77 (22), 59(15).

2-(2-Methoxybenzyl)-2-methyl-[1,3]oxathiolane (**1c**);^{1}H-NMR δ $CDCl_3$(ppm); 1.52(3H, s, -$CH_2CC\underline{H}_3$), 3.00(2H, m, -$C\underline{H}_2CCH_3$), 3.05(2H, m, -$C\underline{H}_2CCH_3$), 3.20(2H,m, -$SC\underline{H}_2$-), 3.78(3H, s, -OCH_3), 4.18(2H, m, -OCH_2-), 7.07(4H, m, ϕ).
MSm/z(rel.int%) ; 224(M^+,7), 121(17), 103(100), 77 (4), 43(50).

2-(2-Methoxybenzyl)-2-methyl-[1,3]dioxane (**1d**);
^{1}H- NMR δ $CDCl_3$(ppm) ; 1.26(3H, s, -$CH_2CC\underline{H}_3$), 1.72(2H, m, -$OCH_2C\underline{H}_2$-), 3.13(2H, s, -$C\underline{H}_2CCH_3$), 3.79(3H, s, -$OC\underline{H}_3$), 3.98(4H, m, -$OC\underline{H}_2CH_2$-×2), 6.93(4H, m, ϕ).
MSm/z(rel.int %) ; 222(M^+,7), 207(15), 121(56), 101 (100), 73(64), 43(75).

2-(2-Methoxybenzyl)-2-methyl-[1,3]dithiane (**1e**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.54(3H, s, -$CH_2CC\underline{H}_3$), 1.98(2H, m, -$SCH_2C\underline{H}_2$-), 2.94(4H, m, -$SC\underline{H}_2$-×2), 3.33(2H, s, -$C\underline{H}_2CCH_3$), 3.80(3H, s, -$OC\underline{H}_3$), 7.06(4H, m, ϕ).
MSm/z(rel.int %) ; 254(M^+,15), 133(100), 121(7), 91 (14), 59(18).

2-(3-Methoxybenzyl)-2-methyl-[1,3]dithiolane(**2b**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.71(3H, s, -$CH_2CC\underline{H}_3$), 3.20(2H, s, -$C\underline{H}_2CCH_3$), 3.27(4H, m, -SCH_2-×2), 3.79(3H, s, -OCH_3), 6.99(4H, m, ϕ).
MSm/z(rel.int%) ; 240(M^+,25), 119(100), 91(14), 77(9), 59(62).

2-(3-Methoxybenzyl)-2-methyl-[1,3]oxathiolane (**2c**);^{1}H-NMR δ $CDCl_3$(ppm) ; 1.54(3H, s, -$CH_2CC\underline{H}_3$), 3.09(2H, m,-$C\underline{H}_2CCH_3$), 3.12(2H,m, -$C\underline{H}_2CCH_3$), 2.98(2H,m,-$SC\underline{H}_2$-), 3.78(3H,s,-OCH_3), 4.16(2H, m, -OCH_2-), 7.01(4H, m, ϕ).
MSm/z(rel.int %) ; 224(M^+,15), 121(15), 103(100), 77(6), 43(76).

2-(3-Methoxybenzyl)-2-methyl-[1,3]dioxane (**2d**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.29(3H, s, -$CH_2CC\underline{H}_3$), 1.72(2H, m, -$OCH_2C\underline{H}_2$-), 2.99(2H, s, -$C\underline{H}_2CCH_3$), 3.78(3H, s, -$OC\underline{H}_3$), 3.96(4H, m, -$OC\underline{H}_2$-×2), 6.98 (4H, d, m, ϕ).
MSm/z(rel.int %) ; 222(M^+,92), 207(100), 121(32), 101(100), 73(59), 43(81).

2-(3-Methoxybenzyl)-2-methyl-[1,3]dithiane (**2e**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.57(3H, s, -$CH_2CC\underline{H}_3$), 1.99(2H, m, -$SCH_2C\underline{H}_2$-), 2.90(4H, m, -$SC\underline{H}_2CH_2$-×2), 3.20(2H, s, -$C\underline{H}_2CCH_3$), 3.79(3H, s, -$OC\underline{H}_3$), 6.02 (4H, m, ϕ).
MSm/z(rel.int %) ; 254(M^+,25), 133(100), 91(10), 77(6), 59(54).

2-(4-Methoxybenzyl)-2-methyl-[1,3]dithiolane(**3b**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.72(3H, s, -$CH_2CC\underline{H}_3$), 3.15(2H, s, -$C\underline{H}_2CCH_3$), 3.22(4H, m, -SCH_2-×2), 3.78(3H, s, -OCH_3), 7.05(4H, d, J=8.8Hz, ϕ).
MSm/z(rel.int %) ; 240(M^+,12), 119(100), 91(7), 77 (12), 59(37).

2-(4-Methoxybenzyl)-2-methyl-[1,3]oxathiolane (**3c**);^{1}H-NMR δ $CDCl_3$(ppm) ; 1.52(3H, s, -$CH_2CC\underline{H}_3$), 2.87(2H, m, -$C\underline{H}_2CCH_3$), 3.00(2H, m, -$C\underline{H}_2CCH_3$), 3.04(2H, m, -$SC\underline{H}_2$-), 3.76(3H, s, -OCH_3), 4.12(2H, m, -OCH_2-), 7.02(4H, d, J=8.8Hz, ϕ).
MSm/z(rel.int %) ; 224(M^+,14), 121(42), 103(100), 77 (7), 43(53).

2-(4-Methoxybenzyl)-2-methyl-[1,3]dioxane (**3d**);
^{1}H-NMR δ $CDCl_3$(ppm) ; 1.27(3H, s, -$CH_2CC\underline{H}_3$), 1.75(2H, m, -$OCH_2C\underline{H}_2$-), 2.95(2H, s, -$C\underline{H}_2CCH_3$), 3.77(3H, s, -$OC\underline{H}_3$), 3.95(2H×2, m, -$OC\underline{H}_2CH_2$-), 6.99(4H, d, J=8.8 Hz, ϕ).
MSm/z(rel.int %) ; 222(M^+,11), 207(100), 121(65),

101(100), 73(62), 43(76).
2-(4-Methoxybenzyl)-2-methyl-[1,3]dithiane (**3e**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 1.55(3H, s, -CH_2CCH_3), 1.98(2H, m, -SCH_2CH_2-), 3.17(2H, s, -CH_2CCH_3), 3.78(3H, s, -OCH_3), 7.01(4H, d, J=8.8Hz, φ).
MSm/z(rel.int %) ; 254(M^+,18), 133(100), 121(28), 77(7), 59(29).
2-(2-Ethyl-[1,3]dithiolan-2-yl)phenol (**4b**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.96(3H, t, J=7.3Hz, -CH_2-CH_3), 2.45(2H, q, J=7.3Hz, -CH_2CH_3), 3.31(4H, m, -SCH_2-×2), 7.25(4H, m, φ), 7.66(1H, s, -OH).
MSm/z(rel.int %) ; 226(M^+,28), 197(100), 137(18), 77 (4), 65(4).
2-(2-Ethyl-[1,3]oxathiolan-2-yl)phenol (**4c**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.93(3H, t, J=7.6Hz, -CH_2-CH_3), 2.16(2H, q, J=7.6Hz, -CH_2CH_3), 3.09(2H, m, -SCH_2-), 4.19(2H, m, -OCH_2-), 7.08(4H, m, φ), 8.28 (1H, s, -OH).
MSm/z(rel.int %) ; 210(M^+,4), 181(65), 121(100), 93(9), 77(3), 65(9).
2-(2-Ethyl-[1,3]dioxan-2-yl)phenol (**4d**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.86(3H, t, J=7.6Hz, -CH_2-CH_3), 1.82 (2H, q, J=7.6Hz, -CH_2CH_3), 2.15(2H, m, -OCH_2CH_2-), 3.91(4H, m, -OCH_2-×2), 7.06(4H, m, φ), 8.19(1H, s, -OH).
MSm/z(rel.int %) ; 208(M^+,18), 179(78), 121(100), 93(23), 65(28).
2-(2-Ethyl-[1,3]dithian-2-yl)phenol (**4e**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.86(3H, t, J=7.2Hz, -CH_2 -CH_3), 1.99(2H, m, -SCH_2CH_2-), 2.12(2H, q, J=7.6Hz, -CH_2CH_3), 2.79(4H, m, -SCH_2-×2), 7.38(4H, m, φ), 8.72(1H, s, -OH).
MSm/z(rel.int %) ; 240(M^+,93), 211(82), 166(90), 137(100), 77(18), 65(14).
4-(2-Ethyl-[1,3]dithiolan-2-yl)phenol (**5b**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.88(3H, t, J=7.2Hz, -CH_2-CH_3), 2.33(2H, q, J=7.2, -CH_2CH_3), 3.28(4H, m, -SCH_2-×2), 5.24(1H, s, -OH), 7.14(4H, d, J=8.8Hz, φ).
MSm/z(rel.int %) ; 226(M^+,28), 197(100), 137(25), 77(4), 65(6).
4-(2-Ethyl-[1,3]oxathiolan-2-yl)phenol (**5c**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.87(3H, t, J=7.2Hz, -CH_2-CH_3), 2.12(2H, q, J=7.2Hz, -CH_2CH_3), 3.08(2H, m, -SCH_2-), 4.06(2H, m, -OCH_2-), 6.26(1H, s, -OH), 7.06(4H, d, J=8.8Hz, φ).
MSm/z(rel.int %) ; 210(M^+,1), 181(32), 121(100), 93(15), 77(3), 65(12).
4-(2-Ethyl-[1,3]dioxan-2-yl)phenol (**5d**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.80(3H, t, J=7.6Hz, -CH_2-CH_3), 1.71 (2H, m, -OCH_2CH_2-), 1.80(2H, q, J=7.6Hz, -CH_2CH_3), 3.88(4H, m, -OCH_2-×2), 6.84(1H, s, -OH), 7.10(4H, d, J=8.8Hz, φ).
MSm/z(rel.int %) ; 208(M^+,1), 165(100), 121(65), 93(7), 65(10).
2-(2-Ethyl-[1,3]dithian-2-yl)phenol (**5e**); ^{1}H-NMR δ $CDCl_3$(ppm) ; 0.81(3H, t, J=7.6Hz, -CH_2-CH_3), 1.93 (2H, m, -SCH_2CH_2-), 2.04(2H, q, J=7.6Hz, -CH_2CH_3), 2.70(4H, m, -SCH_2-×2), 6.52(1H, s, -OH), 7.31(4H, d, J=8.8Hz, φ).
MSm/z(rel.int %) ; 240(M^+,51), 211(100), 166(62).

3. Physiological Activity Tests

3.1 Tyrosinase Activity Inhibition Test Using Tyrosine as a Substrate

A 0.08 mM solution of each individual compound was tested for its ability to inhibit tyrosinase activity as described previously [11] using tyrosinase as a substrate. Absorbance readings were taken at 475nm with a spectrophotometer.

3.2 Tyrosinase Activity Inhibition Test Using *L*-DOPA as a Substrate

A solution of *L*-DOPA was prepared at the concent-

Table 2 Inhibition of Tyrosinase Activity, Scavenging of Superoxide and Hyaluronidase Activity Inhabitory for (1a)～(3e)

Compound[a]	Tyrosinase		S.S [c]	H.A [d]
	Tyrosine	*L*-DOPA		
(1a)	- 2.4[b]	9.5[b]	4.8 [b]	7.8 [b]
(1b)	57.5	18.9	19.0	10.8
(1c)	- 1.5	0.8	8.8	6.3
(1d)	-0.1	2.4	3.0	4.8
(1e)	6.7	14.7	26.9	8.0
(2a)	- 1.2	- 1.4	1.0	2.7
(2b)	54.0	20.2	18.8	17.8
(2c)	29.4	10.1	11.7	2.0
(2d)	- 0.7	-2.5	1.0	3.1
(2e)	2.2	6.8	9.3	6.1
(3a)	6.2	14.0	6.8	4.3
(3b)	82.0	46.1	22.9	5.2
(3c)	19.8	36.5	26.0	10.8
(3d)	1.7	1.1	4.0	11.8
(3e)	84.1	76.4	34.8	28.3
Arbutin	63.0	7.7	0.8	-30.2
Kojic acid	91.2	82.3	25.4	—

a) Concentration ; 0.1mM b) Inhibitory rate(%)
c) Scavenging of Superoxide
d) Hyaluronidase Activity Inhibitory

Table 3 Inhibition of Tyrosinase Activity, Scavenging of Superoxide and Hyaluronidase Activity Inhibitory

Compound[a]	Tyrosinase		S.S [c]	H.A [d]
	Tyrosine	*L*-DOPA		
(4a)	1.1[b]	- 11.6[b]	3.4 [b]	6.7 [b]
(4b)	0.7	- 7.4	7.0	10.1
(4c)	0.8	- 1.6	4.3	6.3
(4d)	0.5	- 3.7	20.1	4.5
(4e)	96.0	95.5	12.7	8.7
(5a)	5.9	- 5.5	1.0	3.5
(5b)	66.0	3.5	0.8	2.4
(5c)	96.7	- 7.5	0.9	1.0
(5d)	1.2	- 4.5	0.9	1.6
(5e)	16.7	- 3.0	32.7	12.5
Arbutin	63.0	7.7	0.8	- 30.2
Kojic acid	91.2	82.3	25.4	—

a) Concentration ; 0.1mM b) Inhibitory rate(%)
c) Scavenging of Superoxide
d) Hyaluronidase Activity Inhibitory

ration determined previously (1.66 mM), and the synthesized compounds were tested for their ability to inhibit tyrosinase activity as described previously using *L*-DOPA as a substrate[11].

3.3 Reactive Oxygen-Species Scavenging Effect Test

A solutions of each individual compound was prepared at the concentration determined previously (0.714 mM) and tested for its ability to scavenge reactive oxygen-species as described previously [11] using the superoxide dismutase (SOD) test and Wako reagent (Wako Pure Chemical Industries Ltd.). The concentration of diformazan generated by the compounds was measured by absorbance at 560 nm with a spectrophotometer.

3.4 Hyaluronidase Activity Inhibition Test

Phosphate buffer (pH 6.0) was prepared containing 0.4% hyaluronic acid and each individual compound was tested for its ability to inhibit hyaluronidase activity as described previously [12] using the hyaluronidase (Sigma Chemical Co., EC 3.2.1.35). Readings were taken with an Ostwald viscosimeter (No.4).

4. Results and Discussion

At present, arbutin, ellagic acid, lucinol and vitamin C, among others are used as raw materials in commercially available skin whitening cosmetics [13-17]. In addition, cysteine and glutathione containing a sulfur atom are used as raw materials [18]. We undertook a condensation reaction between starting material (1), which is considered to be an important intermediate from which to synthesize a *trans*-anethole derivative that has favorable inhibitory activity against the tyrosinase originating from mushroom, and diol (**a**, **d**), 2-mercaptoethanol (**c**) or dithiol (**b**, **e**) and obtained (**2a**) to (**5e**). In addition, in order to clarify the influence of a functional radical located in a benzene ring and its different orientations on the skin whitening ability, similar condensation reactions were undertaken by the method shown in Fig.1 using phenylpropanoid compounds as a control to obtain (**1a**) to (**5a**). The *in vitro* tyrosinase inhibitory activity, reactive oxygen scavenging ability and hyaluronidase inhibitiory activity were evaluated for compounds (**1a**) to (**5e**) synthesized by these methods and compared with the activities of arbutin, an established skin whitening cosmetic. In other words, we tested for inhibition of tyrosinase activity (against tyrosine and *L*-DOPA) to evaluate the inhibitory effects on melanin formation, which causes skin spots, and tested for scavenging of reactive oxygen species to evaluate the effects on skin generated by external causes such as ultraviolet. We also tested for inhibition of hyaluronidase activity to evaluate the activity of hyaluronidase, which is a catabolic enzyme of hyaluronic acid and has the functions of intercellular water retention or shock absorption, and inhibitory and moisturizing effects for small wrinkles and dry skin. The results are shown in Tables 2 and 3.

As a result, in the *in vitro* tyrosinase activity inhibition tests using the tyrosinase originating from mushroom, it was found that the compounds that inhibit the formation of melanin between tyrosine and DOPA and between DOPA and DOPA quinone were (**3b**) and (**3e**),which have a dithial ring on the side chain of (**3**). More than 80% of the tyrosinase activity against tyrosine was inhibited by these compounds. Also, it was confirmed that compounds (**1b**), (**2b**) and (**5e**), which were derived from the control materials (**2**) and (**5**), also had high rates of tyrosinase inhibition (54.0 to 66.0%).

Among them, compounds (**3e**) and (**4e**), which have a 2,6-dithia-3,4,5-dihydro ring on the side chain as a 1,2- and 1,4-disubstitution benzene derivatives, showe dextremely high rates of

tyrosinase inhibition (76.4 to 96.7%) when both tyrosine and *L*-DOPA were used as the substrate. On the other hand, it was confirmed that as an agent for inhibiting tyrosinase activity at the initial stage, compounds (**1b**), (**2b**), (**3b**) and (**4b**), which have a 2,5-dithia-3,4-dihydro ring on the side chain, showed rates of tyrosinase inhibition that were equivalent or higher than those of the commercial product arbutin. Then, the effects of these compounds against reactive oxygen species were tested. Usually, an oxygen molecule in the ground state exists as a triplet state in stable conditions.

However, it can be reduced to superoxide anion, hydrogen peroxide (H_2O_2), hydroxyradical ($\cdot$OH), singlet oxygen ($^1O_2$), lipid radical peroxide (LOO $\cdot$, LO $\cdot$) and nitric oxide (NO $\cdot$) in stages. These harmful reactive oxygen materials are formed inside the skin and on its surface. On the other hand, although oxygen in the from of superoxide dismutase or catalase exists in living body, the living body tends to be easily attacked due to aging. Under the circumstances, the compounds (**1e**), (**2b**), (**3b**), (**3c**), (**3e**), (**4d**) and (**5e**) showed favorable effects for controlling active oxygen species. Especially, both (**3e**) and (**5e**) indicated more than 30% of control effect rate. These results reflect the active oxygen control described in our previous report [3], which is equivalent to a compound where a monoterpenyl radical was introduced on the side chain of phenylpropanoid. As both compounds have a *p*-orientation, we consider that this orientation influences the active oxygen control effect.

On the other hand, the moisture retention due the moisture content inside the stratum corneum of the skin is considered to be an important factor in cosmetics and a hydrophilic moisturizing material called NMF (natural moisturizing factor) exists inside the skin. Polyhydric alcohols such as glycerin, propylene glycol and sorbitol are used as components in moisturizing cosmetics. We therefore searched for the possible alternatives of these compounds and found that only compound (**3e**) showed almost a 30% rate of inhibition of hyaluronidase. Taken together, these results indicate that the dithial compound (**3e**) is the most effective at inhibiting the formation of melanin. It is expected that this compound can be used as a new raw material for cosmetics.

5. References

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