NOVEL ANTIOXIDANTS FROM ROASTED PERILLA SEED

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Novel antioxidants, 5-(3,4-dihydroxyphenylmethyl)oxazolidine-2,4-dione (**1**) and 3-(3,4-dihydroxyphenyl)lactamide (**2**), have been isolated from roasted perilla seed. Compound **1** was the first example of non-synthetic oxazolidinedione.

KEY WORDS *Perilla frutescens*; roasted perilla seed; antioxidant; 5-(3,4-dihydroxyphenylmethyl)oxazolidine-2,4-dione; 3-(3,4-dihydroxyphenyl)lactamide

Antioxidants are major ingredients that protect the quality of oil. As synthetic antioxidants such as BHA (butylhydroxyanisole) have possible activity as promoters of carcinogenesis,[2)] utilization of natural antioxidants is desired. Although natural antioxidants such as tocopherols and ascorbic acid are widely used, investigations are continuously carried out in order to discover more potent and safer antioxidants, especially from plants.

In the course of our investigation of natural antioxidants, we focused on the roasted perilla seed. Perilla (*Perilla frutescens* var. *japonica* Hara) seed oil includes *ca.* 60 % of α-linolenate,[3)] which has received much attention for its various biological activity.[4)] Although perilla seed oil is easily oxidized because of its high degree of unsaturation, the oil from the roasted seed is commonly known to be oxidized less rapidly. This fact indicates that the antioxidants are produced by roasting of perilla seed. In this paper, we report isolation and identification of two novel antioxidants (**1** and **2**) produced by roasting.

Perilla seed (500 g) roasted at 200°C for 20 min was ground and extracted with hexane, then MeOH. The MeOH extract (50 g) was partitioned between hexane and MeOH, and the MeOH-soluble material was further partitioned between AcOEt and water. Unroasted perilla seed was similarly ground, extracted and partitioned. The TLCs of the fractions from roasted and unroasted seed were compared with each other, and the spots of **1** and **2** were detected only on the TLC of the AcOEt-soluble material from roasted seed. As the AcOEt-soluble material showed antioxidative activity, the fraction (13.5 g) was chromatographed on SiO_2 ($CHCl_3 \rightarrow$ MeOH) to give crude fractions of **1** and **2**.

Crude **1** fraction was further chromatographed on SiO_2 ($CHCl_3$-acetone, 1:1) and ODS (MeOH-H_2O, 4:6), followed by HPLC (YMC-Pack, ODS-AQ, MeOH-H_2O, 35:65) separation to give **1** (12.0 mg).[5)] Compound **1** showed positive $FeCl_3$ test. A high-resolution EI-MS of **1** indicated that the molecular formula was $C_{10}H_9NO_5$ (+0.3 mmu error). The ^{1}H-NMR spectrum of **1** exhibited the aromatic ABX-type signals (δ6.54, δ6.67, and δ6.54), which suggested the 1,2,4-trisubstituted benzene and the aliphatic ABX-type signals (δ2.96, δ3.11, and δ5.07). In the ^{13}C-NMR spectrum, two carbonyl signals appeared at δ_C158.6 and δ_C176.8. The HMBC spectrum showed long-range correlations as indicated in Table 1, which suggested that **1** was phenylpropanoid. In order to confirm the positions of NH and OH groups, acetylation and methylation of **1** were carried out. Treatment of **1** with acetic anhydride in pyridine gave a diacetate (**1a**).[6)] The FAB-MS of **1a** showed quasimolecular peaks at 308 (MH^+) and 330 (MNa^+). The ^{1}H-

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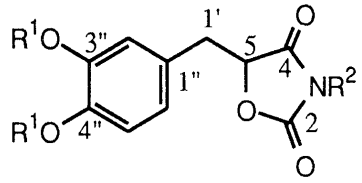
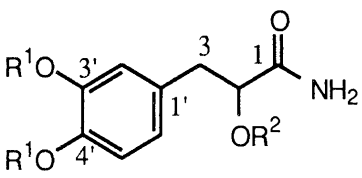


1 $R^1=R^2=H$
1a $R^1=Ac, R^2=H$
1b $R^1=R^2=Me$



2 $R^1=R^2=H$
2a $R^1=R^2=Ac$
2b $R^1=Me, R^2=H$

Fig. 1 Chemical Structures of **1**, **2** and Their Derivatives

Table 1. ^1H- and ^{13}C-NMR Spectral Data for **1** and **2** (^1H: 500 MHz, ^{13}C: 125 MHz)

C. no	**1** (in CD_3OD) δ_C	δ_H (mult., *J*, Hz)	HMBC[a)]	C. no	**2** (in CD_3OD) δ_C	δ_H (mult., *J*, Hz)	HMBC[a)]
2	158.6			1	180.6		
4	176.8			2	74.9	4.13 (dd, 8.3, 3.6)	1, 3
5	83.0	5.07 (dd, 5.0, 4.3)	2, 4, 1' 1"	3	42.0	2.95 (dd, 14.1, 3.6)	1, 1',
1'	37.0	3.11 (dd, 14.8, 4.3)	4, 1",			2.66 (dd, 14.1, 8.3)	2', 6'
		2.96 (dd, 14.8, 5.0)	2", 6"	1'	131.5		
1"	126.8			2'	118.6	6.72 (d,1.8)	3, 6'
2"	118.2	6.67 (d, 2.0)		3'	146.8		
3"	146.5			4'	145.7		
4"	146.0			5'	116.6	6.68 (d, 7.9)	1', 3'
5"	116.6	6.67 (d, 8.3)	1"	6'	122.8	6.58 (dd, 7.9,1.8)	3, 2', 4'
6"	122.6	6.54 (dd, 8.3, 2.0)	1', 2", 4", 5"				

a) Carbon number to which the proton was correlated. (J_{C-H} = 8.3Hz)

NMR spectrum exhibited methyl signal of the acetyl groups at δ2.28 (6H, s) which indicated the existence of two phenolic OH groups in **1**. Treatment of **1** with diazomethane yield trimethylated **1b**,[7)] which was confirmed by FAB-MS spectrum. The ^1H-NMR spectrum of **1b** exhibited the signal of *N*-methyl group at δ2.88, which suggested the existence of imide in **1**.[8)] HMBC experiment (J_{C-H} = 8.3 Hz) of **1b** showed the correlation from *N*-methyl protons to two carbonyl carbons, and the structure of **1** was confirmed as a novel oxazolidinedione as indicated in Fig. 1.

Crude **2** fraction was purified by SiO_2 column chromatography (AcOEt-EtOH-H_2O, 27:2:1 and $CHCl_3$-MeOH-H_2O, 7:3:1, lower layer) and HPLC (YMC-Pack, ODS-AQ, MeOH-H_2O, 3:7) to give **2** (16 mg).[9)] Compound **2** also showed positive $FeCl_3$ test. The ^1H-NMR spectrum of **2** showed signals of both aromatic (δ6.58, δ6.68, and δ6.72) and aliphatic (δ2.66, δ2.95, and δ4.13) ABX-systems similar to those of **1**. These data and ^{13}C-NMR and HMBC spectra indicated that **2** was also a phenylpropanoid. Then **2** was led to a triacetyl derivative (**2a**)[10)] and a dimethyl derivative (**2b**),[11)] to confirm the position of OH and NH groups. The structure of **2a** was clarified by FAB-MS and ^1H-NMR spectrum which showed the signals of two aromatic and an aliphatic acetoxyl groups, a methyne signal shifted downfield, and two exchangeable proton signals of an amide. The structure of **2b** was also clarified by FAB-MS and ^1H-NMR spectrum which indicated the signals of two methoxyl groups, two exchangeable protons of an amide and one exchangeable proton (δ2.43) of an aliphatic hydroxyl group. Thus, the structure of **2** was clarified to be novel 3-(3,4-dihydroxyphenyl)lactamide (Fig. 1).

The antioxidative and radical scavenging activities of **1** and **2** were determined by the ferric thiocyanate method[12,13)] and 1,1-diphenyl-2-picrylhydrazyl (DPPH) method,[12,14)] respectively. Compounds **1** and **2** were both antioxidative, but their activity was weaker than those of BHA and α-tocopherol (Fig. 2). Radical scavenging activities of **1** and **2** were stronger than those of BHA , and **2** was also stronger in the activity than α-tocopherol (Fig. 3).

In summary, we isolated novel antioxidants, 5-(3,4-dihydroxyphenylmethyl)oxazolidine-2,4-dione (**1**) and 3-(3,4-dihydroxyphenyl)lactamide (**2**). Compound **1** was, as far as we know, the first example of the non-synthetic oxazolidinedione. Compounds **1** and **2** were optically active. Determination of their configurations is now in progress.

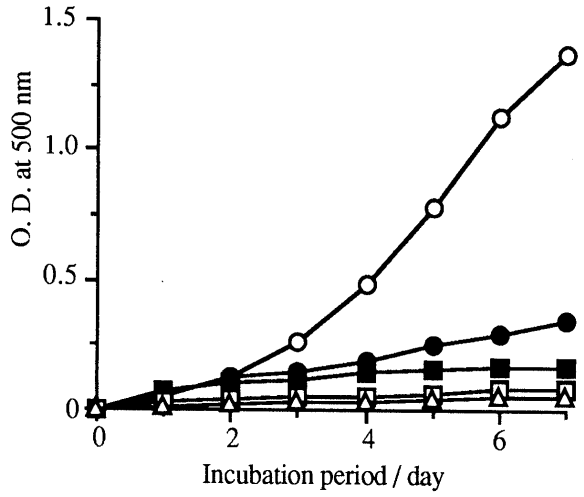


Fig. 2 Antioxidative Activity of **1** and **2** Determined by the Ferric Thiocyanate Method

○, control; ●, 1; ■, 2; □, α-tocopherol; △, BHA

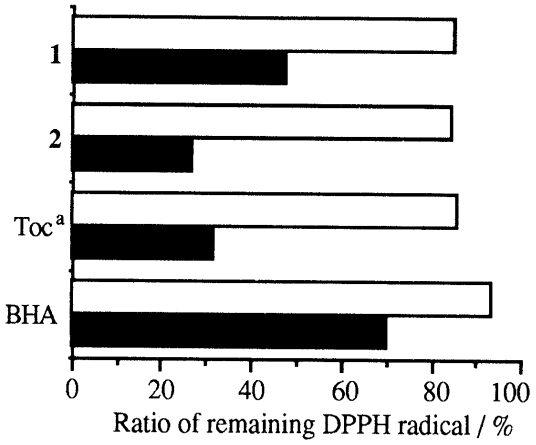


Fig. 3 Scavenging Activity of **1** and **2** on DPPH Radical (1.5×10^{-5} M)

Concentrations of the compounds were 1×10^{-6} M (open column) and 5×10^{-6} M (closed column). [a] α-Tocopherol.

As the AcOEt-soluble material from roasted perilla seed included other antioxidative fractions, the isolation and identification of the components are also now in progress.

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5) **1**: Colorless syrup. $[\alpha]_D^{25}$: +13.0° (*c*=0.105, MeOH). IR (film, cm^{-1}): 3210, 1740.
6) **1a**: ^{1}H-NMR (270 MHz, $CDCl_3$): δ ppm (mult., *J*, Hz): 2.28 (6H, s, Ar-OAc), 3.15 (dd, 14.9, 5.6, 1'-H), 3.31 (dd, 14.9, 4.1, 1'-H), 5.07 (dd, 5.6, 4.1, 5-H), 7.13 (3H, m, Ar-H), 8.10 (br.s, NH).
7) **1b**: FAB-MS (*m/z*): 266 (MH^+), 288 (MNa^+). ^{1}H-NMR (270 MHz, $CDCl_3$): δ ppm (mult., *J*, Hz): 2.88 (3H, s, NMe), 3.11 (dd, 14.8, 5.3, 1'-H), 3.27 (dd, 14.8, 4.3, 1'-H), 3.86 (6H, s, Ar-OMe), 5.02 (dd, 5.3, 4.3, 5-H), 6.72 (d, 2.8, 2"-H), 6.76 (dd, 9.6, 2.8, 6"-H), 6.79 (d, 9.6, 5"-H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C ppm: 25.7 (NMe), 36.2 (C-1'), 55.8 (OMe), 55.9 (OMe), 80.1 (C-5), 111.2 (C-5"), 112.6 (C-2"), 122.0 (C-6"), 125.3 (C-1"), 148.5 (C-4"), 148.9 (C-3"), 155.3 (C-2), 172.2 (C-4). HMBC correlations: NMe → C-2, C-4; 5-H → C-2, C-4, C-1', C-1"; 1'-H → C-4, C-1', C-1"; 2"-H → C-1', C-6".
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9) **2**: Colorless syrup. $[\alpha]_D^{25}$: +29.5° (*c*=0.402, MeOH). IR (film, cm^{-1}): 3290, 1670. FAB-MS (*m/z*): 198 (MH^+), 220 (MNa^+).
10) **2a**: FAB-MS (*m/z*): 324 (MH^+), 346 (MNa^+). ^{1}H-NMR (270 MHz, $CDCl_3$): δ ppm (mult., *J*, Hz): 2.11 (3H, s, 2-OAc), 2.28, 2.28 (each 3H, both s, Ar-OAc), 3.20 (d, 5.6, 3-H), 5.37 (t, 5.6, 2-H), 5.47, 5.93 (each 1H, both br, NH_2), 7.03 (d,1.8, 2'-H), 7.03 (d, 8.2, 5'-H), 7.07 (dd, 8.2, 1.8, 6'-H).
11) **2b**: FAB-MS (*m/z*): 226 (MH^+), 248 (MNa^+). ^{1}H-NMR (270 MHz, $CDCl_3$): δ ppm (mult., *J*, Hz): 2.43 (d, 4.3, OH), 2.89 (dd, 13.9, 8.6, 3-H), 3.19 (dd, 13.9, 4.3, 3-H), 3.88, 3.88 (each 3H, both s, Ar-OMe), 4.30 (ddd, 8.6, 4.3, 4.3, 2-H), 5.44, 6.39 (each 1H, both br, NH_2), 6.80 (d, 2.5, 2'-H), 6.80 (dd, 8.6, 2.5, 6'-H), 6.84 (d, 8.6, 5'-H).
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