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Antioxidant Activity of Chemical Components from Sage (*Salvia officinalis* L.) and Thyme (*Thymus vulgaris* L.) Measured by the Oil Stability Index Method

KAYOKO MIURA, HIROE KIKUZAKI, AND NOBUJI NAKATANI*

Division of Food and Health Science, Graduate School of Human Life Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan

A new abietane diterpenoid, 12-*O*-methyl carnosol (**2**), was isolated from the leaves of sage (*Salvia officinalis* L.), together with 11 abietane diterpenoids, 3 apianane terpenoids, 1 anthraquinone, and 8 flavonoids. Antioxidant activity of these compounds along with 4 flavonoids isolated from thyme (*Thymus vulgaris* L.) was evaluated by the oil stability index method using a model substrate oil including methyl linoleate in silicone oil at 90 °C. Carnosol, rosmanol, epirosmanol, isorosmanol, galdosol, and carnosic acid exhibited remarkably strong activity, which was comparable to that of α-tocopherol. The activity of miltirone, atuntzensin A, luteolin, 7-*O*-methyl luteolin, and eupafolin was comparable to that of butylated hydroxytoluene. The activity of these compounds was mainly due to the presence of *ortho*-dihydroxy groups. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of these compounds showed the similar result.

KEYWORDS: ***Salvia officinalis*** **L.; sage;** ***Thymus vulgaris*** **L.; thyme; 12-*O*-methyl carnosol; apianane terpenoid; abietane diterpenoid; flavonoid; antioxidative activity; oil stability index; OSI; DPPH radical scavenging activity**

INTRODUCTION

Sage (*Salvia officinalis* L.) is now widely cultivated in various parts of the world and popularly used as a culinary herb for flavoring and seasoning. The botanical name of sage is attributed to its medicinal importance. *Salvia* comes from "*salvare*" meaning "to cure" in Latin and "*officinalis*" means medicinal. Chipault et al. (*1*) reported that rosemary (*Rosmarinus officinalis*) and sage were remarkably effective inhibitors of lipid oxidation. Many nonvolatile components such as diterpenoids, triterpenoids, and flavonoids have been isolated from rosemary or sage by Brieskorn et al. (*2*−*5*). We also found that spices belonging to the Labiatae family exhibited the highest antioxidative activity among tested spices, and abietane diterpenoids such as rosmanol and their related compounds were isolated from rosemary and sage (*6*−*10*). It was reported that rosmanol and epirosmanol were obtained as artifacts from carnosol when treated in alcohols (*11*−*12*). Antioxidative compounds from rosemary and sage have been well-established (*11*−*21*), and rosemary extracts are marketed in the U. S., France, Germany, and Japan as natural antioxidants for food.

Antioxidative properties are evaluated by various methods. In this study, the oil stability index (OSI) method was applied for the evaluation of antioxidative activity. It is adopted as the official method by the American Oil Chemists' Society (*22*). The OSI value is defined as the point of maximal change of the rate of oxidation, attributed to the increase of conductivity by the formation of volatile organic acids during lipid oxidation. We have reported the establishment of a standard procedure using a standardized model oil system for the determination of the antioxidant activity by the OSI method (*23*).

In continuation of our chemical investigation of sage, we recently reported the isolation of glycosides (*24*) and terpenoids (*25*). In this study, we now describe the isolation and determination of chemical constituents from sage leaves and their antioxidant activity using a standardized model oil system by the OSI method in comparison with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

MATERIALS AND METHODS

General Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus (Kyoto, Japan) and are uncorrected. Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) and a Union PM-101 (Tokyo, Japan) polarimeter. UV spectra were recorded on a Shimadzu UV-2500PC (Kyoto, Japan) and a Hitachi 220 (Tokyo, Japan) spectrophotometer. IR spectra were run on a Perkin-Elmer FTIR 1720X (Wellesley, MA) and a JASCO IR-S (Tokyo, Japan) spectrometer. ^{1}H, ^{13}C, and 2D nuclear magnetic resonance (NMR; H−H COSY, ^{1}H−^{1}H correlation spectroscopy; HMQC, ^{1}H-detected multiple quantum coherence spectrum; HMBC, ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectrum; NOESY, nuclear Overhauser and exchange spectroscopy) spectra were obtained on a Varian Unity plus 500 instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz; Varian Inc., Palo Alto, CA) and ^{1}H NMR (400Mz) and ^{13}C NMR (100Mz) spectra were obtained on a JEOL GX-400 (Tokyo, Japan)

* To whom correspondence should be addressed. Tel.: +81-6-6605-2812. Fax: +81-6-6605-3086. E-mail: nakatani@life. osaka-cu.ac.jp.

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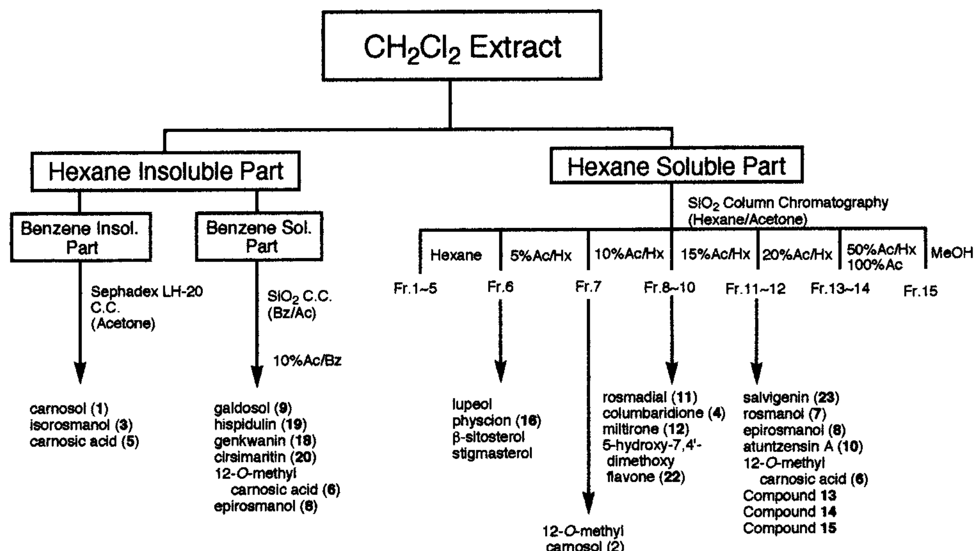

Figure 1. Purification and isolation of the CH_2Cl_2 extract from sage.

and referenced to tetramethylsilane and the residual proton solvent resonance ($((CD_3)_2CO$ at 2.04 ppm for ^1H and 29.3 and 206.3 ppm for ^{13}C NMR). Electron impact mass spectrometry (EIMS) analysis was performed on Hitachi M-2000 and Hitachi M80 mass spectrometers (Tokyo, Japan). Column chromatography was performed using silica gel 60 (70−230 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia Biotech AB), and Chromatorex ODS DM1020T (100−200 mesh; Fuji Silysia Chemical, Tokyo, Japan). Silica gel 60 F254 plates (Merck) and ODS plates (Merck) were used for thin-layer chromatography (TLC). The Omnion Oxidative Stability Instrument (Omnion Inc., Rockland, MA) was used for the OSI method. For measuring the DPPH radical scavenging activity, a Wallac 1420 ARVOSX Multilavel counter (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used.

Plant Material. *S. officinalis* and *T. vulgaris* were kindly provided by Takasago Perfumery Co., Ltd. (Tokyo, Japan) and Nippon Terpene Chemical Co., Ltd. (Kobe, Japan), respectively.

Chemicals. Methyl linoleate (99% grade) was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). 2,6-Di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, BHT), DPPH, l-(+)-ascorbic acid and α-tocopherol were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Silicone oil (TSF451-100) was purchased from Toshiba Silicone Co. Ltd. (Tokyo, Japan).

Extraction from Sage and Isolation. Air-dried and ground leaves of *S. officinalis* (1.0 kg) were successively extracted with methylene chloride (CH_2Cl_2 , 4000 mL × 3) and 70% aqueous acetone (70% aqueous Me_2CO, 2500 mL × 5) at room temperature. Evaporation of organic solvent from each solution under reduced pressure gave the CH_2Cl_2 extract (129.7 g) and aqueous residue, respectively. The resulting aqueous residue was successively partitioned with CH_2Cl_2, ethyl acetate (EtOAc), and butanol (BuOH) to afford CH_2Cl_2 soluble (45.3 g), EtOAc soluble (12.0 g), BuOH soluble (44.8 g), and H_2O soluble (82.3 g) fractions, respectively. The CH_2Cl_2 extract (27.7 g) was dissolved in *n*-hexane, and the hexane soluble part (21.8 g) was subjected to column chromatography on silica gel (Ø50 mm × 680 mm, 500 g) using *n*-hexane with increasing amounts of Me_2CO as an eluent to afford 15 fractions by monitoring with TLC (fractions 1−5 (eluted with *n*-hexane, 5000 mL), fraction 6 (eluted with *n*-hexane−Me_2CO (95:5), 2600 mL), fraction 7 (eluted with *n*-hexane−Me_2CO (90:10), 3000 mL), fractions 8−10 (eluted with *n*-hexane−Me_2CO (85:15), 4000 mL), fraction 11 (eluted with *n*-hexane−Me_2CO (80:20), 1600 mL), fraction 12 (eluted with *n*-hexane−Me_2CO (75:25), 2000 mL), fraction 13 (eluted with *n*-hexane−Me_2CO (50:50), 2800 mL), fraction 14 (eluted with Me_2CO, 2000 mL), and fraction 15 (eluted with MeOH, 2000 mL)). The structures of isolated compounds are shown in **Figures 3** and **4**. Repeated chromatography of the less polar fraction (fraction 6, 1.23 g) and recrystallization afforded known compounds, lupeol (4.7 mg), physcion (**16**, 2.6 mg), *β*-sitosterol (50 mg), and stigmasterol (5 mg). Fraction 7 (0.42 g) was chromatographed on a Sephadex LH-20 column (Ø33 mm × 350 mm) with Me_2CO, followed by on an ODS column (Ø13 mm × 175 mm) with MeCN (100%) to afford 12-*O*-methyl carnosol (**2**, 3.2 mg). Fraction 8 (0.9 g) was successively rechromatographed on a Sephadex LH-20 column (Ø30 mm × 400 mm) eluted with Me_2CO and a silica gel column (Ø15 mm × 240 mm) eluted with C_6H_6−Me_2CO to give rosmadial (**11**, 4.4 mg), columbaridione (**4**, 2 mg), miltirone (**12**, 1.4 mg), and 5-hydroxy-7,4′-dimethoxy flavone (**22**, 3 mg). The mixture (4.6 g) of fractions 11 and 12 was rechromatographed on a Sephadex LH-20 column (Ø30 mm × 365 mm) with Me_2CO to separate 9 fractions (fractions A−I). Furthermore, fraction C was subjected to column chromatography (Ø15 mm × 200 mm) on silica gel eluted with C_6H_6−Me_2CO (97:3) to afford **13** (10.1 mg), **14** (2.5 mg), and **15** (1.5 mg). Repeated chromatography and recrystallization of fractions D and E afforded salvigenin (**23**, 23 mg), atuntzensin A (**10**, 3.8 mg) and 12-*O*-methyl carnosic acid (**6**, 400 mg), rosmanol (**7**, 475 mg), and epirosmanol (**8**, 5 mg). The *n*-hexane insoluble part (0.4 g) of the CH_2Cl_2 extract was rechromatographed on Sephadex LH-20 (Ø30 mm × 400 mm) with Me_2CO as an eluent to afford carnosol (**1**, 40 mg) and isorosmanol (**3**, 11 mg).

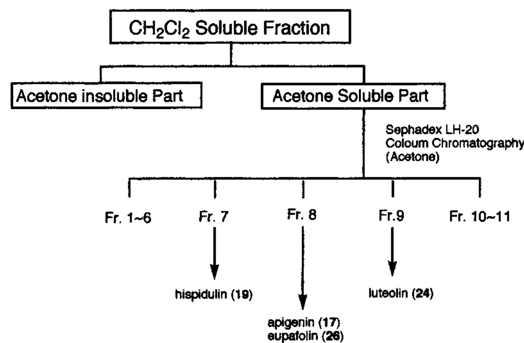

Figure 2. Purification and isolation of the CH_2Cl_2 soluble fraction from sage.

Another part of the CH_2Cl_2 extract (100 g) was dissolved in *n*-hexane to separate the *n*-hexane soluble fraction (84.5 g) and the insoluble fraction (15.5 g), respectively. The *n*-hexane insoluble fraction was dissolved in benzene to give the benzene soluble part (7.3 g), which was subject to column chromatography on silica gel (Ø60 mm × 400 mm, SiO_2, 450 g) and eluted with benzene−Me_2CO (90:10) to give 12-*O*-methyl carnosic acid (**6**, 130 mg), epirosmanol (**8**, 14 mg), galdosol (**9**, 2 mg), hispidulin (**19**, 5 mg), cirsimaritin (**20**, 20 mg), and genkwanin (**18**, 2 mg). The benzene insoluble part (8.2 g) was chromatographed on Sephadex LH-20 with Me_2CO (Ø25 mm × 330 mm) as an eluent to afford carnosic acid (**5**, 9 mg) and isorosmanol (**3**, 16 mg).

Table 1. ^{1}H, ^{13}C, and HMBC NMR Data for **2** ($CDCl_3$)

position	δ H	δ C	HMBC (H→C)
1α	2.46 ddd (4.4, 13.9, 14.2)	28.8	C(2, 3, 5, 9, 10, 20)
1β	2.88 dddd (1.7, 3.2, 4.9, 14.2)		C(3, 5, 10)
2α	1.65 ddddd (3.6, 4.4, 4.9, 4.9, 13.9)	18.9	
2β	1.98 ddddd (3.2, 3.3, 13.6, 13.9, 13.9)		C(3)
3α	1.30 ddd (3.6, 13.2, 13.6)	41.1	C(2, 4, 18)
3β	1.54 dddd (1.7, 3.3, 4.9, 13.2)		C(1, 2, 4, 5)
4	-	34.5	
5	1.74 dd (5.7, 10.6)	45.4	C(3, 4, 6, 7, 9, 10, 18, 19, 20)
6α	2.22 ddd (4.0, 5.7, 13.7)	29.5	C(4, 5, 7, 8)
6β	1.88 ddd (1.7, 10.6, 13.7)		C(4, 5, 10)
7	5.38 dd (1.7, 4.0)	77.7	C(5, 8, 9, 14, 20)
8		136.4	
9		121.5	
10		48.4	
11	5.98 s (OH)	146.0	C(9, 11, 12)
12		144.8	
13		140.1	
14	6.66 s	112.0	C(7, 9, 12, 15)
15	3.22 sept (6.8)	26.6	C(12, 13, 14, 16, 17)
16	1.21 d (6.8)	23.7	C(13, 15, 17)
17	1.21 d (6.8)	23.7	C(13, 15, 16)
18	0.86 s	31.7	C(3, 4, 5, 19)
19	0.91 s	19.7	C(3, 4, 5, 18)
20		175.9	
21	3.75 s	62.0	C(12)

[a] Coupling constants, *J* (Hz), are in parentheses.

The CH_2Cl_2 soluble fraction (4.0 g) was subjected to column chromatography on Sephadex LH-20 eluted with Me_2CO to separate 11 fractions. Fractions 7−9 were repeatedly chromatographed on Sephadex LH-20 with Me_2CO (Ø25 mm × 330 mm) to give hispidulin (**19**, 7 mg), apigenin (**18**, 4 mg), eupafolin (**26**, 4 mg), and luteolin (**24**, 5 mg).

Carnosol (1) (***6***)**.** Colorless needles, mp 230 °C.

12-*O*-Methyl Carnosol (2). Colorless solid, mp 69.0 °C. Anal. calcd for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.6; H, 8.12. Electron impact mass spectrometry (EIMS): *m/z* (%) 344 ([M]$^+$, 22), 300 (100), 298 (17), 285 (21), 283 (16), 231 (14), 229 (20), 218 (18), 215 (18). UV λ_{max}^{EtOH} nm (log ε): 207 (4.53), 284 (3.44). IR ν_{max}^{Nujol} (cm^{-1}): 3320, 1728, 1615, 1580, 1245, 1232, 1220, 1160, 1120, 1040, 1025, 997. ^{1}H NMR (500Mz, $(CD_3)_2CO$), see **Table 1**. ^{13}C NMR (125Mz, $(CD_3)_2CO$), see **Table 1**.

Isorosmanol (3) (***9***)**.** Colorless needles, mp 227 °C.

Columbaridione (4) (***28***)**.** Orange-yellow needles, mp 179 °C. ^{1}H NMR (400Mz, $CDCl_3$): δ 2.31 (1H, ddd, *J* = 4.4, 13.8, 14.1 Hz, H-1α), 2.71 (1H, dddd, *J* = 1.5, 3.3, 3.3, 14.1 Hz, H-1β), 1.65 (1H, ddddd, *J* = 3.2, 3.3, 3.3, 4.4, 14.1 Hz, H-2α), 1.92 (1H, ddddd, *J* = 3.2, 3.3, 13.8, 13.8, 14.1 Hz, H-2β), 1.25 (1H, ddd, *J* = 3.3, 13.2, 13.8 Hz, H-3α), 1.55 (1H, dddd, *J* = 1.5, 3.2, 3.2, 13.2 Hz, H-3β), 1.69 (1H, dd, *J* = 5.9, 10.6 Hz, H-5), 2.24 (1H, ddd, *J* = 4.2, 5.9, 14.1 Hz, H-6α), 1.84 (1H, ddd, *J* = 1.5, 10.6, 14.1 Hz, H-6β), 5.80 (1H, dd, *J* = 1.5, 4.2 Hz, H-7), 3.18 (1H, sept, *J* = 7.1 Hz, H-15), 1.22 (3H, d, *J* = 7.1 Hz, H-16), 1.22 (3H, d, *J* = 7.1 Hz, H-17), 0.90 (3H, s, H-18), 0.87 (3H, s, H-19). ^{13}C NMR (100Mz, $(CD_3)_2CO$): δ 27.4 (C-1), 18.5 (C-2), 40.7 (C-3), 34.8 (C-4), 45.3 (C-5), 28.0 (C-6), 70.3 (C-7), 138.8 (C-8), 146.9 (C-9), 48.9 (C-10), 179.6 (C-11), 150.7 (C-12), 123.7 (C-13), 181.6 (C-14), 24.4 (C-15), 20.1 (C-16), 20.1 (C-17), 32.4 (C-18), 19.7 (C-19), 172.9 (C-20).

Carnosic Acid (5) (***18***)**.** Off-white solid, mp 190 °C.

12-*O*-Methyl Carnosic Acid (6) (***18***)**.** Off-white solid.

Rosmanol (7) (***9***)**.** Colorless needles, mp 241 °C.

Epirosmanol (8) (***9***)**.** Colorless needles, mp 221 °C.

Galdosol (9) (***27***)**.** Brown oil.

Atuntzensin A (10) (***28***)**.** Yellow solid, mp 220 °C. ^{1}H NMR (500Mz, $CDCl_3$): δ 1.61 (1H, m, H-1α), 3.21 (1H, br d, *J* = 14.1 Hz, H-1β), 1.64 (1H, m, H-2α), 1.58 (1H, m, H-2β), 1.17 (1H, ddd, *J* = 3.2 13.4, 14.6 Hz, H-3α), 1.46 (1H, m, H-3β), 2.20 (1H, s, H-5), 4.60 (1H, dd, *J* = 0.7, 3.2 Hz, H-6), 4.74 (1H, d, *J* = 3.2 Hz, H-7), 3.38 (1H, br s, OH-7), 7.22 (1H, br s, OH-12) 3.16 (1H, sept, *J* = 7.1 Hz, H-15), 1.21 (3H, d, *J* = 7.1 Hz, H-16), 1.22 (3H, d, *J* = 7.1 Hz, H-17), 1.04 (3H, s, H-18), 0.90 (3H, s, H-19). ^{13}C NMR (125Mz, $CDCl_3$): δ 25.5 (C-1), 18.5 (C-2), 37.9 (C-3), 31.4 (C-4), 50.6 (C-5), 75.9 (C-6), 65.5 (C-7), 144.1 (C-8), 140.5 (C-9), 46.2 (C-10), 181.5 (C-11), 151.2 (C-12), 125.1 (C-13), 187.4 (C-14), 24.0 (C-15), 19.6 (C-16 or 17), 19.8 (C-16 or 17), 31.3 (C-18), 21.9 (C-19), 175.5 (C-20).

Rosmadial (11) (***7***)**.** Colorless prisms, mp 225 °C.

Miltirone (12) (***19***, ***29***)**.** Orange solid, mp 100 °C.

rel*-(5*S*,6*S*,7*S*,10*R*,12*S*,13*R*)-7-Hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide (13)** (25***)**.** Colorless needles, mp 245 °C.

rel*-(5*S*,6*S*,7*R*,10*R*,12*S*,13*R*)-7-Hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide (14)** (25***)**.** Amorphous solid.

rel*-(5*S*,6*S*,7*S*,10*R*,12*R*,13*S*)-7-Hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide (15)** (25***)**.** Amorphous solid.

Physcion (16) (***30***)**.** Yellow needles, mp 203 °C.

5,7,4′-Trihydroxyflavone (Apigenin, 17) (***31***, ***39***)**.** Off-white solid.

5,4′-Dihydroxy-7-methoxyflavone (Genkwanin, 18) (***32***, ***33***)**.** Yellow amorphous powder, mp 270 °C.

5,7,4′-Trihydroxy-6-methoxyflavone (Hispidulin, 19) (***34***, ***42***)**.** Yellow solid.

5,4′-Dihydroxy-6,7-dimethoxyflavone (Cirsimaritin, 20) (***33***, ***35***)**.** Yellow needles, mp 256 °C.

5,4′-Dihydroxy-6,7,8-trimethoxyflavone (Xanthomicrol, 21) (***33***, ***35***)**.** Yellow needles, mp 225 °C.

5-Hydroxy-7,4′-dimethoxyflavone (22) (***33***, ***36***)**.** Yellow crystals, mp 173 °C.

5-Hydroxy-6,7,4′-trimethoxyflavone (Salvigenin, 23) (***37***, ***42***)**.** Yellow crystals, mp 188 °C.

5,7,3′4′-Tetrahydroxyflavone (Luteolin, 24) (***38***, ***42***)**.** Yellow solid; mp >300 °C.

5,3′4′-Trihydroxy-7-methoxyflavone (25) (***39***)**.** Yellow solid.

5,7,3′4′-Tetrahydroxy-6-methoxy flavone (Eupafolin, 26) (***34***, ***42***)**.** Yellow solid, mp 277 °C.

5,4′-Dihydroxy-6,7,3′-trimethoxyflavone (Cirsilineol, 27) (***33***, ***40***, ***42***)**.** Yellow crystals, mp 205 °C.

5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone (28) (***33***, ***40***, ***41***, ***42***)**.** Yellow needles, mp 165 °C.

Extraction from Thyme and Isolation. Dried ground leaves of thyme (2 kg) cultivated in Spain were extracted with *n*-hexane (3000 mL × 4) to remove the nonpolar components. The residue was extracted with acetone (3000 mL × 4). Evaporation of the filtlate gave a brown residue (100 g), which was followed by steam distillation to separate the volatile and the nonvolatile components. The nonvolatile fraction was dissolved in ether, which was washed successively with 2 N HCl, saturated $NaHCO_3$(aq), and 1 N NaOH solution in the usual way to separate the basic, strongly acidic, weakly acidic, and neutral fractions. The weakly acidic fraction (14.6% based on the acetone extract) was subjected to chromatgraphy on a silica gel column with benzene−acetone as the eluting solvent to afford 11 fractions. Compounds **21** and **28** were obtained from fraction 3. Compounds **25** and **27** were obtained from fraction 4 (*33*).

Evaluation of Antioxidant Activity by the OSI Method (***23***)**.** Methyl linoleate was added to silicone oil at a concentration of 10%. The mixture was stirred with a vortex mixer for 10 min under nitrogen. This substrate oil (5 g) was weighed and transferred into a glass reaction tube. Sage extract and fractions along with α-tocopherol and BHT were dissolved in $CHCl_3$−MeOH (1:1) solution (100 μL) and added to a substrate oil (0.02% as the final concentration). The $CHCl_3$−MeOH (1:1) solutions (100 μL) of test compounds, α-tocopherol, or BHT were also added to model oils. The same amount of $CHCl_3$−MeOH (1:1) solution alone was added as a control. The solvent was flushed out at 90 °C for 30 min with forced aeration without linking the conductivity measurement tubes, after which the OSI value of each sample was automatically measured at 90 °C under aeration. The OSI value was measured in triplicate.

Radical Scavenging Activity on DPPH Radical (***43***)**.** DPPH radical was prepared in EtOH as a 100 μM solution. This DPPH solution (100 μL) was mixed with different concentrations of samples (1, 10, 31.5, and 100 μM) as the final concentrations in microplates. When the concentration was expressed in logarithm, the concentration of 31.5 μM was the half point between 10 and 100 μM. Then, the solution with test samples was slightly shaken and held for 0.5 h at 22 °C. The

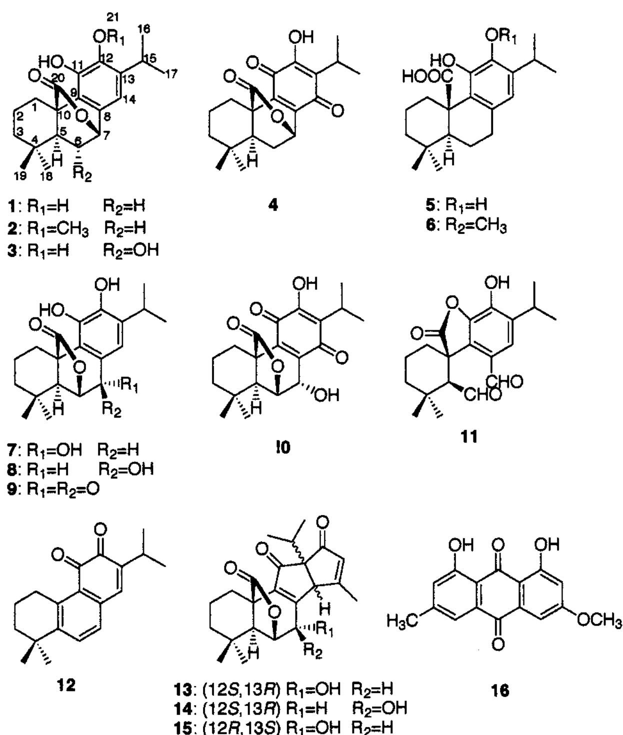

Figure 3. Structure of chemical components isolated from the CH_2Cl_2 extract of sage.

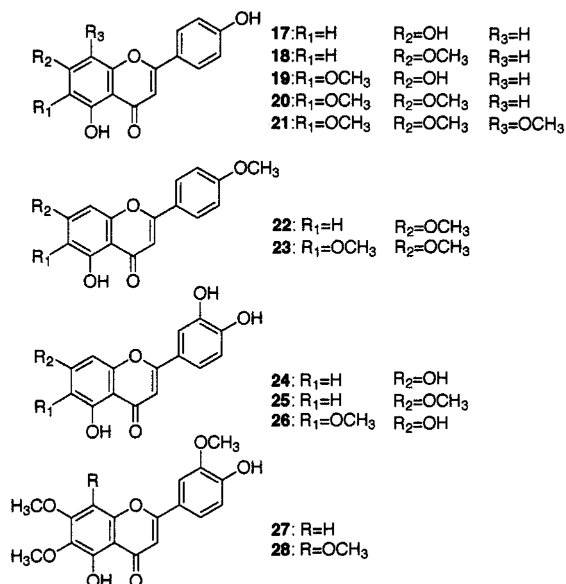

Figure 4. Structure of flavonoids isolated from sage and thyme. Compounds **18**–**20**, **22**, and **23** were isolated from the CH_2Cl_2 extract of sage; **17**, **19**, **24**, and **26** were from the CH_2Cl_2 soluble fraction of sage; and **21**, **25**, **27**, and **28** were isolated from thyme.

absorbance of the samples was measured on a spectrophotometer (Wallac 1420 ARVOSX Multilavel counter) at 520 nm against a blank of EtOH without DPPH. Each value is the mean of triplicate measurements.

RESULTS AND DISCUSSION

The antioxidant activity of each extract and fraction obtained from sage was measured by the OSI method. As shown in **Figure 5**, the CH_2Cl_2 extract and the CH_2Cl_2 soluble fraction showed remarkable activity higher than that of α-tocopherol, a

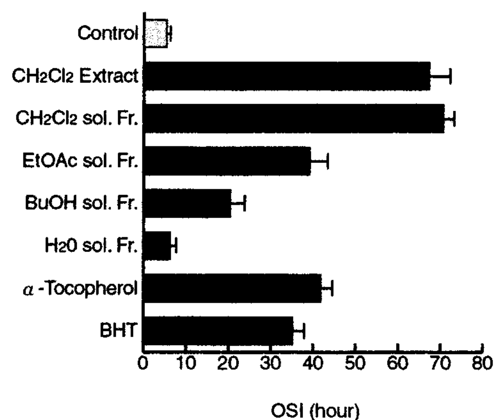

Figure 5. Antioxidant activity of extract and soluble fractions with different polarities from sage measured by the OSI method. The results are expressed as mean value ± standard deviation ($n = 3$).

natural antioxidant. The activity of the EtOAc soluble fraction was comparable to that of α-tocopherol. Then, the CH_2Cl_2 extract was purified by repeated chromatography using silica gel and Sephadex LH-20 to afford the 15 known compounds: carnosol (**1**), isorosmanol (**3**), columbaridione (**4**), carnosic acid (**5**), 12-*O*-methyl carnosic acid (**6**), rosmanol (**7**), epirosmanol (**8**), galdosol (**9**), atuntzensin A (**10**), rosmadial (**11**), miltirone (**12**), new apianane compounds (**13**−**15**), and physcion (**16**). Moreover, the 5 known flavonoids, such as genkwanin (**18**), hispidulin (**19**), cirsimaritin (**20**), 5-hydroxy-7,4′-dimethoxy flavone (**22**), and salvigenin (**23**), were isolated from the same extract (**Figures 1**−**4**). The CH_2Cl_2 soluble fraction of the 70% aqueous Me_2CO extract was also purified by repeated chromatography using Sephadex LH-20 and silica gel to afford the 4 flavonoids apigenin (**17**), hispidulin (**19**), luteolin (**24**), and eupafolin (**26**). Their chemical structures were identified by comparing with spectral data of authentic samples and previous reports (*4*, *7*−*9*, *18*, *19*, *25*−*42*). Compound **2** was obtained from fraction 7 of the CH_2Cl_2 extract by repeated chromatography. The ^{13}C NMR (**Table 1**) and the HMQC spectra of **2** indicated the presence of 21 carbons, which consisted of 4 methyl, 1 methoxy, 4 methylene, 2 methine, 1 oxymethine, 2 quarternary, 6 aromatic, and 1 ester group. The ^{1}H NMR spectrum (**Table 1**) showed signals for 2 geminal methyl groups (δ 0.86 and 0.91), 4 methylene groups (δ 1.30, 1.54, 1.65, 1.88, 1.98, 2.22, 2.46, and 2.88), 1 isopropyl group on an aromatic ring (δ 1.21, 1.21, and 3.22), 1 methoxy group (δ 3.75), 1 methine proton bearing an oxygen function (δ 5.38), 1 hydroxy group (δ 5.98), and 1 aromatic proton (δ 6.66). These data were similar to those of carnosol (**1**) except for the signal due to the methoxy group. Furthermore, the elemental analysis of **2** gave a molecular formula of $C_{21}H_{28}O_4$ and a molecular ion was observed at m/z 344 suggesting that it contained 1 methoxy group instead of the hydroxy group in **1**. The substitution of the methoxy group was confirmed by HMBC (**Table 1**) and NOESY experiments. In the HMBC spectrum, the methoxy protons at δ 3.75 were correlated with the carbon at δ 144.8, while a hydroxy proton at δ 5.98 was correlated with carbons at δ 121.5 (C-9), 146.0, and 144.8. A NOESY experiment of **2** showed a strong cross-peak between the signal of δ 3.75 and the signal of δ 3.22 (H-15). These data indicated that the methoxy group was a substitutent at C-12 and the hydroxy group at C-11. We have previously reported a monomethyl compound (11-*O*- or 12-*O*-methyl carnosol) synthesized from carnosol and its antioxidant activity (*8*), but its structure was not completely

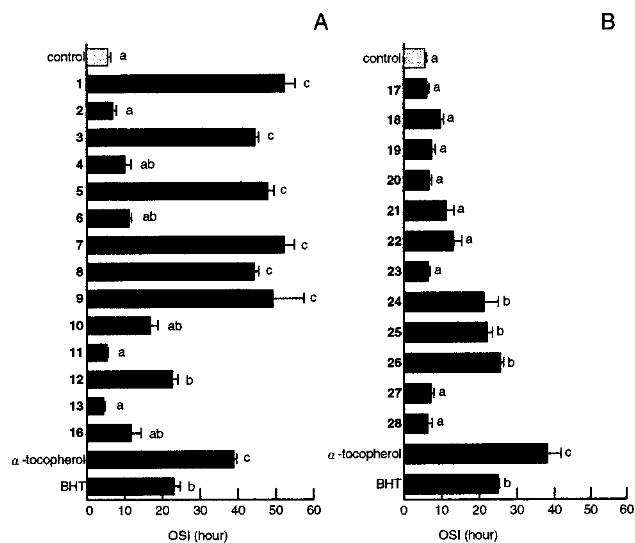

Figure 6. (**A**) Antioxidant activity of compounds isolated from sage measured by the OSI method. The results are expressed as mean value ± standard deviation ($n = 3$). Each column (a–c) with the different letters is significantly different ($p < 0.05$). (**B**) Antioxidant activity of flavonoids measured by the OSI method. The results are expressed as mean value ± standard deviation ($n = 3$). Each column (a–c) with the different letters is significantly different ($p < 0.05$).

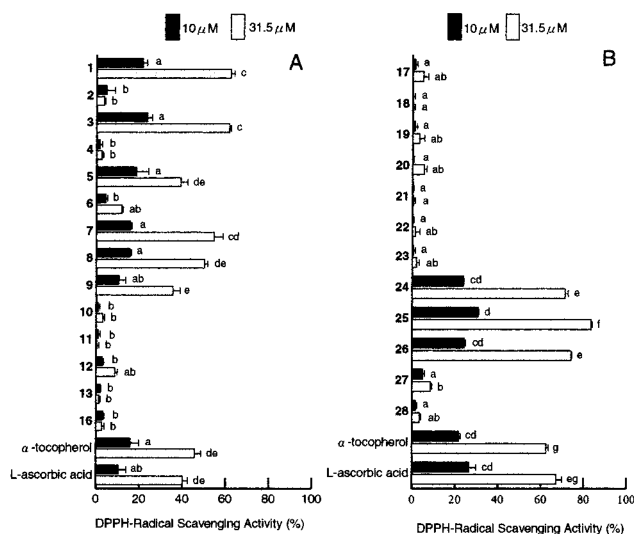

Figure 7. (**A**) DPPH radical scavenging activity of compounds isolated from sage. The results (%) = [(absorbance in the absence of sample − absorbance in the presence of sample)/absorbance in the absence of sample] × 100. Results are expressed as mean value ± standard deviation ($n = 3$). Each column (a–e) with the different letters is significantly different ($p < 0.05$). (**B**) DPPH radical scavenging activity of compounds isolated from sage and thyme. The results (%) = [(absorbance in the absence of sample − absorbance in the presence of sample)/ absorbance in the absence of sample] × 100. Results are expressed as mean value ± standard deviation ($n = 3$). Each column (a–g) with the different letters is significantly different ($p < 0.05$).

clarified at that time. The data for **2** obtained in this study were in good agreement with those of the monomethyl compound synthesized from carnosol. On the basis of these results and already reported data (*7*, *8*), the structure of **2** was determined to be 11-hydroxy-12-methoxy-7,10-(epoxymethano) abieta-8,-11,13-trien-20-one. This is the first report of the natural occurrence of compound **2**.

Antioxidant activity of the compounds isolated from sage except for **14** and **15**, together with 4 flavonoids (**21**, **25**, **27**, and **28**) isolated from thyme (*33*) (**Figures 3** and **4**) was measured by the OSI method using methyl linoleate at 90 °C. Abietane diterpenoids such as carnosol (**1**), isorosmanol (**3**), carnosic acid (**5**), rosmanol (**7**), epirosmanol (**8**), and galdosol (**9**) showed remarkably strong activity, which was comparable to that of α-tocopherol. There was no significance statistically between compounds **1**, **3**, **5**, and **7**−**9** and α-tocopherol (**Figure 6**). Furthermore, there was no difference in the activity between the compounds with a lactone ring in the molecule and those without it. The type of lactone such as γ- and δ-lactone did not show any effect on the activity. However, the compounds showing strong antioxidant activity commonly included an *ortho*-dihydroxy group in the molecule, suggesting that the antioxidant activity is due to the presence of an *ortho*-dihydroxy group on the C-ring. When we measured the antioxidant activity of the derivatives of carnosol (**1**) and rosmanol (**7**) against the autoxidation in an oil−ethanol−water solution by the ferric thiocyanate method and the TBA method, both 12-*O*-methyl carnosol and carnosol showed strong antioxidant activity (*8*). However, when measured by the OSI method in this study, 12-*O*-methyl carnosol (**2**) and 12-*O*-methyl carnosic acid (**6**) exhibited weaker activity, as compared with those of carnosol (**1**) and carnosic acid (**5**). It was deduced that the mechanism of antioxidant activity depends on the type and polarity of the lipid system used as a model substrate and the heat. The antioxidant activity of miltirone (rosmariquinone from rosemary (*19*)) (**12**) and atuntzensin A (**10**) was comparable to that of BHT.

The antioxidant activity of flavonoids (**17**−**28**) was also measured by the OSI method. Luteolin (**24**), 7-*O*-methyl luteolin (**25**), and eupafolin (**26**) exhibited almost the same activity as BHT. It was apparent that these compounds had an *ortho*-dihydroxy phenyl moiety, whose structure played an important role in the antioxidant activity.

Although we have already reported that compounds **27** and **28** possessing an *ortho*-methoxy and hydroxy moiety on the B-ring of flavone showed remarkable antioxidative activity against the autoxidation of linoleic acid in an oil−ethanol−water solution (*33*), they were less active against the oxidation of methyl linoleate measured by the OSI method. This result with flavonoids was similar to that of abietane diterpenoids possessing an *ortho*-methoxy and hydroxy moiety.

Furthermore, the radical scavenging properties of components isolated from sage were evaluated on the DPPH radical by a spectrophotometric assay. As shown in **Figure 7**, compounds **1**, **3**, **5**, **7**−**9**, and **24**−**26** showed radical scavenging activity comparable to that of α-tocopherol and L-ascorbic acid used as positive references. In this study, the isolated compounds possessing *ortho*-dihydroxy groups exhibited strong activity in both the OSI method and the DPPH radical scavenging activity. However, miltirone (**12**), a less polar compound possessing an *ortho*-quinone moiety in the molecule, showed strong activity in the OSI method, whereas it showed lower DPPH radical scavenging activity. Sawai et al. (*44*) reported that the B-ring of (+)-catechin changed to an *ortho*-quinone structure when (+)-catechin was reacted with DPPH by using ^{13}C NMR analysis. It was obvious that there were differences between the results obtained by both methods. Gadow et al. (*45*) reported the results of the antioxidant activity using the Rancimat method operated on the same principle to measure the OSI value and DPPH radical scavenging activity of compounds isolated from

rooibos tea. In the antioxidant activity measured by the Rancimat method, flavonoid glycosides such as rutin and isoquercitrin possessing *ortho*-dihydroxy phenyl groups exhibited less activity than their aglycones against the lipid oxidation, while in the DPPH radical scavenging activity flavonoid glycosides exhibited activity as strong as that of aglycone. In this study, when we measured the antioxidant activity of the crude fractions by the OSI method (**Figure 3**), less polar fractions (CH_2Cl_2 extract and the CH_2Cl_2 soluble fraction) were more effective than polar fractions (BuOH and H_2O soluble fractions). On the basis of the combined results, it is suggested that the antioxidative properties measured by the OSI method are greater in less polar compounds with an *ortho*-dihydroxy phenyl group than polar compounds with the same group.

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