Synthesis and Biological Activity of α-Methylene-γ-lactones as New Aroma Chemicals

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Seven kinds of α-methylene-γ-lactones with an alkyl group at the C-4 position were synthesized according to a previously described method, with yields of 28−34%. These α-methylene-γ-lactones had characteristic and unique odors. All α-methylene-γ-lactones added a roast-like odor to materials. The antimicrobial effects of α-methylene-γ-lactones were investigated by using a paper disk diffusion method. The results showed the α-methylene-γ-lactones inhibited the growth of three bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens*) and two fungi (*Saccharomyces cerevisiae* and *Aspergillus niger*). In particular, α-methylene-γ-undecalactone and α-methylene-γ-dodecalactone exhibited potent inhibition of the growth of these microorganisms compared to butyl *p*-hydroxybenzoate as standard antibiotic. The *umu* test revealed that the α-methylene-γ-lactones suppressed the SOS-inducing activity of three mutagens, furylfuramide, UV irradiation, and Trp-P-1, respectively. The antimicrobial effects and the suppressive effects of SOS induction by α-methylene-γ-lactones had a tendency to intensify as the number of carbons in the side chain increased.

Keywords: *α-Methylene-γ-lactones; odor; antimicrobial activity; SOD-like activity; umu test*

INTRODUCTION

The α-methylene-γ-lactone skeleton is a common grouping in various naturally occurring terpenoid lactones. Their antibiotic (Park et al., 1987; Goren et al., 1990), antitumor (Hall et al., 1978), and insecticidal (Kim et al., 1998) activities and other biological functions have been studied. As a result of various investigations, it was found that one of the structural requirements for significant cytotoxicity was an exocyclic double-bond system as part of an ester, ketone, or lactone (Lee et al., 1975). In addition, γ-lactones are used frequently as fragrance or flavor compounds. However, there are no studies about aroma properties and other biological activities of α-methylene-γ-lactones with an alkyl group at the C-4 position.

In recent years, the occurrence of food poisoning caused by microorganisms, such as enteropathogenic *Escherichia coli* O-157, has increased. Inhibition of microorganisms for food safety is therefore an important issue. Furthermore, ordinary human diets contain several mutagens such as products of heat degradation, food ingredients, and various chemicals. For these reasons, γ-lactones with several functions (e.g., antimicrobial activity, antitumor activity, and so on) maybe valuable as aroma compounds.

In this study, α-methylene-γ-lactones with an alkyl group at the C-4 position were synthesized according to a previously described method (Minato and Horibe, 1967), and their aromas were examined. In addition, α-methylene-γ-lactones were investigated for several biological activities, including antimicrobial activity, SOD-like activity, and suppression of SOS-inducing activity. Structure−property relationships for each biological activity were examined.

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MATERIALS AND METHODS

General Procedures. GC-MS was performed on a Hewlett-Packard 5972 series mass spectrometer interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with an HP-5MS column (30 m × 0.25 mm i. d.). High-resolution MS was carried out with a JEOL-HX100 (with a JEOL JMA-DA 5000 mass data system) apparatus. IR spectra were determined with a Perkin-Elmer 1760-X infrared Fourier transform spectrometer. Nuclear magnetic resonance (NMR) spectra (δ, J in hertz) were recorded on a JEOL GSX 270 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H NMR spectra measured in $CDCl_3$. This solvent was also used for ^{13}C NMR spectra.

Materials. Butyl *p*-hydroxybenzoate was purchased from Tokyo Kasei Kohgyo Co. Ltd. (Tokyo, Japan). Lactose broth and potato dextrose were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Agar powder was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Furylfuramide, Trp-P-1, nitro blue tetrazolium (NBT), xanthine oxidase (XOD), and xanthine were purchased from Wako Pure Chemicals Co. (Osaka, Japan). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). S9 (supernatant of 9000 g) and coenzymes NADPH, NADH, and G-6-P were purchased from Oriental Yeast Co. (Osaka, Japan). All other reagents were of analytical grade.

Microorganisms. The synthetic α-methylene-γ-lactones were tested for antimicrobial activity against the Gram-

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positive bacteria *Staphylococcus aureus* IFO 14462, the Gram-negative bacteria *Escherichia coli* IFO 12734 and *Pseudomonas fluorescens* IFO 3081, and the fungi *Saccharomyces cerevisiae* SW-33 and *Aspergillus niger* IFO 4414.

Synthesis (Minato and Horibe, 1967). A solution of γ-lactone (20 mmol) and ethyl formate (25 mmol) in dry ether (30 mL) was added dropwise to a suspension of sodium hydride (20 mmol) in dry ether (15 mL) with stirring during 15 min in an ice bath. The mixture was stirred for 3 h at room temperature and set aside overnight. The sodium salt of the α-formyl-γ-lactone was separated and added to 2 N hydrochloric acid (30 mL) with stirring in an ice bath. The solution was extracted with ether, washed with saturated sodium chloride solution, dried (Na_2SO_4), and evaporated to give a light yellow oil. The crude product was dissolved in methanol (45 mL) and reduced with sodium borohydride (10 mmol) with stirring for 1 h at room temperature to give a viscous oil. This was chromatographed on silica gel (eluent: CH_2Cl_2/acetone) to give an α-hydroxymethyl-γ-lactone. The α-hydroxymethyl-γ-lactone was tosylated with toluene-*p*-sulfonyl chloride (8 mmol) in pyridine (15 mL). The tosylate was dissolved in pyridine and refluxed for 4−6 h to give crude product. This was chromatographed on silica gel (eluent: CH_2Cl_2/acetone) to give α-methylene-γ-lactone.

α-*Methylene-γ-hexalactone (**8**).* The lactone was obtained as a colorless oil (781 mg, 31%): HRMS *m*/*z* 126.0683 ([M$^+$], calcd for $C_7H_{10}O_2$, 126.0681); El-MS *m*/*z* (rel intensity) 126 [M$^+$] (12), 111 (tr), 97 (100), 69 (35), 41 (32); IR ν_{max} (KBr) cm^{-1} 2971, 1762, 1666, 1278, 1119, 963; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-7a), 5.63 (1H, *dd*, *J* = 3.0 and 2.5, H-7b), 4.47 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.59 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.81−1.63 (2H, *m*, H-5), 1.01 (3H, *t*, *J* = 7.5, H-6); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.7 (*s*, C-2), 121.8 (*t*, C-7), 78.6 (*d*, C-4), 33.0 (*t*, C-3), 29.1 (*t*, C-5), 9.0 (*q*, C-6).

α-*Methylene-γ-heptalactone (**9**).* The lactone was obtained as a colorless oil (952 mg, 34%): HRMS *m*/*z* 140.0836 ([M$^+$], calcd for $C_8H_{12}O_2$, 140.0837); El-MS *m*/*z* (rel intensity) 140 [M$^+$] (3), 125 (tr), 111 (4), 97 (100), 69 (27), 41 (30); IR ν_{max} (KBr) cm^{-1} 2963, 1762, 1667, 1274, 1118, 1003; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-8a), 5.62 (1H, *dd*, *J* = 3.0 and 2.5, H-8b), 4.53 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.77−1.55 (2H, *m*, H-5), 1.55−1.39 (2H, *m*, H-6), 0.97 (3H, *t*, *J* = 7.5, H-7); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.8 (*s*, C-2), 121.8 (*t*, C-8), 77.3 (*d*, C-4), 38.3 (*t*, C-3), 33.5 (*t*, C-5), 18.1 (*t*, C-6), 13.7 (*q*, C-7).

α-*Methylene-γ-octalactone (**10**).* The lactone was obtained as a colorless oil (864 mg, 28%): HRMS *m*/*z* 154.0995 ([M$^+$], calcd for $C_9H_{14}O_2$, 154.0994); El-MS *m*/*z* (rel intensity) 154 [M$^+$] (tr), 139 (tr), 125 (3), 111 (2), 97 (100), 69 (27), 41 (26); IR ν_{max} (KBr) cm^{-1} 2931, 1763, 1666, 1278, 1117, 1005; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-9a), 5.63 (1H, *dd*, *J* = 3.0 and 2.5, H-9b), 4.52 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.82−1.54 (2H, *m*, H-5), 1.52−1.28 (4H, *m*, H-6, 7), 0.92 (3H, *t*, *J* = 7.0, H-8); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.7 (*s*, C-2), 121.8 (*t*, C-9), 77.5 (*d*, C-4), 35.9 (*t*, C-3), 33.5 (*t*, C-5), 26.9 (*t*, C-6), 22.3 (*t*, C-7), 13.8 (*q*, C-8).

α-*Methylene-γ-nonalactone (**11**).* The lactone was obtained as a colorless oil (1042 mg, 31%): HRMS *m*/*z* 168.1150 ([M$^+$], calcd for $C_{10}H_{16}O_2$, 168.1151); El-MS *m*/*z* (rel intensity) 168 [M$^+$] (tr), 153 (tr), 139 (3), 111 (3), 97 (100), 69 (25), 41 (25); IR ν_{max} (KBr) cm^{-1} 2922, 1734, 1667, 1457, 937, 814; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-lOa), 5.63 (1H, *dd*, *J* = 3.0 and 2.5, H-lOb), 4.52 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.50−1.41 (2H, *m*, H-5), 1.36−1.28 (6H, *m*, H-6, 7, 8), 0.90 (3H, *t*, *J* = 7.0, H-9); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.8 (*s*, C-2), 121.8 (*t*, C-10), 76.6 (*d*, C-4), 36.2 (*t*, C-3), 33.5 (*t*, C-5), 31.4 (*t*, C-6), 24.5 (*t*, C-7), 22.4 (*t*, C-8), 13.9 (*q*, C-9).

α-*Methylene-γ-decalactone (**12**).* The lactone was obtained as a colorless oil (1201 mg, 33%): HRMS *m*/*z* 182.1305 ([M$^+$], calcd for $C_{11}H_{18}O_2$, 182.1307); EI-MS *m*/*z* (rel intensity) 182 [M$^+$] (tr), 153 (2), 140 (14), 111 (3), 97 (100), 69 (26), 41 (32); IR ν_{max} (KBr) cm^{-1} 2930, 1762, 1667, 1276, 1118, 1010; ^{1}H NMR ($CDCl_3$) δ_H 6.21 (1H, *dd*, *J* = 3.0 and 2.5, H-11a), 5.62 (1H, *dd*, *J* = 3.0 and 2.5, H-11b), 4.51 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.78−1.68 (2H, *m*, H-5), 1.36−1.22 (8H, *m*, H-6, 7, 8, 9), 0.89 (3H, *t*, *J* = 7.0, H-10); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.8 (*s*, C-2), 121.8 (*t*, C-11), 76.5 (*d*, C-4), 36.2 (*t*, C-3), 33.5 (*t*, C-5), 31.6 (*t*, C-6), 28.9 (*t*, C-7), 24.7 (*t*, C-8), 22.5 (*t*, C-9), 14.0 (*q*, C-10).

α-*Methylene-γ-undecalactone (**13**).* The lactone was obtained as a colorless oil (917 mg, 29%): HRMS *m*/*z* 196.1466 ([M$^+$], calcd for $C_{12}H_{20}O_2$, 196.1464); El-MS *m*/*z* (rel intensity) 196 [M$^+$] (tr), 178 (tr), 163 (tr), 140 (13), 111 (7), 97 (100), 69 (27), 41 (28); IR ν_{max} (KBr) cm^{-1} 2931, 1767, 12667, 1278, 1118, 1000; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-12a), 5.62 (1H, *dd*, *J* = 3.0 and 2.5, H-12b), 4.51 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.05 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-12a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-12b), 1.36−1.20 (12H, *m*, H-5, 6, 7, 8, 9, 10), 0.88 (3H, *t*, *J* = 7.0, H-11); ^{13}C NMR ($CDCl_3$) δ_C 170.0 (*s*, C-1), 136.0 (*s*, C-2), 122.0 (*t*, C-12), 83.8 (*d*, C-4), 41.3 (*t*, C-3), *26.5* (*t*, *C-5*), 39.4 (*t*, C-6), 31.6 (*t*, C-7), 29.4 (*t*, C-8), 23.6 (*t*, C-9), 22.5 (*t*, C-10), 14.0 (*q*, C-11).

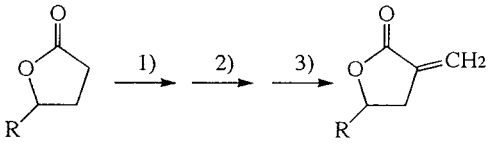
α-*Methylene-γ-dodecalactone (**14**).* The lactone was obtained as a colorless oil (882 mg, 31%): HRMS *m*/*z* 210.1619 ([M$^+$], calcd for $C_{13}H_{22}O_2$, 210.1620); El-MS *m*/*z* (rel intensity) 210 [M$^+$] (tr), 192 (tr), 153 (1), 140 (12), 111 (6), 97 (100), 69 (31), 41 (35); IR ν_{max} (KBr) cm^{-1} 2927, 1767, 1667, 1278, 1118, 1003; ^{1}H NMR ($CDCl_3$) δ_H 6.22 (1H, *dd*, *J* = 3.0 and 2.5, H-13a), 5.62 (1H, *dd*, *J* = 3.0 and 2.5, H-13b), 4.52 (1H, *tt*, *J* = 7.5 and 6.0, H-4), 3.06 (1H, *dddd*, *J* = 17.0, 7.5, 3.0, and 2.5, H-3a), 2.58 (1H, *dddd*, *J* = 17.0, 6.0, 3.0, and 2.5, H-3b), 1.36−1.18 (14H, *m*, H-5, 6, 7, 8, 9, 10, 11), 0.88 (3H, *t*, *J* = 7.0, H-12); ^{13}C NMR ($CDCl_3$) δ_C 170.3 (*s*, C-1), 134.8 (*s*, C-2), 121.8 (*t*, C-13), 76.5 (*d*, C-4), 36.3 (*t*, C-3), 33.5 (*t*, C-5), 31.7 (*t*, C-6), 29.4 (*t*, C-7), 29.3 (*t*, C-8), 29.1 (*t*, C-9), 24.8 (*t*, C-10), 22.6 (*t*, C-11), 14.1 (*q*, C-12).

Evaluation of Antimicrobial Activity. The paper disk diffusion method was used to determine the activity of synthetic compounds on test microorganisms. The overnight cultures of microorganisms were spread over (10^6 cfu/plate) the appropriate media (bacteria, lactose broth; fungi, potato dextrose) in Petri plates (90 mm). Paper disks (8 mm) impregnated with DMSO solutions of the test compounds were placed on the air-dried surface of the media seeded with the respective microorganisms. After incubation (bacteria, 37 °C for 24 h; fungi, 30 °C for 48 h), the zones of inhibition around the disks were measured. The antimicrobial effects of compounds that produced zones of inhibition >2 mm against bacteria were quantified using the broth dilution method (Gotou et al., 1983) and minimum inhibitory concentration (MIC) values were determined. Broths of serial 2-fold dilution of the compound dissolved in DMSO (maximum concentration = 800 μg/mL) were made, and the tubes were inoculated with microorganisms at a density of 10^5 cfu/mL. After incubation (37 °C for 18 h), the tubes were visually examined for growth of bacteria. In both methods, DMSO was used as a control.

SOD-like Activity Test. The method for the SOD-like activity assay of test compounds was carried out according to the NBT (IV) improved method of Beauchamp et al. (1971). A buffer of 0.3 M Na_2CO_3−$NaHCO_3$ (pH 10.2) containing 0.6 mM EDTA·2Na, 0. 15 mM NBT (nitro blue tetrazolium), 0.3 mM xanthine, and 0.15 w/v % BSA was mixed. The solution was subdivided into 2.5 mL portions in each test tube. The test compounds (0. 1 mL in DMSO, end concentration = 0.33 mg/mL), ethanol (0.1 mL), and water (0.2 mL) were added to each test tube. In the tube without xanthine oxidase (XOD), 0.3 mL of water was added. After 10 min of incubation at 25 °C, the enzyme reaction was initiated by the addition of XOD (0. 1 mL). After 20 min of incubation at 25 °C, the absorbance at 560 nm was measured. The intensity of the SOD-like activity was calculated as

$$\text{intensity of activity} = [B - (A - C)]/B \times 100\ (\%)$$

Scheme 1. Synthesis of α-Methylene-γ-lactones[a]



(**1**) - (**7**) (**8**) - (**14**)

(**1**) R = C_2H_5 (**8**) R = C_2H_5
(**2**) R = C_3H_7 (**9**) R = C_3H_7
(**3**) R = C_4H_9 (**10**) R = C_4H_9
(**4**) R = C_5H_{11} (**11**) R = C_5H_{11}
(**5**) R = C_6H_{13} (**12**) R = C_6H_{13}
(**6**) R = C_7H_{15} (**13**) R = C_7H_{15}
(**7**) R = C_8H_{17} (**14**) R = C_8H_{17}

[a] 1, NaH, HCO_2Et; 2, $NaBH_4$; 3, TsCl.

where *A* is the absorbance at 560 nm of sample with XOD, *B* is the absorbance at 560 nm of DMSO with XOD, and *C* is the absorbance at 560 nm of sample without XOD.

***Umu* Test.** The method for the *umu* test for detecting SOS-inducing activity of chemicals was carried out according to that of Oda et al. (1985) using *Salmonella typhimurium* TA1535/pSK1002, having plasmid pSK1002 carrying a *umuC′-′lac*Z fused gene. The overnight culture of bacterial strain was diluted 50-fold into TGA medium (1% Bactotryptone, 0.5% NaCl, and 0.2% glucose supplemented with 50 mg/L of ampicillin) and incubated at 37 °C until the bacterial density reached 0.25−0.30 at 600 nm. The bacterial culture was subdivided into 2.1 mL portions in test tubes, and the test compound (50 μL), 0.1 M phosphate buffer (300 μL, pH 7.4), and mutagens (50 μL in DMSO) were added to each tube. In the case of Trp-P-1, S9 mix was added in each tube instead of phosphate buffer. After 2 h of incubation at 37 °C with shaking, the culture was centrifuged (3000 rpm) to collect cells, which were resuspended in 2.5 mL of PBS. The level of β-galactosidase activity was measured by using a slight modification of Miller's method (Miller, 1972). Fractions (0.25 mL) of the culture were diluted with 2.25 mL of Z buffer and 0. 1% SDS solution (50 μL) and chloroform (10 μL) were added to each fraction. The enzyme reaction was initiated by the addition of 0.25 mL of 2-nitrophenyl β-D-galactopyranoside solution (ONPG, 4 mg/mL in 0.1 M phosphate buffer, pH 7.4) at 28 °C. After 15 min, 0.1 M Na_2CO_3 was added, and the absorbance at 420 and 500 nm was measured. Using the remainder of culture, the bacterial density was measured at 600 nm. The unit of β-galactosidase activity was calculated according to the method of Miller (1972).

UV Irradiation as Mutagen. The overnight cultured cells (*S. typhimunuium* TA1535/pSK1002) were diluted 50-fold with fresh TGA medium and incubated at 37 °C until the bacterial density at 600 nm reached 0.25−0.30 with 5 mL of 0. 1 M phosphate buffer. The cells were transferred to a Petri plate (40 mm) and UV-irradiated for 5 s (2 J/m^2) with a germicidal lamp at room temperature.

RESULTS AND DISCUSSION

α-Methylene-γ-lactones were easily synthesized according to the method of Minato and Horibe (1967) (Scheme 1). The synthetic α-methylene-γ-lactones had unique odors, which were roast-like in character (described in Table 1). From the results of the antimicrobial test (Table 2), compounds **9—14** were observed to inhibit the growth of bacteria (*S. aureus*, *E. coli*, and *P. fluorescens*) and fungi (*S. cerevisiae* and *A. niger*). α-Methylene-γ-hexalactone (**8**) inhibited the growth of only fungi. Butyl *p*-hydroxybenzoate as a standard antibiotic inhibited the growth of all microorganisms. In particular, α-methylene-γ-undecalactone (**13**) and α-methylene-γ-dodecalactone (**14**) exhibited potent inhibition to the growth of bacteria similar to butyl *p*-hydroxybenzoate as a standard antibiotic. The MIC values of compounds **9—14** against *S. aureus* were 400, 400, 200, 100, 50, and 50 μg/mL, respectively. The MIC values of compounds **9—14** against *E. coli* were 400, 200, 200, 100, 50, and 25 μg/mL, respectively. The MIC values of compounds **9—14** against *P. fluorescens* were 800, 800, 400, 200, 100, and 50 μg/mL, respectively. According to the MIC value, the antimicrobial effects of α-methylene-γ-lactones with respect to bacteria had a tendency to intensify as the number of carbons in the side chain increased. From the diameter of the inhibition zones (Figure 1), the antimicrobial effects of α-methylene-γ-lactones on fungi were greatest when the number of carbons in the side chain was five (α-methylene-γ-nonalactone).

The results of the superoxide dismutase (SOD-like) activity test showed (Table 3) that compounds **9**, **10**, **11**, and **12** exhibited 1, 2, 4, and 1% of SOD-like activity at a concentration of 0.33 mg/mL, respectively, whereas

Table 1. Aroma Characteristics of α-Methylene-γ-lactones

α-methylene-γ-	aroma characteristics
hexalactone (**8**)	roast; coconut, coumarin, herbal
heptalactone (**9**)	roast; nut, coumarin
octalactone (**10**)	roast; coconut, herbal
nonalactone (**11**)	roast; peach, coconut, cream
decalactone (**12**)	roast; mild, peach, cream
undecalactone (**13**)	roast; peach, fatty
dodecalactone (**14**)	roast; peach, fatty

Table 2. Antimicrobial Activity of α-Methylene-γ-lactones

	microorganism[a]				
compd	1	2	3	4	5
8	−	−	−	++++	++++
9	++	++	+	++++	++++
10	++	+++	+++	++++	++++
11	++	+++	+++	++++	++++
12	++	++	+++	++++	++++
13	+++	++	+++	++	++++
14	++	++	++	+	+++
BHB[b]	+++	+++	++	+++	++++

[a] Microorganisms: 1, *S. aureus*; 2, *E. coli*; 3, *P. fluorescens*; 4, *S. cerevisiae*; 5, *A. niger*. The results are the average of three readings. Inhibition zones: ++++, >15 mm; +++, 11−15 mm; ++, 6−10 mm; +, 2−5 mm; −, <2 mm. Dose of the compound: 1 mg/disk. [b] Standard antibiotic: butyl *p*-hydroxybenzoate.

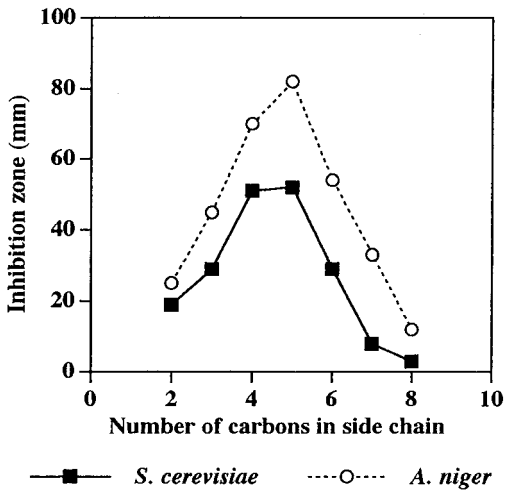


Figure 1. Inhibition zone of α-methylene-γ-lactones against fungi.

Table 3. SOD-like Activity of α-Methylene-γ-lactones by NBT(IV) Method

compd[a]	activity (%)	compd[a]	activity (%)
8	inactive	**12**	1
9	1	**13**	inactive
10	2	**14**	inactive
11	4		
TP[b]	75		

[a] Dose of the compound: 0.33 mg/tube. [b] Standard antioxidant: *dl*-α-tocopherol.

compounds **8**, **13**, and **14** did not exhibit SOD-like activity. *dl*-α-Tocopherol, as a control, exhibited 75% of SOD-like activity at the same concentration. On the basis of these results, α-methylene-γ-lactones had little SOD-like activity. Thus, those compounds did not quench or scavenge free radicals (superoxide anion). From the results of the *umu* test, the suppressive effects of compounds **8—14** were determined (Figure 2). All compounds inhibited the SOS induction of furylfuramide. Compounds **8**, **9**, and **11—13** suppressed 39, 38, 98, 85, and 97% of the SOS-inducing activity of furylfuramide at a concentration of 0.04 mg/mL, respectively. Compounds **10** and **14** suppressed 41 and 92% of the SOS-inducing activity of furylfuramide at a concentration of 0.01 mg/mL, respectively. Additionally, compounds **8—14**, except for **9**, showed suppression of the SOS-inducing activity of UV irradiation. Compounds **8** and **11—13** suppressed 20, 65, 88, and 97% of the SOS-inducing activity of UV irradiation at a concentration of 0.04 mg/mL, respectively. Compounds **10** and **14** suppressed 21 and 65% of the SOS-inducing activity of UV irradiation at a concentration of 0.01 mg/mL, respectively. Furthermore, compounds **8—14** also showed suppression of the SOS-inducing activity of Trp-P-1, which requires metabolic activation. Compounds **8**, **9**, and **11—13** suppressed 14, 66, 83, 43, and 72% of the SOS-inducing activity of Trp-P-1 at a concentration of 0.04 mg/mL, respectively, whereas compounds **10** and **14** suppressed 10 and 14% of the SOS-inducing activity of Trp-P-1 at a concentration of 0.01 mg/mL. This was the maximum concentration of compounds **10** and **14** that could be tested because of their cytotoxicity toward *S. typhimurium*. From these results, compounds **8—14** showed similar suppressive effects on furylfuramide-, UV irradiation-, and Trp-P-1-induced SOS response, and these effects intensified as the number of carbons in the side chain increased. The increase in antibiotic effects and suppressive effects on SOS induction with the increase in the side chain length may reflect the ability of α-methylene-γ-lactones to pass through the phospholipid bilayer as a result of the increasing hydrophobicity.

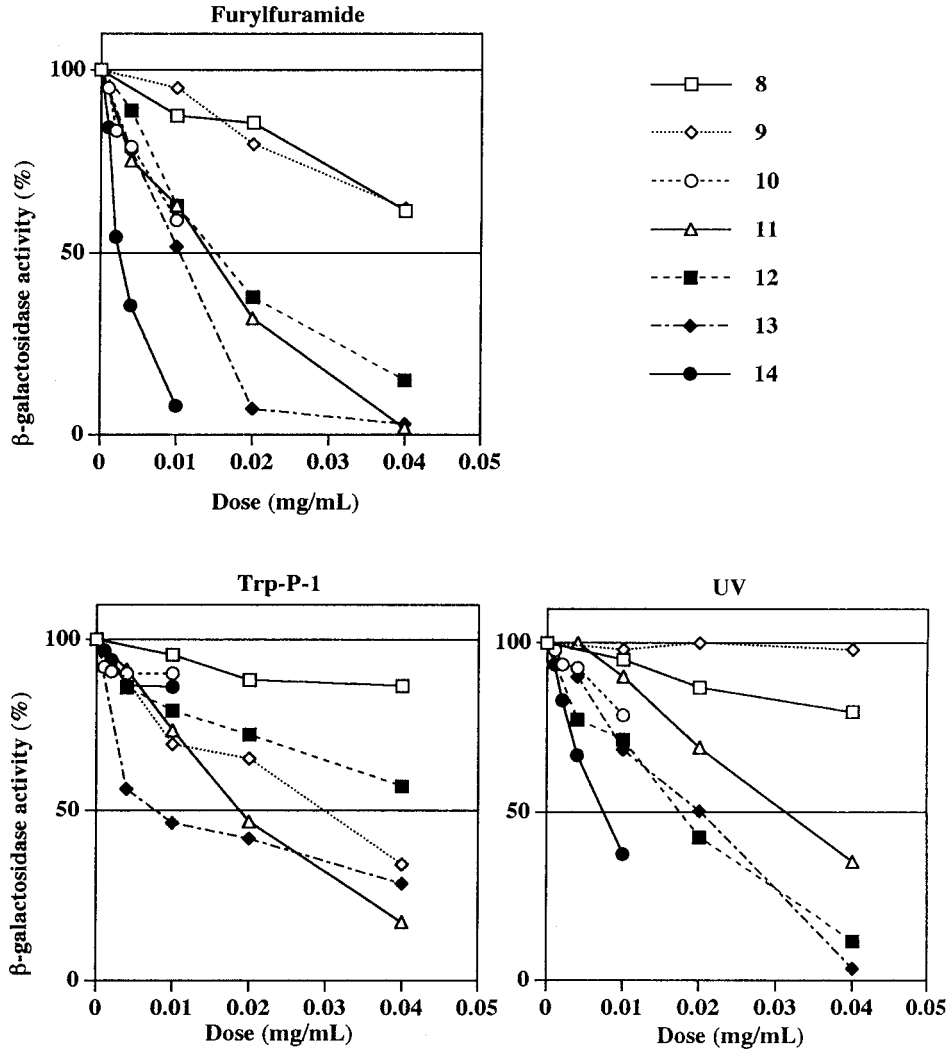


Figure 2. Suppression of furylfuramide-, Trp-P-1-, and UV-induced SOS responses by α-methylene-γ-lactones in *S. typhimurium* TA 1535/pSK1002. Furylfuramide (1 μg/mL in DMSO) was added at 50 μL. Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL. The cells were exposed to UV light (2.0 J/m^2) with a germicidal lamp at room temperature.

Further studies will be needed to clarify the mechanisms of antimicrobial effects and suppressive effects of SOS induction by α-methylene-γ-lactones.

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