JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antimicrobial and Bactericidal Activities of Esters of 2-*endo*-Hydroxy-1,8-cineole as New Aroma Chemicals

MITSUO MIYAZAWA* AND YUYA HASHIMOTO

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan

Fourteen kinds of alkyl esters of 2-*endo*-hydroxy-1,8-cineole were synthesized, with yields of 57.8−98.0%. Each ester had a characteristic and unique odor. Especially, the *tert*-butyl acetate of 2-*endo*-hydroxy-1,8-cineole had the most interesting odor of all the synthetic esters. The antimicrobial and bactericidal activities of these synthetic esters against test bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens*) were examined using the broth dilution method. As a result, the *tert*-butyl acetate of 2-*endo*-hydroxy-1,8-cineole showed the highest antimicrobial and bactericidal activities against all kinds of the test bacteria.

KEYWORDS: Esters of 2-*endo*-hydroxy-1,8-cineole; odor; antimicrobial activity; bactericidal activity

INTRODUCTION

Previously, we have studied the synthesis and biological activity of α-methylene-γ-lactones as new aroma chemicals; we found that these new lactones had a characteristic odor, showed antimicrobial activity, and suppressed SOS-inducing activity (*1*).

The acetate of 2-*endo*-hydroxy-1,8-cineole [named "acetate (**3a**)" in this paper], which is a terpenyl ester, was found as one of the characteristic aroma components of the rhizomes from *Alpinia galanga* Willd (*2*). Furthermore, compound **3a** has been synthesized to investigate the odor in detail (*3*, *4*), but, there has been no discussion about any biological activities of compound **3a**.

A series of esters of 2-hydroxy-1,8-cineole were synthesized and tested for their olfactive character (*5*). All of the tested esters of 2-hydroxy-1,8-cineole in the cited work were mixtures of the 2-*endo* and 2-*exo* forms.

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. For these reasons, esters of 2-*endo*-hydroxy-1,8-cineole with several functions (e.g., antimicrobial and bactericidal activities) may be valuable as aroma compounds.

In this study, 2-*endo*-hydroxy-1,8-cineole and its esters were synthesized by referring to previously described methods (*6*−*8*), and their aromas were examined. In addition, the antimicrobial and bactericidal activities of the synthetic esters were investigated. Finally, structure−property relationships for the antimicrobial and bactericidal activities and the potential use of these esters were examined.

* Author to whom correspondence should be addressed (telephone +81-6-6721-2332, ext. 4153; fax +81-6-6727-4301; e-mail miyazawa@apch.kindai.ac.jp).

MATERIALS AND METHODS

General Procedures. Optical rotations were measured on a Japan Spectroscopic Co. LTD DIP-1000 in $CHCl_3$. GC-MS was performed on a Hewlett-Packard 5972A series mass spectrometer interfaced with a Hewlett-Packard 5890A 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 ^1H NMR spectra measured in $CDCl_3$. This solvent was also used for ^{13}C NMR spectra.

Materials. α-Terpineol (**1**) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *m*-Chloroperoxybenzoic acid was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). The acyl chlorides used in this study were purchased from Tokyo Kasei Kohgyo Co., Ltd. (Tokyo, Japan). Butyl *p*-hydroxybenzoate was also purchased from Tokyo Kasei Kohgyo Co., Ltd. Glucose was purchased from Wako Pure Chemical Industries, Ltd. Yeast extract and nutrient broth were purchased from Difco Laboratories (Detroit, MI). Lactose broth was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Agar powder was purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Microorganisms. The synthetic 2-*endo*-hydroxy-1,8-cineole and its ester derivatives were tested for antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* IFO 14462, and the Gram-negative bacteria *Escherichia coli* IFO 12734 and *Pseudomonas fluorescens* IFO 3081.

Synthesis of 2-*endo*-Hydroxy-1,8-cineole. A solution of α-terpineol (**1**) [3.0 g, $[\alpha]_D^{25}$ −15.0° (*c* 1.00 in $CHCl_3$), 36.3% ee] in CH_2Cl_2 (50 mL) was added dropwise to a suspension of *m*-chloroperbenzoic acid (4.8 g) in CH_2Cl_2 (50 mL) with stirring during 15 min in an ice bath. The mixture was stirred for 2 h at 0 °C. The mixture was filtrated and treated successively three times with 5% $NaHSO_3$ (100 mL each time), three times with 5% $NaHCO_3$ (100 mL each time), and with water (100 mL), then dried with Na_2SO_4, and evaporated to give a colorless oil. This oil was chromatographed on silica gel (eluent: hexane/ether) to give 2-*endo*-hydroxy-1,8-cineole (**2**).

10.1021/jf011555w CCC: $22.00
Published on Web 05/02/2002

*2-endo-Hydroxy-1,8-cineole (**2**) [(1R,2R,4S)-1,3,3-Trimethyloxabicyclo-[2.2.2]octan-6-ol].* The alcohol was obtained as a colorless needle-like crystal (2.0 g, 60.4%): $[\alpha]_D^{25}$ −7.9° (*c* 1.53 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 170.1305 ([M$^+$], calcd for $C_{10}H_{18}O_2$, 170.1307); EI-MS, *m/z* (rel intensity) 170 [M$^+$] (10), 155 (tr), 137 (1), 126 (44), 111 (34), 109 (12), 108 (65), 93 (34), 83 (40), 71 (63), 69 (37), 58 (19), 55 (21), 43 (100); IR ν_{max} (KBr) cm^{-1} 3445, 2966, 1457, 1364, 1063, 1034, 978; ^{1}H NMR ($CDCl_3$) δ_H 3.73 (1H, ddd, *J* = 10.0, 4.0, 2.0 Hz, H-2$_{exo}$), 2.52 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.01−1.85 (2H, m, H-5, H-6), 1.58−1.48 (3H, m, H4, H-5, H-6), 1.31 (1H, ddd, *J* = 15.0, 4.0, 3.0, H-3$_{endo}$), 1.28 (3H, s, H-9), 1.20 (3H, s, H-10), 1.10 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 73.4 (*s*, C-8), 72.5 (*s*, C-1), 71.1 (d, C-2), 34.6 (t, C-3), 34.2 (d, C-4), 29.0 (q, C-10), 28.6 (q, C-9), 24.9 (t, C-6), 24.0 (q, C-7), 22.1 (t, C-5).

Synthesis of Esters of 2-*endo*-Hydroxy-1,8-cineole. Each acyl chloride (1.2 equiv mol) was added dropwise with stirring to 2-*endo*-hydroxy-1,8-cineole (**2**) (100.0 mg, 1.0 equiv mol) and pyridine (56.0 mg, 1.2 equiv mol) in an organic solvent (ether or pyridine) (30 mL). Then, the solution was heated at 25 or 130 °C for 1−5 h. After cooling, the mixture was poured into water (100 mL) and the aqueous solution was extracted thoroughly with ether. The combined ether extracts were washed successively three times with 5% HCl (100 mL each time), three times with 5% $NaHCO_3$ (100 mL each time), and then with water (100 mL), dried with Na_2SO_4, and evaporated to give a colorless oil. This oil was chromatographed on silica gel (eluent: hexane/ether) to give each ester **(3a−3n)** of compound **2**.

*Acetate (**3a**).* The ester was obtained as a colorless oil (122.2 mg, 98.0%): $[\alpha]_D^{25}$ −25.6° (*c* 1.25 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 212.1414 ([M$^+$], calcd for $C_{12}H_{20}O_3$, 212.1413); EI-MS, *m/z* (rel intensity) 212 [M$^+$] (9), 197 (tr), 170 (tr), 155 (2), 137 (2), 126 (17), 111 (15), 109 (17), 108 (36), 93 (19), 82 (17), 71 (24), 55 (10), 43 (100); IR υ_{max} (KBr) cm^{-1} 2973, 1742, 1457, 1376, 1241, 1027; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.06 (3H, s, H-12), 2.05−1.96 (1H, m, H-5), 1.87 (1H, ddd, *J* = 14.0, 11.0, 4.0, H-6$_{endo}$), 1.64−1.57 (1H, m, H-6$_{exo}$), 1.57−1.49 (2H, m, H-4, H-5), 1.32−1.27 (1H, m, H-3), 1.28 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 170.5 (s, C-11), 73.7 (s, C-8), 72.8 (d, C-2), 70.8 (s, C-1), 33.8 (d, C-4), 32.7 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 25.9 (t, C-6), 24.1 (q, C-7), 21.9 (t, C-5), 21.2 (q, C-12).

*Propionate (**3b**).* The ester was obtained as a colorless oil (117.7 mg, 88.5%): $[\alpha]_D^{25}$ −25.0° (*c* 1.21 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 226.1568 ([M$^+$], calcd for $C_{13}H_{22}O_3$, 226.1569); EI-MS, *m/z* (rel intensity) 226 [M$^+$] (13), 211 (1), 168 (1), 152 (3), 137 (5), 126 (46), 111 (31), 109 (38), 108 (100), 93 (42), 82 (44), 71 (48), 57 (95), 43 (100); IR ν_{max} (KBr) cm^{-1} 2976, 1736, 1458, 1364, 1159, 1022; ^{1}H NMR ($CDCl_3$) δ_H 4.70 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.62 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.33 (2H, q, *J* = 8.0, H-12), 2.05−1.96 (1H, m, H-5), 1.91−1.84 (1H, m, H-6), 1.64−1.57 (1H, m, H-6), 1.57−1.48 (2H, m, H-4, H-5), 1.31−1.25 (1H, m, H-3), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.15 (3H, t, *J* = 8.0, H-13), 1.04 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 173.7 (s, C-11), 73.6 (s, C-8), 72.5 (d, C-2), 70.8 (s, C-1), 33.8 (d, C4), 32.7 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 27.9 (t, C-12) 25.9 (t, C-6), 24.1 (q, C-7), 21.9 (t, C-5), 9.1 (q, C-13).

*Butyrate (**3c**).* The ester was obtained as a colorless oil (137.2 mg, 97.2%): $[\alpha]_D^{25}$ −24.2° (*c* 0.99 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 240.1725 ([M$^+$], calcd for $C_{14}H_{24}O_3$, 240.1726); El-MS, *m/z* (rel intensity) 240 [M$^+$] (10), 225 (tr), 183 (tr), 170 (tr), 152 (2), 137 (3), 126 (28), 111 (16), 109 (23), 108 (63), 93 (24), 82 (24), 71 (62), 55 (15), 43 (100); IR ν_{max} (KBr) cm^{-1} 2970, 1735, 1458, 1377, 1185, 1012; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.33 (2H, t, *J* = 8.0, H-12), 2.08−1.80 (2H, m, H-5, H-6), 1.75−1.45 (5H, m, H-4, H-5, H-6, H-13), 1.33−1.20 (1H, m, H-3), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.96 (3H, t, *J* = 7.0, H-14); ^{13}C NMR ($CDCl_3$) δ_C 173.1 (s, C-11), 73.7 (s, C-8), 72.5 (d, C-2), 70.9 (s, C-1), 36.5 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 24.1 (q, C-7), 22.0 (t, C-5), 18.5 (t, C-13), 13.6 (q, C-14).

*Valerate (**3d**).* The ester was obtained as a colorless oil (129.8 mg, 86.9%): $[\alpha]_D^{25}$ −22.2° (*c* 1.13 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 254.1882 ([M$^+$], calcd for $C_{15}H_{26}O_3$, 254.1883); El-MS, *m/z* (rel intensity) 254 [M$^+$] (11), 239 (1), 196 (tr), 168 (tr), 152 (3), 137 (4), 126 (45), 111 (22), 109 (36), 108 (100), 93 (34), 85 (41), 82 (36), 71 (34), 57 (46), 55 (29), 43 (93); IR ν_{max} (KBr) cm^{-1} 2965, 1737, 1458, 1377, 1182, 1017; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.62 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.31 (2H, t, *J* = 8.0, H-12), 2.08−1.93 (1H, m, H-5), 1.93−1.80 (1H, m, H-6), 1.68−1.45 (5H, m, H-4, H-5, H-6, H-13), 1.44−1.20 (3H, m, H-3, H-14), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.93 (3H, t, *J* = 7.0, H-15); ^{13}C NMR ($CDCl_3$) δ_C 173.2 (s, C-11), 73.7 (s, C-8), 72.5 (d, C-2), 70.8 (s, C-1), 34.3 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 27.1 (t, C-13), 26.0 (t, C-6), 24.1 (q, C-7), 22.2 (t, C-14), 22.0 (t, C-5), 13.7 (q, C-15).

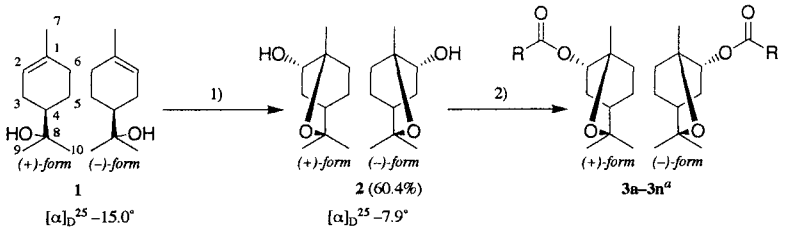
*Hexanoate (**3e**).* The ester was obtained as a colorless oil (134.3 mg, 85.2%): $[\alpha]_D^{25}$ −20.9° (*c* 1.15 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 268.2041 ([M$^+$], calcd for $C_{16}H_{28}O_3$, 268.2040); EI-MS, *m/z* (rel intensity) 268 [M$^+$] (9), 253 (tr), 210 (tr), 182 (tr), 170 (tr), 152 (3), 137 (3), 126 (35), 111 (16), 109 (28), 108 (78), 99 (20), 93 (26), 82 (24), 71 (36), 55 (23), 43 (100); IR ν_{max} (KBr) cm^{-1} 2932, 1736, 1458, 1378, 1181, 1017; ^{1}H NMR (CDCI3) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.44 (2H, t, *J* = 8.0, H-12), 2.08−1.80 (2H, m, H-5, H-6), 1.72−1.45 (5H, m, H-4, H-5, H-6, H-13), 1.42−1.20 (5H, m, H-3, H-14 H-15), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.91 (3H, t, *J* = 7.0, H-16); ^{13}C NMR ($CDCl_3$) δ_C 173.3 (s, C-11), 73.8 (s, C-8), 72.6 (d, C-2), 70.9 (s, C−l), 34.6 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 31.2 (t, C-14) 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 24.7 (t, C-13), 24.1 (q, C-7), 23.9 (t, C-15), 22.0 (t, C-5), 13.8 (q, C-16).

*Heptanoate (**3f**).* The ester was obtained as a colorless oil (134.5 mg, 81.1%): $[\alpha]_D^{25}$ −20.3° (*c* 1.02 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 282.2195 ([M$^+$], calcd for $C_{17}H_{30}O_3$, 282.2197); EI-MS, *m/z* (rel intensity) 282 [M$^+$] (8), 267 (tr), 224 (tr), 196 (tr), 183 (tr), 170 (tr), 152 (3), 137 (3), 126 (39), 111 (16), 109 (29), 108 (82), 93 (25), 82 (24), 71 (21), 55 (25), 43 (100); IR ν_{max} (KBr) cm^{-1} 2926, 1736, 1458, 1379, 1157, 1012; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.44 (2H, t, *J* = 8.0, H-12), 2.08−1.79 (2H, m, H-5, H-6), 1.72−1.44 (5H, m, H-4, H-5, H-6, H-13), 1.44−1.20 (7H, m, H-3, H-14, H-15, H-16), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.89 (3H, t, *J* = 7.0, H-17); ^{13}C NMR ($CDCl_3$) δ_C 173.3 (s, C-11), 73.8 (s, C-8), 72.6 (d, C-2), 70.9 (s, C-l), 34.0 (t, C-12), 33.8 (d, C-4), 32.7 (t, C-3), 31.4 (t, C-14, C-15), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-13), 26.0 (t, C-6), 24.1 (t, C-16), 24.1 (q, C-7), 22.0 (t, C-5), 14.0 (q, C-17).

*Octanoate (**3g**).* The ester was obtained as a colorless oil (138.9 mg, 79.8%): $[\alpha]_D^{25}$ −19.7° (*c* 0.85 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 296.2355 ([M$^+$], calcd for $C_{18}H_{32}O_3$, 296.2354); EI-MS, *m/z* (rel intensity) 296 [M$^+$] (9), 281 (tr), 238 (tr), 210 (tr), 197 (tr), 170 (1), 153 (5), 137 (3), 126 (51), 111 (17), 109 (36), 108 (100), 93 (28), 82 (28), 71 (25), 57 (41), 55 (33), 43 (90); IR ν_{max} (KBr) cm^{-1} 2931, 1737, 1458, 1380, 1157, 1012; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.62 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.30 (2H, t, *J* = 8.0, H-12), 2.08−1.79 (2H, m, H-5, H-6), 1.72−1.44 (5H, m, H-4, H-5, H-6, H-13), 1.38−1.20 (9H, m, H-3, H-14, H-15, H-16, H-17), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.88 (3H, t, *J* = 7.0, H-18); ^{13}C NMR ($CDCl_3$) δ_C 173.2 (s, C-11), 73.7 (s, C-8), 72.6 (d, C-2), 70.9 (s, C-l), 34.6 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 31.6 (t, C-16), 29.1 (t, C-14), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-15), 26.0 (t, C-6), 25.0 (t, C-13), 24.1 (q, C-7), 22.6 (t, C-17), 22.0 (t, C-5), 14.0 (q, C-18).

*Nonanoate (**3h**).* The ester was obtained as a colorless oil (169.0 mg, 92.7%): $[\alpha]_D^{25}$ −18.0° (*c* 0.93 in $CHCl_3$), 36.3% ee; HRMS, *m/z* 310.2510 ([M$^+$], calcd for $C_{19}H_{34}O_3$, 310.2511); EI-MS, *m/z* (rel intensity) 310 [M$^+$] (12), 295 (tr), 267 (tr), 252 (tr), 224 (tr), 185 (tr), 170 (1), 153 (6), 141 (12), 126 (53), 111 (17), 109 (35), 108 (100), 93 (27), 82 (26), 71 (37), 57 (25), 55 (37), 43 (98); IR ν_{max} (KBr) cm^{-1} 2927, 1737, 1458, 1378, 1156, 1012; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.35 (2H, t, *J* = 8.0, H-12), 2.10−1.80 (2H, m, H-5, H-6), 1.70−1.48 (5H, m, H-4, H-5, H-6, H-13), 1.42−1.20 (11H, m, H-3, H-14, H-15, H-16, H-17, H-18), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.88 (3H, t, *J* = 7.0, H-19); ^{13}C NMR ($CDCl_3$) δ_C

Scheme 1. Synthesis of Esters of 2-*endo*-Hydroxy-1,8-cineole: (1) Epoxidation with *m*-Chloroperoxybenzoic acid; (2) Acylation with Acyl Chloride



[a] Shown in **Table 1**.

173.3 (s, C-11), 73.8 (s, C-8), 72.6 (d, C-2), 71.0 (s, C-1), 34.6 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 31.8 (t, C-17), 29.7 (t, C-14), 29.4 (t, C-15), 29.1 (t, C-16), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 25.0 (t, C-13), 24.1 (q, C-7), 22.6 (t, C-18), 22.0 (t, C-5), 14.0 (q, C-19).

*Decanoate (**3i**).* The ester was obtained as a colorless oil (172.1 mg, 90.3%): $[\alpha]_D^{25}$ −16.9° (*c* 1.11 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 324.2666 ([M^+], calcd for $C_{20}H_{36}O_3$, 324.2668); EI-MS, *m*/*z* (rel intensity) 324 [M^+] (8), 309 (tr), 267 (tr), 238 (tr), 223 (tr), 209 (tr), 183 (tr), 170 (1), 155 (10), 135 (4), 126 (56), 111 (16), 109 (34), 108 (100), 93 (26), 82 (26), 71 (32), 55 (34), 43 (96); IR ν_{max} (KBr) cm^{-1} 2922, 1737, 1458, 1378, 1156, 1013; ^1H NMR ($CDCl_3$) δ_H 4.68 (1H, ddd, J = 10.0, 4.0, 2.0, H-2_{exo}), 2.62 (1H, dddd, J = 15.0, 10.0, 4.0, 3.0, H-3_{exo}), 2.30 (2H, t, J = 8.0, H-12), 2.05−1.96 (1H, m, H-5), 1.86 (1H, ddd, J = 14.0, 11.0, 4.0, H-6_{endo}), 1.66−1.47 (5H, m, H-4, H-5, H-6, H-13), 1.36−1.20 (13H, m, H-3, H-14, H-15, H-16, H-17, H-18, H-19), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.88 (3H, t, J = 7.0, H-20); ^{13}C NMR ($CDCl_3$) δ_C 173.3 (s, C-11), 73.7 (s, C-8), 72.6 (d, C-2), 70.9 (s, C-1), 34.7 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 31.8 (t, C-18), 29.7 (t, C-14), 29.4 (t, C-16), 29.3 (t, C-17), 29.1 (t, C-15), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 25.1 (t, C-13), 24.2 (q, C-7), 22.7 (t, C-19), 22.0 (t, C-5), 14.1 (q, C-20).

*Undecanoate (**3j**).* The ester was obtained as a colorless oil (187.5 mg, 94.3%): $[\alpha]_D^{25}$ −15.7° (*c* 0.89 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 338.2826 ([M^+], calcd for $C_{21}H_{38}O_3$, 338.2825); EI-MS, *m*/*z* (rel intensity) 338 [M^+] (6), 323 (1), 252 (tr), 169 (9), 153 (10), 135 (5), 126 (65), 111 (11), 109 (33), 108 (100), 93 (23), 82 (28), 71 (26), 55 (27), 43 (75); IR ν_{max} (KBr) cm^{-1} 2927, 1737, 1458, 1378, 1185, 1014; ^1H NMR ($CDCl_3$) δ_H 4.68 (1H, ddd, J = 10.0, 4.0, 2.0, H-2_{exo}), 2.62 (1H, dddd, J = 15.0, 10.0, 4.0, 3.0, H-3_{exo}), 2.30 (2H, t, J = 8.0, H-12), 2.08−1.79 (2H, m, H-5, H-6), 1.69−1.44 (5H, m, H-4, H-5, H-6, H-13), 1.37−1.19 (15H, m, H-3, H-14, H-15, H-16, H-17, H-18, H-19, H-20), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7), 0.88 (3H, t, J = 7.0, H-21); ^{13}C NMR ($CDCl_3$) δ_C 173.2 (s, C-11), 73.7 (s, C-8), 72.5 (d, C-2), 70.9 (s, C−l), 34.6 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 31.8 (t, C-19), 29.5 (t, C-15), 29.4 (t, C-18), 29.3 (t, C-16), 29.2 (t, C-14), 29.1 (t, C-17), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 25.0 (t, C-13), 24.1 (q, C-7), 22.6 (t, C-20), 22.0 (t, C-5), 14.1 (q, C-21).

*Isobutyrate (**3k**).* The ester was obtained as a colorless oil (125.9 mg, 89.2%): $[\alpha]_D^{25}$ −19.7° (*c* 0.85 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 240.1726 ([M^+], calcd for $C_{14}H_{24}O_3$, 240.1726); EI-MS, *m*/*z* (rel intensity) 240 [M^+] (6), 152 (2), 137 (3), 126 (29), 111 (15), 109 (22), 108 (64), 93 (24), 82 (26), 71 (55), 55 (11), 43 (100); IR ν_{max} (KBr) cm^{-1} 2975, 1736, 1472, 1364, 1154, 1012; ^1H NMR ($CDCl_3$) δ_H 4.68 (1H, ddd, J = 10.0, 4.0, 2.0, H-2_{exo}), 2.66−2.49 (2H, m, H-12, H-3_{exo}), 2.05−1.97 (1H, m, H-5), 1.91−1.84 (1H, ddd, J = 14.0, 11.0, 4.0, H-6_{endo}), 1.65−1.57 (1H, m, H-6), 1.56−1.48 (2H, m, H-4, H-5), 1.29 (3H, s, H-9), 1.28−1.23 (1H, m, H-3), 1.23 (3H, s, H-10), 1.17 (6H, d, J = 7.0, H-13, H-14), 1.04 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 176.4 (s, C-11), 73.8 (s, C-8), 72.4 (d, C-2), 70.9 (s, C-l), 34.1 (d, C-12), 33.8 (d, C-4), 32.7 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 24.1 (q, C-7), 22.0 (t, C-5), 18.9 (q, C-13), 18.8 (q, C-14).

*Pivaloate (**3l**).* The ester was obtained as a colorless oil (86.4 mg, 57.8%): $[\alpha]_D^{25}$ −21.5° (*c* 0.91 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 254.1882 ([M^+], calcd for $C_{15}H_{26}O_3$, 254.1883); EI-MS, *m*/*z* (rel intensity) 254 [M^+] (18), 239 (1), 196 (tr), 169 (tr), 153 (7), 137 (4), 126 (42), 111 (16), 109 (34), 108 (82), 93 (28), 82 (31), 71 (26), 57 (100), 43 (85); IR ν_{max} (KBr) cm^{-1} 2975, 1732, 1458, 1284, 1152, 1011; ^1H NMR ($CDCl_3$) δ_H 4.65 (1H, ddd, J = 10.0, 4.0, 2.0, H-2_{exo}), 2.61 (1H, dddd, J = 15.0, 10.0, 4.0, 3.0, H-3_{exo}), 2.09−1.94 (1H, m, H-5), 1.94−1.81 (1H, m, H-6), 1.68−1.58 (1H, m, H-6), 1.58−1.42 (2H, m, H-4, H-5), 1.29 (3H, s, H-9), 1.28−1.24 (1H, m, H-3), 1.22 (3H, s, H-10), 1.20 (9H, s, H-13, H-14, H-15), 1.04 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 177.7 (s, C-11), 73.7 (s, C-8), 72.3 (d, C-2), 70.9 (s, C-1), 38.5 (s, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 27.0 (q, C-13, C-14, C-15), 26.1 (t, C-6), 24.2 (q, C-7), 22.0 (t, C-5).

*Isovalerate (**3m**).* The ester was obtained as a colorless oil (133.7 mg, 89.5%): $[\alpha]_D^{25}$ −18.6° (*c* 0.94 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 254.1882 ([M^+], calcd for $C_{15}H_{26}O_3$, 254.1883); EI-MS, *m*/*z* (rel intensity) 254 [M^+] (13), 239 (tr), 211 (tr), 196 (tr), 181 (tr), 170 (1), 153 (4), 137 (4), 126 (40), 111 (20), 109 (33), 108 (87), 93 (32), 85 (35), 83 (29), 71 (27), 57 (50), 43 (100); IR ν_{max} (KBr) cm^{-1} 2965, 1735, 1466, 1376, 1158, 1016; ^1H NMR ($CDCl_3$) δ_H 4.68 (1H, ddd, J = 10.0, 4.0, 2.0, H-2_{exo}), 2.63 (1H, dddd, J = 15.0, 10.0, 4.0, 3.0, H-3_{exo}), 2.19 (2H, d, J = 8.0, H-12), 2.15−2.06 (1H, m, H-13), 2.05−1.97 (1H, m, H-5), 1.91−1.83 (1H, ddd, J = 14.0, 11.0, 4.0, H-6_{endo}), 1.64−1.56 (1H, m, H-6), 1.56−1.48 (2H, m, H-4, H-5), 1.31−1.25 (1H, m, H-3), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7),

Table 1. Synthesis of Esters of *2-endo*-Hydroxy-1,8-cineole

ester	R	time (h)	yield (%)	$[\alpha]_D^{25}$ (deg)
acetate (**3a**)	CH_3	1[a]	98.0	−25.6
propionate (**3b**)	C_2H_5	1[a]	88.5	−25.0
butyrate (**3c**)	C_3H_7	1[a]	97.2	−24.2
valerate (**3d**)	C_4H_9	1[a]	86.9	−22.2
hexanoate (**3e**)	C_5H_{11}	1[a]	85.2	−20.9
heptanoate (**3f**)	C_6H_{13}	1[a]	81.1	−20.3
octanoate (**3g**)	C_7H_{15}	1[a]	79.8	−19.7
nonanoate (**3h**)	C_8H_{17}	1[a]	92.7	−18.0
decanoate (**3i**)	C_9H_{19}	1[a]	90.3	−16.9
undecanoate (**3j**)	$C_{10}H_{21}$	2[a]	94.3	−15.7
isobutyrate (**3k**)	$CH(CH_3)_2$	2[a]	89.2	−19.7
pivaloate (**3l**)	$C(CH_3)_3$	1[b]	57.8	−21.5
isovalerate (**3m**)	$CH_2CH(CH_3)_2$	2[a]	89.5	−18.6
tert-butyl acetate (**3n**)	$CH_2C(CH_3)_3$	5[a]	64.7	−20.8

[a] Solvent, diethyl ether; temperature, 25 °C. [b] Solvent, pyridine; temperature, 130 °C.

Table 2. Odor Characteristics of Esters of *2-endo*-Hydroxy-1,8-cineole

ester	R	odor
acetate (**3a**)	CH_3	white, cineole-like, pear
propionate (**3b**)	C_2H_5	fruity, cineole-like, apple
butyrate (**3c**)	C_3H_7	carboxyl acid-like
valerate (**3d**)	C_4H_9	carboxyl acid-like
hexanoate (**3e**)	C_5H_{11}	green note
heptanoate (**3f**)	C_6H_{13}	earthy, heptanol-like
octanoate (**3g**)	C_7H_{15}	wax, weak, watery
nonanoate (**3h**)	C_8H_{17}	white, vegetable, melon-like
decanoate (**3i**)	C_9H_{19}	medicinal
undecanoate (**3j**)	$C_{10}H_{21}$	very weak, dusty
isobutyrate (**3k**)	$CH(CH_3)_2$	carboxyl acid-like
pivaloate (**3l**)	$C(CH_3)_3$	fresh topnote, spicy
isovalerate (**3m**)	$CH_2CH(CH_3)_2$	fruity, green, banana-like
tert-butyl acetate (**3n**)	$CH_2C(CH_3)_3$	apple-like, fresh fruity, green esteric

Table 3. Antimicrobial and Bactericidal Activities of Esters of 2-*endo*-Hydroxy-1,8-cineole

		S. aureus		*E. coli*		*P. fluorescens*	
ester	R[a]	MIC[b]	MBC[b]	MIC[b]	MBC[b]	MIC[b]	MBC[b]
acetate (**3a**)	CH_3	>400		>400		>400	
propionate (**3b**)	C_2H_5	>400		>400		>400	
butyrate (**3c**)	C_3H_7	200	400	200	200	200	200
valerate (**3d**)	C_4H_9	200	200	200	200	200	200
hexanoate (**3e**)	CH	200	200	200	200	200	200
heptanoate (**3f**)	C_6H_{13}	100	200	100	200	100	200
octanoate (**3g**)	C_7H_{15}	100	200	100	200	100	200
nonanoate (**3h**)	C_8H_{17}	100	200	100	200	100	200
decanoate (**3i**)	C_9H_{19}	100	200	100	200	100	200
undecanoate (**3j**)	$C_{10}H_{21}$	100	200	100	200	100	200
isobutyrate (**3k**)	$CH(CH_3)_2$	400	>400	400	>400	400	>400
pivaloate (**3l**)	$C(CH_3)_3$	200	200	200	200	200	200
isovalerate (**3m**)	$CH_2CH(CH_3)_2$	200	400	200	400	200	400
tert-butyl acetate (**3n**)	$CH_2C(CH_3)_3$	100	200	100	200	100	100
butyl *p*-hydroxybenzoate[c]		200	400	200	400	200	400

[a] Chemical structures are pictured in **Scheme 1**. [b] Concentration of chemicals in μg/mL. [c] Standard antibiotic.

0.97 (6H, d, J = 7.0, H-14, H-15); ^{13}C NMR ($CDCl_3$) δ_C 172.5 (s, C-11), 73.7 (s, 11 C-8), 72.6 (d, C-2), 70.8 (s, C-l), 43.7 (t, C-12), 33.8 (d, C-4), 32.8 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 26.0 (t, C-6), 25.8 (d, C-13), 24.2 (q, C-7), 22.4 (q, C-15), 22.3 (q, C-14), 22.0 (t, C-5).

*tert-Butyl Acetate (**3n**).* The ester was obtained as a colorless oil (102.0 mg, 64.7%): $[\alpha]_D^{25}$ −20.8° 14 (*c* 0.88 in $CHCl_3$), 36.3% ee; HRMS, *m*/*z* 268.2039 ([M^+], calcd for $C_{16}H_{28}O_3$, 268.2040); EI-MS, *m*/*z* (rel intensity) 268 [M^+] (11), 253 (1), 225 (tr), 210 (tr), 182 (tr), 169 (1), 153 (17), 135 (7), 126 (47), 111 (18), 109 (37), 108 (92), 99 (31), 93 (33), 82 (30), 71 (36), 57 (67), 55 (30), 43 (100); IR ν_{max} (KBr) cm^{-1} 2956, 1735, 1466, 1230, 1132, 1015; ^{1}H NMR ($CDCl_3$) δ_H 4.65 (1H, ddd, J = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.65 (1H, dddd, J = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.20 (2H, s, H-12), 2.05−1.96 (1H, m, H-5), 1.88 (1H, ddd, J = 14.0, 11.0, 4.0, H-6$_{endo}$), 1.65−1.56 (1H, m, H-6), 1.56−1.47 (2H, m, H-4, H-5), 1.33−1.27 (1H, m, H-3), 1.29 (3H, s, H-9), 1.22 (3H, s, H-10), 1.06 (3H, s, H-7), 1.04 (9H, s, H-14, H-15, H-16); ^{13}C NMR ($CDCl_3$) δ_C 171.8 (s, C-11), 73.7 (s, C-8), 72.7 (d, C-2), 70.8 (s, C−l), 48.3 (t, C-12), 33.8 (d, C-4), 33.0 (t, C-3), 30.9 (s, C-13), 29.7 (q, C-14, C-15, C-16), 28.8 (q, C-10), 28.5 (q, C-9), 26.1 (t, C-6), 24.3 (q, C-7), 22.0 (t, C-5).

Evaluation of Antimicrobial and Bactericidal Activities. The antimicrobial and bactericidal activities of the synthetic esters, which have a characteristic odor, against test bacteria were examined using the broth dilution method (*9*). Then, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Broths (glucose, 0.1%; yeast extract, 0.5%; nutrient broth, 0.8%) including serial 2-fold dilution of the ester dissolved in DMSO (maximum concentration = 400 μg/mL) were prepared in test tubes, and the test bacteria at a density of 10^5 cfu/mL were inoculated into the test tubes. After incubation (37 °C for 18 h), the test tubes were visually examined for growth of bacteria. In addition, the resulting broths in which growth of bacteria was not visually found were spread over the lactose broth agar medium (lactose broth, 1.8%; agar powder, 1.8%) in Petri plates. After incubation (37 °C for 24 h), the Petri plates were visually examined for growth of bacteria. In the antimicrobial and bactericidal tests, DMSO was used as a control and butyl *p*-hydroxybenzoate was used as a standard antibiotic.

RESULTS AND DISCUSSION

2-*endo*-Hydroxy-1,8-cineole (**2**) was synthesized from α-terpineol (**1**) with *m*-chloroperoxybenzoic acid by referring to previously described methods (*6*−*8*), followed by synthesis of esters of compound **2** in an organic solvent (ether or anhydrous pyridine), generally in excellent yields, by reacting compound **2** with 14 kinds of aliphatic acyl chlorides (**Scheme 1**). The reaction conditions, the yields, and the optical rotations of esters are shown in **Table 1**. Except for compounds **3l** and **3n**, almost all of the esters were synthesized at room temperature in diethyl ether at a short reaction time (1−2 h) in good yield (81.1−98.0%). Compound **3n** was synthesized in diethyl ether for 5 h in 64.7% yield. The bulky *tert*-butylacetyl moiety caused small steric hindrance to the reactive site; therefore, synthesis of compound **3n** required a longer reaction time than that of the other esters. Compound **3l** was synthesized at 130 °C in pyridine for 1 h in 57.8% yield. The bulky pivaloyl moiety caused a large steric hindrance to the reactive site; therefore, a higher temperature was needed for synthesis of compound **3l**. Each synthetic ester had a unique odor (described in **Table 2**). Compound **3n** had the most interesting odor (apple-like, fresh fruity, green esteric) of all the synthetic esters.

The antimicrobial and bactericidal activities of the synthetic esters against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas fluorescens* were investigated (shown in **Table 3**). The MIC values of compounds **3a**−**3j** against all of the test bacteria were >400, >400, 200, 200, 200, 100, 100, 100, 100, and 100 μg/mL, respectively. The MBC values of compounds **3c**−**3j** against *S. aureus* were 400, 200, 200, 200, 200, 200, 200, and 200 μg/mL, respectively. The MBC values of compounds **3c**−**3j** against *E. coli* were 200, 200, 200, 200, 200, 200, 200, and 200 μg/mL, respectively. The MBC values of compounds **3c**−**3j** against *P. fluorescens* were 200, 200, 200, 200, 200, 200, 200, and 200 μg/mL, respectively. The MIC values of compounds **3c** and **3k**, which have three carbons in the side chain (except for the carbonyl carbon), against all of the test bacteria were 200 and 400 μg/mL, respectively. The MIC values of compounds **3d**, **3l**, and **3m**, which have four carbons in the side chain, against all of the test bacteria were 200, 200, and 200 μg/mL, respectively. The MIC values of compounds **3e** and **3n**, which have five carbons in the side chain, against all of the test bacteria were 200 and 100 μg/mL, respectively. The MBC values of compounds **3c** and **3k**, which have three carbons in the side chain, against all of the test bacteria were 400 and >400 μg/mL, respectively. The MBC values of compounds **3d**, **3l**, and **3m**, which have four carbons in the side chain, against all of the test bacteria were 200, 200, and 400 μg/mL, respectively.

LITERATURE CITED

(1) Miyazawa, M.; Shimabayashi, H.; Hayashi, S.; Hashimoto, S.; Nakamura, S.; Kosaka, H.; Kameoka, H. Synthesis and biological activity of α-methylene-γ-lactones as new aroma chemicals. *J. Agric. Food Chem.* **2000**, *48*, 5406−5410.

(2) Mori, H.; Kubota, K.; Kobayashi, A. Potent aroma components of rhizomes from *Alpinia galanga* Willd. L. *Nippon Shokuhin Kagaku Kaishi* **1995**, *42*, 989−995.
(3) Kubota, K.; Nakamura (Murayama), K.; Kobayashi, A. Acetoxy-1,8-cineoles as aroma constituents of *Alpinia galanga*. *J. Agric. Food Chem.* **1998**, *46*, 5244−5247.
(4) Kubota, K.; Someya, Y.; Yoshida, R.; Kobayashi, A.; Morita, T.; Koshino, H. Enantiomeric purity and odor characteristics of 2- and 3-acetoxy-1,8-cineole in the rhizomes of *Alpinia galanga* Willd. *J. Agric. Food Chem.* **1999**, *47*, 685−689.
(5) Mariani, E.; Neuhoff, C.; Bargagna, A.; Longobardi, M.; Ferro, M.; Gelardi, A. Ester of 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ols: synthesis, odor evaluation and in vitro preliminary toxicity assays. *Int. J. Cosmet. Sci.* **1995**, *17* (5), 187−196.
(6) Kopperman, H. L.; Hallcher, R. C.; Riehl, A., Sr.; Carlson, R. M.; Caple, R. Aquous chlorination of α-terpineol. *Tetrahedron* **1976**, *32*, 1621−1626.
(7) MacRae, I. C.; Alberts, V.; Carman, R. M.; Shaw, I. M. Products of 1,8-cineole oxidation by a Pseudomonad. *Aust. J. Chem.* **1979**, *32*, 917−922.
(8) Carman, R. M.; Fletcher, M. T. The isomeric 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ols (2-hydroxy-1,8-cineoles). *Aust. J. Chem.* **1984**, *37*, 1117−1122.
(9) Gotou, S.; Kaneko, Y. Measurement method for medicines' sensibility. *Rinshou-Kensa* **1983**, *27*, 1397−1406.

Received for review November 26, 2001. Revised manuscript received March 17, 2002. Accepted March 18, 2002.

JF011555W