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Synthesis of 4-Hydroxy-3(2*H*)-furanone Acyl Derivatives and Their Anti-cataract Effect on Spontaneous Cataract Rats (ICR/f)

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4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone (EHMF) are known to inhibit cataract development in spontaneous cataract rats (ICR/f). Forty-five acylated hydroxyfuranone derivatives were designed and synthesized for an anti-cataract test, and their hydrophobic constants were also tested. Among these derivatives, 2,5-dimethyl-4-pivaloyloxy-3(2*H*)-furanone (HDMF pivalate) exerted a marked protective effect against the development of cataract in a galactose-induced model using cultured rat lens (*in vitro*). When tested on an ICR/f cataract model (*in vivo*), HDMF pivalate showed more significant inhibition of cataract development than parent compound HDMF. This derivative is more lipophilic than HDMF, so that HDMF pivalate can penetrate the cornea more easily than HDMF. The inhibition of cataract development by HDMF converted from HDMF pivalate is supported by the fact that HDMF was observed in the lens of ICR/f rats treated with HDMF pivalate.

Key words: acyl derivatives; furanone; anti-cataract; partition coefficient; spontaneous cataract rat

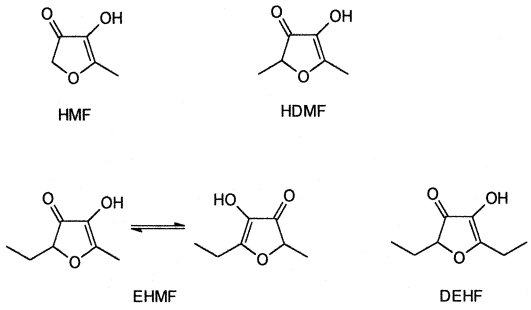

Fig. 1. 4-Hydroxy-3(2*H*)-furanones Which Having Antioxidative Activity.

4-Hydroxy-5-methyl-3(2*H*)-furanone (HMF), one of the flavors of beef broth.[4] 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), a flavor isolated from pineapple and strawberry.[4] 2(or 5)-Ethyl-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone (EHMF), the typical flavor of fermented soy sauce.[5] 2,5-Diethyl-4-hydroxy-3(2*H*)-furanone (DEHF), a synthetic compound that is similar to the natural flavor.[6]

It is well known that cataract involves opacity of the lens in connection with diabetes or aging. It was been reported that one of the causes of cataract formation is oxidation of the lens,[1] so that many antioxidants have been examined as potential anti-cataract drug.[2,3] 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methy-3(2*H*)-furanone (EHMF) are shown in Fig. 1. Our previous study confirmed that these furanones inhibited cataract development in spontaneous cataract (ICR/f) rats.[6] We then attempted to synthesize furanone derivatives as prodrugs that would inhibit cataract development more significantly than the lead compounds. It is known that corneal permeability increases with increasing hydrophobicity of the drug,[7] so more hydrophobic compounds were designed. We tried to synthesize acyl derivatives for prodrugs, because of presence of esterase in the cornea.[8] HDMF and EHMF were observed to inhibit cataract development, while 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF) was an antioxidant similar to HDMF and EHMF.[6] These three hydroxyfuranones were thus chosen as lead compounds. Each furanone has two parts of keto-enol tautomeric groups which could be acylated, and the diacyl derivatives were synthesized in addition to the monoacyl derivatives. Both the partition coefficient and anti-cataract activity of each of these derivatives were examined. The partition coefficient as a hydrophobic parameter was determined by using 1-octanol and water. The anti-cataract activity was determined by an *in vitro* lens culture experiment (the galactose-induced cataract model) and by an *in vivo* spontaneous cataract rat (ICR/f rat) experiment.

Materials and Methods

Apparatus. ^{1}H-NMR spectra were recorded with a JEOL EX-90A spectrometer, IR spectra were recorded with a Jasco FT/IR-7300 infrared spectrometer, and mass spectra were recorded with a Hitachi M-80B instrument. The HPLC system consisted of a Toyo Soda CCMP pump, a Jasco UV-970 detector, a Gilson model 231 injector, and a Hewlett Packard Chemistation system data processor.

Materials. HDMF was purchased from Aldrich Chem-

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ical Co. (Milwaukee, United States), and EHMF was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). The TC199 culture medium was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan), dipalmitoylphosphatidylcholine (DPPC) was from Nihon Yushi Co. (Japan), and mydrin P® was from Santen Pharmaceutical Co. (Osaka, Japan). HMF[9] and DEHF[6] were synthesized from D-(+)-xylose and 1-pentyn-3-ol, respectively. All other chemicals and solvents were of reagent grade.

Monoesterification by acid chlorides. A two-necked flask (100-ml volume) equipped with a calcium chloride drying tube and a dropping funnel was charged with 14 mmol of 4-hydroxy-3(2*H*)-furanone, 20 ml of dry dichloromethane, 20 ml of pyridine and 15 mg (0.08 mmol) of 4-(dimethylamino) pyridine. The solution was stirred and cooled in an ice bath at 0°C, while 14 mmol of an acid chloride was added over a 10-min period. After a further 10-min period at 0°C, the ice bath was removed and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was poured into 50 ml of ice-cooled 0.5 M hydrochloric acid, and extracted with three 20-ml portions of diethyl ether. The organic layer was successively washed with two 20-ml portions of 0.5 M hydrochloric acid and two 20-ml portions of a saturated sodium bicarbonate solution. The organic solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography.

4-Acetoxy-5-methyl-3(2H)-furanone (***1a***). IR ν_{max} (film) cm^{-1}: 3000, 1780, 1710, 1640, 1420, 1370, 1320, 1190, 1120, 1060, 1000, 930; NMR δ_H ($CDCl_3$): 2.19 (3H, s), 2.28 (3H, s), 4.59 (2H, s); MS *m/z* (%): 156 (15), 114 (43), 69 (44), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_7H_8O_4$, 156.0423; found, 156.0347.

5-Methyl-4-propionyloxy-3(2H)-furanone (***2a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1640, 1420, 1360, 1270, 1200, 1130, 1080, 1000, 970, 930; NMR δ_H ($CDCl_3$): 1.23 (3H, t, J=7.5 Hz), 2.18 (3H, s), 2.58 (2H, q, J=7.5 Hz), 4.59 (2H, s); MS *m/z* (%): 171 (39), 143 (36), 115 (25), 57 (100), 30 (46); HR-MS *m/z* (M^+): calcd. for $C_8H_{10}O_4$, 170.0579; found, 170.0583.

4-Butyryloxy-5-methyl-3(2H)-furanone (***3a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1650, 1420, 1320, 1200, 1140, 1100, 1050, 1010, 930; NMR δ_H ($CDCl_3$): 1.02 (3H, t, J=7.3 Hz), 1.55–1.96 (2H, m), 2.18 (3H, s), 2.53 (2H, t, J=7.3 Hz), 4.59 (2H, s); MS *m/z* (%): 184 (27), 114 (41), 71 (100), 43 (48); HR-MS *m/z* (M^+): calcd. for $C_9H_{12}O_4$, 184.0736; found, 184.0631.

4-Isobutyryloxy-5-methyl-3(2H)-furanone (***4a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1470, 1420, 1390, 1360, 1260, 1200, 1130, 1090, 1010, 930, 910; NMR δ_H ($CDCl_3$): 1.29 (6H, d, J=7.1 Hz), 2.17 (3H, s), 2.81 (1H, m), 4.58 (2H, s); MS *m/z* (%): 184 (100), 114 (19), 71 (22), 43 (10); HR-MS *m/z* (M^+): calcd. for $C_9H_{12}O_4$, 184.0736; found, 184.0727.

5-Methyl-4-valeryloxy-3(2H)-furanone (***5a***). IR ν_{max} (film) cm^{-1}: 2950, 1760, 1720, 1640, 1420, 1310, 1190, 1130, 1110, 1000, 920; NMR δ_H ($CDCl_3$): 0.95 (3H, t, J=6.0 Hz), 1.25–1.79 (4H, m), 2.18 (3H, s), 2.55 (2H, t, J=7.2 Hz), 4.59 (2Hs); MS *m/z* (%): 198 (22), 114 (99), 85 (99), 57 (100); HR-MS *m/z* (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0882.

4-Isovaleryloxy-5-methyl-3(2H)-furanone (***6a***). IR ν_{max} (film) cm^{-1}: 2970, 1770, 1720, 1640, 1420, 1320, 1290, 1190, 1120, 1090, 1000, 920; NMR δ_H ($CDCl_3$): 1.04 (6H, d, J=6.6 Hz), 2.18 (3H, s), 2.42 (2H, d, J=6.2 Hz), 2.05–2.48 (1H, m) 4.59 (2H, s); MS *m/z* (%): 198 (38), 143 (30), 114 (22), 85 (100), 57 (69); HR-MS *m/z* (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0871.

5-Methyl-4-pivaloyloxy-3(2H)-furanone (***7a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1480, 1420, 1370, 1320, 1280, 1190, 1130, 1110, 1010, 930, 880; NMR δ_H ($CDCl_3$): 1.33 (9H, s), 2.15 (3H, s), 4.57 (2H, s); MS *m/z* (%): 198 (99), 114 (69), 85 (99), 41 (99); HR-MS *m/z* (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0898.

4-Acetoxy-2,5-dimethyl-3(2H)-furanone (***8a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1630, 1410, 1310, 1190, 1130, 1010, 1100, 1070, 1040, 930, 880, 860; NMR δ_H ($CDCl_3$): 1.50 (3H, d, J=7.3 Hz), 2.17 (3H, s), 2.27 (3H, s), 4.57 (1H, q, J=7.3 Hz); MS *m/z* (%): 170 (11), 128 (29), 85 (15), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_8H_{10}O_4$, 170.0579; Found, 170.0580.

2,5-Dimethyl-4-propionyloxy-3(2H)-furanone (***9a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1640, 1420, 1310, 1200, 1140, 1070, 1070, 1050, 1000; NMR δ_H ($CDCl_3$): 1.23 (3H, t, J=7.5 Hz), 1.50 (3H, d, J=7.3 Hz), 2.16 (3H, s), 2.58 (2H, q, J=7.5 Hz), 4.57 (H, q, J=7.3 Hz); MS *m/z* (%): 184 (23), 157 (10), 128 (57), 85 (13), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_9H_{12}O_4$, 184.0736; found, 184.0758.

4-Butyryloxy-2,5-dimethyl-3(2H)-furanone (***10a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1640, 1420, 1370, 1310, 1240, 1200, 1140, 1100, 1040, 1000, 940, 910; NMR δ_H ($CDCl_3$): 1.02 (3H, t, J=7.5 Hz), 1.50 (3H, d, J=7.3 Hz), 1.62–2.10 (2H, m), 2.17 (3H, s), 2.53 (2H, t, J=7.2 Hz), 4.58 (1H, q, J=7.3 Hz); MS *m/z* (%): 198 (43), 157 (45), 128 (99), 71 (99), 28 (100); HR-MS *m/z* (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0911.

4-Isobutyryloxy-2,5-dimethyl-3(2H)-furanone (***11a***). IR ν_{max} (film) cm^{-1}: 2980, 1770, 1710, 1640, 1420, 1310, 1200, 1140, 1090, 1000; NMR δ_H ($CDCl_3$): 1.29 (6H, d, J=6.8 Hz), 1.50 (3H, d, J=7.3 Hz), 2.15 (3H, s), 2.65–2.96 (1H, s), 4.57 (1H, q, J=7.3 Hz); MS *m/z* (%): 198 (98), 157 (99), 128 (99), 71 (99), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0910.

2,5-Dimethyl-4-valeryloxy-3(2H)-furanone (***12a***). IR ν_{max} (film) cm^{-1}: 2970, 1770, 1720, 1640, 1410, 1310, 1190, 1140, 1100, 1050, 1000; NMR δ_H ($CDCl_3$): 0.94

(3H, t, J=6.9 Hz), 1.50 (3H, d, J=7.3 Hz), 1.22–1.86 (4H, m), 2.16 (3H, s), 2.54 (2H, t, J=7.2 Hz), 4.57 (1H, q, J=7.3 Hz); MS m/z (%): 212 (61), 157 (87), 127 (99), 85 (99), 57 (100), 28 (100); HR-MS m/z (M^+): calcd. for $C_{11}H_{16}O_4$, 212.1049; found: 212.1060.

4-Isovaleryloxy-2,5-dimethyl-3(2H)-furanone (***13a***). IR ν_{max} (film) cm^{-1}: 2960, 1770, 1710, 1640, 1420, 1370, 1310, 1200, 1150, 1100, 1000; NMR δ_H ($CDCl_3$): 1.04 (6H, d, J=6.6 Hz), 1.50 (3H, d, J=7.1 Hz), 2.17 (3H, s), 2.10–2.47 (3H, m), 4.57 (1H, q, J=7.3 Hz); MS m/z (%): 211 (100), 185 (38), 157 (16), 128 (100), 85 (100), 43 (99); HR-MS m/z (M^+): calcd. for $C_{11}H_{16}O_4$, 212.1049; found, 212.1089.

2,5-Dimethyl-4-pivaloyloxy-3(2H)-furanone (***14a***). IR ν_{max} (film) cm^{-1}: 2980, 1760, 1720, 1640, 1480, 1410, 1310, 1270, 1190, 1140, 1100, 1030, 1000; NMR δ_H ($CDCl_3$): 1.32 (9H, s), 1.49 (3H, d, J=7.3 Hz), 2.13 (3H, s), 4.56 (1H, q, J=7.3 Hz); MS m/z (%): 212 (12), 128 (48), 85 (30), 57 (100), 29 (64); HR-MS m/z (M^+): calcd. for $C_{11}H_{16}O_4$, 212.1049; found, 212.1081.

4-Acetoxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (***15a***). IR ν_{max} (film) cm^{-1}: 3000, 1780, 1710, 1630, 1420, 1370, 1320, 1190; NMR δ_H ($CDCl_3$): 1.01 (1.8H, t, J=7.5 Hz), 1.22 (1.2H, t, J=7.5 Hz), 1.45 (1.2H, d, J=7.5 Hz), 1.80–2.04 (1.2H, m), 2.18 (1.8H, s), 2.27 (3H, s), 2.64 (0.8H, q, J=7.5 Hz), 4.47–4.53 (1H, m); MS m/z (%): 184 (42), 142 (52), 101 (8), 43 (100); HR-MS m/z (M^+): calcd. for $C_9H_{12}O_4$, 184.0736; found, 184.0720.

2(or 5)-Ethyl-5(or 2)-methyl-4-propionyloxy-3(2H)-furanone (***16a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1630, 1420, 1320, 1190; NMR δ_H ($CDCl_3$): 1.01 (1.8H, t, J=7.5 Hz), 1.23 (4.2H, t, J=7.5 Hz), 1.50 (1.2H, d, J=7.0 Hz), 1.72–2.04 (1.2H, m), 2.19 (1.8H, s), 2.39–2.71 (1.6H, m), 2.46–2.71 (1.2H, m), 4.43–4.62 (1H, m); MS m/z (%): 198 (10), 142 (21), 101 (8), 57 (100); HR-MS m/z (M^+): calcd. for $C_{10}H_{14}O_4$, 198.0892; found, 198.0908.

4-Butyryloxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (***17a***). IR ν_{max} (film) cm^{-1}: 3000, 1760, 1710, 1630, 1420, 1320, 1190, 1130; NMR δ_H ($CDCl_3$): 1.00 (4.8H, t, J=7.6 Hz), 1.21 (1.2H, t, J=7.6 Hz), 1.42–1.97 (2H, m), 1.49 (1.2H, d, J=7.0 Hz), 1.70–2.00 (1.2H, m), 2.16 (1.8H, s), 2.35–2.60 (2.8H, m), 4.40–4.60 (1H, m); MS m/z (%): 212 (20), 142 (31), 71 (100), 29 (19); HR-MS m/z (M^+): calcd. for $C_{11}H_{16}O_4$, 212.1049; found, 212.1056.

2(or 5)-Ethyl-4-isobutyryloxy-5(or 2)-methyl-3(2H)-furanone (***18a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1640, 1460, 1320, 1190, 1130, 1090; NMR δ_H ($CDCl_3$): 1.01 (1.8H, t, J=7.6 Hz), 1.22 (1.2H, t, J=7.9 Hz), 1.29 (6H, d, J=7.1 Hz), 1.49 (1.2H, d, J=7.0 Hz), 1.72–2.04 (1.2H, m), 2.17 (1.8H, s), 2.50 (0.8H, q, J=7.9 Hz), 2.77 (1H, q, J=7.1 Hz), 4.42–4.54 (1H, m); MS m/z (%): 212 (70), 142 (75), 99 (11), 43 (100); HR-MS m/z (M^+): calcd. for $C_{11}H_{16}O_4$, 212.1049; found, 212.1097.

2(or 5)-Ethyl-5(or 2)-methyl-4-valeryloxy-3(2H)-furanone (***19a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710, 1640, 1420, 1320, 1190, 1130; NMR δ_H ($CDCl_3$): 0.93 (3H, t, J=6.2 Hz), 1.00 (1.8H, t, J=7.1 Hz), 1.21 (1.2H, t, J=7.1 Hz), 1.21–1.99 (5.2H, m), 1.49 (1.2H, d, J=7.0 Hz), 2.16 (1.8H, s), 2.50 (0.8H, q, J=7.1 Hz), 2.54 (2H, t, J=7.1 Hz), 4.41–4.52 (1H, m); MS m/z (%): 226 (13), 185 (15), 142 (31), 101 (9), 57 (100); HR-MS m/z (M^+): calcd. for $C_{12}H_{18}O_4$, 226.1205; found, 226.1241.

2(or 5)-Ethyl-4-isovaleryloxy-5(or 2)-methyl-3(2H)-furanone (***20a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1190, 1150, 1090; NMR δ_H ($CDCl_3$): 1.00 (1.8H, t, J=6.2 Hz), 1.03 (6H, d, J=6.2 Hz), 1.13 (1.2H, t, J=5.8 Hz), 1.49 (1.2H, d, J=7.0 Hz), 1.79–2.04 (2.2H, m), 2.18 (1.8H, s), 2.35 (0.8H, q, J=5.8 Hz), 2.42 (2H, d, J=5.9 Hz), 4.40–4.65 (1H, m); MS m/z (%): 226 (30), 185 (6), 142 (45), 99 (6), 57 (100); HR-MS m/z (M^+): calcd. for $C_{12}H_{18}O_4$, 226.1205; found, 226.1232.

2(or 5)-Ethyl-5(or 2)-methyl-4-pivaloyloxy-3(2H)-furanone (***21a***). IR ν_{max} (film) cm^{-1}: 2980, 1760, 1720, 1640, 1480, 1410, 1310, 1270, 1190, 1140, 1100, 1030, 1000; NMR δ_H ($CDCl_3$): 1.01 (3H, t, J=7.3 Hz), 1.33 (9H, s), 1.79–2.04 (2H, m), 2.15 (3H, s), 4.46 (1H, t, J=5.7 Hz); MS m/z (%): 226 (62), 185 (15), 142 (56), 85 (81), 43 (100); HR-MS m/z (M^+): calcd. for $C_{12}H_{18}O_4$, 226.1205; found, 226.1302.

Esterification by fatty acids with dicyclohexylcarbodiimide. A round-bottomed flask (100-ml volume) equipped with a calcium chloride drying tube was charged with a fatty acid (15 mmol), 50 ml of dichloromethane, 14 mmol of 4-hydroxy-3(2*H*)-furanone, and 30 mg (0.16 mmol) of 4-(dimethylamino)pyridine. The solution was stirred and cooled in an ice bath to 0°C, while 3.1 g (15 mmol) of dicyclohexylcarbodiimide was added over a 5-min period. After a further 5 min at 0°C, the reaction mixture was stirred overnight at room temperature. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was successively washed with 0.5 M hydrochloric acid (50 ml × 2) and two 50-ml portions of a saturated sodium bicarbonate solution. The organic solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting oil was purified by silica gel column chromatography.

2(or 5)-Ethyl-5(or 2)-methyl-4-palmitoyloxy-3(2H)-furanone (***22a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1730, 1690, 1600, 1460, 1200; NMR δ_H ($CDCl_3$): 0.68–1.05 (4.8H, m), 1.05–1.71 (28.4H, m), 1.79–1.96 (1.2H, m), 2.18 (1.8H, s), 2.35–2.54 (2.8H, m), 4.40–4.55 (1H, m); MS m/z (%): 381 (8), 340 (5), 240 (70), 142 (100), 97 (18), 57 (85); HR-MS m/z (M^+): calcd. for $C_{23}H_{40}O_4$, 380.2927; found, 380.2921.

2(or 5)-Ethyl-5(or 2)-methyl-4-stearoyloxy-3(2H)-furanone (***23a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1710,

1640, 1460, 1380, 1200, 1140; NMR δ_H ($CDCl_3$): 0.82–2.05 (38.4H, m), 2.17 (1.8H, s), 2.45–2.65 (2.8H, m), 4.40–4.65 (1H, m); MS *m/z* (%): 409 (7), 268 (52), 185 (8), 142 (100), 71 (33), 28 (80); HR-MS *m/z* (M^+): calcd. for $C_{25}H_{44}O_4$, 408.3240; found, 408.3229.

2(or 5)-Ethyl-5(or 2)-methyl-4-oleoyloxy-3(2H)-furanone (***24a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1460, 1420, 1320, 1190, 1130; NMR δ_H ($CDCl_3$): 0.80–1.44 (26.2H, m), 1.01 (1.8H, t, *J*=7.1 Hz), 1.49 (1.2H, d, *J*=7.0 Hz), 1.60–1.95 (1.2H, m), 1.96–2.10 (4H, m), 2.17 (1.8H, s), 2.45–2.60 (2.8H, m), 4.35–4.60 (1H, m), 5.30–5.45 (2H, m); MS *m/z* (%): 407 (15), 266 (100), 142 (100), 97 (14), 55 (28); HR-MS *m/z* (M^+): calcd. for $C_{25}H_{42}O_4$, 406.3083; found, 406.3061.

2(or 5)-Ethyl-4-linoloxy-5(or 2)-methyl-3(2H)-furanone (***25a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1190; NMR δ_H ($CDCl_3$): 0.89 (3H, t, *J*=6.7 Hz), 1.01 (1.8H, t, *J*=7.0 Hz), 1.13–2.03 (23.6H, m), 2.17 (1.8H, s), 2.30–2.90 (2.8H, m), 2.50 (0.8H, q, *J*=7.5 Hz), 2.53 (1.2H, t, *J*=7.3 Hz), 4.35–4.60 (1H, m), 5.20–5.55 (4H, m); MS *m/z* (%): 404 (100), 362 (4), 264 (32), 142 (28), 67 (66); HR-MS *m/z* (M^+): calcd. for $C_{25}H_{40}O_4$, 404.2927; found, 404.2904.

2(or 5)-Ethyl-4-linoleonyloxy-5(or 2)-methyl-3(2H)-furanone (***26a***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1720, 1640, 1190; NMR δ_H ($CDCl_3$): 0.98 (3H, t, *J*=7.3 Hz), 1.01 (1.8H, t, *J*=7.4 Hz), 1.10–2.15 (18.4H, m), 2.18 (1.8H, s), 2.52 (2H, t, *J*=7.5 Hz), 2.70–2.90 (4H, m), 4.40–4.60 (1H, m), 5.15–5.55 (6H, m); MS *m/z* (%): 402 (17), 262 (18), 142 (100), 95 (42), 55 (72); HR-MS *m/z* (M^+): calcd. for $C_{25}H_{38}O_4$, 402.2770; found, 402.2679.

Diesterification by acid anhydrides. A two-necked flask (200-ml volume) equipped with a calcium chloride drying tube and dropping funnel was charged with 39 mmol of 4-hydroxy-3(2*H*)-furanone, 50 ml of dry dichloromethane, 50 ml of pyridine and 30 mg (0.16 mmol) of 4-(dimethylamino)pyridine. The solution was stirred and cooled in a ice bath to 0°C, while 78 mmol of an acid anhydride was added over a 10-min period. After a further 10 min at 0°C, the ice bath was removed, and the reaction mixture was stirred for 6 h at 80°C in an oil bath. The reaction mixture was poured into 60 ml of ice-cooled 0.5 M hydrochloric acid, and extracted with three 60-ml portions of diethyl ether. The organic layer was successively washed with two 60-ml portions of 0.5 M hydrochloric acid and two 60 ml portions of a saturated sodium bicarbonate solution. The organic solution was dried over anhydrous magnesium sulfate and evaporated *in vacuo*. The residue was purified by silica gel column chromatography.

3,4-Diacetoxy-2-methylfuran (***1b***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1660, 1570, 1480, 1400, 1370, 1280, 1240, 1170, 1130, 1110, 1030, 940, 890; NMR δ_H ($CDCl_3$): 2.16 (3H, s), 2.23 (3H, s), 2.29 (3H, s), 7.53 (1H, s); MS *m/z* (%): 198 (15), 149 (23), 114 (27), 85 (15), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_9H_{10}O_5$, 198.0528; found, 198.0512.

2-Methyl-3,4-di(propionyloxy)furan (***2b***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1660, 1570, 1440, 1350, 1280, 1240, 1160, 1080, 980, 930, 880, 810; NMR δ_H ($CDCl_3$): 1.20 (3H, t, *J*=7.6 Hz), 1.26 (3H, t, *J*=7.6 Hz), 2.16 (3H, s), 2.51 (2H, q, *J*=7.6 Hz), 2.57 (2H, q, *J*=7.6 Hz), 7.54 (1H, s); MS *m/z* (%): 198 (15), 149 (23), 114 (27), 85 (15), 43 (100); HR-MS *m/z* (M^+): calcd. for $C_{11}H_{14}O_5$, 226.0841; found, 226.0891.

3,4-Di(butyryloxy)-2-methylfuran (***3b***). IR ν_{max} (film) cm^{-1}: 3000, 1820, 1770, 1710, 1660, 1570, 1440, 1280, 1240, 1160, 1100, 1030, 940; NMR δ_H ($CDCl_3$): 0.98 (3H, t, *J*=7.5 Hz), 1.04 (3H, t, *J*=7.4 Hz), 1.55–1.98 (4H, m), 2.16 (3H, s), 2.46 (2H, t, *J*=7.2 Hz), 2.52 (2H, t, *J*=7.2 Hz), 7.54 (1H, s); MS *m/z* (%): 254 (15), 213 (24), 184 (36), 149 (52), 114 (35), 71 (100), 43 (57); HR-MS *m/z* (M^+): calcd. for $C_{13}H_{18}O_5$, 254.1154; found, 254.1143.

3,4-Di(isobutyryloxy)-2-methylfuran (***4b***). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1660, 1580, 1470, 1450, 1390, 1350, 1280, 1240, 1120, 1040, 940, 910, 850; NMR δ_H ($CDCl_3$): 1.24 (6H, d, *J*=6.8 Hz), 1.30 (6H, d, *J*=6.8 Hz), 2.16 (3H, s), 2.56–2.96 (2H, m), 7.54 (1H, s); MS *m/z* (%): 254 (31), 219 (32), 181 (46), 131 (49), 69 (100), 43 (66); HR-MS *m/z* (M^+): calcd. for $C_{13}H_{18}O_5$, 254.1154; found, 254.1163.

2-Methyl-3,4-di(valeryloxy)furan (***5b***). IR ν_{max} (film) cm^{-1}: 2980, 1770, 1660, 1570, 1440, 1380, 1280, 1230, 1160, 1100, 940, 890; NMR δ_H ($CDCl_3$): 0.93 (3H, t, *J*=6.4 Hz), 0.97 (3H, t, *J*=6.4 Hz), 1.25–1.80 (8H, m), 2.16 (3H, s), 2.47 (3H, t, *J*=6.8 Hz), 2.55 (3H, t, *J*=6.8 Hz), 7.54 (1H, s); MS *m/z* (%): 283 (13), 199 (100), 143 (43), 114 (23), 85 (67), 57 (55); HR-MS *m/z* (M^+): calcd. for $C_{15}H_{22}O_5$, 282.1467; found, 282.1432.

3,4-Di(isovaleryloxy)-2-methylfuran (***6b***). IR ν_{max} (film) cm^{-1}: 2970, 1820, 1770, 1660, 1570, 1470, 1370, 1280, 1240, 1160, 1040, 970; NMR δ_H ($CDCl_3$): 1.00 (12H, d, *J*=6.4 Hz), 2.16 (3H, s), 2.33 (2H, d, *J*=5.5 Hz), 2.35 (2H, d, *J*=5.7 Hz), 2.07–2.45 (2H, m), 7.55 (1H, m); MS *m/z* (%): 282 (12), 198 (54), 114 (78), 85 (100), 57 (81), 30 (10); HR-MS *m/z* (M^+): calcd. for $C_{15}H_{22}O_5$, 282.1467; found, 282.1421.

2-Methyl-3,4-di(pivaloyloxy)furan (***7b***). IR ν_{max} (film) cm^{-1}: 3000, 1760, 1660, 1570, 1480, 1400, 1370, 1280, 1240, 1170, 1130, 1110, 1030, 1110, 1030, 940, 890; NMR δ_H ($CDCl_3$): 1.27 (9H, s), 1.35 (9H, s), 2.14 (3H, s), 7.56 (1H, s); MS *m/z* (%): 282 (35), 198 (55), 131 (26), 85 (99), 57 (100), 29 (78); HR-MS *m/z* (M^+): calcd. for $C_{15}H_{22}O_5$, 282.1467; found, 282.1469.

3,4-Diacetoxy-2,5-dimethylfuran (***8b***). IR ν_{max} (film) cm^{-1}: 2930, 1770, 1670, 1640, 1440, 1370, 1190, 1110, 1010, 930, 890; NMR (H ($CDCl_3$): 2.14 (6H, s), 2.23 (6H, s); MS *m/z* (%): 212 (11), 170 (16), 128 (75), 101

(8), 69 (10), 43 (100); HR-MS m/z (M^+): calcd. for $C_{10}H_{12}O_5$, 212.0685; found, 212.0726.

2,5-Dimethyl-3,4-di(propionyloxy)furan (**9b**). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1670, 1620, 1450, 1350, 1250, 1140, 1070, 1020, 980, 930, 870, 855; NMR δ_H ($CDCl_3$): 1.22 (6H, t, J=7.5 Hz), 2.13 (6H, s), 2.51 (4H, q, J=7.5 Hz); MS m/z (%): 240 (43), 215 (6), 184 (100), 157 (16), 127 (98), 57 (98), 30 (22); HR-MS m/z (M^+): calcd. for $C_{12}H_{16}O_5$, 240.0998; found, 240.0999.

3,4-Di(butyryloxy)-2,5-dimethylfuran (**10b**). IR ν_{max} (film) cm^{-1}: 2870, 1760, 1670, 1620, 1450, 1360, 1250, 1140, 1090, 1030, 930, 860; NMR δ_H ($CDCl_3$): 1.00 (6H, t, J=7.3 Hz), 1.61–1.93 (4H, m), 2.13 (6H, s), 2.46 (4H, q, J=7.1 Hz); MS m/z (%): 268 (30), 198 (74), 171 (35), 128 (99), 71 (100), 43 (99); HR-MS m/z (M^+): calcd. for $C_{14}H_{20}O_5$, 268.1311; found, 268.1310.

3,4-Di(isobutyryloxy)-2,5-dimethylfuran (**11b**). IR ν_{max} (film) cm^{-1}: 2980, 1770, 1710, 1670, 1470, 1390, 1350, 1250, 1140, 1040; NMR δ_H ($CDCl_3$): 1.25 (12H, t, J=6.8 Hz), 2.12 (3H, s), 2.58–2.89 (2H, m); MS m/z (%): 268 (6), 297 (24), 171 (18), 128 (24), 71 (100), 43 (99); HR-MS m/z (M^+): calcd. for $C_{14}H_{20}O_5$, 268.1311; found, 268.1353.

2,5-Dimethyl-3,4-di(valeryloxy)furan (**12b**). IR ν_{max} (film) cm^{-1}: 2950, 1770, 1670, 1620, 1450, 1380, 1250, 1140, 930; NMR δ_H ($CDCl_3$): 0.94 (6H, t, J=7.0 Hz), 1.27–1.77 (4H, m), 2.13 (3H, s), 2.48 (2H, t, J=7.0 Hz); MS m/z (%): 296 (10), 212 (34), 181 (21), 128 (100), 69 (100), 29 (64); HR-MS m/z (M^+): calcd. for $C_{16}H_{24}O_5$, 296.1624; found, 296.1614.

3,4-Di(isovaleryloxy)-2,5-dimethylfuran (**13b**). IR ν_{max} (film) cm^{-1}: 2960, 1770, 1670, 1620, 1450, 1370, 1290, 1250, 1150, 1120, 1090, 960; NMR δ_H ($CDCl_3$): 1.01 (12H, d, J=6.4 Hz), 2.13 (6H, s), 1.97–2.41 (6H, m); MS m/z (%): 296 (11), 212 (35), 181 (14), 128 (100), 85 (62), 57 (57), 29 (26); HR-MS m/z (M^+): calcd. for $C_{16}H_{24}O_5$, 296.1624; found, 296.1636.

3,4-Diacetyloxy-2-ethyl-5-methylfuran (**15b**). IR ν_{max} (film) cm^{-1}: 3000, 1770, 1670, 1670, 1440, 1370, 1280, 1200, 1160; NMR δ_H ($CDCl_3$): 1.17 (3H, t, J=7.5 Hz), 2.13 (3H, s), 2.22 (6H, s), 2.49 (2H, q, J=7.5 Hz); MS m/z (%): 226 (74), 184 (85), 142 (96), 101 (10), 43 (100); HR-MS m/z (M^+): calcd. for $C_{11}H_{14}O_5$, 226.0841; found, 226.0843.

2-Ethyl-5-methyl-3,4-di(propionyloxy)furan (**16b**). IR ν_{max} (film) cm^{-1}: 3000, 1780, 1670, 1440, 1350, 1280, 1140; NMR δ_H ($CDCl_3$): 1.17 (3H, t, J=7.6 Hz), 1.21 (6H, t, J=7.6 Hz), 2.13 (3H, s), 2.50 (6H, q, J=7.6 Hz); MS m/z (%): 254 (8), 197 (31), 171 (6), 142 (40), 57 (100), 30 (35); HR-MS m/z (M^+): calcd. for $C_{13}H_{18}O_5$, 254.1154; found, 254.1122.

3,4-Di(butyryloxy)-2-ethyl-5-methylfuran (**17b**). IR ν_{max} (film) cm^{-1}: 2970, 1760, 1670, 1620, 1440, 1360, 1280, 1240, 1140, 1090; NMR δ_H ($CDCl_3$): 1.00 (6H, t, J=7.6 Hz), 1.17 (3H, t, J=7.6 Hz), 1.53–1.99 (4H, m), 2.13 (3H, s), 2.46 (4H, t, J=7.3 Hz), 2.50 (2H, q, J=7.6 Hz); MS m/z (%): 281 (8), 211 (36), 171 (28), 142 (43), 71 (100), 29 (100); HR-MS m/z (M^+): calcd. for $C_{15}H_{22}O_5$, 282.1467; found, 282.1468.

2-ethyl-3,4-Di(isobutyryloxy)-5-methylfuran (**18b**). IR ν_{max} (film) cm^{-1}: 2900, 1770, 1670, 1620, 1470, 1450, 1390, 1350, 1290, 1230, 1140, 1100, 1070, 1050, 960, 920; NMR δ_H ($CDCl_3$): 1.17 (3H, t, J=7.6 Hz), 1.25 (12H, d, J=6.8 Hz), 2.13 (3H, s), 2.49 (2H, q, J=7.6 Hz), 2.58–2.89 (2H, m); MS m/z (%): 282 (12), 212 (29), 181 (14), 142 (99), 71 (100), 29 (99); HR-MS m/z (M^+): calcd. for $C_{15}H_{22}O_5$, 282.1467; found, 282.1468.

2-Ethyl-5-methyl-3,4-di(valeryloxy)furan (**19b**). IR ν_{max} (film) cm^{-1}: 2980, 1760, 1660, 1610, 1440, 1280, 1220, 1130, 1090, 950; NMR δ_H ($CDCl_3$): 0.94 (6H, t, J=7.0 Hz), 1.17 (3H, t, J=7.5 Hz), 1.30–1.77 (8H, t, J=7.5 Hz), 2.13 (3H, s), 2.48 (4H, t, J=7.2 Hz), 2.49 (2H, q, J=7.5 Hz); MS m/z (%): 310 (8), 226 (27), 185 (22), 142 (97), 85 (99), 57 (99), 30 (100); HR-MS m/z (M^+): calcd. for $C_{17}H_{26}O_5$, 310.1780; found, 310.1786.

2-Ethyl-3,4-di(isovaleryloxy)-5-methylfuran (**20b**). IR ν_{max} (film) cm^{-1}: 2960, 1770, 1670, 1620, 1470, 1370, 1290, 1240, 1150, 1120, 1090, 960; NMR δ_H ($CDCl_3$): 1.01 (12H, d, J=6.4 Hz), 1.17 (3H, t, J=7.5 Hz), 2.13 (3H, s), 1.96–2.41 (2H, m), 2.35 (4H, d, J=7.3 Hz), 2.49 (2H, q, J=7.5 Hz); MS m/z (%): 311 (8), 281 (6), 226 (34), 181 (16), 142 (100), 69 (88), 29 (99); HR-MS m/z (M^+): calcd. for $C_{17}H_{26}O_5$, 310.1780; found, 310.1699.

Partition coefficients of the hydroxyfuranone derivatives. The partition coefficients were determined by the 1-octanol-water system.[10] A sample (1.0–2.0 mg) was weighed into a screw-capped sample bottle (12-ml volume), and dissolved in a mixture of 1-octanol (1.0 ml) and water (10.0 ml). The mixture was shaken on a mechanical shaker for 1.5 h, after which the aqueous layer was drawn off and centrifuged at 2500 rpm for 1.5 h. The amount of compound in each layer was measured by HPLC. Separation was accomplished with an acetonitrile-water mobile phase delivered at 1 ml/min by a Shiseido ODS Capcellpak column (UG 120 C_{18}, 4.6 mm i.d. × 250 mm, 120 Å, 5 μm). Solvents of (I) acetonitrile-water (20:80, v/v) and (II) acetonitrile were used as a gradient system: 0–5 min (I:100%–100%), 5–35 min (I:100%–0%), 35–40 min (I:0%–0%). The sample injection volume was varied from 2–5 μl, and the analytes were detected *via* UV at 290 nm for hydroxyfuranones, at 262 nm for monoacyl derivatives, and at 219 nm for diacyl derivatives.

Some partition coefficients could not be calculated by this method, because the concentration of the 1-octanol layer could not measured. These partition coefficients were evaluated by the retention time of HPLC by the method of McCall.[11]

Anti-cataract effects of the hydroxyfuranone derivatives on cataract induced by galactose in organ-cultured rat lenses. Lenses enucleated from male Wistar/Crj strain rats (7 weeks) were immersed in the TC199 culture medium (10 ml) supplemented with 24 mM $NaHCO_3$ and 30 mM galactose, and cultured in the presence of 5% CO_2 at 37°C with daily replacement of the medium. A hydroxyfuranone derivative was dissolved in dimethyl sulfoxide (DMSO) by which the final concentration was brought to 0.1 mg/ml, and this solution was added to the TC199 medium at the rate of 50 μl/10 ml. After 7 d, the degree of opacity in each group was scored by the method of Hattori *et al.*[12] A lens with no opacity scored 0, one with a low degree of opacity on the cortex scored 1, one with opacity all over the cortex and deeper scored 3, while one with an intermediate degree of opacity scored 2.

Anti-cataract effects of the hydroxyfuranone derivatives in spontaneous cataract rats (ICR/f rats). A hydroxyfuranone derivative (1.0%) was dissolved in saline, and DPPC was added at a concentration of 0.5% to form liposomes. Topical eye drops were prepared for each hydroxyfuranone derivative containing the liposome solution, while the vehicle alone was used for the control group. In this experiment, seven to nine male ICR/f rats (Kiwa Laboratory Animal Co. Ltd.) were purchased at 6 weeks old and were used at 7 weeks old in each group. The topical eye drops were applied three times a day (morning, afternoon and evening) in both eyes of the experimental animals for 3 weeks.

The rat lenses were observed and documented by using a photo slit lamp microscope (NIKON FS-3) in maximal mydriasis (Mydrin P®). The score for lens opacity was evaluated from stage 0 to stage 5 (6 degrees), according to the method of Nishida *et al.*[13] Stage 4, at which the lens opacity became clearly recognizable with the naked eye, without having to use a slit lamp biomicroscope, was judged to be the cataract condition.

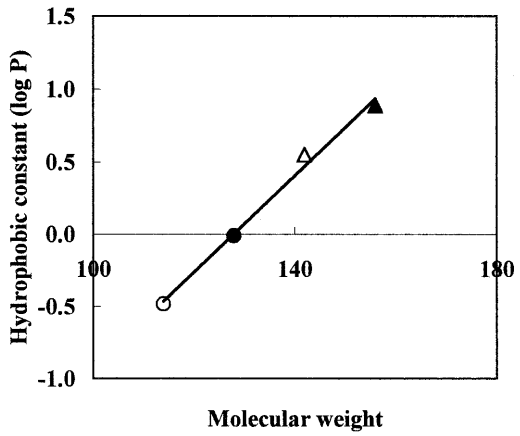

Fig. 2. Hydrophobic Constants and Molecular Weights of the 4-Hydroxy-3(2*H*)-furanones.
4-Hydroxy-3(2*H*)-furanone: HMF (○), HDMF (●), EHMF (△), DEHF (▲).

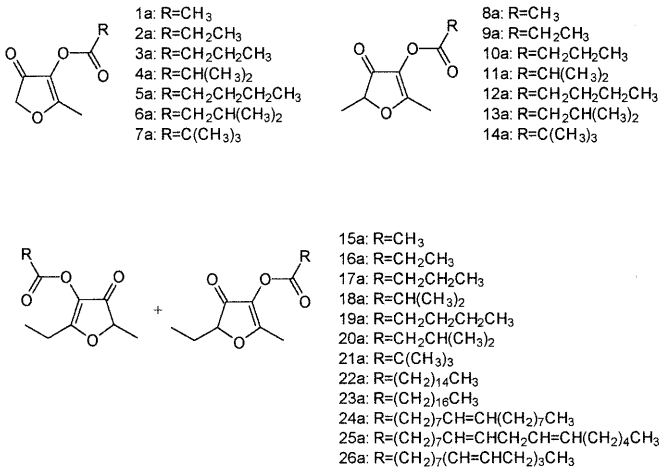

Fig. 3. Monoacyl Derivatives of the 4-Hydroxy-3(2*H*)-furanones.

Results

The hydrophobic constants of four hydroxyfuranones were examined (Fig. 2) which were expected to be the lead compounds (Fig. 1), the logarithm of the partition coefficient being defined as the hydrophobic constant. The hydrophobic constants of HMF, HDMF, EHMF, and 2,5-diethyl-4-hydroxy-3(2*H*)-furanone (DEHF) were correlated closely with their molecular weights. HMF is more soluble in water than 1-octanol, and HDMF is soluble in water to the same degree as in 1-octanol. EHMF and DEHF are more soluble in 1-octanol than water. The anti-cataract activity of each of the four furanones was examined, and HDMF and EHMF were found to inhibit the formation of cataract in the ICR/f rat model.[6] As a result, HDMF and EHMF were chosen as lead compounds after considering their penetrability into the cornea.

These 4-hydroxy-3(2*H*)-furanones have two enolates of ketones, and their monoacyl derivatives and diacyl derivatives can be synthesized. Twenty-one monoacyl derivatives (**1a–21a**) were synthesized by using an equimolar amount of each acid chloride, and the other large derivatives (**22a–26a**) were synthesized by using an equimolar amount of a fatty acid and DCC as a condensing agent (Fig. 3). Of these compounds, each HEMF acyl derivative (**15–26a**) was a mixture of two compounds. On the other hand, 19 of the diacyl derivatives (**1b–20b**) were synthesized by using double the molar quantity of a carboxylic anhydride (Fig. 4). All derivatives whose carbon number for each acyl group is less than 6 were synthesized, except for 2,5-dimethyl-3,4-di(pivaloyloxy) furane and 2-ethyl-5-methyl-3,4-di(pivaloyloxy)furane.

The acyl derivatives were synthesized for two purposes: one is that the acyl derivatives are for more lipophilic than the lead compound, and the other is that the acyl derivatives are more effective antioxidants such as an unsaturated carboxylate ester. As typical unsaturated fatty acids, oleic acid, linoleic acid, and linolenic acid were used.

It is known that penetration into the cornea is related to hydrophobicity and molecular weight.[14] Before the anti-cataract tests, the physical data for these acyl deriva-

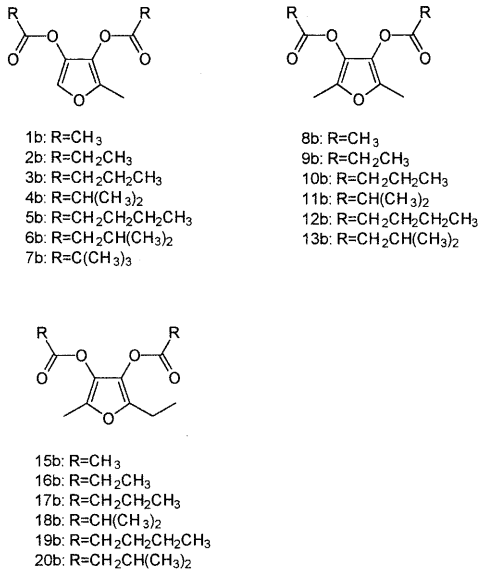

Fig. 4. Diacyl Derivatives of the 4-hydroxy-3(2*H*)-furanones.

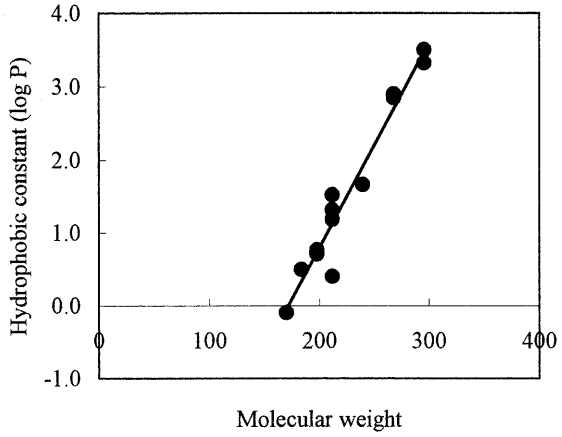

Fig. 6. Relationship between the Hydrophobic Constant and Molecular Weight of Each Acyl Derivative of HDMF.

There was a correlation between the hydrophobic constant of an HDMF derivative and its molecular weight: $Y=0.028X-4.80$, $R^2=0.948$, $n=13$.

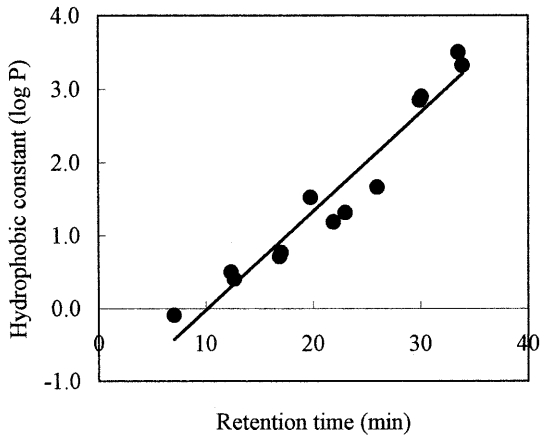

Fig. 5. Relationship between the Hydrophobic Constant and Retention Time of Each Acyl Derivative of HDMF.

There was a correlation between the hydrophobic constant of an HDMF derivative and its retention time by HPLC: $Y=0.135X-1.38$, $R^2=0.939$, $n=13$.

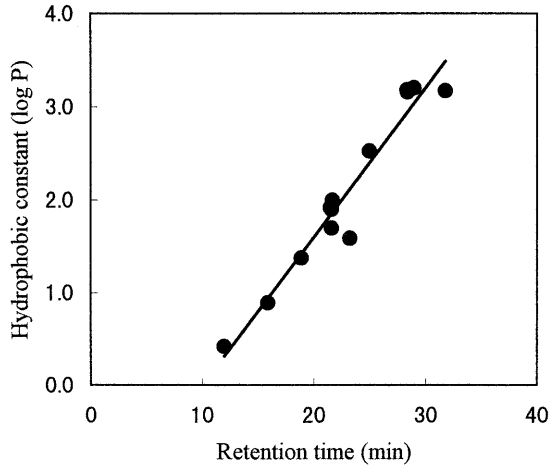

Fig. 7. Relationship between the Hydrophobic Constant and Retention Time of Each Acyl Derivative of EHMF.

There was a correlation between the hydrophobic constant of an EHMF derivative and its molecular weight: $Y=0.1605X-1.6124$, $R^2=0.9417$, $n=13$.

tives were measured. The hydrophobic constant (log P), molecular weight and retention time (HPLC) of each acyl derivative are shown in Table 1. The relationships of the molecular weight and retention time of each HDMF derivative against the hydrophobic constant are shown in Figs. 5 and 6. Almost all hydrophobic constants of the EHMF derivatives were in proportion to their retention times by HPLC (Fig. 7), so we measured the other hydrophobic constants by using the values for the EHMF derivatives. The acyl derivatives showed no antioxidative activity (data not shown), although antioxidative activity could be shown after hydrolysis in human plasma.[15] The hydrolysis of 2,5-dimethyl-4-propionyloxy-3(2*H*)-furanone (**9a**) in a Söensen buffer (pH 7.4) is shown in Fig. 8. After 24 h, less than 40% of **9a** remained, and more than 30% of **9a** had been converted to HDMF. It is assumed that HDMF produced by hydrolysis was oxidized. The hydrolysis of 2,5-dimethyl-3,4-dipropionyloxy-3(2*H*)-furanone (**9b**) is shown in Fig. 9. After 24 h, more than 70% of **9b** still existed, the remaining **9b** being converted to **9a** and HDMF. The conversion rate to **9a** and HDMF was more than 10% of the starting material. After 100 h, **9b** continued decreasing and HDMF continued increasing, although the amount of **9a** was almost same at about 10% of the starting material.

The anti-cataract activity of each acyl derivative was determined by the galactose-induced cataract model, using rat lenses, and is shown in Fig. 10. Among these derivatives, 2,5-dimethyl-4-pivaloyloxy-3(2*H*)-furanone (**14a**, 0.1 mg/ml) and a mixture of 2-ethyl-5-methyl-4-pivaloyloxy-3(2*H*)-furanone and 5-ethyl-2-methyl-4-pivaloyloxy-3(2*H*)-furanone (**21a**, 0.1 mg/ml) inhibited cataract formation. The anti-cataract activity

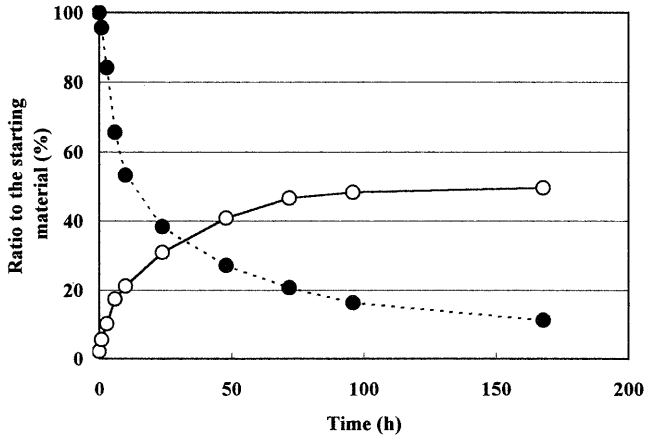

Fig. 8. Time-course Plots for of the Hydrolysis of 2,5-Dimethyl-4-propionyloxy-3(2*H*)-furanone (**9a**) in Sörensen Buffer (pH 7.4).
HDMF (○), **9a** (●). The solution was shaken at 140 rpm and 30°C.

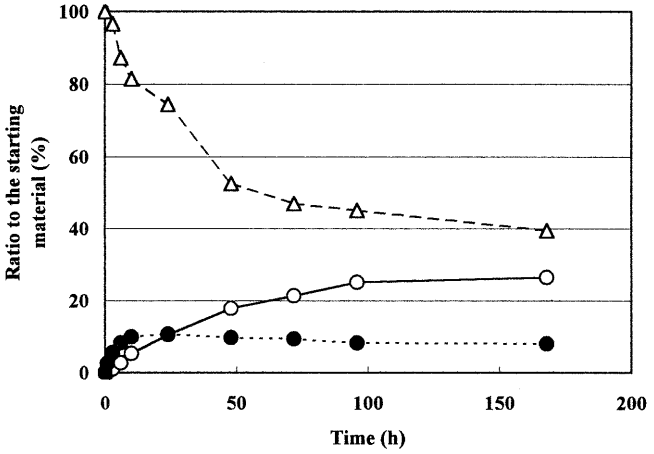

Fig. 9. Time-Course Plots for the Hydrolysis of 2,5-Dimethyl-3,4-di(propionyloxy)furane (**9b**) in a Sörensen buffer (pH 7.4).
HDMF (○), **9a** (●), **9b** (△). The solution was shaken at 140 rpm and 30°C.

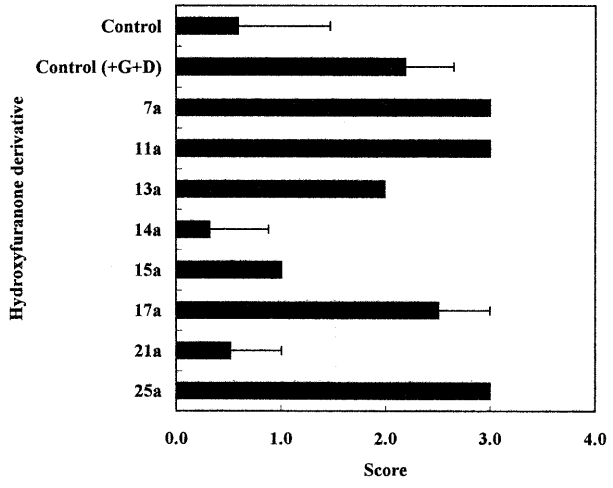

Fig. 10. Anti-cataract Activity of Each Acylated Derivative by the Galactose-induced Cataract Model *in vitro*.

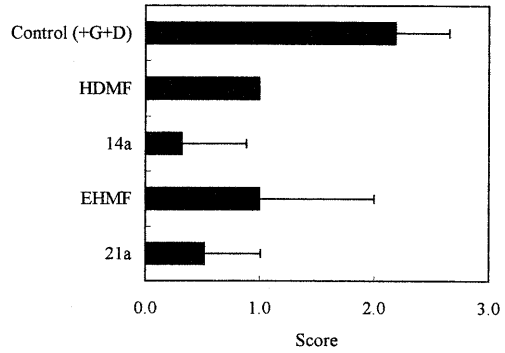

Fig. 11. Comparison of the Anti-cataract Activity between Each Opivaloyl Derivative and Its Parent Compound.

Table 1. Physical Data for the Synthesized Furanone Derivatives

HMF monoacylate	RT	MW	log *P*	HMF diacylate	RT	MW	log *P*
1a	4.8	156	−0.607	**1b**	17.2	198	1.180
2a	8.0	170	0.013	**2b**	21.8	226	2.344
3a	13.5	184	0.538	**3b**	26.4	254	3.136
4a	13.0	184	0.493	**4b**	27.2	282	3.171
5a	17.2	198	1.298	**5b**	27.1	282	3.208
6a	17.4	198	1.060	**6b**	29.6	282	3.506
7a	21.2	198	1.772	**7b**	29.3	282	3.035
HDMF monoacylate	RT	MW	log *P*	HDMF diacylate	RT	MW	log *P*
8a	7.1	170	−0.093	**8b**	12.7	212	0.403
9a	12.4	184	0.497	**9b**	26.0	240	1.663
10a	17.1	198	0.770	**10b**	29.9	268	2.847
11a	16.9	198	0.710	**11b**	30.1	268	2.896
12a	21.9	212	1.186	**12b**	33.9	296	3.326
13a	23.0	212	1.314	**13b**	33.5	296	3.505
14a	19.8	212	1.523				
EHMF monoacylate	RT	MW	log *P*	HEMF diacylate	RT	MW	log *P*
15a	12.0	184	0.420	**15b**	21.6	226	1.698
16a	15.9	198	0.890	**16b**	25.0	254	2.525
17a	21.6	212	1.898	**17b**	28.4	282	3.180
18a	18.9	212	1.373	**18b**	28.4	282	3.159
19a	21.7	226	1.994	**19b**	31.8	310	3.173
20a	21.5	226	1.915	**20b**	29.0	310	3.204
21a	23.3	226	1.588				
22a	38.9	380	4.627*				
23a	41.0	408	4.964*				
24a	39.5	406	4.724*				
25a	35.6	404	4.098*				
26a	39.7	402	4.763*				

RT, retention time by HPLC; MW, molecular weight of derivative; log *P*, hydrophobic constant (logarithm of partition coefficient).
* log *P* was calculated from the RT value ($Y=0.1604X-1.6121$).

of each of these pivalates was more effective than that of the lead compound (Fig. 11).

The anti-cataract activities of the acyl derivatives *in vivo* were examined by the spontaneous cataract rat (ICR/f) model, and are shown in Table 2. On the 21st day of this experiment, 56% of lenses in the control group had opacity and were judged to be the cataract condition. **14a** most significantly inhibited cataract for-

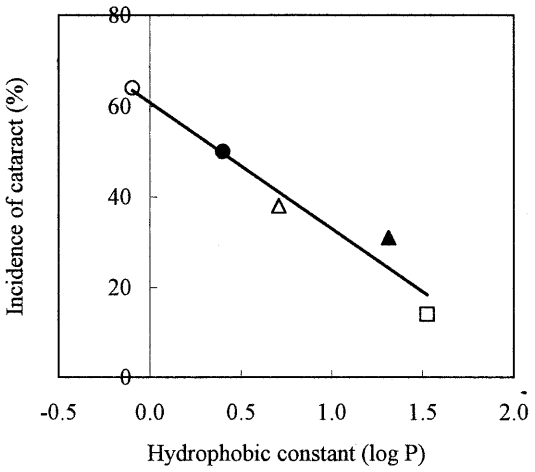

Fig. 12. Hydrophobic Constant and Incidence of Cataract in Spontaneous Cataract Rats (ICR/f).

4-Acetoxy-2,5-dimethyl-3(2*H*)-furanone (○), 3,4-diacetoxy-2,5-dimethylfurane (●), 4-isobutyl-2,5-dimethyl-3(2*H*)-furanone (△), 4-isovaleryl-2,5-dimethyl-3(2*H*)-furanone (▲), 2,5-dimethyl-4-pivaloyloxy-3(2*H*)-furanone (□). There was a correlation between the hydrophobic constant of each HDMF derivative and the incidence of cataract: $Y=-27.911X+60.93$, $R^2=0.9477$.

Table 2. 4-Hydroxy-3(2*H*)-furanone Derivatives and Their Effect on the Incidence of Cataract Development in ICR/f Rats

Acyl derivatives of 4-hydroxy-3(2*H*)-furanones	Incidence of cataract on the 21st day (%)	Number of cataract Lenses/Total number of lenses
7a	39	7/18
8a	64	9/14
11a	38	6/16
13a	31	5/16
14a	14	2/14
15a	36	5/14
17a	42	5/12
21a	36	5/14
25a	50	7/14
1b	39	7/18
Saline (control)	56	10/18

mation with 14% of cataract incidence. The compounds which controlled the incidence of cataract to less than 40% were isovalerate **13a**, acetate **15a**, pivalate **21a**, isobutyrate **11a**, diacetate **1b**, and pivalate **7a**. A comparison among the HDMF derivatives showed that the more hydrophobic the derivative was, the more effectively it inhibited cataract development in ICR/f rats. Linoleate **25a**, however, one of the most hydrophobic derivatives and having an unsaturated acyl group, had no effect on preventing galactose-induced cataract formation *in vitro* and cataract development in the ICR/f rats.

Half an hour after administering **14a**, the lenses were removed from one of the ICR/f rats that had been treated with **14a**, and were homogenized to measure HDMF. Consequently, we observed the same peak (same retention time and same wavelength absorbance) as that of HDMF by HPLC. The existence of HDMF in the lens was gauged from this result.

Discussion

Dipivaloyl epinephrine (DPE) is known as a clinically useful prodrug of epinephrine for the treatment of glaucoma.[16] A prodrug is an agent that undergoes biotransformation prior to exhibiting its pharmacologic action.[4] Although the human cornea is anatomically composed of 5 distinct tissue types, only the epithelium, stroma and endothelium present barriers to absorption.[4] The epithelial barrier resists penetration because it is lipophilic and has low porosity and high tortuosity. On the other hand, the stroma allows relatively free diffusion of a drug because of its aqueous environment, low tortuosity and high porosity. The resistance of the epithelium substantially limits the penetration of hydrophilic drugs, whereas the stroma and endothelium have only minimal effect. In contrast, the epithelium was minimal effect, while the stroma and endothelium become significant barriers to most lipophilic drugs. Consequently, highly lipophilic prodrugs have the potential to accumulate in the epithelium. However, once a prodrug has been converted by esterases residing in the epithelium to the hydrophilic drug species, it can potentially accumulate in the stroma and slowly penetrate into the crystalline lens across the lipophilic endothelial barrier. Acyl derivatives were synthesized which could potentially penetrate into the cornea more easily than the lead compounds and could be hydrolyzed by esterase in the cornea.

We synthesized 45 of the possible acyl derivatives as prodrugs and examined their hydrophobic constants and anti-cataract effects. Of these derivatives, 2,5-dimethyl-4-pivaloyloxy-3(2*H*)-furanone (**14a**) was observed to have a marked protective effect against cataract development in the galactose-induced cataract model (*in vitro*). Furthermore, **14a** showed more significant inhibition of cataract development than the parent compound HDMF did in spontaneous cataract rats (ICR/f). It was found that not only **14a**, but also several hydroxyfuranones derivatives, inhibited cataract formation in rats (Table 2). Therefore, we investigated the properties of the HDMF derivatives. Figure 12 shows that the incidence of cataract development in spontaneous cataract rats (ICR/f) was in proportion to the hydrophobic constant of the HDMF derivatives examined. The larger the hydrophobic constant (log *P*) of the HDMF derivatives was, the more easily it could penetrate into the cornea. **14a** was the most effective anti-cataract drug of these compounds. We assume that **14a** penetrated into the cornea most efficiently and was converted into HDMF to inhibit cataract development in the lens. The more hydrophobic a derivative was did not necessary inhibit cataract development, because one of the most hydrophobic derivatives, linoleoyl HDMF, had no effect in the anti-cataract test on ICR/f rats.

Hydroxyfuranones were modified to become more suitable for penetrating the cornea. Therefore, **14a** is concluded to be the best compound of the 45 furanone derivatives synthesized from the results of our anti-

cataract tests. There are probably three reasons why **14a** had the best performance among our derivatives: first is the required hydrophobicity to penetrate the cornea, second is the compact molecule to penetrate the cell membrane, and third is the possibility of hydrolysis in the cornea. The hydrolysis of **14a** in the cornea was suggested by the fact that HDMF was identified by HPLC in lenses which had been removed from ICR/f rat that had been administered. It is assumed that **14a** that had penetrated into the cornea was hydrolyzed by esterase in cornea and performed as an anti-cataract compound in the lens.

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