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Studies on Styrene Derivatives. I. Synthesis and Antiinflammatory Activities of α-Benzylidene-γ-butyrolactone Derivatives[1)]

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Two α-benzylidene-γ-butyrolactones, α-(3,5-dimethyl-4-hydroxybenzylidene)-γ-butyrolactone and α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone (KME-4), were found to have platelet aggregation inhibitory activity; the latter also had potent antiinflammatory activity and inhibited not only prostaglandin synthetase (PGS) but also 5-lipoxygenase. Further α-benzylidene-γ-butyrolactones were synthesized, and tested for antiinflammatory activity in carrageenin-induced rat paw edema assay (CPE) and for PGS inhibitory activity. It was found that the structural combination of a *tert*-butyl group at the 3 position, an alkyl group at the 5 position and an oxygen atom at the 4 position in α-benzylidene-γ-butyrolactone is necessary for antiinflammatory activity, and that rather broad structural variation is possible for inhibitors of PGS. The structural requirements for antiinflammatory activity in the CPE assay also seem to be partial requirements for inhibitory activity against PGS.

Keywords——α-benzylidene-γ-butyrolactone; α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone (KME-4); antiinflammatory activity; prostaglandin synthetase inhibition; 5-lipoxygenase inhibition; carageenin-induced rat paw edema test; structure–activity relationship

Introduction

γ-Butyrolactone derivatives have various biological activities,[2)] and an α,β-unsaturated carbonyl skeleton is often involved in the structure of biologically active substances[3)] (*e.g.* analogues of cinnamic acid).[4)] α-Methylene-γ-butyrolactone[5)] has some biological activities, and this compound contains both γ-butyrolactone and α,β-unsaturated carbonyl moieties. We therefore synthesized several α-benzylidene-γ-butyrolactones, and tested them for biological activities. As a result, α-(3,5-dimethyl-4-hydroxybenzylidene)-γ-butyrolactone was found to have platelet aggregation inhibitory activity. Other α-(3,5-dialkyl-4-hydroxybenzylidene)-γ-butyrolactones were further prepared and tested for this activity. Among them, a new compound which we designated as KME-4, α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone, was the most active and also had potent antiinflammatory activity in the carrageenin paw edema test. Moreover, it inhibited not only prostaglandin synthetase (PGS) but also 5-lipoxygenase.[6)] Some work has been done on the relationships between structural factors and antiinflammatory activity or PGS inhibitory activity.[5d,7)] We therefore prepared more α-benzylidene-γ-butyrolactones in the hope of finding new and potent antiinflammatory agents and to identify the important functional groups in relation to antiinflammatory activity.

Chemistry

The compounds were synthesized by three general methods. Most of the compounds were prepared as shown in Chart 1. Aldehydes were condensed with γ-butyrolactone in the presence of a base such as sodium methoxide,[2g,8)] or were reacted with α-triphenylphosphoranylidene-γ-butyrolactone by using the Wittig reaction.[5b,9)]

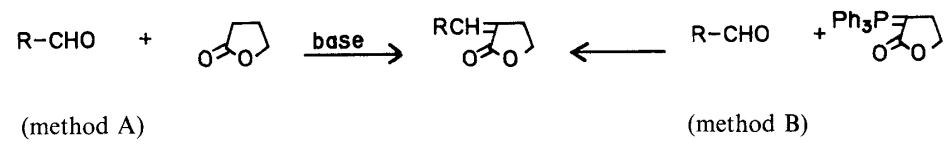


Chart 1

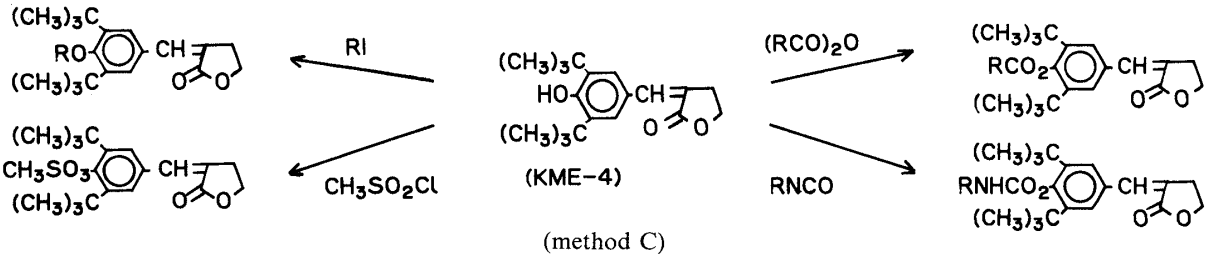
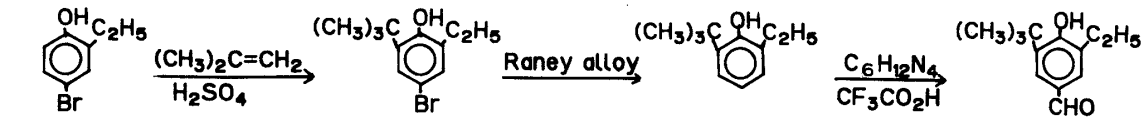
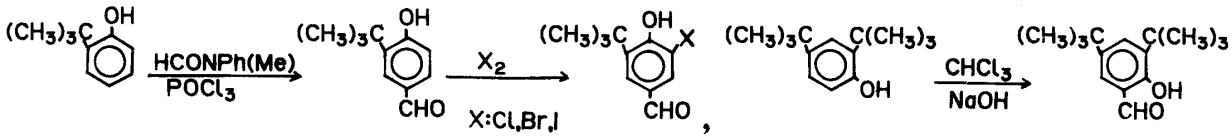
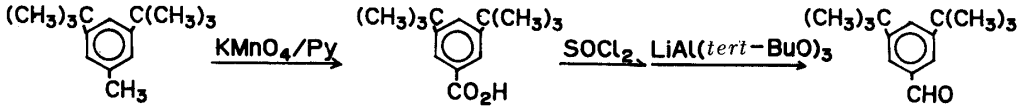


Chart 2







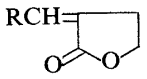
Py = pyridine

Chart 3

Other preparative methods are shown in Chart 2. Derivatives of α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone were obtained by reaction with acid anhydrides, isocyanates, alkyl iodides, or methanesulfonyl chloride.

Some of the starting aldehydes in Chart 1 were commercially available and other aldehydes were synthesized. For example, as shown in Chart 3, 3-*tert*-butyl-5-ethyl-4-hydroxybenzaldehyde was prepared by the procedures of Hart and Haglund,[10] and Smith.[11] That is, it was obtained by Friedel–Crafts alkylation of 4-bromo-2-ethylphenol with isobutene in the presence of sulfuric acid, followed by debromination with Raney nickel, and finally the improved Duff reaction. 3-*tert*-Butyl-4-hydroxybenzaldehyde was obtained by Vilsmeier reaction of *o*-*tert*-butylphenol and *N*-methylformanilide in the presence of phosphorus oxychloride. Halogenation of this compound gave 3-*tert*-butyl-4-hydroxy-5-halobenzaldehyde. 3,5-Di-*tert*-butyl-2-hydroxybenzaldehyde was synthesized by Reimer–Tiemann formylation. The preparation of 3,5-di-*tert*-butylbenzaldehyde was achieved by oxidation of 3,5-di-*tert*-butyltoluene followed by reduction of the resulting 3,5-di-*tert*-butylbenzoic acid.

TABLE I. Antiinflammatory Activity and PGS Inhibitory Activity of α-Benzyliden-γ-butyrolactones



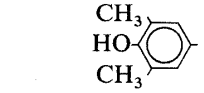
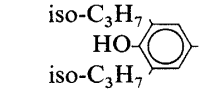
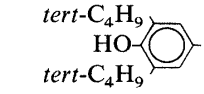
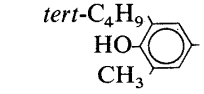
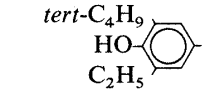
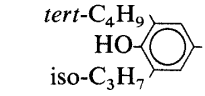
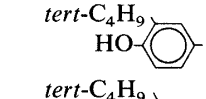
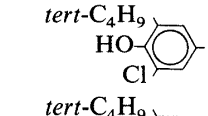
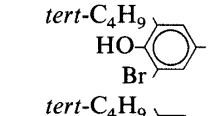
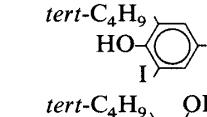
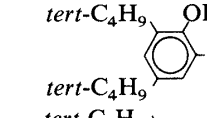
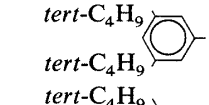
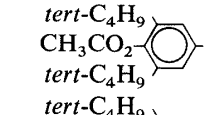
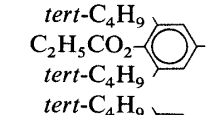
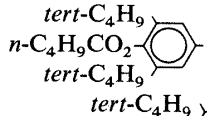
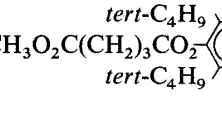
Compd. No.	R	Method[a]	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	CPE[b] (%)	PGS IC_{50} (μM)
1		B	48.5	180—182	$C_{13}H_{14}O_3$	71.54 (71.28	6.47 6.53)		9	17
2		B	34.6	169—171	$C_{17}H_{22}O_3$	74.42 (74.41	8.08 8.10)		−7	11
3		B	77.4	155—156	$C_{19}H_{26}O_3$	75.46 (75.41	8.67 8.76)		35	0.28
4		B	48.4	201—203	$C_{16}H_{20}O_3$	73.82 (73.74	7.74 7.77)		24	0.89
5		B	67.7	180—182	$C_{17}H_{22}O_3$	74.42 (74.31	8.08 8.31)		21	0.19
6		B	45.8	155—157	$C_{18}H_{24}O_3$	74.97 (75.09	8.39 8.34)		27	0.36
7		B	71.7	239—241	$C_{15}H_{18}O_3$	73.14 (72.74	7.37 7.56)		6	1.4
8		B	60.5	224—226	$C_{15}H_{17}ClO_3$	64.18 (63.91	6.10 6.15)		9	0.90
9		B	41.0	233—235	$C_{15}H_{17}BrO_3$	55.40 (55.37	5.27 5.22)		4	
10		B	66.3	219—221	$C_{15}H_{17}IO_3$	48.41 (48.20	4.60 4.55)		5	0.69
11		B	14.2	195—197	$C_{19}H_{26}O_3$	75.46 (75.29	8.67 8.85)		5	6.9
12		B	68.1	112—114	$C_{19}H_{26}O_2$	[c]			11	
13		C	79.0	144—146	$C_{21}H_{28}O_4$	73.22 (73.11	8.19 8.22)		37	1.9
14		C	41.0	107—108	$C_{22}H_{30}O_4$	73.71 (73.57	8.44 8.64)		14	
15		C	48.6	142—144	$C_{24}H_{34}O_4$	74.57 (74.07	8.87 8.55)		9	7.3
16		C	64.1	103—105	$C_{25}H_{34}O_6$	69.74 (69.69	7.96 7.78)		2	

TABLE I. (continued)

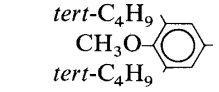
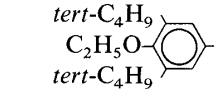
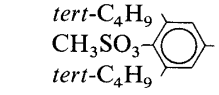
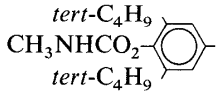
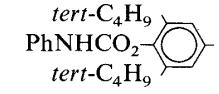
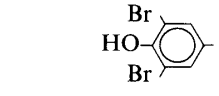
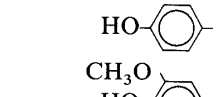
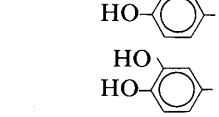
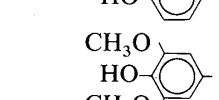
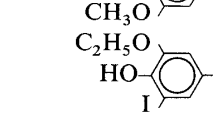
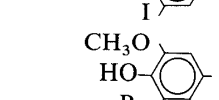
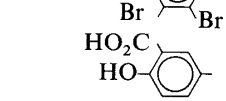
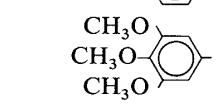
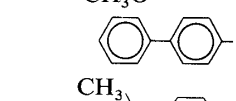
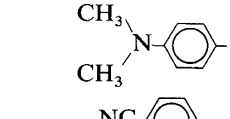
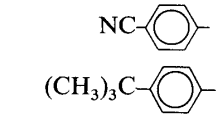
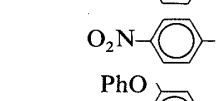
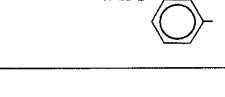
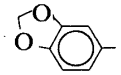
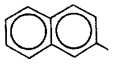
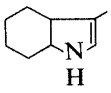
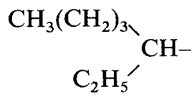
Compd. No.	R	Method[a]	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	CPE[b] (%)	PGS IC_{50} (uM)
17		C	76.0	131—132	$C_{20}H_{28}O_3$	75.91 (75.99	8.92 8.70)		25	1.2
18		C	32.1	122—125	$C_{21}H_{30}O_3$	76.32 (76.33	9.15 8.90)		24	
19		C	1.7	187—189	$C_{20}H_{28}O_5S$	63.14 (63.19	7.42 7.21)		4	14
20		C	50.4	185—187	$C_{21}H_{29}NO_4$	70.17 (69.70	8.13 8.15	3.90 3.86)	5	
21		C	74.2	176—178	$C_{26}H_{31}NO_4$	74.08 (73.88	7.41 7.40	3.32 3.28)	10	
22		B	73.9	233—235	$C_{11}H_8Br_2O_3$	37.97 (37.80	2.32 2.42)		−12	>100
23		A	1.8	180—181	$C_{11}H_{10}O_3$		[d]		11	>100
24		B	55.1	155—156	$C_{12}H_{12}O_4$		[d]		−9	>100
25		B	16.1	206—208	$C_{11}H_{10}O_4$	64.07 (63.90	4.89 4.80)		5	35
26		B	77.0	156—158	$C_{13}H_{14}O_5$	62.39 (62.18	5.64 5.82)		−1	
27		B	16.3	215—217	$C_{13}H_{13}IO_4$	43.36 (43.11	3.64 3.69)		12	>100
28		B	20.1	204—206	$C_{12}H_{10}Br_2O_4$	38.13 (38.04	2.67 2.48)		−22	>100
29		B	7.7	251—253	$C_{12}H_{10}O_5$	61.54 (61.38	4.30 4.19)		11	>100
30		B	59.4	150—151	$C_{14}H_{16}O_5$		[d]		17	
31		B	89.0	201—203	$C_{17}H_{14}O_2$	81.58 (81.63	5.64 5.57)		−9	>100
32		B	55.7	192—193	$C_{13}H_{15}NO_2$		[d]		−3	>100
33		B	56.8	158—160	$C_{12}H_9NO_2$	72.35 (72.03	4.55 4.30	7.03 7.01)	−4	>100
34		B	32.2	132—134	$C_{15}H_{18}O_2$	78.23 (78.29	7.88 8.06)		−3	>100
35		B	74.0	200—202	$C_{11}H_{19}NO_4$	60.27 (60.18	4.14 4.01	6.39 6.37)	1	
36		B	62.8	91—93	$C_{17}H_{14}O_3$	76.67 (76.46	5.30 5.17)		17	100

TABLE I. (continued)

Compd. No.	R	Method[a]	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	CPE[b] (%)	PGS IC_{50} (μM)
37	CH_3CONH- 	B	15.7	197—198	$C_{13}H_{13}NO_3$		[d]		−13	
38		A	34.3	176—177	$C_{12}H_{10}O_4$		[d]		19	
39		B	84.9	169—171	$C_{16}H_{12}O_2$	80.33 (80.05	5.39 5.31)		−18	
40		B	44.0	110—112	$C_{10}H_9NO_2$	68.56 (68.47	5.18 5.18	8.00 7.80)	10	
41		B	19.6	219—220	$C_{13}H_{11}NO_2$	73.22 (73.32	5.20 4.96	6.57 6.58)	−8	
42		B	75.1	92—94	$C_9H_8O_3$		[e]		−2	
43	$CH_3(CH_2)_3$ 	B	39.8	Oil	$C_{12}H_{20}O_2$	73.43 (73.47	10.27 10.07)		−8	
44	H	B	54.2	Oil	$C_5H_6O_2$		[f]		3	
Indomethacin									33[g]	0.53
Phenylbutazone									22	
Ibuprofen									32	

a) See Charts 1 and 2. *b*) Inhibition (%) of edema formation induced by carrageenin in rats (50 mg/kg *p.o.*). *c*) High-resolution mass spectrum data *m/e*: 286.193 (M^+). *d*) See ref 8*a*. *e*) See ref 9*c*. *f*) See ref 5*b*, 9*d*. *g*) 5 mg/kg *p.o.*

Results and Discussion

Antiinflammatory activities of the compounds were evaluated by using the carrageenin-induced edema test in rats. Some compounds were examined *in vitro* for their effects on microsomal prostaglandin synthetase and cytosol 5-lipoxygenase activities.

The results are summarized in Table I. The synthesized α-benzylidene-γ-butyrolactones are pure isomers (either *cis* or *trans*) judging from the narrow range of melting point and the nuclear magnetic resonance (NMR) spectrum. Compound **3** is considered to be the *trans* isomer; in the NMR spectrum of **3**, the chemical shift of the olefinic proton shifted to lower field, when the solvent was changed from $CDCl_3$ to C_6H_6-d_6, whereas other signals shifted to higher field. This result suggests that the olefinic proton is *cis* oriented with respect to the carbonyl group, as mentioned by Minami *et al.*[12] The other compounds are also considered to be *trans* isomers, because the products are generally *trans* in the Wittig reaction of a resonance-stabilized phosphorane with an aldehyde[13] or in aldol-type condensation.[14]

Though α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone (**3**) had potent antiinflammatory and PGS inhibitory activity, α-(3,5-dimethyl-4-hydroxybenzylidene)-γ-butyrolactone (**1**) and α-(3,5-diisopropyl-4-hydroxybenzylidene)-γ-butyrolactone (**2**) showed no antiinflammatory activity and only moderate PGS inhibitory activity. The other 3,5-dialkyl-4-hydroxyphenyl compounds in which the *tert*-butyl group at the 5 position of **3** is replaced with a methyl (**4**), ethyl (**5**) or isopropyl group (**6**) showed considerable antiinflammatory activity. Hydrogen, chlorine, bromine or iodine in place of the *tert*-butyl group at the 5 position of **3** (**7**—**10**) resulted in loss of this activity. On the other hand, these

Table II. Inhibition of 5-Lipoxygenase

Compd. No.	IC_{50} (μM)
3	1.05
4	10.7
13	>100
17	>100
Indomethacin	>100

compounds (**4**—**10**) showed excellent or moderate PGS inhibitory activity. Compounds **11** and **12** (lacking the hydroxy group at position 4) exhibited a striking loss of antiinflammatory activity and a decrease in PGS inhibitory activity. As regards the antiinflammatory activity of derivatives of **3**, the acetyl derivative (**13**) retained potent activity, but the derivatives (**14**—**16**) having a bulky acyl group showed weak activity. Alkoxy compounds (**17**, **18**) showed slightly diminished activity. Carbamates (**20**—**21**) and the mesylate (**19**) were inactive. Some of these compounds (**13**—**21**) had significant or moderate PGS inhibitory activity. Other phenol derivatives without a *tert*-butyl group (**23**—**29**), phenyl analogues substituted with a variety of groups (**30**—**39**), heterocyclic compounds (**40**—**42**) and others (**43**, **44**) had little or no activity in both tests (carageenin-induced rat paw edema and PGS).

These results suggested that the structural combination of a *tert*-butyl group at the 3 position, an alkyl group at the 5 position and an oxygen atom at the 4 position in the α-benzylidene-γ-butyrolactones plays an important role in enhancing antiinflammatory activity, and that the combination of a *tert*-butyl group at the 3 position and an oxygen atom at the 4 position, or of a hydroxy group at the 4 position and two alkyl groups at the 3 and 5 positions is necessary for activity as an inhibitor of PGS. Thus, broad structural variations are possible among these compounds without loss of activity as inhibitors of PGS, and the structure at the 3, 4 and 5 positions in the compounds, as described above, seems to be important for potent antiinflammatory activity. As regards antiinflammatory activity, **3** and α-(3,5-di-*tert*-butyl-4-acetoxybenzylidene)-γ-butyrolactone (**13**) were the most potent compounds in this series. They were more potent than ibuprofen and phenylbutanone, but less potent than indomethacin in inhibiting the inflammation induced by carrageenin. Compounds such as **4** and **17** showed moderate activity, but not more than the reference drugs. Compounds **3**, **4**, **13** and **17** were about as effective as indomethacin in inhibiting PGS.

As shown in Table II, **3** and **4** had potent inhibitory activity against 5-lipoxygenase, but **13**, **17** and indomethacin were inactive.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-30IR spectrometer. NMR spectra were recorded on a Varian EM-390 NMR spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed at Sagami Chemical Research Center and high-resolution mass spectrum was determined at the National Chemical Laboratory for Industry.

Preparation of α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone (3; KME-4) (Method B)——A solution of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (18 g), α-triphenylphosphoranylidene-γ-butyrolactone (27 g) in dimethyl sulfoxide (DMSO) was stirred at 80 °C for 20 h. After cooling of the mixture to room temperature, 800 ml of chloroform was added, and the resulting mixture was washed 5 times with the same volume of water. The chloroform layer was evaporated to dryness *in vacuo* and the residue was crystallized by addition of ethanol. Recrystallization from ethanol afforded compound **3** (18 g 77.4%). IR (KBr): 3505 (OH), 1735 (C=O), 1640 (C=C) cm^{-1}. NMR ($CDCl_3$) δ: 1.47 (18H, s, $(CH_3)_3C$), 3.21 (2H, dt, J=3, 7 Hz, $=CCH_2-$), 4.43 (2H, t, J=7 Hz, CH_2O-), 5.50 (1H, s, OH), 7.31 (2H, s, ArH), 7.49 (1H, t, J=3 Hz, CH=); (C_6D_6) δ: 1.33 (18H, s, $(CH_3)_3C$), 2.42 (2H, dt, J=7, 3 Hz,

$=CCH_2-$), 3.66 (2H, t, $J=7$ Hz, CH_2O-), 5.25 (1H, s, OH), 7.21 (2H, s, ArH), 7.79 (1H, t, $J=3$ Hz, CH=).

Compounds **1**, **2**, **4**—**12**, **22**, **24**—**37** and **39**—**44** were prepared by a similar procedure in 8—89% yields.

Preparation of α-(3,5-Di-*tert*-butyl-4-acetoxybenzylidene)-γ-butyrolactone (13)——A drop of sulfuric acid was added to a suspension of **3** (2.0 g) in acetic anhydride (3.0 g) and the mixture was stirred at 80 °C for 3 h, then poured onto crushed ice. The solid product was collected by filtration, washed with water, and recrystallized from ethanol to give **13** (1.8 g 79%). IR (KBr): 1750 (C=O), 1660 (C=C) cm^{-1}. NMR ($CDCl_3$) δ: 1.33 (18H, s, $(CH_3)_3C$), 2.19 (3H, s, CH_3COO-), 3.18 (2H, dt, $J=3$, 7 Hz, $=CCH_2-$), 4.41 (2H, t, $J=7$ Hz, CH_2O), 7.41 (2H, s, ArH), 7.48 (1H, t, $J=3$ Hz, CH=).

Compounds **14**—**16** were obtained by a similar method in 41—64% yields.

Preparation of α-(3,5-Di-*tert*-butyl-4-methoxybenzylidene)-γ-butyrolactone (17)——Sodium hydride (1 g, 60% mineral oil dispersion) and then methyl iodide (5 ml) were added to a solution of **3** (3.02 g) in tetrahydrofuran (15 ml). The mixture was heated under reflux for 7 h, and after cooling, poured into water (100 ml). The resulting mixture was acidified with dilute sulfuric acid, and extracted twice with chloroform (100 ml). The chloroform layer was washed twice with water and evaporated under reduced pressure. The residue was crystallized from *n*-hexane to give **17** (2.4 g 76%). IR (KBr): 1745 (C=O), 1640 (C=C) cm^{-1}. NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 3.62 (2H, dt, $J=3$, 7 Hz, $=CCH_2-$), 3.70 (3H, s, CH_3O), 4.43 (2H, t, $J=7$ Hz, CH_2O), 7.37 (2H, s, ArH), 7.50 (1H, t, $J=3$ Hz, CH=).

Compound **18** was prepared by a similar procedure in 32% yield.

Preparation of α-(3,5-Di-*tert*-butyl-4-methylsulfonyloxybenzylidene)-γ-butyrolactone (19)——Methanesulfonyl chloride (1.09 g) was added to a solution of the potassium salt of **3** in tetrahydrofuran (THF) (50 ml), and the mixture was heated under reflux for 9.5 h, then concentrated to dryness under reduced pressure. *n*-Hexane was added to the residue. The resulting mixture was washed with water, and dried over anhydrous sodium sulfate, then the solvent was evaporated off. The residue was purified by preparative thin layer chromatography (TLC) (silica gel; eluent, $CHCl_3$) and recrystallized from acetone to give **19** (61 mg 1.7%). IR (KBr): 1740 (C=O), 1655 (C=C) cm^{-1}. NMR ($CDCl_3$) δ: 1.45 (18H, s, $(CH_3)_3C$), 3.1—3.4 (5H, m, CH_3SO_3, $=CCH_2$), 4.37 (2H, t, $J=6$ Hz, CH_2O), 7.47 (2H, s, ArH), 7.53, (1H, t, $J=3$ Hz, CH=).

Preparation of α-(3,5-Di-*tert*-butyl-4-phenylcarbamyloxybenzylidene)-γ-butyrolactone (21)——A solution of **3** (3.20 g), triethylamine (0.2 ml) and phenylisocyanate (1.09 ml) in toluene (5 ml) was stirred at 70 °C for 5 h. The precipitated crystals were filtered, washed with toluene and recrystallized from ethanol to give **21** (2.89 g 74%). IR (KBr): 3330 (NH), 1750 (C=O), 1720 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.42 (18H, s, $(CH_3)_3C$), 3.26 (2H, dt, $J=3$, 7 Hz, =CH–CH), 4.47 (2H, t, 7 Hz, CH_2O), 7.0—7.6 (9H, m, CH=, aromatic H, NH).

Compound **20** was obtained in the same way in 50% yield.

Preparation of α-(3,4-Methylenedioxybenzylidene)-γ-butyrolactone (38) (Method A)——Sodium methoxide was added to a solution of piperonal (15.07 g) and γ-butyrolactone (17.22 g) in toluene (100 ml), and the mixture was stirred at 40—50 °C for 1 h. After cooling of the mixture, 10% sulfuric acid was added under stirring, and the whole was stirred at room temperature for 0.5 h. The resulting precipitate was filtered by suction, washed with water, and recrystallized from ethanol to give **38** (7.50 g 34.3%). IR (KBr): 1745 (C=O), 1660 (C=C) cm^{-1}. NMR (DMSO-d_6) δ: 3.22 (2H, dt, $J=3$, 7 Hz, $=CCH_2$), 4.42 (2H, t, $J=7$ Hz, CH_2O), 6.05 (2H, s, OCH_2O), 6.9—7.2 (3H, m, ArH), 7.3 (1H, t, $J=3$ Hz, CH=).

Compound **23** was prepared in the same manner in 1.8% yield.

Preparation of 4-Bromo-2-*tert*-butyl-6-ethylphenol——Isobutene (15 g) was passed through a mixture of 5-bromo-2-ethylphenol (36 g), benzene (48 ml) and sulfuric acid (1.2 ml) at 65 °C for 7.5 h. The mixture was washed with water, 5% $NaHCO_3$, and water. After removal of the solvent, the residue was distilled at 118—120 °C/4 mmHg to give the desired phenol (28.1 g 61.1%). NMR ($CDCl_3$) δ: 1.20 (3H, t, $J=7$ Hz, $C\underline{H}_3CH_2$), 1.40 (9H, s, $(CH_3)_3C$), 2.53 (2H, q, $J=7$ Hz, $CH_3C\underline{H}_2$), 4.78 (1H, s, OH), 7.07 (1H, d, $J=2$ Hz, ArH), 7.21 (1H, d, $J=2$ Hz, ArH).

This product was used for the next step without further purification.

Preparation of 2-*tert*-Butyl-6-ethylphenol——A solution of 10% NaOH (750 ml) was added slowly (over 2.5 h) to a mixture of 4-bromo-2-*tert*-butyl-6-ethylphenol (27.5 g), Raney nickel–aluminum alloy and 95% EtOH (125 ml). The resultant mixture was stirred at room temperature for 1 h, then refluxed for 2 h, and filtered. The nickel catalyst was washed with 10% NaOH followed by benzene. The filtrate and washing were poured into concentrated hydrochloric acid (625 ml) and extracted with $CHCl_3$. The organic layer was washed with water. After removal of the solvent, the residue was purified by column chromatography (silica gel; eluent $CHCl_3$) to give the desired phenol (11 g 57.8%). IR (neat): 3575 (OH), 1595 (Ar) cm^{-1}. NMR ($CDCl_3$) δ: 1.26 (3H, t, $J=8$ Hz, $C\underline{H}_3CH_2$), 1.43 (9H, s, $(CH_3)_3C$), 2.59 (2H, q, $J=8$ Hz, $CH_3C\underline{H}_2$), 4.79 (1H, s, OH), 6.67—7.16 (3H, m, ArH).

This product was used for the next step without further purification.

Preparation of 3-*tert*-Butyl-5-ethyl-4-hydroxybenzaldehyde——A mixture of 6-*tert*-butyl-2-ethylphenol (7.0 g), hexamethylenetetramine (5.5 g) and trifluoroacetic acid (60 ml) was stirred at 80—90 °C for 17 h. The mixture was concentrated, poured onto crushed ice, neutralized with Na_2CO_3, and extracted with $CHCl_3$. The organic extract was washed with water. After the removal of $CHCl_3$, the residue was recrystallized from benzene to afford the desired benzaldehyde (4.5 g 59.0%), mp 124—126 °C. IR (KBr): 3270 (OH), 1660 (C=O), 1575 (Ar) cm^{-1}. NMR ($CDCl_3$) δ: 1.30 (3H, t, $J=8$ Hz, $C\underline{H}_3CH_2$), 1.45 (9H, s, $(CH_3)_3C$), 2.72 (2H, q, $J=8$ Hz, $CH_3C\underline{H}_2$), 5.88 (1H, s, OH), 7.56 (1H, d,

J=2 Hz, ArH), 7.68 (1H, d, J=2 Hz, ArH), 9.84 (1H, s, CHO).

Preparation of 3-*tert*-Butyl-4-hydroxybenzaldehyde——Phosphorus oxychloride (21.4 g) was added dropwise to *N*-methyl formanilide (20.3 g) with stirring and ice-water cooling, and the reaction mixture was stirred at room temperature for 1 h. Next, *o*-*tert*-butylphenol (13.5 g) was added dropwise with stirring at room temperature. After additional stirring at room temperature for 1 h and at 50—60 °C for 5 h, the mixture was poured onto crushed ice, and extracted with $CHCl_3$. The organic layer was shaken with 5% KOH (400 ml). The alkaline layer was acidified with dil. HCl, and the resultant oil was extracted with $CHCl_3$. The organic layer was washed with water, and concentrated under reduced pressure, and the residue was recrystallized from toluene to afford the desired benzaldehyde (2.8 g 17.5%), mp 140—142 °C. IR (KBr): 3320 (OH), 1660 (C=O), 1580 (Ar) cm^{-1}. NMR ($CDCl_3$–DMSO-d_6 (3:1)) δ: 1.40 (9H, s, $(CH_3)_3C$), 6.89 (1H, d, J=8 Hz, ArH), 7.49 (1H, dd, J=2, 8 Hz, ArH), 7.69 (1H, d, J=2 Hz, ArH), 9.74 (1H, s, CHO), 9.60—10.30 (1H, br, OH).

Preparation of 3-Bromo-5-*tert*-butyl-4-hydroxybenzaldehyde——Bromine (4.7 g) was added to a solution of 3-*tert*-butyl-4-hydroxybenzaldehyde (3.0 g) in acetic acid (30 ml) with stirring and ice-water cooling. The reaction mixture was stirred at room temperature for 5 h, then poured onto crushed ice. The resulting precipitate was filtered by suction, washed with water, 0.1 N $Na_2S_2O_3$ and water, and recrystallized with water–ethanol to give the desired benzaldehyde (1.2 g 27.5%). IR (KBr): 3255 (OH), 1680 (C=O), 1590 (Ar) cm^{-1}. NMR ($CDCl_3$) δ: 1.44 (9H, s, $(CH_3)_3C$), 6.38 (1H, s, OH), 7.76 (1H, d, J=2 Hz, ArH), 7.90 (1H, d, J=2 Hz, ArH), 9.83 (1H, s, CHO).

This product was used for the next step without further purification.

Preparation of 3,5-Di-*tert*-butylbenzoic Acid——Potassium permanganate (39.1 g) was added over 2 h to a mixture of 3,5-di-*tert*-butyltoluene (20.4 g), pyridine (55 ml), KOH (8.61 g) and water (19 ml) with stirring at 90—95 °C, and the whole was stirred at 90—95 °C for 3.5 h. After cooling, the mixture was filtered and the filtrate was washed with 2 N KOH and water. The filtrate and the washings were combined, acidified with sulfuric acid, and extracted with ether. The ether extract was dried over Na_2SO_4, and concentrated to dryness. The residue was recrystallized from EtOH–H_2O to afford the desired benzoic acid (2.21 g 52.1%). IR (KBr): 3000—2500 (COOH), 1690 (C=O), 1600 (Ar) cm^{-1}. NMR ($CDCl_3$) δ: 1.39 (18H, s, $(CH_3)_3C$), 7.69 (1H, t, J=2 Hz, ArH), 8.00 (2H, d, J=2 Hz, ArH).

This product was used for the next step without further purification.

Preparation of 3,5-Di-*tert*-butylbenzaldehyde——A solution of the 3,5-di-*tert*-butylbenzoyl chloride in THF (10 ml) was added dropwise over 40 min to a slurry of $LiAl[OC(CH_3)_3]_3H$ (2.50 g) and THF (10 ml) with stirring at −78 °C. The mixture was stirred at −78 °C for 0.5 h and at room temperature for 2 h, then water (100 ml) was added. The whole was neutralized with dil. sulfuric acid, and extracted with $CHCl_3$. The organic extract was washed with water and dried over Na_2SO_4. After removal of the $CHCl_3$, the residue was purified by column chromatography (silica gel; eluent CCl_4–$CHCl_3$ (49:1)) to give the desired benzaldehyde (0.43 g 2.2%). IR (CCl_4): 1700 (C=O) cm^{-1}. NMR (CCl_4) δ: 1.39 (18H, s, $(CH_3)_3C$), 7.61 (3H, s, ArH), 9.93 (1H, s, CHO).

This product was used for the next step without further purification.

Preparation of 3,5-Di-*tert*-butyl-2-hydroxybenzaldehyde——Chloroform (6.2 g) was added over 0.5 h to a mixture of 2,4-di-*tert*-butylphenol (10.0 g) in EtOH (25 ml), NaOH (14.0 g) in water (30 ml), and a small amount of benzyltriethylammonium chloride, with stirring at 70—80 °C. The whole was stirred for 50 min, then the solvent was evaporated off. The residue was acidified (pH 1) with dil. sulfuric acid and extracted with ether. The ether extract was washed with water and satd. NaCl, then dried over Na_2SO_4. After removal of the ether, the residue was subjected to column chromatography (silica gel; eluent $CHCl_3$) to give a crude benzaldehyde (1.03 g) which contained a small amount of impurities. This crude product was used for the next step without further purification.

Pharmacological Tests——Carrageenin-induced Paw Edema in Rats: Antiinflammatory activities of the compounds were evaluated by the method of Winter *et al.*[15] The test compounds were administered orally to groups of 4—6 male Wistar rats weighing 160—220 g. One hour later, 1% carrageenin (0.1 ml/rat) in 0.9% NaCl was injected subcutaneously into a hind paw. Paw volumes were measured 5 h after the injection of carrageenin. The results were expressed as percent inhibition of edema in comparison with the control.

Preparation of Enzymes: The microsomal fraction from kidney medullas of male rabbits was obtained according to the method of Tai *et al.*[16] and used as the prostaglandin synthetase preparation.

Peritoneal polymorphonuclear leucocytes from male guinea pigs (2% casein 5 ml/100 g body weight, intraperitoneal injection, 15 h) were prepared essentially according to the method of Ochi *et al.*[17] The cells suspended in 50 mM phosphate buffer (pH 7.4) containing 1 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) and 0.1% gelatin were sonicated, then centrifuged at 105000 ***g*** for 60 min. The cytosol fraction was used as the 5-lipoxygenase preparation.

Enzyme Assays: The test compounds were dissolved in ethanol and the final concentration of ethanol was kept at 2% in each assay. The reaction mixture for prostaglandin synthetase assay consisted of 100 mM Tris–HCl (pH 7.6), 4 mM glutathione (GSH), 4 mM epinephrine bitartrate, 20 μM [1-^{14}C]arachidonic acid (0.05 μCi), microsomal enzyme (110 μg protein) and a test compound in a total volume of 0.2 ml. The mixture was incubated for 15 min at 37 °C with shaking and the reaction was terminated by the addition of 2.5 ml ethyl acetate and 25 μl of 1 N formic acid.

For the 5-lipoxygenase assay, the cytosol fraction (protein, 600 μg/0.2 ml/tube) was preincubated with a test

compound in the presence of 1 mM $CaCl_2$ and 1 mM GSH for 5 min at 30 °C, and then the mixture was incubated with 20 μM [1-^{14}C]arachidonic acid (0.1 μCi) for 5 min at 30 °C with shaking. The reaction was terminated by the addition of 2.5 ml of chloroform–methanol (2 : 1) and 0.3 ml of 40 mM citric acid.

In both cases, the metabolites in the extract (solvent layer) were separated by TLC with ethyl acetate–2,2,4-trimethylpentane–acetic acid–water (11 : 5 : 2 : 10 solvent layer). Radioactive zones were located by radioautography, scraped off and counted in a liquid scintillation counter. Microsomal prostaglandin synthetase activity was measured in terms of both PGE_2 and $PGF_{2\alpha}$ production, and cytosol 5-lipoxygenase was measured in terms of 5-hydroxyeicosatetraenoic acid (5-HETE) production.

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