2,4-DIHYDROXYACETOPHENONE DERIVATIVES AS ANTILEUKOTRIENICS WITH A MULTIPLE ACTIVITY MECHANISM

Miroslav KUCHAR, Katerina CULIKOVA, Vladimira PANAJOTOVOVA, Bohumila BRUNOVA, Antonin JANDERA and Vojtech KMONICEK

Research Institute for Pharmacy and Biochemistry, 130 60 Prague 3, Czech Republic; e-mail: vufbchs@mbox.vol.cz

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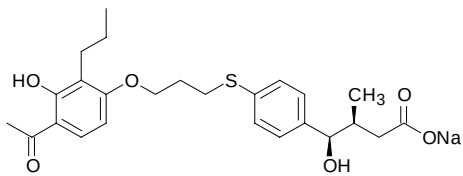
A series of 2,4-dihydroxy-3-propylacetophenone derivatives containing alkylthiobenzoic or arylacetic acid moiety was prepared. The antileukotrienic activity of the substances was assessed in terms of inhibition of the biosynthesis of LTB_4 and bonding to LTB_4 and LTD_4 receptors. Some of the substances were found to exert a complex antileukotrienic effect in all of the three tests. The compounds also displayed an antiinflammatory effect, as manifested by a significant inhibition of LTD_4-induced bronchospasm.

Key words: 2,4-Dihydroxy-3-propylacetophenone derivatives; Antileukotrienic activity; Antiasthmatic effect.

Affecting the enzyme system which catalyzes the oxidative metabolism of arachidonic acid can serve as a fruitful basis for the development of efficient chemotherapeutics. Initially, this primarily concerned inhibitors of cyclooxygenase, interfering with the biosynthesis of prostaglandins (PG) and thromboxanes (Tx), which are used as anti-inflammatory agents and peripheral analgetics[1] and influence significantly the behaviour of some blood particles, thrombocytes in particular[2]. An alternative biotransformation pathway of arachidonic acid, during which another class of important metabolites of this acid, *viz.* leukotrienes (LT), are formed as a result of the action of 5-lipoxygenase (5-LO), was discovered more recently[3]. The peptidoleukotrienes C_4 and D_4 were characterized[4] as components of the "slowly reacting substance" SRS-A which induces anaphylactic reaction and has been known for nearly half a century[5]. SRS-A has been found[6] to be an inflammation mediator in bronchial constriction and to increase vascular permeability in diseases associated with acute hypersensitivity. Furthermore, it has been observed that LTB_4 stimulates aggregation and degranulation of human neutrophils and promotes chemotaxis and chemokinesis of leukocytes and other cells which are involved in the development of the inflammatory process. LTB_4 is a mediator in the release of lysosomal enzymes and stimulates the formation of superoxide[7,8]. Therefore, this substance is supposed to be an important mediator in many inflammatory diseases[9].

Major progress in the investigation of LT antagonists has been achieved throughout the past decade. The discovery[10] of the substance FPL 55,712 (**1**) has opened up a way to the development of many efficient antagonists of leukotrienes containing the hydroxyacetophenone moiety in their molecules[11–13]. Structural modifications of this substance afforded a series of very efficient antagonists of LTD_4. Among the best known substances in this class ranks[14] LY 171,883, 5-[4-(4-acetyl-3-hydroxy-2-propylphenoxy)butyl]tetrazole. This compound was employed as a reference standard in the present study. Most of the substances in this class exhibit mainly an antagonistic effect towards LTD_4, accompanied by insignificant inhibition of 5-LO. Antagonism towards LTB_4, associated with a modification of the dihydroxyacetophenone moiety by methylation of hydroxy groups, was observed exceptionally[15]. Inhibition of 5-LO is exceptional with dihydroxyacetophenone derivatives; such inhibition has been observed, for instance, with substance WY-44329 (**2**), where a simultaneous antagonistic effect towards LTD_4 has been found[16]. The present work was aimed at preparing 2,4-dihydroxyacetophenone derivatives carrying a carbonyl group at the other aromatic ring and exhibiting a broader spectrum of antileukotrienic activities.



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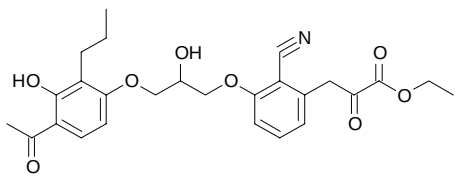

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RESULTS AND DISCUSSION

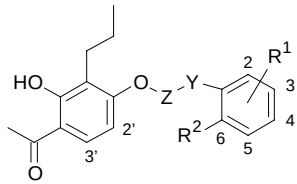
The series of 2,4-dihydroxyacetophenone derivatives (**3**) was synthesized using fragments of 2-alkylthiobenzoic, 3-alkylthiobenzoic, and arylacetic acids as the carbonyl carriers. 2,4-Dihydroxy-3-propylacetophenone was prepared following ref.[17]. Compounds **3** (Y = $(CH_2)_n$) were obtained by reacting mercaptobenzoic acids or methyl 4-hydroxyarylacetates with 2-haloalkoxyacetophenones **4a–4c** (Method *A*). In the case of esters **3h**, **3k**, **3m**, and **3o**, the corresponding acids were also isolated (Method *B*). To obtain the hydroxy analogs **3p** and **3r**, 4-(2,3-epoxypropoxy)-2-hydroxy-3-propylacetophenone (**5**) was prepared and reacted with the corresponding mercaptobenzoic acid (Method *C*). The physicochemical data of compounds **3** are given in Table I.

TABLE I
Physicochemical data of 2,4-dihydroxy-3-propylacetophenone derivatives **3**

Compound	M.p., °C Solvent[a]	Method Yield, %	Formula M.w.	Calculated/Found		
				%C	%H	%S or %Cl
3a	153–155 AA	*A* 32	$C_{20}H_{22}O_5S$ 374.4	64.15 63.93	5.92 5.88	8.56 8.62
3b	161–163 MA	*A* 41	$C_{21}H_{24}O_5S$ 388.5	64.92 64.79	6.23 6.41	8.25 8.03
3c	111–113 CH	*A* 54	$C_{22}H_{26}O_5S$ 402.5	65.64 65.39	6.51 6.71	7.97 7.99
3d	112–113.5 AA	*A* 32	$C_{20}H_{22}O_5S$ 374.4	64.15 64.07	5.92 6.06	8.56 8.60
3e	137–138.5 MA	*A* 67	$C_{21}H_{24}O_5S$ 388.5	64.92 64.95	6.23 6.28	8.25 7.94
3f	113–115 MA	*A* 60	$C_{22}H_{26}O_5S$ 402.5	65.64 65.81	6.51 6.72	7.97 7.63
3g	110–112 MA	*B* 75	$C_{22}H_{26}O_6$ 386.4	68.38 68.21	6.78 6.73	
3h	64–65.5 MA	*A* 48	$C_{23}H_{28}O_6$ 400.5	68.98 68.85	7.05 7.18	
3i	73–76 NM	*A*, *B* 48[b]	$C_{23}H_{28}O_6 \cdot H_2O$ 418.5	66.02 66.12	7.22 7.08	
3j	128–130 –	*B* 82	$C_{21}H_{23}ClO_6$ 406.8	62.00 62.16	5.70 5.92	8.71 8.61
3k	90–91.5 iPA	*A* 32	$C_{22}H_{25}ClO_6$ 420.9	62.78 62.87	5.99 6.09	8.42 8.51
3l	123–124 iPA	*B* 89	$C_{22}H_{25}ClO_6$ 420.9	62.78 62.49	5.99 6.03	8.43 8.47
3m	75–76.5 iPA	*A* 59	$C_{23}H_{27}ClO_6$ 434.9	63.52 63.41	6.26 6.40	8.15 8.13
3n	108–109 MA	*B* 91	$C_{23}H_{27}ClO_6 \cdot CH_3OH$ 466.9	61.73 62.01	6.69 6.60	7.59 7.92
3o	69.5–71.5 MA	*A* 80	$C_{24}H_{29}ClO_6$ 448.9	64.20 64.36	6.51 6.63	7.90 8.30
3p	108–110 NM	*C* 69	$C_{21}H_{24}O_6S$ 404.5	62.35 61.79	5.98 5.95	7.93 7.66
3r	132–134 NM	*C* 52	$C_{21}H_{24}O_6S$ 404.5	62.35 61.91	5.98 5.92	7.93 7.72

[a] AA acetic acid, MA methanol, CH chloroform, NM nitromethane, iPA propan-2-ol, EA ethyl alcohol, E diethyl ether; [b] total yield of reactions A and B.

The antileukotrienic effect of compounds **3** was assessed in three tests measuring their influence on the inhibition of LTB_4 biosynthesis (and hence, 5-LO inhibition) and affinity for LTB_4 and LTD_4 receptors (which is a prerequisite for antagonistic activity



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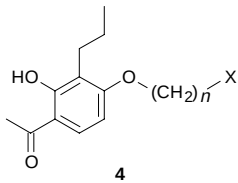
3	Y	Z	R^1	R^2
a	S	$(CH_2)_2$	2-COOH	H
b	S	$(CH_2)_3$	2-COOH	H
c	S	$(CH_2)_4$	2-COOH	H
d	S	$(CH_2)_2$	3-COOH	H
e	S	$(CH_2)_3$	3-COOH	H
f	S	$(CH_2)_4$	3-COOH	H
g	O	$(CH_2)_3$	4-CH_2COOH	H
h	O	$(CH_2)_3$	4-CH_2COOCH_3	H
i	O	$(CH_2)_4$	4-CH_2COOH	H
j	O	$(CH_2)_2$	4-CH_2COOH	H
k	O	$(CH_2)_2$	4-CH_2COOCH_3	Cl
l	O	$(CH_2)_3$	4-CH_2COOH	Cl
m	O	$(CH_2)_3$	4-CH_2COOCH_3	Cl
n	O	$(CH_2)_4$	4-CH_2COOH	Cl
o	O	$(CH_2)_4$	4-CH_2COOCH_3	Cl
p	S	$CH_2CH(OH)CH_2$	2-COOH	H
r	S	$CH_2CH(OH)CH_2$	3-COOH	H



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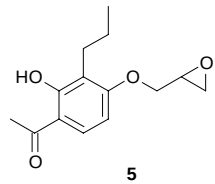

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4	*n*	X
a	2	Cl
b	3	Cl
c	4	Br

towards those LT). The results (Table II) showed that for the majority of derivatives, the presence of the mercaptobenzoic and hydroxyarylacetic acid fragments resulted in a broadening of the spectrum of their antileukotrienic effects. Mostly, the fragments enhanced the inhibition of 5-LO as compared to the LY 171,883 reference standard while leaving the affinity for LTD_4 at the level corresponding to that standard (with a few exceptions). Although the affinity for the LTB_4 receptors is not significant, for some derivatives (**3e**, **3f**, **3g**, **3p**) the IC_{50} value is also at the level of units of micromoles.

The affinity for the LTD_4 receptors seems to be appreciably affected by the distance between the carboxyl group and the lipophilic centre in the acetophenone moiety, represented by the propyl group. Out of acids **3a–3c** derived from 2-mercaptobenzoic acid, the derivative **3c** with a four-carbon spacer is the most efficient. In the next group, **3d–3f**, the most efficient is compound **3e**, with a three-carbon spacer, which is consist-

TABLE II
Inhibition of LTB_4 biosynthesis by compounds **3** and their affinity for LTB_4 and LTD_4 receptors

Compound	Inhibition of LTB_4 biosynthesis		Affinity to LTB_4		Affinity to LTD_4	
	Inh[a]	IC_{50}[b]	Inh[c]	IC_{50}[b]	Inh[a]	IC_{50}[b]
3a	97/10	2.6	57/10	14	57/100	43
3b	100/10	0.42	64/10	14	78/100	24
3c	95/10	0.2	32/20	24	97/100	7.7
3d	70/30	0.75	56/20	16	96/100	3.5
3e	100/10	1.2	98/20	0.9	96/100	2.5
3f	96/30	0.35	53/20	7.3	100/100	4
3g	100/30	0.39	85/20	3.8	98/100	3.9
3h	100/10	1.3	0/20	nd[d]	48/100	9.5
3i	95/30	0.62	34/20	27	47/100	10
3j	100/30	0.55	32/20	30	95/100	3.2
3k	98/10	0.23	11/20	nd[d]	65/100	19
3l	99/30	0.46	28/20	nd[d]	98/100	0.73
3m	0/10[e]	nd	0/10	nd[d]	72/100[e]	25
3n	100/30	0.46	58/20	11	100/100	8.1
3o	0/10[e]	nd	0/10	nd[d]	49/100[e]	92
3p	89/30	1.38	60/20	5.8	32/100	440
3r	95/30	0.86	45/20	28	72/100	22.6
St_A[f]	83/10	5.3	15/20	81	92/100	5.8
St_B[g]	100/30	0.01	nd[d]	nd[d]	100/30	nd[d]

[a] Percentage inhibition/dose (μg); [b] in μM; [c] percentage inhibition/dose (μM); [d] nd not determined; [e] partly soluble; [f] compound LY 171,883 served as standard A; [g] Zilleuton served as standard B.

ent with the displacement of the carboxyl to the *meta* position of the aromatic ring. Also in derivatives **3g** and **3i**, with the acetic acid moiety in position 4, substance **3g** possessing a shorter (three-carbon) spacer was more efficient than the other. Derivative **3l** with a three-carbon spacer is also the most efficient within the group of substances **3j**, **3l**, and **3n**. The overall lipophilicity of the acids seems to affect the bonding to the LTD_4 receptors as well. Substitution with a hydroxy group at the spacer in derivatives **3p** and **3r** caused a significant decrease in the interaction of the substances with the LTD_4 receptor.

The LTB_4 biosynthesis inhibition is nearly unaffected by the spacer length, except that acid **3a** with the shortest distance between the carboxy group and the lipophilic propyl group is the weakest inhibitor. The remaining IC_{50} values differ so little that the effect of structural changes cannot be specified in more detail. The presence of the hydroxy group and introduction of a double bond in the spacer do not appreciably influence the inhibiting activity either.

In the groups of benzoic and phenylacetic acids, the derivatives **3b**, **3e**, and **3g** with three-carbon spacers are most efficient in the bonding to the LTB_4 receptors. This may be in a relation to the fact that the distance between the two polar groups – the thioether or ether group in the aliphatic chain and the hydroxy group at the aromatic ring – can simulate the distance between the two hydroxy groups in positions 5 and 12 in the molecule of LTB_4. The fact that the group of 3-chlorophenylacetic acids **3j**, **3l**, and **3n** fails to follow the pattern can be due to the ether group being excluded from the interaction by the steric or electronic effect of the chlorine.

The substances were also tested for their antiinflammatory effect in inflammation models *in vivo*. The results are given in Table III. The compounds which are most efficient in the three inflammation models, *viz.* **3d**, **3f**, **3g**, and **3n**, were also found to exhibit the highest antileukotrienic activity *in vitro*. In the correlation between the *in vitro* and *in vivo* activities, **3e** is an outlier, being the most efficient agent in the antileukotrienic activity but a weak antiinflammatory agent. We evaluated the inhibition of LTD_4-induced bronchospasm, believing that here the antileukotrienic activity would manifest itself most markedly. The results are summarized in Table IV. Again, the value for compound **3e** is beyond the correlation with the *in vivo* activities, whereas the substances **3f**, **3g**, and **3n**, exerting a high antileukotrienic effect, inhibit also appreciably the LTD_4-induced bronchospasm.

From the results it can be concluded that a high antileukotrienic activity is a prerequisite but not the sole precondition for the inhibition of LTD_4-induced bronchospasm, which is accompanied by a significant antiinflammatory effect. Analysis of the effects of the physicochemical and structural parameters is the objective of a follow-up study.

EXPERIMENTAL

The melting temperatures were determined on a Boetius type Kofler stage and are not corrected. The ^{1}H NMR spectra of 6% solutions of the compounds in deuteriochloroform (or in hexadeuteriodimethyl sulfoxide for substances **3a**, **3b**, **3p**, and **3r**) containing tetramethylsilane as the internal standard were scanned on a TESLA BS 487s – 80 MHz spectrometer (Czech Republic); the data are given in Tables V and VI. The purity of the intermediates **4** and **5** was examined by gas chromatography on a Perkin–Elmer 8700 instrument using a fused silica capillary column 30 m long, 0.32 mm i.d., wetted with a layer of OV 1701 (0.25 mm thick).

4-(2-Chloroethoxy)-2-hydroxy-3-propylacetophenone (**4a**)

A constantly stirred mixture of 2-chloroethyl toluene-4-sulfonate (54.7 g, 0.23 mol), 2,4-hydroxy-3-propylacetophenone[17] (45.3 g, 0.23 mol), anhydrous potassium carbonate (32.0 g, 0.23 mol), and po-

TABLE III
In vivo inflammatory activity of compounds **3**

Compound	CE[a] % inh.	Experimental pleuritis, % inh.[b]			Ear edema, % inh.[c]	
		A	B	C	D	E
3a	22	12	7[d]	2[d]	10	4[d]
3b	6[d]	17	28	17[d]	8	3[d]
3c	14	1[d]	15	18	6[d]	6[d]
3d	30	34	37	3[d]	25	0
3e	14	20	20	3[d]	9	7[d]
3f	25	38	33	9[d]	18	0
3g	56	32	54	33	18	0
3h	16	10[d]	17	8[d]	0	0
3i	39	23	17	7[d]	7[d]	5[d]
3j	21	9[d]	15	8[d]	16	2[d]
3k	26	18	36	32	0	0
3l	16	11[d]	33	25	2[d]	0
3m	26	21	17	12[d]	10	2[d]
3n	36	20	41	26	16	3[d]
3o	34	25	26	8[d]	5[d]	2[d]
3p	11	0	37	38	10	5[d]
3r	5[d]	8[d]	8[d]	0	7[d]	0
St_B[e]	46	36	49	20	9[d]	13
St_C[f]	67	55	51	13	22	68

[a] Inhibition of carrageenan edema after a dose of 100 mg/kg; [b] inhibition after a dose of 100 mg/kg: A volume of exudate in pleural cavity, B number of cells, C cellularity in volume unit; [c] inhibition: D ear lobe weight, E degree of ear lobe hyperemia; [d] level of significance $P > 0.05$; [e] piroxicam served as standard C; [f] Zilleuton served as standard B.

tassium iodide (93.5 g, 0.023 mol) in acetone (300 ml) was heated to the boil for 16 h. After cooling down to 20 °C, the solid was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in dichloromethane (400 ml), and the solution was washed with 2% NaOH (100 ml) and water (2 × 100 ml). The solution was dried, solvent was removed by evaporation, and the crystalline residue was recrystallized from methanol. Compound **4a** was obtained as a pure substance (GLC) in a yield of 21.0 g (36%); m.p. 72–73.5 °C. ^{1}H NMR spectrum: 2.59 s (CH_3CO); 3.84 t, J = 6.0 (H-1″); 4.30 t, J = 6.0 (H-2″); 6.40 d, J = 9.0 (H-2′); 7.60 d, J = 9.0 (H-3′); 12.75 s (OH). For $C_{13}H_{17}ClO_3$ (256.7) calculated: 60.81% C, 6.67% H, 13.81% Cl; found: 60.94% C, 6.72% H, 13.80% Cl.

4-(3-Chloropropoxy)-2-hydroxy-3-propylacetophenone (**4b**)

A solution of 2,4-dihydroxy-3-propylacetophenone (15.5 g, 0.08 mol) in acetone (80 ml) was added to a boiling mixture of 1-bromo-3-chloropropane (50.4 g, 0.32 mol), potassium carbonate (11.1 g, 0.08 mol), and potassium iodide (1.2 g, 0.008 mol) in the same solvent (70 ml). After 5 h of boiling, the mixture was cooled down to 20 °C and poured slowly into water (300 ml). The oil which separated was dissolved in boiling propan-2-ol. Filtration with activated charcoal and cooling gave crystals of pure **4b** (GLC) in a yield of 14.6 g (67%); m.p. 39–41 °C (ref.[18]: 37–38 °C). ^{1}H NMR spectrum: 2.30 m (H-2″); 2.58 s (CH_3CO); 3.77 t, J = 6.0 (H-1″); 4.10 t, J = 6.0 (H-3″); 6.46 d, J = 9.0 (H-2′); 7.60 d, J = 9.0 (H-3′); 12.76 s (OH).

4-(4-Bromobutoxy)-2-hydroxy-3-propylacetophenone (**4c**)

This compound was prepared by the same procedure as the chloro derivative **4b** from 2,4-dihydroxy-3-propylacetophenone and 1,4-dibromobutane, and isolated by vacuum distillation in an 87% yield; b.p. 175–180 °C/40 Pa (ref.[19]: 180 °C/33 Pa), purity 95% (GLC). ^{1}H NMR spectrum: 2.05 m (H-2″, H-3″); 2.58 s (CH_3CO); 3.50 t, J = 6.0 (H-1″); 4.10 t, J = 6.0 (H-4″); 6.43 d, J = 9.0 (H-2′); 7.60 d, J = 9.0 (H-3′); 12.78 s (OH).

TABLE IV
Inhibition of LTD_4-induced bronchospasm

Compound	Dose, mg/kg	Inhibition, %	Compound	Dose, mg/kg	Inhibition, %
3c	10	0	**3g**	30	78
	30	12		100	98
3d	10	0	**3i**	30	74
	30	24		100	92
	100	26	**3n**	10	88
3e	30	15		30	83
	100	8		100	85
3f	10	23	St_A[a]	30	92
	30	75	St_B[g]	100	75
	100	95			

[a] LY 171,883 served as standard A; [b] Zilleuton served as standard B.

4-(Oxiranylmethyl)-2-hydroxy-3-propylacetophenone (**5**)

A mixture of 2,4-dihydroxy-3-propylacetophenone (19.4 g, 0.1 mol), potassium carbonate (20.7 g, 0.15 mol), and 1-chloro-2,3-epoxypropane (27.8 g, 0.3 mol) in butan-2-ol (130 ml) was heated to the boil for 10 h. The insoluble fraction was filtered off and the filtrate was evaporated to dryness. The partly crystalline residue was dissolved in dichloromethane (100 ml), washed with water (2 × 100 ml), and the solvent was removed by evaporation. The crude product was purified by chromatography on silica gel (250 g) using dichloromethane as the eluent. The solution was then concentrated, and the oily residue was dissolved in 25 ml of boiling propan-2-ol. After filtration and cooling, the crystal-

TABLE V
^{1}H NMR data (δ, ppm; *J*, Hz) of compounds **3**, aromatic parts

Compound	H-2′ (d)[a]	H-3′ (d)[a]	H-2 to H-6[a]	arom. OH (s)
3a	6.67	7.80	7.92 brd (H-3); 7.10–7.60 m (H-4, H-5, H-6)	12.05
3b	6.70	7.82	7.87 dd, *J* = 7.9, 1.3 (H-3); 7.48 m (H-4, H-6); 7.23 dd, *J* = 8.7, 1.3 (H-5)	–
3c	6.49	7.96	8.13 brd, *J* = 8.0 (H-3); 7.10–7.50 m (H-4, H-5, H-6	–
3d	6.44	7.62	8.05 s (H-2); 7.84 d, *J* = 7.8 (H-4); 7.33 t, *J* = 7.8 (H-5); 7.48 d, *J* = 7.8 (H-6)	–
3e	6.44	7.60	8.10 brs, (H-2); 7.92 brd, *J* = 7.0 (H-4); 7.20–7.40 m (H-5, H-6)	–
3f	6.43	7.58	8.06 brs, (H-2); 7.92 bd, *J* = 7.0 (H-4); 7.40 m (H-5, H-6)	–
3g	6.44	7.58	6.86 d (H-2, H-6); 7.19 d (H-3, H-5)	–
3h	6.47[b]	7.58[b]	6.86 d (H-2, H-6); 7.20 d (H-3, H-5)	12.75
3i	6.45	7.60	6.85 d (H-2, H-6); 7.20 d (H-3, H-5)	–
3j	6.52	7.60	6.96 d, *J* = 8.0 (H-2); 7.16 dd, *J* = 8.0, 2.0 (H-3); 7.32 d, *J* = 2.0 (H-5)	–
3k	6.52	7.60	6.90–7.40 m (H-2, H-3, H-5)	–
3l	6.50	7.60	6.90 d, *J* = 8.0 (H-2); 7.15 dd, *J* = 8.0, 2.0 (H-3); 7.30 d, *J* = 2.0 (H-5)	–
3m	6.49	7.60	6.85 d, *J* = 8.0 (H-2); 7.13 dd, *J* = 8.0, 3.0 (H-3); 7.30 d, *J* = 3.0 (H-5)	–
3n	6.44[b]	6.57[b]	6.87 d, *J* = 8.0 (H-2); 7.10 dd, *J* = 8.0, 2.0 (H-3); 7.28 brd (H-5)	12.72
3o	6.45[b]	7.60[b]	6.88 d, *J* = 8.0 (H-2); 7.14 dd, *J* = 8.0, 2.0 (H-3); 7.31 t, *J* = 2.0 (H-5)	12.76
3p	6.42	7.57	8.07 brd, *J* = 7.8 (H-3); 7.12–7.51 m (H-4, H-5, H-6)	12.72
3r	6.42[b]	7.20–7.62 m	8.05 s (H-2); 7.82 bd, *J* = 7.8 (H-4); 7.20–7.62 m (H-5, H-6)	12.72

[a] Unless stated otherwise, *J* in doublets is 9.0 Hz; [b] *J* = 8.5 Hz.

line product was filtered off giving compound **5** of a 95% purity (GLC) in a yield of 14.5 g (62%); m.p. 52–56.5 °C (ref.[20]: 54–55 °C). ^{1}H NMR spectrum: 2.56 s (CH_3CO); 2.58–3.00 m (H-2″); 3.37 m (H-2″); 4.16 m (H-3″); 6.44 d, *J* = 9.0 (H-2′); 7.58 d, *J* = 9.0 (H-3′); 12.75 s (OH).

2,4-Dihydroxy-3-propylacetophenone Derivatives (**3**)

Method A. To a constantly stirred mixture of a haloalkoxyacetophenone **4** (26 mmol), anhydrous potassium carbonate (7.2 g, 52 mmol), and potassium iodide (0.39 g, 2.6 mmol) in butan-2-one (50 ml), a solution of the corresponding mercaptobenzoic acid (26 mmol) or methyl arylacetate (26 mmol) in

TABLE VI
^{1}H NMR data (δ, ppm; *J* Hz) of compounds **3**; aliphatic parts

Compound	Aliphatic fragment[a] Z	CH_3CO (s)	Propyl			CH_2COO (s)
			H-1	H-2	H-3	
3a	3.42 brt (H-1″); 4.36 brt (H-2″)	2.58	2.56 t	1.50 m	0.86 t	
3b	3.12 brt (H-1″); 2.10 m (H-2″); 4.22 brt (H-3″)	2.56	2.63 brt	1.50 m	0.92 t	
3c	3.05 brt (H-1″); 2.00 brm (H-2″,H-3″); 4.08 brt (H-4″)	2.56	2.58 brt	1.50 m	0.92 t	
3d	3.05 brt (H-1″); 4.42 brt (H-2″)	2.56	2.62 brt	1.52 m	0.90 t	
3e	3.22 t, *J* = 6.0 (H-1″); 2.20 m (H-2″); 4.17 t, *J* = 6.0 (H-3″)	2.56	2.60 brt	1.50 m	0.94 t	
3f	3.10 brt (H-1″); 1.95 m (H-2″,H-3″); 4.06 brt (H-4″)	2.55	2.62 t	1.52 m	0.92 brt	
3g	4.19 q, *J* = 6.0 (H-1″,H-3″); 2.28 m (H-2″)	2.55	2.64 brt	1.53 m	0.92 t	3.58
3h	4.20 q, *J* = 6.0 (H-1p,H-3p); 2.28 m (H-2″)	2.56	2.64 brt	1.54 m	0.92 t	3.46[b]
3i	4.08 brm (H-1″,H-4″); 2.00 brm (H-2″,H-3″)	2.56	2.65 brt	1.54 m	0.93 t	3.58
3j	4.41 s (H-1″,H-2″)	2.58	2.63 t	1.50 m	0.88 t	3.58
3k	4.40 s (H-1″,H-2″)	2.56	2.64 t	1.60 m	0.90 brt	3.75[c]
3l	4.28 t, 4.23 t, *J* = 6.0 (H-1″,H-3″); 2.34 m (H-2″)	2.56	2.63 brt	1.50 m	0.91 t	3.56
3m	4.20 t, 4.22 t, *J* = 6.0 (H-1″,H-3″); 2.34 m (H-2″)	2.56	2.64 t	1.52 m	1.12 t	3.54[d]
3n	4.10 m (H-1″,H-4″); 2.03 m (H-2″,H-3″)	2.54	2.63 t	1.49 m	0.90 t	3.55
3o	4.13 m (H-1″,H-4″); 2.06 m (H-2″,H-3″)	2.58	2.66 brt	1.56 m	0.94 t	3.55[e]
3p	3.28 m (H-1″); 4.24 m (H-2″,H-3″)[f]	2.55	2.66 brt	1.55 m	0.93 t	
3r	3.26 brt (H-1″); 4.12 brs (H-2″,H-3″)[g]	2.55	2.60 brt	1.50 m	0.88 t	

[a] The spacer Z is numbered starting from the aromatic moiety. Chemical shifts of ester methyl groups: [b] 3.64 s; [c] 3.88 s; [d] 3.68 s; [e] 3.70 s; [f] 6.20 brs (aliph. OH); [g] 6.41 d, *J* = 8.5 (aliph. OH).

the same solvent (50 ml) was added under nitrogen, and the whole was allowed to boil for 24 h. The solvent was then removed by evaporation in a vacuum and the residue was treated as follows. For acids **3a–3f**, the residue was dissolved in water (100 ml), the solution was filtered with activated charcoal, the cool filtrate was made acidic (pH 1) with dilute hydrochloric acid, and the crude product so obtained was recrystallized. For esters **3h**, **3k**, **3m**, and **3o**, the residue was stirred with 100 ml of dichloromethane, the insoluble fraction was filtered off, the filtrate was evaporated to dryness, and the solid residue was either purified by recrystallization or directly subjected to hydrolysis to give the corresponding acid (**3g**, **3i**, **3j**, **3l**, **3n**) (see Table I).

Method B. Methyl ester **3h**, **3k**, **3m**, or **3o** (0.014 mol) was dissolved in methanol (35 ml) and mixed with a solution of potassium hydroxide (3.9 g, 0.07 mol) in 75% methanol (70 ml), and the whole was heated to the boil for 4 h. After cooling to 20 °C, the mixture was poured onto 170 ml of a stirred ice–water mixture, and the turbid solution was filtered with activated charcoal and made acidic (pH 1) with dilute hydrochloric acid. The crude product obtained was purified by recrystallization.

Method C. A stirred mixture of substance **5** (5.0 g, 0.02 mol) and 2- or 3-mercaptobenzoic acid (3.08 g, 0.02 mol) in methanol (100 ml) was heated to the boil for 20 h. After cooling down, the mixture was filtered with activated charcoal and the filtrate was evaporated. The oily residue, which crystallized on standing, was purified by recrystallization.

Biological Evaluation

Inhibition of carrageenan edema was evaluated by the method of Winter[21]. The experimental conditions have been described elsewhere[22]. Inhibition of experimental pleuritis was evaluated by the method of Hidaka[23] in a group of Wistar Han female rats pretreated with 0.5% carrageenan in saline by intrapleural injection. The tested compounds, in suspension with gum arabic, were applied orally in a single dose 1 h before application of carrageenan. The volume of exudate from the pleural activity and the cell counts (determined with a Sysmex counter) were compared with those of the untreated animals. Arachidonic acid-induced ear inflammation in mice was achieved by using the method of Opas and coworkers[24], ear lobe inflammation was induced by application of 20 μl of arachidonic acid solution in acetone. The substance was administered orally 16 h before edema induction. The degree of ear-lobe hyperemia and the ear lobe weight were evaluated 1 h after application of arachidonic acid. The results were expressed in terms of percentage inhibition relative to the untreated control. Inhibition of allergic bronchospasm induced by LTD_4 was evaluated by the modified method of Kreutner and coworkers[25]. Bronchoconstriction was induced by intravenous injection of 1 μg/kg LTD_4 to guinea-pig. The animals were premedicated with indomethacin, mepyramine and propranolol, and the compound tested was administered in doses of 10, 30, and 100 mg/kg *p.o.* 5 min before the application of LTD_4. The effect was expressed in percentage inhibition of bronchospasm relative to the untreated control.

Inhibition of LTB_4 biosynthesis: the production of LTB_4 was determined in rat polymorphonuclear cells from pleural exudate elicited by heat-activated rat serum[26]. The cells were stimulated with the Ca ionophore A23187 (Sigma) and incubated with various concentrations of the drugs tested. LTB_4 was determined in supernatant using a commercial RIA kit (Amersham). A slight modification of the method of Cheng and coworkers[27] was used in the LTB_4 receptor binding study. The membrane fraction was prepared from male guinea-pig spleen; 2 mg of membranes were incubated with 0.3 nM ^{3}H-LTB_4 at 25 °C for 30 min in 100 μl of the incubated mixture. The nonspecific binding was determined in the presence of 0.1 μM LTB_4. The membranes were filtered through Whatman GF/C paper and washed triply with buffer. The radioactivity was measured by liquid scintillation spectrometry, and the specific binding of ^{3}H-LTB_4 to the receptor was determined. The LTD_4 receptor binding

study relied on the method of Bruns and coworkers[28]. The membrane fraction was prepared from male guinea-pig lungs, and 4 mg of this fraction were incubated with 0.4 nM ^{3}H-LTD_4 for 60 min at 25 °C in 100 μl of the incubated mixture. The nonspecific binding was determined in the presence of 0.1 μM LTD_4. The filtration of the membranes, washing, and radioactivity measurement were as above. The biological activity of the compounds **3** is given in Tables II–IV.

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