After 15 min, MeOH (10 mL) was added and the mixture was stirred for an additional 15 min. Removal of the solvent left a residue, which was purified on a silica gel column using $CHCl_3$/MeOH (6:1) to give the corresponding unprotected dipeptides. Yields, physical characteristics, and UV data of compounds **12**, **13**, **17**, **23**, and **24**, obtained by this method, are recorded in Table I. ^1H NMR data of **12** and **17** are recorded in Table II, while those of **13**, **23**, and **24** are in Table III.

Analgesia Assay. Analgesia was evaluated in male ICR Swiss albino mice weighing 20–25 g by means of the tail-flick test carried out in the manner described by Nott,[19] using a cutoff time of 10 s. The pain reaction was recorded 30 min before the administration of any drug or saline and at various times later. The control reaction time was in the range of 1.8–2 s. The peptides were dissolved in 0.01 N HCl, neutralized with 0.1 M NaOH, and injected intracerebroventricularly into conscious animals at a constant volume of 5 μL. The Student's *t* test was used for statistical comparisons.

Acknowledgment. We thank the Consejo Superior de Investigaciones Cientificas and the Comisiôn Asesora de Investigaciôn Cientifica y Tecnica for financial support.

Registry No. **2**, 109064-70-2; **3**, 109064-71-3; **4**, 109064-72-4; **5**, 109064-73-5; **6**, 109064-74-6; **7**, 109064-75-7; **8**, 109064-76-8; **9**, 109064-77-9; **10**, 109064-78-0; **11**, 109064-79-1; **12**, 109064-80-4; **13**, 109064-81-5; **14**, 109064-82-6; **15**, 109064-83-7; **16**, 109064-84-8; **17**, 109064-85-9; **18**, 51440-62-1; **19**, 109064-86-0; **20**, 109064-87-1; **21**, 109064-88-2; **22**, 109064-89-3; **23**, 109064-90-6; **24**, 109064-91-7; $(o\text{-}MeO_2CC_6H_4S)_2$, 5459-63-2; CmPS-Cl, 78880-71-4; PS-Cl, 931-59-9; pNPS-Cl, 937-32-6; DNPS-Cl, 528-76-7; NPS-Cl, 7669-54-7; AcCysOMe, 7652-46-2; *t*-BuSH, 75-66-1; $HSCH_2CH_2CO_2Me$, 2935-90-2; ZLys(Z)OSu, 21160-83-8; ZLys(Z)OH, 405-39-0; HONSu, 6066-82-6.

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5-Acyl-3-substituted-benzofuran-2(3*H*)-ones as Potential Antiinflammatory Agents

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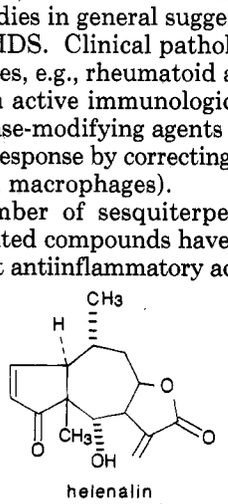
A series of 5-acyl-3-substituted-benzofuran-2(3*H*)-ones and their respective ring-opened *o*-hydroxy acids were synthesized. The antiinflammatory activity was evaluated in terms of their ability to improve adjuvant induced arthritis in rats. Their effect on the production of both cyclooxygenase (CO) and lipoxygenase (LO) metabolites of arachidonic acid in guinea pig peritoneal polymorphonuclear neutrophils (PMNs) was also examined. No correlation between the antiinflammatory activity and increasing stability of the lactones could be found. The degree of activity in general shown by the benzofuranones was similar to that of their corresponding *o*-hydroxy acids. This, coupled with the evidence from studies on opening of the lactone ring, suggests an in vivo transformation of the former into the latter. Benzofuranones displayed a dual inhibition of CO and LO products, while a moderate reduction in CO metabolites was shown by their acids.

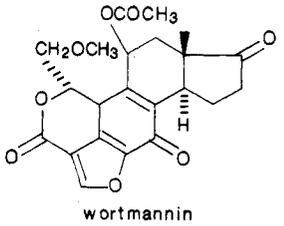
In a search for an effective disease-modifying agent, a considerable amount of research has been done over the last decade to understand the mode of action of the nonsteroidal antiinflammatory drugs (NSAIDS). Antiinflammatory activity has been associated with the inhibition of prostaglandin synthetase[1] (cyclooxygenase), stabilization of lysosomal membranes,[2] suppression of mononuclear leucocyte migration,[3] and other cellular and biochemical events. These studies in general suggest the multiplicity of actions of NSAIDS. Clinical pathology in chronic inflammatory diseases, e.g., rheumatoid arthritis, indicates the presence of an active immunological response. It is believed that disease-modifying agents interfere with this aberrant immune response by correcting inflammatory cell functions (T-cells, macrophages).

Recently a number of sesquiterpene lactones (e.g., helenalin) and related compounds have been reported[4] to possess very potent antiinflammatory activity. The mode of action of these lactones appeared to be at multiple sites. They inhibited lysosomal enzyme activity, suppressed stimulated migration of human polymorphonuclear neutrophils (PMNs), and inhibited delayed hypersensitivity reactions. The 2-methylene γ-lactone skeleton has been associated with their activity.



helenalin

Recent work[5] on potent antiinflammatory mold metabolites, e.g., wortmannin, suggested that the intact furan ring was essential for activity. Following this lead, Close et al.[6] have developed a series of 2,3-dihydrobenzofuran-2-ones. Some of them with an attached cyclohexyl or phenyl group showed strong antiinflammatory activity in rats. These were also potent inhibitors of prostaglandin synthesis (in vitro). It was, however, not determined whether these lactones were active per se or as prodrugs.



wortmannin

A notable variation of the linear biaryl arrangement would be to attach the second aryl group through an angular carbonyl linkage as envisaged in structure A. This

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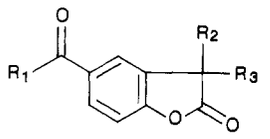


A

incorporates a hydrophobic noncoplanar chain similar to that found in clinically effective drugs (ketoprofen, suprofen) and also essential pharmacophores, as discussed above. The γ-lactone ring contained in A may open to (*o*-hydroxyaryl)alkanoic acids; it was thus of interest to examine both the lactones as well as the hydroxy acids. We report here the synthesis of a series of 5-acylbenzofuran-2(3*H*)-ones and their corresponding (*o*-hydroxyphenyl)alkanoic acids. The antiinflammatory activity of these compounds has been evaluated in terms of their effect on adjuvant induced arthritis in rats.

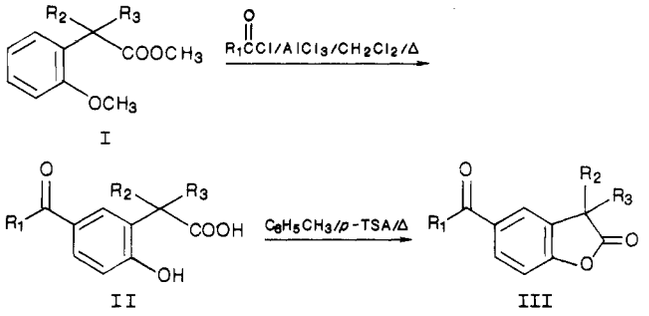
Many NSAI drugs exert their influence by inhibiting prostaglandin synthesis. Prostaglandins (PGs) are formed from arachidonic acid (AA) via the enzyme cyclooxygenase (CO). Until recently, PGs were considered as proinflammatory mediators. It has now been shown that some PGs, particularly PGE_2, can act as inhibitors of the cellular immune response.[7] This may be beneficial in the chronic state of the disease. AA is also metabolized by lipoxygenase (LO) to generate hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs). These are highly potent biologically active mediators, believed to be involved in anaphylactic and inflammatory responses. Metabolites such as 5-HETE and 5,12-diHETE, including LTB_4, are implicated in a number of cell functions, e.g., chemotaxis[8] and aggregation.[9] Thus, we have also studied the in vitro effect of the benzofuranones and their respective acids on the production of both CO and LO metabolites of AA in guinea pig peritoneal polymorphonuclear leukocytes (PMNs).

Chemistry

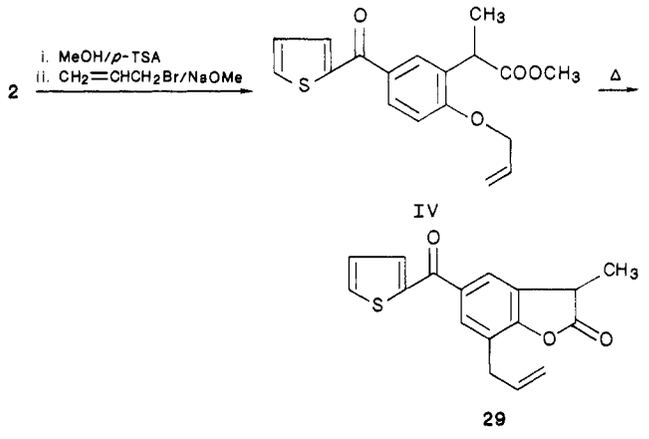
The *o*-hydroxy acids **1–7** and **10–13** (Table I) and the benzofuranones **17–21** and **24–28** (Table II) were obtained by following the route shown in Scheme I. The acids II, **1–5** and **10–13**, were prepared by Friedel–Crafts acylation of appropriately 2-substituted methyl (2-methoxyphenyl)acetates (I). In some cases, however, complete demethylation did not occur to give the hydroxy acids. The resultant esters, 5-acyl methyl 2-(2-hydroxyphenyl)propanoates, were hydrolyzed to produce the acids **10** and **11**. Similarly, the products containing ethers, 5-acyl 2-(2-methoxyphenyl)propanoic acids, on treatment with HBr–acetic acid underwent demethylation and ring closure to yield the lactones **22** and **23**. Acids **6** and **7** were obtained by base hydrolysis of **22** and **23**, respectively. The *o*-hydroxy acids II were dehydrated to give the corresponding lactones III, **17–21** and **24–28**.

The starting methyl 2-(2-methoxyphenyl)-2-alkylacetates I, R_2 = H, R_3 = alkyl, were obtained from methyl (2-methoxyphenyl)acetate by alkylation.[10] The second alkyl group was introduced by a similar procedure using a second molar equivalent of LDA and alkyl iodide to give the geminally substituted dialkyl products I, $R_2 = R_3$ = alkyl.

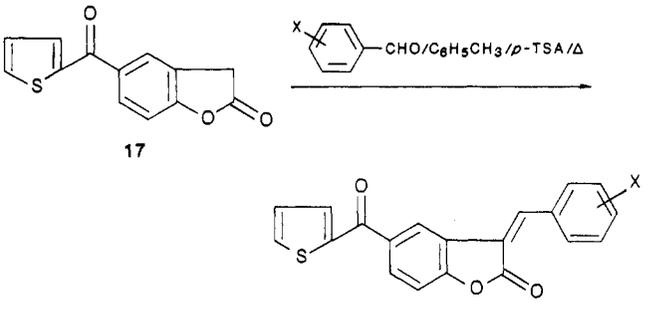
Scheme I



Scheme II



Scheme III



31: X = H
32: X = 4-Cl
33: X = 4-CF_3
34: X = 4-OH, 3,5 di-*t*-Bu

Compounds **9** and **29** were prepared as shown in Scheme II. The methyl ester of **2** was treated with allyl bromide in the presence of sodium methoxide to give IV, which was hydrolyzed to the acid **9**. The ester IV was subjected to Claisen rearrangement conditions to yield the lactone **29**.

The syntheses of compounds **15**, **16**, and **31–34** are shown in Scheme III. The lactone **17** was reacted with an appropriately substituted benzaldehyde to give the corresponding benzylidene derivatives V, **31–34**. Compounds **31**, on mild hydrolysis with aqueous NaOH, gave **16**. Compound **15** was also obtained from **31** upon catalytic reduction and subsequent hydrolysis.

The carboxanilide **30** was synthesized by treating the lactone **17** with phenyl isocyanate.[11]

Results and Discussion

The antiinflammatory activity of compounds **1–34** was determined in terms of their ability to improve the adjuvant induced arthritis in rats. Their in vitro effect on the

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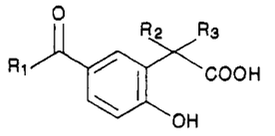
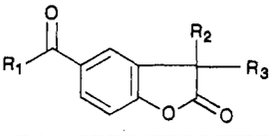
Table I. 2-(5-Acyl-2-hydroxyphenyl)-2-substituted-acetic Acids



no.	R_1	R_2	R_3	meth	yield, %	mp, °C	crystn solv	formula
1	2-thienyl	H	H	A	64	171–172	EtOAc–hex.[f]	$C_{13}H_{10}O_4S$
2	2-thienyl	CH_3	H	A	73	203–204	EtOAc	$C_{14}H_{12}O_4S$
3	2-thienyl	CH_3CH_2	H	A	50	160–161	EtOAc–hex.	$C_{15}H_{14}O_4S$
4	2-thienyl	$CH_3CH_2CH_2$	H	A	49	166–167	EtOAc–hex.	$C_{16}H_{16}O_4S$
5	2-thienyl	$CH_3CH_2CH_2CH_2$	H	A	28	125–127	toluene	$C_{17}H_{18}O_4S$
6	2-thienyl	CH_3	CH_3	C	99	182–183	EtOH–petroleum ether	$C_{15}H_{14}O_4S$
7	2-thienyl	CH_3CH_2	CH_3CH_2	C	99	157–158	EtOAc–hex.	$C_{17}H_{18}O_4S$
8[b]	2-thienyl	CH_3CH_2	CH_3CH_2	*a*	17	188–189	EtOAc	$C_{18}H_{20}O_4S$
9[c]	2-thienyl	CH_3	H	D	95	147–148	EtOAc	$C_{17}H_{16}O_4S$
10	5-Me-2-thienyl	CH_3	H	A	16	183–184	EtOAc–EtOH	$C_{15}H_{14}O_4S$
11	2-furanyl	CH_3	H	A	9	178–179	EtOAc	$C_{14}H_{12}O_5$
12	phenyl	CH_3	H	A	50	184–186	EtOAc–$CHCl_3$	$C_{16}H_{14}O_4$
13	4-F-phenyl	CH_3	H	A	44	183–185	EtOAc	$C_{16}H_{13}FO_4$
14	4-OMe-phenyl	CH_3	H	*d*	82	190–192	EtOAc	$C_{17}H_{16}O_5$
15	2-thienyl	$C_6H_5CH_2$	H	C	38	168–170	toluene	$C_{20}H_{16}O_4S$[e]
16	2-thienyl	$C_6H_5CH=$		C	70	204–206	EtOH–H_2O	$C_{20}H_{14}O_4S$[e]

[a] Obtained as a byproduct from **23**. [b] 2-OCH_3. [c] 2-O-Allyl. [d] See Experimental Section. [e] Supported by high-resolution mass spectrum. [f] hex. = *n*-hexane.

Table II. 5-Acyl-3-substituted-benzofuran-2(3*H*)-ones



no.	R_1	R_2	R_3	meth	yield, %	mp, °C	crystn solv	formula	pseudo-half-life, min
17	2-thienyl	H	H	B	81	174–175	EtOAc	$C_{13}H_8O_3S$	27.5
18	2-thienyl	CH_3	H	B	97	125–126	EtOAc–hex.[d]	$C_{14}H_{10}O_3S$	32.5
19	2-thienyl	CH_3CH_2	H	B	92	70–71	EtOH–hex.	$C_{15}H_{12}O_3S$	52.5
20	2-thienyl	$CH_3CH_2CH_2$	H	B	92	91–92	EtOH	$C_{16}H_{14}O_3S$	70.0
21	2-thienyl	$CH_3CH_2CH_2CH_2$	H	B	100	*a*	—	$C_{17}H_{16}O_3S$[c]	84.0
22	2-thienyl	CH_3	CH_3	A	72	168–169	EtOH–hex.	$C_{15}H_{12}O_3S$	151.0
23	2-thienyl	CH_3CH_2	CH_3CH_2	A	35	128–129	EtOAc	$C_{17}H_{16}O_3S$	2910.0
24	5-Me-2-thienyl	CH_3	H	B	77	96–97	EtOAc	$C_{15}H_{12}O_3S$	
25	2-furanyl	CH_3	H	B	80	96–97	Et_2O–toluene	$C_{14}H_{10}O_4$	
26	phenyl	CH_3	H	B	66	109–111	EtOAc–hex.	$C_{16}H_{12}O_3$	
27	4-F-phenyl	CH_3	H	B	50	125–128	EtOAc–hex.	$C_{16}H_{11}FO_3$	
28	4-OMe-phenyl	CH_3	H	B	74	148–149	EtOAc	$C_{17}H_{14}O_4$	
29[b]	2-thienyl	CH_3	H	D	40	67	—	$C_{17}H_{14}O_3S$[c]	
30	2-thienyl	C_6H_5NHCO	H	11	42	180 dec	dioxane-H_2O	$C_{20}H_{13}NO_4S$	
31	2-thienyl	$C_6H_5CH=$		E	93	174–175	EtOAc	$C_{20}H_{12}O_3S$	
32	2-thienyl	4-$ClC_6H_4CH=$		E	41	161–163	toluene	$C_{20}H_{11}ClO_3S$	
33	2-thienyl	4-$CF_3C_6H_4CH=$		E	39	146–147	benzene–petroleum ether	$C_{21}H_{11}F_3O_3S$[c]	
34	2-thienyl	4-OH-3,5-di-*t*-$BuC_6H_2CH=$		E	70	165–166	toluene–hex.	$C_{28}H_{28}O_4S$	

[a] Viscous oil. [b] 7-Allyl. [c] Supported by high-resolution mass spectrum. [d] hex. = *n*-hexane.

production of both CO and LO metabolites of AA in guinea pig PMNs was also evaluated (Table III).

Both the benzofuranones and their respective (*o*-hydroxyphenyl)alkanoic acids were examined in the adjuvant arthritis model to determine whether the in vivo activity was due to the intact lactone or its ring-opened acid. Since the equilibrium content of γ-lactones would be expected to increase with an alkyl substitution on the α-position, various alkyl groups were introduced in the 3-position. It is evident from Table II that the stability of the lactones, as measured by their "pseudo-half-lives" at pH 7.4, increased with the size of the alkyl group. However, no correlation between the stability of these lactones and their antiinflammatory activity was found. Compound **19** is almost twice as active as **22**, yet it is 3 times less stable than **22**. The adjuvant data for the benzofuranones in most cases is of the same order of magnitude, particularly in the secondary C lesions, as for their corresponding hydroxy acids. Studies on the ring opening of **19** at various pH levels (8.3 to 1.1) show a conversion of the lactone to the *o*-hydroxy acid. It is reasonable to assume that an *o*-hydroxy acid would be prevalent in a biological system. The compounds with an acyl group, either a 2-thienylcarbonyl or a benzoyl, with a small alkyl group at the 3-position showed good activity. A substitution of the phenyl ring with a F atom (**13**, **27**) did not improve on activity. Electron-donating groups on the aryl ring (**14**, **28**, **10**, **24**) diminished activity. A small alkyl group at the 3-position (**2**, **3**, **18**, **19**) seems to be beneficial. The compounds substituted with an ethyl group (**3**, **19**) showed enhanced activity. This is interesting, since a number of NSAI drugs contain a phenylpropionic acid chain. Geminally substituted dialkyl compounds (**6**, **7**, **22**, **23**) did not offer much advantage over their monosubstituted counterparts, except compound **22**, which showed dual inhibition of the products of CO and LO.

Table III. Effect on Adjuvant Arthritis and Arachidonic Acid (AA) Metabolites

	% reduction:[a] adjuvant arthritis				% reduction[b]				
					CO products			LO products	
no.	A	B	C	J	$PGF_{2\alpha}$	PGE_2	TxB_2	5-HETE	5,12-diHETE
1	11	20	10	1	74[c]	74[c]	89[c]	−72[c,e]	−62[c]
2	39[c]	34	35	−15	76[c]	76[d]	89[d]	−68[d]	−84[c]
3	20	64[c]	76[c]	60	65[d]	ND[f]	92[c]	16	−33[c]
4	26[c]	30	52[d]	33	50[d]	87[c]	86	−33[d]	−42[c]
5	5	29	53[c]	14	51	44	64[d]	60[c]	60[c]
6	4	20	46	26	55[c]	80[c]	90[c]	−20	−23[d]
7	6	22	45[d]	38	50[d]	54[d]	90[c]	−21[d]	−18
8	−11	16	42[d]	42	ND	ND	ND	ND	ND
9	18	−8	3	−14	11	6	62[c]	−25[d]	−21[d]
10	13	11	29	−16	49[c]	54[c]	82[c]	−69[c]	−36[d]
11	−31	−5	13	7	61[c]	87[c]	87[c]	−14	−17[d]
12	30[c]	37[d]	61[d]	38	52[d]	77[d]	75[c]	−27[d]	−55[d]
13	22[d]	32[d]	56[d]	33	49[d]	46	74[c]	−1	−8
14	12	31	22	7	58[d]	ND	89[c]	5	−10
15	29	30	10	6	ND	ND	ND	ND	ND
16	15	11	−15	5	ND	ND	ND	ND	ND
17	25	9	−35	5	57[d]	54[d]	65[d]	−33	−44
18	31[c]	40[c]	48	31	15	61[d]	67[c]	37	39[d]
19	13	51[c]	81[c]	52	62[c]	79[c]	90[c]	20	9
20	23[d]	41[d]	67[d]	61	49[c]	85[c]	81[c]	−2	7
21	4	−22	−55[d]	−1	62[d]	68[d]	89[c]	73[c]	61[c]
22	28[d]	36[d]	50[d]	44	−37	81[c]	81[c]	52[d]	49[d]
23	18	17	41	23	48	62[c]	90[c]	2	−7
24	10	19	36	15	28[d]	65[c]	86[c]	−3	15
25	−5	12	25	2	ND	81[c]	81[c]	20[d]	3
26	5	35[d]	52[d]	29	56[d]	71[d]	82[c]	10	−10
27	5	10	31	−1	47[d]	46	76[c]	6	−25
28	−9	5	22	−9	51[d]	ND	70[d]	50[d]	44[c]
29	1	6	17	5	77[c]	88[c]	93[c]	14	−24
30	8	6	25	1	86[c]	93[c]	93[c]	94[c]	85[d]
31	21	48[d]	76[d]	46	60[c]	81[c]	92[c]	−18[d]	−10
32	−5	−18	−14	12	69[d]	77[c]	93[c]	71[c]	73[c]
33	3	19	15	4	67[d]	79[c]	95[c]	67[c]	68[c]
34	−28[d]	−7	8	17	6	−14	26	−11	−8
indomethacin	37[d]	56[c]	77[c]	91	70[c]	84[c]	91[c]	−12	−81[d]
BW755C					82[c]	79[c]	90[d]	45[d]	40[d]

[a] At oral doses, 33 mg/kg, except indomethacin (3 mg/kg). Adjuvant: A = right primary lesion, volume change in the injected paw measured from day 0 to day 8; B = right secondary lesion, volume change in the injected paw measured from day 9 to day 18; C = left secondary lesion, volume change in the uninjected paw measured from day 9 to day 18; J = joint mobility of the paw. [b] At 30 μM concentration, except indomethacin (3 μM). [c] $P < 0.001$. [d] $P < 0.01$. [e] (−) = increase. [f] ND = not done.

Since 3-methylene benzofuran-2(3*H*)-ones are known[12] to polymerize readily, a few 3-benzylidene derivatives (**16**, **31**–**34**) were examined. These were found to be stable compounds. The lactone ring in them can also open to a cinnamic acid. Cinnamic acid derivatives,[13] e.g., caffeic acids, are reported to be potent antiinflammatory agents. However, compound **16** was ineffective in reducing adjuvant arthritis. Interestingly, the corresponding benzofuranone (**31**) in this case displayed good activity. It also reduced CO metabolites of AA in vitro. It is, however, surprising that the 4-Cl and 4-CF_3 analogues (**32**, **33**) had very little antiinflammatory activity, although they inhibited both CO and LO products in vitro. The benzofuranones in general exhibited dual CO and LO enzyme inhibition; the most active compound (**30**), however, showed only weak antiinflammatory activity. It has been suggested[14] that dual enzyme inhibitors may have properties similar to antiinflammatory corticosteroids, which inhibit the production of PGs and LTs by suppressing the release of AA from phospholipids.[15]

Modest reduction of CO metabolites has been seen with most of the (*o*-hydroxyphenyl)alkanoic acids. They also tend to show some increase in LO products. A similar effect is also observed with indomethacin. This is consistent with its effect observed previously in rabbit neutrophils[16] and guinea pig PMNs.[17] The moderately weak inhibition of CO activity as compared with the effect observed with indomethacin may well predict a low ulcerogenic potential of these compounds.

The *o*-allyl derivative **9**, similar to alclofenac, and its rearranged product **29** were inactive on the adjuvant test, but showed some activity in the reduction of CO metabolites.

It is considered that the acidic enolate anion is stabilized by internal H bonding with the amide proton in highly potent 1,2-benzothiazine carboxanilides, e.g., piroxicam and related compounds.[11] The benzofuranone 3-carboxanilide **30**, with similar structural features, was disappointingly weak on the adjuvant test, but was active and equipotent in the inhibition of AA metabolites.

Compounds containing a 2,6-di-*tert*-butyl phenol moiety are reported[18] to demonstrate potent antiinflammatory activity with antioxidant properties. Compound **34**, how-

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ever, was inactive in our tests.

Experimental Section

Chemistry. Melting points were determined on a Reichert Köfler apparatus and were uncorrected. All compounds and intermediates gave satisfactory NMR, IR, UV, and mass spectra. $MgSO_4$ was used as a drying agent. Elemental analyses were within ±0.4% of the theoretical values, except where stated otherwise.

Methyl 2-(2-methoxyphenyl)propanoate and other monoalkylated esters I were obtained from methyl (2-methoxyphenyl)acetate by alkylation (LDA, alkyl iodide).[10] Geminally substituted alkyl derivatives were prepared from the respective monoalkyl precursors by using a similar procedure. All the mono- and dialkyl derivatives I were obtained in excellent yields and were of sufficient purity for further use.

[2-Hydroxy-5-(2-thienylcarbonyl)phenyl]acetic Acid (1). Method A. To a stirred suspension of anhydrous $AlCl_3$ (60.5 g, 0.457 mol) in dry CH_2Cl_2 (380 mL) was added methyl (2-methoxyphenyl)acetate (25.54 g, 0.14 mol) dropwise at 0 °C. A solution of thiophene-2-carbonyl chloride (15.2 mL, 0.14 mol) in CH_2Cl_2 (50 mL) was added dropwise at 0–5 °C. The mixture was stirred at ambient temperature for 15 min and then heated to reflux for 12 h. The solution was poured into 2 M HCl (500 mL) and ice, and $CHCl_3$ was added to promote two phases, which were filtered and separated. The organic phase was washed with brine and extracted with 2 M NaOH. The resulting basic solution, on neutralization with dilute HCl, gave a gum, which subsequently crystallized (23.7 g): ^{1}H NMR δ 3.55 (2 H, s), 6.85 (1 H, d), 7.10 (1 H, t), 7.60 (4 H, m); IR (KBr) ν_{max} 1700 (C=O, acid), 1625 cm^{-1} (C=O); UV (MeOH) λ_{max} 307 nm (ε 16 296); MS, m/z 244 ((M − H_2O)$^+$).

The product from similar experiments, where a methyl ester, methyl 2-(5-acyl-2-hydroxyphenyl)propanoate, was obtained, was refluxed in ethanol with excess KOH for 2 h. The solvent was evaporated, and the residue was dissolved in water and neutralized with 5 M HCl to give the corresponding acids **10** and **11**.

The methoxy ether 2-[2-methoxy-5-(2-thienylcarbonyl)-phenyl]-2-methylpropanoic acid obtained from the above acylation reaction was dissolved in HBr (45% w/v) in acetic acid and heated to reflux for 24 h. The mixture, when cold, was poured into brine and extracted with ethyl acetate. The extract was washed with 2 M NaOH and brine, dried, and evaporated. The residue, dissolved in CH_2Cl_2, was chromatographed on a silica gel column eluting with CH_2Cl_2 to give **22**. Compound **23** was obtained in a similar manner. In the case of **23**, however, further elution with CH_2Cl_2 and methanol afforded the compound **8**.

5-(2-Thienylcarbonyl)benzofuran-2(3*H*)-one (17). Method B. Hydroxy acid **1** (19.6 g, 0.075 mol) was dehydrated (water was removed by the use of a Dean–Stark trap) in boiling toluene in the presence of *p*-toluenesulfonic acid (*p*-TSA) (0.75 g). The solution, while hot, was filtered and evaporated to a solid, which was dissolved in $CHCl_3$. The solution was washed with 0.1 M NaOH and brine, dried, and evaporated (14.8 g; recrystallized): ^{1}H NMR δ 3.85 (2 H, s), 7.20 (1 H, t), 7.25 (1 H, d), 7.80 (4 H, m); IR (KBr) ν_{max} 1810 (C=O, lactone) 1625 cm^{-1} (C=O); UV (MeOH) λ_{max} 301 nm (ε 15 899); MS, m/z 244 (M$^+$).

2-[2-Hydroxy-5-(2-thienylcarbonyl)phenyl]-2-methylpropanoic Acid (6). Method C. A solution of benzofuranone **22** (2.6 g, 0.0095 mol) in methanol (25 mL) was refluxed with 2 M NaOH (9.5 mL) for 1 h. The solution was poured into ice and water and was washed with $CHCl_3$. The aqueous solution was acidified with 2 M HCl. The resulting solid was filtered, washed with water, and dried to yield the acid: ^{1}H NMR δ 1.55 (6 H, s), 6.90 (1 H, d), 7.15 (1 H, t), 7.5–7.95 (4 H, m); IR (KBr) ν_{max} 1700 (C=O, acid), 1620 cm^{-1} (C=O, ketone); UV (MeOH) λ_{max} 301 nm (ε 16 223); MS, m/z 277 ((M − H_2O)$^+$).

2-[2-(Allyloxy)-5-(2-thienylcarbonyl)phenyl]propanoic Acid (9). Method D. The hydroxy acid **2** (5.52 g, 0.02 mol) was refluxed in methanol (50 mL) with *p*-TSA (0.5 g) for 4 h. The solution was cooled to ambient temperature and added dropwise to a freshly prepared solution of sodium (0.6 g, 0.026 mol) in methanol (20 mL) with stirring. Allyl bromide (3 mL, 0.035 mol) in methanol (10 mL) was added dropwise. The mixture was heated to reflux for 48 h. The solution was evaporated, and the residue was dissolved in $CHCl_3$, washed with brine, 0.2 M NaOH, and brine again, dried, and evaporated to give methyl 2-[2-(allyloxy)-5-(2-thienylcarbonyl)phenyl]propanoate (IV) as an oil in an almost pure state (5.9 g, 90%). This ester (4.0 g, 0.012 mol) was hydrolyzed by using method C to give **9**: yield, 3.59 g (crystallized); ^{1}H NMR δ 1.50 (3 H, d), 4.10 (1 H, q), 4.70 (2 H, d), 5.45 (2 H, m), 6.15 (1 H, m), 7.00 (1 H, d), 7.20 (1 H, t), 7.88 (4 H, m), 7.20 (1 H, exchangeable); IR (KBr) ν_{max} 1690 (C=O, acid), 1620 cm^{-1} (C=O); UV (MeOH) λ_{max} 304.6 nm (ε 17 891); MS, m/z 316 (M$^+$).

7-Allyl-3-methyl-5-(2-thienylcarbonyl)benzofuran-2-(3*H*)-one (29). The above methyl ester IV (5.9 g, 0.018 mol) was heated with stirring to 180–200 °C under N_2 for 48 h. The product was dissolved in CH_2Cl_2. The solution was washed with 0.2 M NaOH and brine, dried, and evaporated. The residue in the CH_2Cl_2 solution was chromatographed on a silica gel column, eluting with CH_2Cl_2. On evaporation of the solvent from combined fractions, an oil (2.1 g) was obtained, which on long standing crystallized: ^{1}H NMR δ 1.63 (3 H, d), 3.50 (2 H, q), 3.85 (1 H, q), 5.15 (2 H, m), 6.00 (1 H, m), 7.20 (1 H, t), 7.75 (4 H, m); IR (KBr) ν_{max} 1810 (C=O, lactone), 1635 cm^{-1} (C=O); MS, m/z 298 (M$^+$).

3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxybenzylidene]-5-(2-thienylcarbonyl)benzofuran-2(3*H*)-one (34). Method E. A mixture of the lactone **17** (1.22 g, 0.005 mol), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (1.17 g, 0.005 mol), and *p*-TSA (0.3 g) was refluxed in toluene (100 mL) for 48 h. The solution was evaporated under reduced pressure to yield an oil, which was dissolved in CH_2Cl_2. The solution was passed through a silica gel column, eluting with CH_2Cl_2. The combined fractions containing the product were evaporated to an oil, which crystallized on standing. The product was then triturated with petroleum ether and recrystallized (1.6 g): ^{1}H NMR δ 1.45 (18 H, s), 5.75 (1 H, s, OH), 7.55 (2 H, s), 8.50 (1 H, d), 7.0–8.0 (7 H, arom); IR (KBr) ν_{max} 1768 (C=O, lactone), 1630 cm^{-1} (C=O); MS, m/z 460 (M$^+$).

2-[2-Hydroxy-5-(4-methoxybenzoyl)phenyl]propanoic Acid (14). Compound **13** (8 g, 0.028 mol) in dry Me_2SO (72 mL) was added to freshly prepared NaOMe from Na (2.56 g, 0.111 mol) and MeOH. The mixture was stirred at 115 °C for 5.5 h. The solution was poured onto ice–water and acidified with concentrated HCl to pH 2. The resulting solid was filtered, washed with water, and dried: yield 2.24 g (ethyl acetate). Anal. ($C_{17}H_{16}O_5$) C, H; C, H: calcd, 67.99, 5.37; found, 67.04, 5.73.

Hydrolysis of Benzofuranones. The hydrolysis rates were determined on a Pye-Unicam SP8-100 UV spectrophotometer using the repeat scan mode. A stock solution of the benzofuranone (0.005 g) in dioxane (1 mL) was prepared. An aliquot solution (10 μL) was added to a phosphate buffer (2.5 mL, pH 7.4) containing 25% dioxane at 37 °C. With the above buffer solution as a reference, the spectrum was scanned from 400 to 200 nm at 5- or 10-min intervals. Measurements at 328 and 240 nm led to the construction of absorption/time curves, from which "pseudo-half-life" values were obtained (Table II). Pseudo-half-life was invoked because the ring-opening process of benzofuranones gives rise to more than one ionic species in slightly basic medium.

The opening of the lactone ring of compound **19** was studied over a pH range 8.3–1.1. By comparison of initial rates and measurement of approximate half-lives, the rates of reaction were shown to be in the following order: pH 8.3 > 7.4 >> 5.2 ≃ 3.3 ≃ 2.6 < 1.1. The slight increase in rate at pH 1.1 is consistent with the high concentration of hydrogen ions in solution. A complete conversion to the *o*-hydroxy acid was shown by the coincidence of the UV curve, particularly absorbance maximum, with that of the corresponding acid **3**.

Biological Methods. Acute Toxicity. The compounds were given orally to mice in doses up to 1600 mg/kg. The mice were kept under observation over 48 h for mortalities and other gross behavioral changes. Approximate LD_{50} values were calculated from the results. Values from 1200 to >1600 mg/kg were recorded for all compounds except for compounds **5** and **13**, for which values were >800 mg/kg.

Adjuvant Arthritis.[19] Female Sprague–Dawley rats (150–170

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g) were used in groups of six. Arthritis was induced by subcutaneous injection into the plantar surface of the right hind paw of 0.125 mg of dead tubercle bacillus (comprising human strains PN, DT, and C) homogenized in 0.05 mL of liquid paraffin.

The course of the subsequent inflammatory response was monitored over 18 days by measurement of the volumes of the hind paws by mercury plethysmography. The volume of the right injected paw was measured initially and then every 2 or 3 days over the 18 days of the test. Volume of the left uninjected paw was measured every 2 or 3 days from the 9th day onward. Compounds were administered in 14 daily oral doses (33 mg/kg) beginning on the day before adjuvant injection. Indomethacin was used (3 mg/kg po) as a standard drug.

Areas under the time-course curves were calculated and represented the progress and the severity of the inflammatory response. Following administration of drugs, the difference in the areas under the time-course curves of drug-treated animals from the corresponding areas for the controls was statistically evaluated by analysis of variance.

Joint involvement was assessed by measuring the angle through which the hind paws could be moved easily and expressed as a percentage change over nontreated controls.

Assay of Cyclooxygenase and Lipoxygenase Metabolites of Arachidonic Acid. The method used for detecting inhibitors of arachidonic acid (AA) metabolism was described in a previous paper.[17] Guinea pig peritoneal PMNs, incubated with [^{14}C]AA, were stimulated with the calcium ionophore A23187. Compounds were used at 30 μM concentration in 0.1 N NaOH–Krebs Ringer bicarbonate buffer solution except for indomethacin (3 μM). Indomethacin and BW755C were used as standards. The radiolabeled products, as cyclooxygenase and lipoxygenase metabolites, were identified by using HPLC and GC–MS. A TLC system was used to separate these metabolites, which were then assayed by automatic quantitative scanning. Results were expressed as a percentage change of the radioactivity in each metabolite compared with the stimulated controls. The results in terms of $PGF_{2\alpha}$, PGE_2, and TxB_2 as cyclooxygenase products and 5-HETE and 5,12-diHETE as lipoxygenase metabolites are shown in Table III.

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Registry No. **1**, 109217-20-1; **2**, 109217-21-2; **3**, 109217-22-3; **4**, 109217-23-4; **5**, 109241-76-1; **6**, 109217-24-5; **7**, 109217-25-6; **8**, 109217-26-7; **9**, 109217-27-8; **10**, 109217-28-9; **11**, 109217-29-0; **12**, 109217-30-3; **13**, 109217-31-4; **14**, 109217-32-5; **15**, 109217-33-6; **16**, 109217-34-7; **17**, 109217-35-8; **18**, 109217-36-9; **19**, 109217-37-0; **20**, 109217-38-1; **21**, 109217-39-2; **22**, 109217-40-5; **23**, 109217-41-6; **24**, 109217-42-7; **25**, 109217-43-8; **26**, 109217-44-9; **27**, 109217-45-0; **28**, 109217-46-1; **29**, 109217-47-2; **30**, 109217-48-3; **31**, 109217-49-4; **32**, 109217-50-7; **33**, 109217-51-8; **34**, 109217-52-9; IV, 109217-53-0; methyl (2-methoxyphenyl)acetate, 27798-60-3; thiophene-2-carbonyl chloride, 5271-67-0; 2-[2-methoxy-5-(2-thienylcarbonyl)phenyl]-2-methylpropanoic acid, 109241-77-2; 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 1620-98-0; phenyl isocyanate, 103-71-9.

Synthesis and Evaluation of Melphalan-Containing *N,N*-Dialkylenkephalin Analogues as Irreversible Antagonists of the δ Opioid Receptor[1]

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N,N-Dialkylated leucine enkephalin analogues containing melphalan (Mel) in place of Phe^4 were synthesized as potentially irreversible antagonists of the δ opioid receptor. These compounds, along with the corresponding Phe^4 peptides, were tested for both agonist and antagonist activity in the GPI and MVD smooth muscle preparations. All but two of the eight compounds showed antagonist activity at 1 μM against [D-Ala^2,D-Leu^5]enkephalin (DADLE) in the MVD when tested under reversible conditions; in all cases the Mel^4 peptide had lower activity against DADLE than did the corresponding Phe^4 peptide. At higher concentrations (10 μM) the two active Mel^4 analogues, (benzyl)$_2$Tyr-Gly-Gly-Mel-Leu (**2a**) and (allyl)$_2$Tyr-Aib-Aib-Mel-Leu (**3a**), both showed weak irreversible antagonism at the δ receptor. Compound **2a** was a selective irreversible δ opioid antagonist while **3a** was an irreversible antagonist at both the μ and δ opioid receptors.

Nonequilibrium opioid receptor antagonists are valuable pharmacological tools. Since they do not dissociate from the receptor, they have advantages over reversible ligands in studies such as receptor isolation. They can also be useful in sorting out various opioid receptor types and in determining their relative importance in various opiate-induced pharmacological effects. The ligands that have been employed extensively for these purposes are β-chlornaltrexamine[3,4] (β-CNA) and β-funaltrexamine[4,5] (β-FNA). In vitro, β-CNA irreversibly blocks three opioid receptor types, whereas β-FNA's irreversible antagonism is highly μ-selective. Both ligands show similar activity in vivo, displaying ultralong-acting opioid antagonist activity.

Several compounds have been prepared as potential affinity labels for the δ opioid receptor. Most of these are enkephalin derivatives.[4,6] In addition, the nonpeptide ligands fentanyl isothiocyanate (FIT) and fumaramido-

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