Chemicals, Hicksville, NY) and shaken overnight with large glass beads. All compounds were administered orally, in milligrams per kilogram of body weight, according to the respective dosing regimens described in the table legends. Normal rats and the respective control groups received water plus Tween-80.

Diuretic Assays. A primary assay for diuretic activity was conducted in male rats weighing approximately 160 g. The rats were deprived of food 24 h and water $1^1/_2$ h before test time. During testing both food and water were withheld. Testing was initiated by simultaneous hydration and oral administration of test agent. This was accomplished by gavage with 25 mL/kg of normal saline (0.9%) containing (carboxymethyl)cellulose (0.5%) and test substance. Rats were placed in metabolism cages and urine collected over the ensuing 5 h. In all instances, the test dose was 40 mg/kg. Criteria for declaring test substances active or inactive were established from a two-stage test as described by Roseberry and Gehan.[15] For each stage, the ratio (denoted T/C) of urine volume in treated animals to urine volume in control rats was determined. When the product of T/C for stage 1 and stage 2 was 3.34 or greater, the test substance was declared active. Secondary testing was carried out on selected pyrimidinones. These agents were subjected to dose–response analyses using a wide range of doses (0.3–100 mg/kg) and tested for effects on urinary Na^+ and K^+ excretion. Urinary Na^+ and K^+ were measured by flame photometry. In all other respects, i.e., tests animals, route of administration, hydration, and collection periods, secondary testing was identical to primary testing.

Hypotensive Assay—Blood Pressure in Rats. The arterial blood pressures of restrained female Sprague–Dawley rats were measured directly from indwelling aortic cannulas exteriorized at the nape of the neck.[16] The rats were restrained in a towel when observations were made with a Statham transducer (P23G) and Grass polygraph. Mean arterial pressure was obtained by electrical integration of the phasic pressure. Observations were made before, as well as 4 and 24 h after, oral treatment. Compounds were suspended in a (carboxymethyl)cellulose vehicle to provide a dose of 50 mg/kg in a volume of 10 mL/kg. Mean arterial blood pressure values of two animals were averaged at each of the three observation periods. An average change of at least 5 mmHg was required posttreatment to attain statistical significance ($P < 0.05$).

Acknowledgment. We thank S. A. Mizsak for assistance in setting parameters for NMR experiments, J. E. Rogers for blood pressure measurements, and R. A. Zandt for conducting the diuretic tests.

Registry No. 1, 56741-94-7; 2 (R^1 = H, R^2 = CH_3), 92519-10-3; 2 (R^1 = Br, R^2 = CH_3), 92519-08-9; 2 (R^1 = I, R^2 = H), 72943-43-2; 2 (R^1 = I, R^2 = CH_3), 92519-09-0; 2 (R^1 = I, R^2 = CH_2CHCH_2), 102649-71-8; 3 (R^1 = H, R^2 = CH_3), 100008-30-8; 3 (R^1 = Br, R^2 = CH_3), 102649-63-8; 3 (R^1 = I, R^2 = $COCH_3$), 102649-68-3; 3 (R^1 = I, R^2 = CH_3), 102649-70-7; 4 (R^1 = I), 102649-67-2; 4 (R^1 = H), 31937-04-9; 4 (R^1 = Br), 102649-64-9; 5 (R^1 = H), 102649-58-1; 5 (R^1 = Br), 102649-65-0; 5 (R^1 = I), 102649-69-4; 6, 56741-95-8; 7, 74856-68-1; 8, 102649-59-2; 9, 36822-11-4; 9 (dione), 102649-77-4; 10, 102649-60-5; 11, 74303-68-7; 12, 102649-61-6; 14, 102649-66-1; 15, 61736-36-5; 15 (5-bromo), 102649-72-9; 15 (5-iodo), 102649-73-0; 16, 42542-57-4; 16 (5-bromo), 102649-74-1; 16 (5-iodo), 102649-75-2; 16 (5-chloro), 102649-76-3; H_2NCONH_2, 57-13-6; $C_6H_5COCH_2CO_2CH_2CH_3$, 94-02-0; $H_3CNHCONH_2$, 598-50-5; H_2NCSNH_2, 62-56-6; $H_3CNHC(NH)NH_2 \cdot {}^1/_2H_2SO_4$, 598-12-9; $(CH_3)_2NC(NH)NH_2 \cdot HCl$, 1186-46-5; 6-phenyl-2,4-pyrimidinedione, 13345-09-0; 1,3-dimethyl-5-bromo-6-phenyl-2,4-pyrimidinedione, 98854-09-2; 5-bromo-6-phenyl-2,4-pyrimidinedione, 16290-56-5; 2-amino-1-methyl-6-phenyl-4-pyrimidinone, 102649-62-7; biguanide, 56-03-1; 1-hydroxy-2-amino-5-bromo-6-phenyl-4-pyrimidinone, 78222-48-7.

Topical Nonsteroidal Antipsoriatic Agents. 1. 1,2,3,4-Tetraoxygenated Naphthalene Derivatives[1]

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Institutes of Bio-Organic Chemistry, Organic Chemistry, and Biological Sciences, Syntex Research, Palo Alto, California 94304. *Received December 3, 1985*

On the basis of previous observations that both 2,3-dihydro-2,2,3,3-tetrahydroxy-1,4-naphthoquinone (oxoline, **1**) and 6-chloroisonaphthazarin (**2**) had demonstrated antipsoriatic activity in vivo, a series of structural derivatives of **2** were prepared and examined in the Scholtz–Dumas topical psoriasis bioassay. Of these six (**5**, **6**, **9a**, **10**, **11a**, **11b**), the most effective compound was found to be 6-chloro-1,4-diacetoxy-2,3-dimethoxynaphthalene (RS-43179, lonapalene, **11a**). An extensive series of 1,2,3,4-tetraoxygenated naphthalenes (**16–74**) incorporating variations of the ester, ether, and aryl substituents were prepared as analogues of **11a** to examine the structural requirements for activity and were screened in vivo as inhibitors of arachidonic acid induced mouse ear edema, a topical bioassay capable of detecting 5-lipoxygenase inhibitors. Net lipophilicity, hydrolytic stability, and ring substitution play significant roles in determining the observed in vivo activity. Lonapalene (**11a**) is currently in clinical development as a topically applied nonsteroidal antipsoriatic agent.

Psoriasis is a chronic, relapsing hyperproliferative and inflammatory disease of the skin characterized by symmetrically distributed silvery red, scaling plaques found on the scalp, the extremities, back, buttocks, and especially the knees and elbows. In severe cases, most of the body surface may be involved. An estimated 6–8 million persons in the United States have psoriasis, contributing to a 2–6% incidence worldwide. While the exact etiology of the disease remains elusive, there now exist a number of treatments which provide temporary relief,[2] including coal tar and UV irradiation (the Goeckermann regimen), psoralen and UV-A irradiation (PUVA), oral retinoids, corticosteroids, and antimetabolites. Probably the most widespread treatment used, however, is topical anthralin, an irritating and mutagenic compound that leaves a brown

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[§] Institute of Organic Chemistry.

(1) Contribution No. 212 from the Institute of Bio-Organic Chemistry. Simpson, R. J.; Jones, G. H.; Young, J. M.; Venuti, M. C.; Loe, B. E.; Scholtz, J. R.; Tanenbaum, L.; Akers, W. A. Presented in part at the 188th National Meeting of the American Chemical Society, Philadelphia, PA, 26–31 Aug 1984; Abstract MEDI 22.

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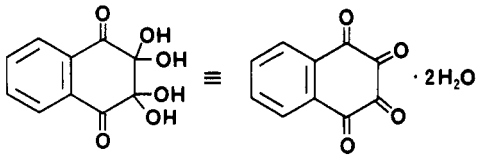
0022-2623/86/1829-1504$01.50/0

stain after use. Recent advances in anthralin treatment include the so-called "minutes" therapy, utilizing short-term applications of a suberythematous dose to minimize the untoward effects,[3] and chemical modification of the agent itself.[4] Thus far, unfortunately, all of these methods possess limitations, reducing patient compliance and attesting to the difficulty inherent in the long-term treatment of chronic psoriasis.

As part of a program to develop topical nonsteroidal antipsoriatic agents,[1,5] a number of 2,3-dihydroxy-1,4-naphthoquinones (isonaphthazarins) and other structures related to 2,3-dihydro-2,2,3,3-tetrahydroxy-1,4-naphthoquinone (oxoline, 1) possessing varied biological activities[6] were tested in the modified Scholtz–Dumas screen,[7] a human topical bioassay designed to assess the effectiveness of potential antipsoriatic agents. Among these compounds, 6-chloroisonaphthazarin (2) was found to exhibit signifi-



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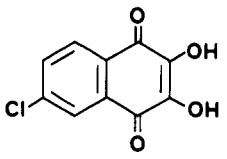


2

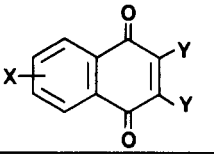
cant potency as an antipsoriatic agent when topically applied to humans. This compound, however, suffered from three drawbacks: the material is highly colored, imparting a deep blue stain to the skin; the inherent instability of the compound precluded development of sufficiently stable topical formulations; and the compound initiated a moderate sensitization/irritation reaction in approximately one-third of the patients.[5]

With these results in hand, we sought a modified form of 2 that eliminated some or all of the cited deficiencies. The compounds studied were of two types. The first category maintained the quinone oxidation state, while masking the 2,3-diol as ethers or esters. The other, a second-generation modification, masked the quinone as a hydroquinone diester, releasing a derivative of 2 upon ester hydrolysis and oxidation. Herein are described the initial modifications of 2 defined by the above criteria and also a full range of analogues of one of these, found to be the most active structural type.[5,8]

Chemistry

The structural derivatives of 2 (Table I) were uniformly available from the known 6-chloronaphthoquinone (3; Scheme I), which can be prepared in quantity by minor variation of the published procedure.[9] In a modification of our original procedure,[6] 2 could be prepared from 3 via epoxidation to 4 using commercial bleach in tetrahydrofuran, followed by acid-mediated epoxide opening and oxidation. Acetylation of 2 with acetic anhydride and acid catalyst afforded 2,3-diacetoxy-6-chloro-1,4-naphthoquinone (5). Direct opening of epoxide 4 under similar conditions gave the corresponding 2,3-diacetoxy-2,3-dihydronaphthoquinone 6. Chlorination of 3 in acetic acid in the absence of catalyst[9] gave the dihydro dichloro derivative 7, which could be converted to 2,3,6-trichloronaphthoquinone (8) by chlorination in the presence of sodium acetate.[9] This two-step sequence could be reduced to one, however, by using iodine as a catalyst,[10] to give 8 directly from 3. Subsequent conversion of 8 to naphthoquinone 2,3-diethers (9a,b) was carried out with use of the

Table I. Chemical Data for Naphthoquinone Synthetic Intermediates



compd	X	Y	mp,[a,b] °C	anal. or lit. mp, °C
2	6-Cl	OH	228–229	$C_{10}H_5ClO_4$
3	6-Cl	H	109–110	107[c]
4[d]			141–142	$C_{10}H_5ClO_3$
5	6-Cl	$OCOCH_3$	103–104	$C_{14}H_9ClO_6$
6[d]			222–223	$C_{14}H_{11}ClO_6$
7[d]			149–150	$C_{10}H_5Cl_3O_2 \cdot 0.5H_2O$
8	6-Cl	Cl	147.5–148.5	$C_{10}H_3Cl_3O_2$
9a	6-Cl	OCH_3	124–125 (B)	$C_{12}H_9ClO_4$
10d	6-Cl	$OCOCH_3$	184–185	$C_{18}H_{15}ClO_8$
13a	$5\text{-}CH_3$	H	121–122	122.5–123[e]
13b	$6\text{-}CH_3$	H	88–89	91–92[e]
13c	$6,7\text{-}(CH_3)_2$	H	116–117	118–119[f]
14a	$5\text{-}CH_3$	Cl	169–170	$C_{11}H_6Cl_2O_2$
14b	$6\text{-}CH_3$	Cl	147–148	$C_{11}H_6Cl_2O_2$
14c	$6,7\text{-}(CH_3)_2$	Cl	222–223	$C_{12}H_8Cl_2O_2$
15a	H	OCH_3	116–117 (B)	115[g]
15b	5-Cl	OCH_3	120–121 (A)[h]	$C_{12}H_9ClO_4$
15c	6-F	OCH_3	110–111 (A)[h]	$C_{12}H_9FO_4$
15d	6-Br	OCH_3	112–113 (A)[h]	$C_{12}H_9BrO_4$
15e	6-Cl	OEt	41.5-42.5 (B)	$C_{14}H_{13}ClO_4$
15f	6-Cl	O-*n*-Pr	oil (B)	$C_{16}H_{17}ClO_4$
15g	6-Cl	O-*i*-Pr	41–42 (B)	$C_{16}H_{17}ClO_4$
15h	$5\text{-}OCH_3$	OCH_3	105–106 (B)[i]	$C_{13}H_{12}O_5$
15i	$6\text{-}OCH_3$	OCH_3	116–117 (B)[i]	$C_{13}H_{12}O_5$
15j	5-CN	OCH_3	173.5–174 (A)[h]	$C_{13}H_9NO_4$
15k	6-CN	OCH_3	150–151 (A)[h]	$C_{13}H_9NO_4$
15l	$5\text{-}NH_2$	OCH_3	116–117	$C_{12}H_{11}NO_4$
15m	$5\text{-}CH_3$	OCH_3	86–87 (B)	$C_{13}H_{12}O_4$
15n	$6\text{-}CH_3$	OCH_3	88–89 (B)	$C_{13}H_{12}O_4$
15o	$6,7\text{-}(CH_3)_2$	OCH_3	121–122 (B)	$C_{14}H_{14}O_4$
15p	H	OPh	202–203 (B)	204[j]
15q	6-Cl	OPh	166–167 (B)	$C_{22}H_{13}ClO_4$
15r	5-SPh	OCH_3	79–81 (B)	$C_{18}H_{14}SO_4$
15s	6-Cl	SCH_3	117–118 (B)	$C_{12}H_9ClS_2O_2$

[a] Uncorrected. [b] Method A or B indicated for preparation 2,3-dioxynaphthoquinones. [c] Reference 9. [d] See Scheme I for structure. [e] Reference 14. [f] Reference 15. [g] Reference 16. [h] Isonaphthazarin from ref 5. [i] From the corresponding nitro-2,3-dichloronaphthoquinone and 3 equiv of sodium methoxide. [j] Reference 17.

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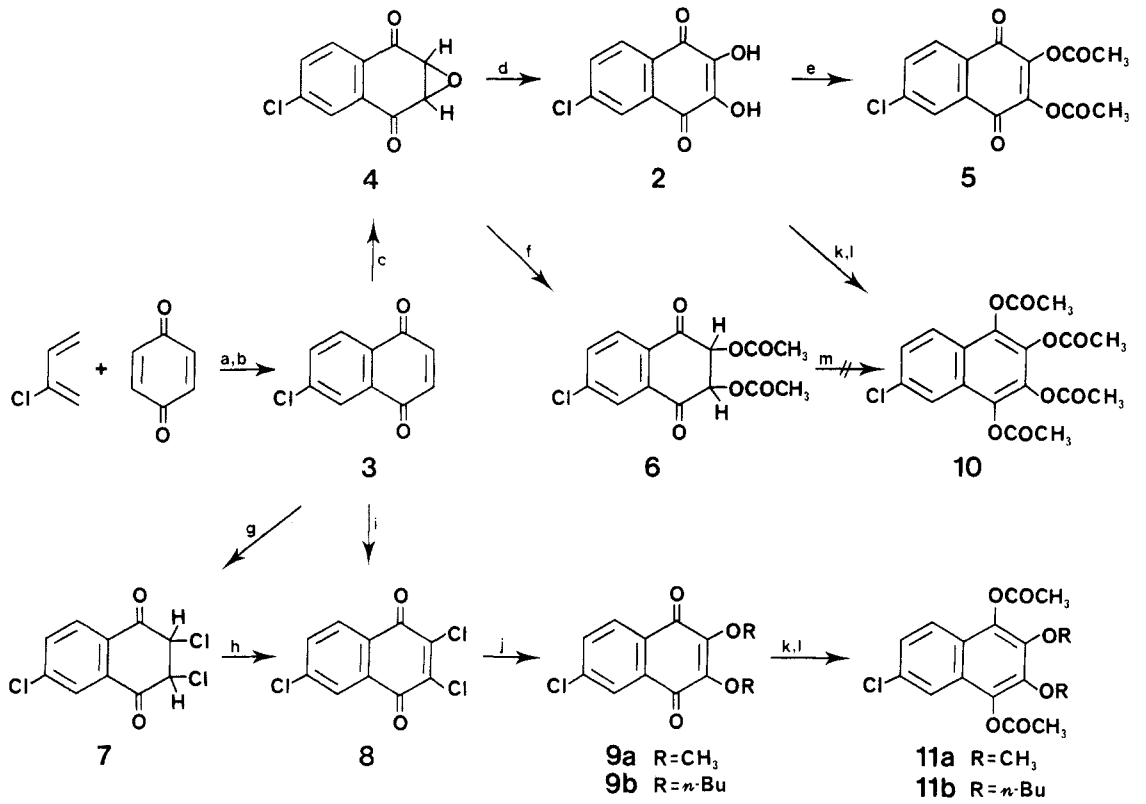
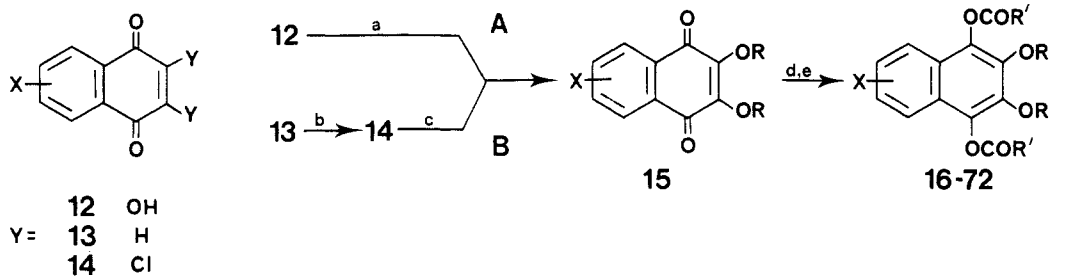
(10) Many catalysts have been used for the conversion of naphthoquinone to 2,3-dichloronaphthoquinone. As an example of the use of iodine, see: Inoue, A.; Nomura, Y.; Kuroki, N.; Konishi, K. *Yuki Gosei Kagaku Kyokai Shi* **1958**, *16*, 536; *Chem. Abstr.* **1959**, *53*, 1301*i*.

Scheme I[a]



[a] Key: a, HOAc, 48–72 h; b, $Na_2Cr_2O_7$/aqueous H_2SO_4/HOAc; c, NaOCl/aqueous $HClO_4$; e, Ac_2O/H_2SO_4; f, Ac_2O/HOAc/H_2SO_4; g, Cl_2/HOAc; h, Cl_2/NaOAc/HOAc; i, Cl_2/I_2/HOAc; j, RONa/MeOH or DMF; k, H_2/Pd–C/THF; l, Ac_2O/py/DMAP; m, $AcOC(CH_2)CH_3$/H_2SO_4/$PhCH_3$.

Scheme II[a]



[a] Key: a, CH_2N_2/THF; b, Cl_2/I_2/HOAc; c, RONa/ROH or DMF; d, H_2/Pd–C/THF; e, $R'CO_2O$/py/DMAP.

desired alkoxide and either the corresponding alcohol or DMF as the solvent.

The second general type of derivative was prepared by quinone reduction, followed by acylation. Compounds **2**, **9a**, and **9b** were reduced to the corresponding hydroquinones with hydrogen and 10% palladium on carbon in dry tetrahydrofuran. Subsequent in situ acylation with acetic anhydride, pyridine, and a catalytic amount of 4-(dimethylamino)pyridine afforded **10**, **11a**, and **11b**, respectively. However, attempted conversion of **6** to **10** using isopropenyl acetate under acid catalysis failed.

Upon identification of **11a** (RS-43179) as the leading clinical candidate[5] (vide infra), a series of analogues (Table II) was prepared from the appropriately substituted isonaphthazarins **12** or naphthoquinones **13** (Table I), as shown in Scheme II. Isonaphthazarins **12**[6] were treated with diazomethane[11] in tetrahydrofuran to give the corresponding 2,3-dimethoxy-1,4-naphthoquinone analogues **15** (method A). Alternatively, the available naphthoquinones **13**[6] were converted as described above to the corresponding 2,3-dichloronaphthoquinones **14** and then to the 2,3-dialkoxy- or -bis(aryloxy)naphthoquinones **15** (method B), sequentially reduced and acylated as above to give the analogues **16–72**. Finally, the reduction of two sulfur-containing naphthoquinones (**15r,s**) was carried out by catalytic transfer hydrogenation,[12] followed by acylation to provide **73** and **74**, respectively. As a measure of lipophilicity of these analogues, octanol–water partition coefficients were determined by reported HPLC or extrapolation methods[13] and are expressed as log P values (Table II).

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Biological Evaluation

There currently exists no generalized animal model of psoriasis.[19] While the Scholtz–Dumas topical human psoriasis bioassay[7] does definitively demonstrate activity in vivo, it cannot be used for the routine screening of large numbers of compounds. Since the cause of the misregulated epidermal cell growth in psoriasis is unknown, it becomes necessary to screen potential antipsoriatic agents as inhibitors of one or more of the putative mediators of the inflammatory response associated with the disease state. The biochemical systems that modulate this response include the cyclic nucleotide, diamine/polyamine, and the arachidonic acid regulatory mechanisms.[20] Inhibitors of both cyclic AMP phosphodiesterase (e.g., Ro 20–1724[21]) and ornithine decarboxylase (e.g., glucocorticoids, retinoids, and others[22]) have shown efficacy in the treatment of psoriasis. Recent reports, however, have increasingly highlighted the important role of lipoxygenase products derived from arachidonic acid in the pathophysiology of the disease. Elevated levels of both 12-hydroxyeicosatetraenoic acid[23] and leukotriene B_4 (LTB_4) like material[24] have been detected in psoriatic lesions. In addition, cell-free preparations from psoriatic epidermis produce significantly more LTB_4-like material than preparations from normal skin.[25] It thus seems quite likely that modulation or inhibition of lipoxygenase in psoriatic lesions might provide an alternative route to treatment of the disease.

Detailed examination of specificity, time course, and mediators involved in the mouse ear inflammatory response to topical arachidonic acid supports the utility of this model for the rapid in vivo screening of agents by topical application showing selective inhibition of arachidonic acid 5-lipoxygenase.[26,27] Pretreatment of the mouse ear with an acetone solution of the test compound is followed in 60 min by treatment with arachidonic acid. The resulting ear edema is measured 1 h later by the increase in weight of a 8-mm-diameter plug vs. both a negative (acetone) control and a positive (arachidonic acid) control carried out simultaneously. The results for tetraoxygenated naphthalenes **11a,b** and **16–74** are listed in Table II. RS-43179 (**11a**) exhibited a dose-dependent inhibition of the edema response (Figure 1).

As an adjunct to the arachidonic acid induced mouse ear edema model we also established a method for determination of the topical inflammatory response intrinsic to each test agent as a preliminary indication of proinflammatory potential. Daily application of the test compound in acetone solution (1 mg/ear) and measurement of ear

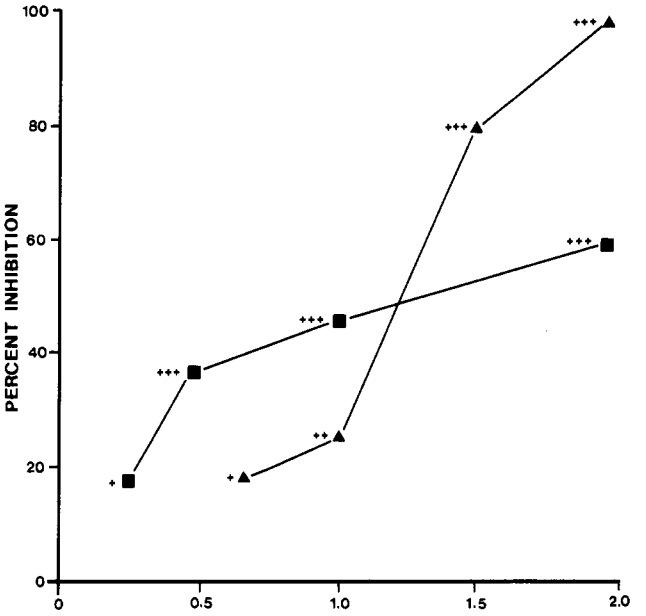

Figure 1. Dose-dependent inhibition of arachidonic acid induced mouse ear edema (■, total dose per ear, mg) and human PNM 5-lipoxygenase (▲, log concentration, μM) by **11a**. Statistical significance key given in footnote *d*, Table II.

thickness vs. a negative (acetone) control was carried out over 28 days. Of the representative compound types tested (**2, 5, 6, 9a, 10, 11a,b**), only diacetate **5** elicited an inflammatory response significantly above control. In subsequent studies, representative tetraoxygenated analogues from among **16–74** have also been found inactive in this screen.

The activity of **11a** as an inhibitor of 5-lipoxygenase has been confirmed by in vitro studies[28] using calcium ionophore stimulated human PMN leukocytes ($IC_{50} \simeq 15$ μM) and cell-free preparations from rat basophilic leukemic cells ($IC_{50} \simeq 0.5$ μM).[29] At concentrations up to 200 μM, **11a** was inactive or slightly stimulatory toward human platelet cyclooxygenase, thromboxane synthetase, prostaglandin synthase, and 12- and 15-lipoxygenase from a variety of sources.[30,31] These results, and those derived from more detailed studies to be published,[32] indicate that **11a** and analogues are potent and selective 5-lipoxygenase inhibitors, further implicating leukotriene B_4 and/or the peptido leukotrienes as mediators of psoriasis.[33]

In clinical trials using either gel or ointment formulations (0.5% b.i.d.), **11a** has produced good to excellent clearing of stable psoriatic plaques in >75% of the patients (n = 80), a rate comparable to that observed with fluocinolone acetonide and other potent corticosteroids. The incidence of irritation and sensitization has been low.[34,35] These

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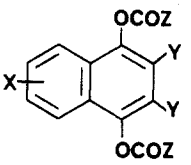
Table II. Chemical and Biological Screening Data for Tetraoxygenated Naphthalene Derivatives (11a,b, 16–74)



compd	X	Y	Z	mp,[a] °C	anal.	log P[b]	% inhibn[c]	sig[d]
11a	6-Cl	OCH_3	CH_3	91–92	$C_{16}H_{15}ClO_6$	3.26[e]	54	+++
11b	6-Cl	O-*n*-Bu	CH_3	75–76	$C_{22}H_{27}ClO_6$	5.9	23	++
16	H	OCH_3	CH_3	138–139	135–137[f]	2.39[e]	19	+
17	H	OCH_3	Et	111–112	$C_{18}H_{20}O_6$	3.44[g]	33	++
18	H	OCH_3	*n*-Pr	53–54	$C_{20}H_{24}O_6$	4.64[g]	59	+++
19	H	OCH_3	*i*-Pr	95–96	$C_{20}H_{24}O_6$	4.61[g]	53	+++
20	H	OCH_3	*t*-Bu	178–180	$C_{22}H_{28}O_6$	5.69[g]	0	NS
21	H	OPh	CH_3	242–243	$C_{26}H_{20}O_6$	4.8	–[h]	–[h]
22	H	OPh	Et	164–165	$C_{28}H_{24}O_6$	5.8	–[h]	–[h]
23	H	OPh	*t*-Bu	218–219	$C_{32}H_{32}O_6$	7.8	–[h]	–[h]
24	H	OPh	Ph	201–202	$C_{36}H_{24}O_6$	7.9	–[h]	–[h]
25	5-Cl	OCH_3	CH_3	133–134	$C_{16}H_{15}ClO_6$	3.16[e]	11	NS
26	5-Cl	OCH_3	*t*-Bu	128–130	$C_{22}H_{27}ClO_6$	6.1	6	NS
27	6-F	OCH_3	CH_3	102–103	$C_{16}H_{15}FO_6$	2.75[e]	40	+++
28	6-Br	OCH_3	CH_3	102–103	$C_{16}H_{15}BrO_6$	3.39[e]	42	+++
29	6-Cl	OCH_3	Et	84–85	$C_{18}H_{19}ClO_6$	4.30	49	+++
30	6-Cl	OCH_3	*n*-Pr	62–63	$C_{20}H_{23}ClO_6$	5.50	58	+++
31	6-Cl	OCH_3	*i*-Pr	64–65	$C_{20}H_{23}ClO_6$	5.40	23	++
32	6-Cl	OCH_3	*t*-Bu	132–133	$C_{22}H_{27}ClO_6$	6.60	11	NS
33	6-Cl	OCH_3	Ph	161–162	$C_{26}H_{19}ClO_6$	6.7	18	+
34	6-Cl	OEt	CH_3	91–92	$C_{18}H_{19}ClO_6$	4.0	38	+++
35	6-Cl	OEt	Et	81–82	$C_{20}H_{23}ClO_6$	5.2	50	+++
36	6-Cl	OEt	*i*-Pr	77–78	$C_{22}H_{27}ClO_6$	6.4	11	+
37	6-Cl	OEt	*t*-Bu	104–105	$C_{24}H_{31}ClO_6$	7.4	19	+++
38	6-Cl	O-*n*-Pr	CH_3	53–54	$C_{20}H_{23}ClO_6$	5.2	50	+++
39	6-Cl	O-*n*-Pr	Et	39–40	$C_{22}H_{27}ClO_6$	6.4	30	+++
40	6-Cl	O-*n*-Pr	*n*-Pr	28–29	$C_{24}H_{31}ClO_6$	7.6	2	NS
41	6-Cl	O-*n*-Pr	*i*-Pr	51–52	$C_{24}H_{31}ClO_6$	7.5	28	++
42	6-Cl	O-*n*-Pr	*t*-Bu	59–60	$C_{26}H_{35}ClO_6$	8.5	16	+
43	6-Cl	O-*i*-Pr	CH_3	132–133	$C_{20}H_{23}ClO_6$	4.6	19	+++
44	6-Cl	O-*i*-Pr	Et	58–59	$C_{22}H_{27}ClO_6$	5.8	16	NS
45	6-Cl	O-*i*-Pr	*t*-Bu	137–138	$C_{26}H_{35}ClO_6$	7.9	26	+++
46	6-Cl	OPh	CH_3	144–145	$C_{26}H_{19}ClO_6$	5.6	5[i]	NS
47	6-Cl	OPh	Et	136–137	$C_{28}H_{23}ClO_6$	6.6	–15[i]	NS
48	6-Cl	OPh	*n*-Pr	109–110	$C_{30}H_{27}ClO_6$	7.7	–5[i]	NS
49	6-Cl	OPh	*i*-Pr	116–117	$C_{30}H_{27}ClO_6$	7.7	0[i]	NS
50	6-Cl	OPh	*t*-Bu	164–165	$C_{32}H_{31}ClO_6$	8.7	–[h]	
51	6-Cl	OPh	Ph	173–174	$C_{36}H_{23}ClO_6$	8.7	–1[i]	NS
52	5-OCH_3	OCH_3	CH_3	69–70	$C_{17}H_{18}O_7$	2.43[e]	47	++
53	5-OCH_3	OCH_3	Et	72–75	$C_{19}H_{22}O_7$	3.44[e]	57	+++
54	5-OCH_3	OCH_3	*t*-Bu	153–154	$C_{23}H_{30}O_7$	5.6	29	++
55	6-OCH_3	OCH_3	CH_3	77–78	$C_{17}H_{18}O_7$	2.39[e]	53	+++
56	6-OCH_3	OCH_3	Et	86–87	$C_{19}H_{22}O_7$	3.28[g]	49	++
57	6-OCH_3	OCH_3	*t*-Bu	45–46	$C_{23}H_{30}O_7$	5.4	3	NS
58	5-CN	OCH_3	CH_3	152–153	$C_{17}H_{15}NO_6$	2.51[e]	24	++
59	6-CN	OCH_3	CH_3	124–125	$C_{17}H_{15}NO_6$	2.28[e]	7	NS
60	5-NHAc	OCH_3	CH_3	177–178	$C_{18}H_{19}NO_7$	2.00[e]	6[i]	NS
61	5-CH_3	OCH_3	CH_3	114–115	$C_{17}H_{18}O_6$	2.8	22	+++
62	5-CH_3	OCH_3	Et	91–92	$C_{19}H_{22}O_6$	3.9	24	++
63	5-CH_3	OCH_3	*i*-Pr	80–81	$C_{21}H_{26}O_6$	5.0	12	NS
64	5-CH_3	OCH_3	*t*-Bu	130–131	$C_{23}H_{30}O_6$	6.1	10	NS
65	6-CH_3	OCH_3	CH_3	70–71	$C_{17}H_{18}O_6$	2.86[e]	40	+++
66	6-CH_3	OCH_3	Et	57–58	$C_{19}H_{22}O_6$	3.9	39	+++
67	6-CH_3	OCH_3	*t*-Bu	110–111	$C_{23}H_{30}O_6$	6.1	11	NS
68	6,7-$(CH_3)_2$	OCH_3	CH_3	124–125	$C_{18}H_{20}O_6$	3.14[e]	19	+++
69	6,7-$(CH_3)_2$	OCH_3	Et	108–109	$C_{20}H_{24}O_6$	4.2	26	+++
70	6,7-$(CH_3)_2$	OCH_3	*n*-Pr	69–70	$C_{22}H_{28}O_6$	5.4	14	NS
71	6,7-$(CH_3)_2$	OCH_3	*i*-Pr	98–99	$C_{22}H_{28}O_6$	5.3	21	+++
72	6,7-$(CH_3)_2$	OCH_3	*t*-Bu	124–125	$C_{24}H_{32}O_6$	6.5	6	NS
73	5-SPh	OCH_3	CH_3	138–140	$C_{22}H_{20}SO_6$	4.2	42	+++
74	6-Cl	SCH_3	CH_3	113–114	$C_{16}H_{15}ClS_2O_4$		–2	NS

[a] Uncorrected. [b] Observed or extrapolated lipophilicity according to ref 13 (see the Experimental Section). [c] Percent inhibition (mean ± 3% SE, n = 8) of arachidonic acid induced mouse ear edema at 2 mg of total dose of test compound, according to ref 26 (see the Experimental Section). [d] Statistical significance, p values as compared to positive control by Student's t-test: +, ≤0.05; ++, 0.01; +++, 0.01; +++, ≤0.001; NS, not statistically significant. [e] 1-Octanol system. [f] Literature mp, ref 18. [g] Calculated. [h] Insoluble. [i] Tested as suspension of insoluble material in acetone.

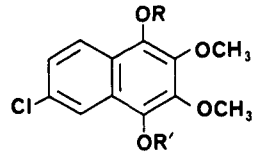
clinical results support the use of the topical arachidonic acid mouse ear edema model as a screening bioassay for agents useful against psoriasis acting primarily by inhibition of 5-lipoxygenase.

Structure-Activity Correlations

Three significant and interrelated determinants of the structure–activity relationship within the analogue series **16–74** are quickly evident on examination of the arachidonic acid induced mouse ear edema bioassay data (Table II). Net lipophilicity, as indicated by log P values, plays a highly important role, since skin penetration is an obvious requirement for activity. Compounds too lipophilic to penetrate the stratum corneum, with log P values in general greater than 5.5 were, if not too insoluble to test, significantly less active than compounds otherwise functionally similar in structure. The importance of the lipophilicity factor was amply illustrated by examination of **11a** and **11b** (log P = 3.26 and 5.9, respectively, and both fully solubilized) in the Scholtz–Dumas topical human bioassay. Whereas **11a** proved to be the most potent compound of those tested, the di-*n*-butoxy homologue **11b** exhibited no detectable antipsoriatic activity, demonstrating the importance of net lipophilicity as a determinant of skin penetration, and thus of in vivo activity.[5]

Another factor clearly important in determination of topical activity in the mouse ear edema model is the hydrolytic lability of the 1,4-diester functionality. Within the series of 6-chloro-2,3-dimethoxy-1,4-diacylates (**11a, 29–33**), a precipitous drop in activity is observed as ester hydrolytic stability increases from acetate to pivalate and benzoate. While at first glance this factor is somewhat difficult to separate from net lipophilicity, the observation that ester lability is a necessary component of the SAR profile has been independently demonstrated. Thus, the in vitro 5-lipoxygenase inhibition studies mentioned above[30] revealed a time-dependent *increase* in activity upon incubation of **11a** with human PMN preparations, indicating that a primary or later hydrolysis product is likely to be the active inhibitor of 5-lipoxygenase.

Confirmation was obtained by in vitro examination of the two possible monohydrolysis products of **11a**, namely isomers **75a** and **75b**, prepared by pH-controlled hydrolysis of **11a** and separated by preparative HPLC.[36] Inhibition of human PMN 5-lipoxygenase by either **75a** or **75b** revealed that, after the standard 5-min incubation, these monoesters exhibited inhibitory activity comparable to that of **11a** upon 20-min incubation (IC_{50} = 9 μM). Further hydrolysis of monoesters **75a** and **75b** should afford hydroquinone **76**, independently prepared by dithionite reduction of **9a**[37] and shown to be chemically quite prone to oxidation. Since both quinone **9a**, the product of this oxidative hydrolysis cascade, and 6-chloroisonaphthazarin (**2**), a potential product of further degradation, are significantly less potent (IC_{50} = 57 and 150 μM, respectively), monoesters such as **75** are implicated as the putative 5-lipoxygenase inhibitory species. This series of chemical and biological observations thus lends support to the hypothesis that ester hydrolysis is an obligatory step in any proposed mechanism of action.[32]



75a R=Ac R′=H
75b R=H R′=Ac
76 R=R′=H

The final factor differentiating analogues **16–74** is the nature and position of B-ring substitution, which will necessarily impact upon both net lipophilicity and the susceptibility of the 1,4-diesters to hydrolysis. Within the series of 6-halo analogues (**11a, 27, 28**) no major differences are observed in the bioassay data. However, upon movement of the 6-chloro substituent to position 5 (**25**) a significant drop in activity in the mouse ear edema model is observed. Other changes in general indicate that the substitution contribution to ester lability is significant. Within the series of analogues substituted at position 6 with chloro (**11a**), hydrogen (**16**), methoxy (**55**), cyano (**59**), and methyl (**65**), activity is severely affected by lack of π-electron-donating capability (i.e., CN and H). The corresponding effects on the stability (or reactivity) of the putative active species (e.g., monoesters **75a,b**) can only be speculated upon until the exact mode of inhibition of 5-lipoxygenase by these compounds is established.[32,38]

Conclusion

An observation of the antipsoriatic activity of **1** over a decade ago prompted a search for stable derivatives of this lead compound that maintained the desired activity while eliminating a number of untoward chemical and biological properties. The products of this search, **11a** and analogues, are a set of structurally novel and biologically active compounds that fit these criteria. Analysis of the analogue series (**16–74**) has revealed a combination of factors affecting in vivo topical antiinflammatory activity and has led to the choice of **11a** (lonapalene) as a clinical candidate for the topical treatment of psoriasis.

Experimental Section

Melting points were obtained on a Fisher Johns hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on either a Varian EM-390 (90-MHz) or a Bruker WM 300 (300-MHz) instrument. ^{13}C NMR spectra were obtained on a Bruker WH 90 instrument (22.62 MHz). Infrared spectra were recorded as KBr pellets with a Perkin-Elmer 237 grating spectrometer. Mass spectra were determined on Atlas CH-4 or CH-7 instruments. All compounds exhibited NMR, IR, and mass spectral data consistent with the proposed structures. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, or Analytical Services, Syntex Research.

6-Chloro-1,4-naphthoquinone (3). 6-Chloro-1,4-naphthoquinone was prepared by the method of Fieser.[9] A large-scale preparation was carried out as follows. 2-Chlorobutadiene (2 kg of 50% solution in xylene, Pfaltz & Bauer) was distilled to give purified diene [bp 60–75 °C, yield 768 g (8.88 mol, 79%, d = 0.958)] and was added to a suspension of *p*-benzoquinone (873 g, 8.07 mol; Aldrich) in acetic acid (3.6 L). After being stirred mechanically at room temperature for 3 days, the dark mixture was filtered to remove solids, and the residue was washed with acetic acid, giving a total filtrate volume of 4.8 L. The filtrate was divided into four equal portions, and *each* was treated as follows.[39] To 1.2 L of the above filtrate warmed to 50 °C in a 12-L flask with stirring and internal thermometer was added a warmed (50 °C) solution of sodium dichromate dihydrate (800 g) in water (500 mL) and concentrated sulfuric acid (40 mL) in

(35) Lassus, A.; Forsstrom, S. *Br. J. Dermatol.* **1985**, *113*, 103. Reports on subsequent trials are in preparation.

(36) The pH–rate profile of the hydrolysis of **11a** and conditions for HPLC separation of **75a** and **75b** were determined by Dr. Michael Powell and Adina Magill, Institute of Pharmaceutical Sciences, Syntex Research.

(37) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley: New York, 1967; Vol. 1, p 1081.

(38) A complete QSAR examination based on these qualitative observations and other parameters has been developed and will be published in due course: Unger, S. H., private communication.

(39) The extremely exothermic nature of this oxidation using the conditions noted by Fieser[9] necessitated that the reaction be carried out on lots no larger than described when working on a laboratory scale.

a slow stream. The temperature was maintained at 60–65 °C by periodic cooling as required. When the addition was complete, cooling was maintained until the temperature dropped to 40 °C, at which time 4 L of ice–water was added with rapid stirring. The brown precipitate was collected and air-dried overnight. The three additional runs were carried out in the same way to obtain crude product. Completion of the oxidation and recrystallization was carried out by dissolution in hot acetic acid (700 mL/100 g of crude product) followed by the addition of 10% aqueous dichromate (100–150 mL/100 g of crude product). After heating for 15 min at 60–70 °C, the mixture was diluted with 1 volume of water and allowed to cool overnight. Filtration and thorough drying afforded **3**: 1235 g, 6.41 mol, 79.4% based on benzoquinone; mp 109–110 °C (lit.[9] mp 107 °C).

6-Chloro-1,4-naphthoquinone 2,3-Oxide (4). Commercial bleach (1.2 L) was added to a solution of **3** (118 g, 612 mmol) in tetrahydrofuran (1.2 L) over 5 min. The reaction was stirred for 1 h at ambient temperature and was then diluted with water (6 L). The resulting precipitate was collected by filtration and dried thoroughly to give **4**: 120 g, 575 mmol, 94%; mp 141–142 °C. Anal. ($C_{10}H_5ClO_3$) C, H, Cl.

6-Chloro-2,3-dihydroxy-1,4-naphthoquinone (2). A solution of **4** (20 g, 96 mmol) in acetic acid (240 mL), perchloric acid (70%, 40 mL), and water (40 mL) was heated on a steam bath with a stream of air introduced below the surface of the solution. After 7 h, the mixture was diluted with water (1 L) and stored overnight in the refrigerator. The resulting precipitate was collected by filtration and washed with water. The moist solid was dissolved in methanol (100 mL), and to the solution was added potassium acetate (10 g, 102 mmol) in water (100 mL). The resulting thick gel was stirred at room temperature for 1 h and then filtered. The solid material was washed with methanol (3 × 50 mL) and then heated at 100 °C for 1 h with 50% acetic acid (200 mL). After cooling overnight in the refrigerator, the product was collected, washed with water (4 × 100 mL), and air-dried to yield **2**: 9.1 g, 40.5 mmol, 42%; mp 228–229 °C. Anal. ($C_{10}H_5ClO_4$) C, H, Cl.

2,3-Diacetoxy-6-chloro-1,4-naphthoquinone (5). Sulfuric acid (1 mL) was added to a suspension of **2** (10.0 g, 44.5 mmol) in acetic anhydride (50 mL). The mixture was stirred for 10 min at ambient temperature and was then poured into saturated aqueous sodium bicarbonate (500 mL). The resulting orange precipitate was collected by filtration and dried over phosphorus pentoxide to yield **5**: 13.1 g, 42.5 mmol, 95%; mp 103–104 °C. Anal. ($C_{14}H_9ClO_6$) C, H, Cl.

2,3-Diacetoxy-6-chloro-2,3-dihydro-1,4-naphthoquinone (6). A suspension of **4** (25 g, 120 mmol) in acetic anhydride (200 mL) and acetic acid (200 mL) was treated with concentrated sulfuric acid (25 mL). After the mixture was stirred overnight, the precipitate was collected, washed with ether, and dried. Recrystallization from dichloromethane gave **6**: 13.50 g, 43.5 mmol, 36%; mp 222–223 °C. Anal. ($C_{14}H_{11}ClO_6$) C, H, Cl.

2,3,6-Trichloro-1,4-naphthoquinone (8). Preparation of **8** could be carried out in two stages according to the method of Fieser,[9] via the intermediate dihydro dichloro derivative **7**, mp 149–150 °C. Anal. ($C_{10}H_5Cl_3O_2 \cdot 0.25H_2O$) C, H, Cl. However, the preferred procedure to prepare **8** from **3** is as follows.[10]

Chlorine gas was bubbled into a suspension of **3** (100 g, 520 mmol) in acetic acid (800 mL) heated to 70 °C with mechanical stirring. Heating was increased to bring the mixture to reflux, at which time solid iodine (13.2 g, 104 mmol, 0.2 equiv) was added. Chlorine addition at reflux was continued until TLC (CH_2Cl_2) showed complete conversion to product (2–10 h total). The reaction was cooled to give a thick precipitate, which was collected by filtration. A second crop was obtained by concentration and/or dilution with water, to give a total yield of dried product **8**: 121.2 g, 464 mmol, 89%; mp 147.5–148.5 °C. Anal. ($C_{10}H_3Cl_3O_2$) C, H, Cl.

6-Chloro-2,3-dimethoxy-1,4-naphthoquinone (9a). Quinone **8** (130 g, 0.5 mol) was added as rapidly as possible to a mechanically stirred solution of sodium methoxide (55.5 g, 1.025 mol) in anhydrous methanol (1.5 L) under a blanket of nitrogen. The temperature rose to 50 °C during the addition, and the reaction was then heated to reflux for 1 h. The mixture was cooled and acidified with methanol saturated with hydrogen chloride (13.5 M) to give a brilliant yellow color. After the addition of water (300 mL), the reaction mixture was filtered, and the precipitate was washed with water–methanol (4:1) until the filtrate was yellow-orange. The precipitate was air-dried to yield **9a**: 102 g, 404 mmol, 81%; mp 125–126 °C. Anal. ($C_{12}H_9ClO_4$) C, H, Cl.

1,2,3,4-Tetraacetoxy-6-chloronaphthalene (10). A solution of **2** (25 g, 75 mmol) in tetrahydrofuran (250 mL) was hydrogenated at atmospheric pressure over Pd–C (10%, 2.5 g) until the solution was colorless, approximately 2 h. A solution of acetic anhydride (35 mL) and pyridine (35 mL) was added, and the solution was stirred overnight under hydrogen. The solution was filtered to remove catalyst and evaporated to give a thick oil. The residue was dissolved in ethyl acetate (250 mL), washed with 1 M HCl (3 × 100 mL) and brine (2 × 100 mL), dried over sodium sulfate, filtered, and evaporated. Trituration with ether followed by recrystallization from toluene–dichloromethane afforded **10**: 12.4 g, 31.3 mmol, 42%; mp 184–185 °C. Anal. ($C_{18}H_{15}ClO_8$) C, H, Cl.

1,4-Diacetoxy-6-chloro-2,3-dimethoxynaphthalene (11a). A solution of **9a** (50.5 g, 200 mmol) in THF (500 mL) was hydrogenated at atmospheric pressure over Pd–C (10%, 5.0 g) until the solution was colorless, approximately 4 h. While still under a blanket of hydrogen, a solution of acetic anhydride (47 mL, 500 mmol), pyridine (40 mL, 500 mmol), and DMAP (1.22 g, 10 mmol) in THF (50 mL) was added to the mixture. After stirring for 1 h, the mixture was filtered to remove the catalyst and then evaporated. The residue was dissolved in ether (500 mL) and was washed with 1 M HCl (3 × 250 mL) and with brine (2 × 250 mL). The organic layer was dried over sodium sulfate, filtered, and evaporated to give an oil that crystallized under high vacuum. Recrystallization from ether–petroleum ether afforded **11a**: 50.0 g, 166 mmol, 83%; mp 91–92 °C. Anal. ($C_{16}H_{15}ClO_6$) C, H, Cl.

1,4-Diacetoxy-2,3-di-*n*-butoxy-6-chloronaphthalene (11b) was prepared from unstable **9b** by a procedure analogous to that for **11a**. Compound **11b**, mp 75–76 °C, was obtained in 66% yield. Anal. ($C_{22}H_{27}ClO_6$) C, H, Cl.

2,3-Dialkoxy- or -Bis(aryloxy)-1,4-naphthoquinones (15). Method A. Where available,[6] isonaphthazarins **12** were converted to 2,3-dimethoxy-1,4-naphthoquinones **15** by the addition of 2.2 equiv of ethereal diazomethane to a solution of **12** in tetrahydrofuran. The mixture, which turned from dark red to yellow on completion of the reaction, was evaporated to afford **15** quantitatively.

Method B. Naphthoquinones **13** were chlorinated in the presence of iodine under conditions identical with the conversion of **3** to **8** to give 2,3-dichloro-1,4-naphthoquinones **14**. Subsequent reaction with the desired alkoxide (2.2 equiv) in the corresponding alcohol (for C_1–C_3 alkoxides) or DMF afforded the 2,3-dialkoxy-1,4-naphthoquinones **15** after evaporation, aqueous extraction, and silica gel chromatography using dichloromethane as eluant. Yields were essentially identical with those in the sequence described above for **11a**.

1,4-Bis(acyloxy)-2,3-dialkoxynaphthalenes or 1,4-Bis(acyloxy)-2,3-bis(aryloxy)naphthalenes (16–72). The diacylated hydroquinones **16–72** were uniformly prepared from the appropriate quinone (**15**) by the method described for the parent compound (**11a**) in yields consistently above 80%. In the cases where Z = *tert*-butyl (dipivalates), the reaction mixture was maintained at reflux under an inert atmosphere for 2–72 h to complete the acylation reaction.

Sulfur-Containing Compounds 73 and 74. Reduction of quinones **15r** and **15s** to the corresponding hydroquinones was carried out under catalytic transfer hydrogenation conditions,[12] using 10% palladium on carbon and 1,4-cyclohexadiene in tetrahydrofuran. Acylation proceeded as before to give **73** and **74** in 46 and 33% yields, respectively.

Controlled Hydrolysis of 11a to Monoester Isomers 75a and 75b. Diacetate **11a** (10 g) was heated at 80 °C in a mixture of acetonitrile and 0.005 M Na_2HPO_4 buffer (150 mL each) adjusted to pH 8.5.[36] Additional solid Na_2HPO_4 was added as required to maintain pH 8.5. When **11a** was consumed by TLC (CH_2Cl_2), the mixture was cooled, evaporated to remove acetonitrile, acidified to pH 3 with 2 N HCl, and extracted with ethyl acetate (3 × 100 mL). The organic extract was washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered, and evaporated. Crystallization of the residue from petroleum ether gave isomer **75a**, mp 165–166 °C. Anal. ($C_{14}H_{13}ClO_5$) C, H, Cl. Preparative HPLC of the mother liquor (Rainin Dynamax Si column, 4%

anhydrous methanol in hexane) gave isomer **75b**, mp 106–107 °C, Anal. ($C_{14}H_{13}Cl_5$) C, H, Cl.[40] HPLC retention time = 30.5 min, and for additional amounts of **75a**, HPLC retention time = 33.5 min.

6-Chloro-2,3-dimethoxynaphthalene-1,4-diol (76). A mixture of quinone **9a** (5.0 g, 19.8 mmol) in diethyl ether (30 mL) and sodium dithionite (7.58 g, 43.5 mmol) in water (30 mL) was refluxed with stirring for 3 h. After cooling, the aqueous layer was removed and the organic layer was washed with saturated brine. Evaporation of the ether and recrystallization of the residue from ether–petroleum ether afforded **76**: 1.85 g, 8.4 mmol, 43%; mp 106–107 °C. Anal. ($C_{12}H_{11}ClO_4$) C, H, Cl. Unless stored in the dark under an inert atmosphere, this compound slowly reoxidizes to **9a**. In solution, the degradation is complete in minutes, as evidenced by the characteristic yellow color of **9a**.

Determination of Lipophilicity. Three methods were used for the determination of lipophilicity. For the more hydrophilic analogues, the standard 1-octanol-coated reversed-phase high-performance liquid chromatography procedure was used.[13a] Phosphate-buffered saline (PBS), pH 7.4, 0.15 μM, saturated with 1-octanol was used as the mobile phase on either 2 × 30 mm Lichrosorb RP-18 or Corosil C-18 packing materials. Compounds too lipophilic to be chromatographed on the 1-octanol system were next examined on a standard reversed-phase system (40% acetonitrile/60% PBS, 0.15 μM, pH 7.4) on a 2 × 10 or 2 × 30 mm Lichrosorb RP18 or C-18, respectively,[13b] to provide log k' values ($k' = (t - t_0)/t_0$; where t = retention time in minutes). By the use of overlapping series with the first system, log P values were derived. Since these compounds have a constant number of hydrogen binding sites, such comparisons are reasonably accurate. Finally for the most lipophilic compounds that could not be detected by the second method, a series of lipophilic substituent constants were derived by simple Free–Wilson methods and the lipophilicities were calculated using additivity. Since the very lipophilic compounds were all uniformly inactive, this was deemed adequate. log P values are detailed in Table II.

Biological Screening.[26] Edema Induction and Measurement. Female mice (Sim:(SW)fBR; Simonsen Labs, Gilroy, CA) 6–10 weeks old were caged together in treatment groups of eight. Animals were acclimated for 1 week before use. All experimental procedures were performed without anesthesia, and animals were killed by cervical dislocation. Arachidonic acid (Sigma Chemical Co., St. Louis, MO; Grade 1, approximately 99%) was dissolved in reagent-grade acetone at 100 mg/mL and stored at −20 °C for no longer than 1 week. Test compound solutions were also prepared in acetone. Arachidonic acid (AA) in acetone was applied in volumes of 0.01 mL to each of the inner and outer surfaces of the right ear by means of an automatic microliter pipet. This volume was found to spread evenly over the whole surface of the ear with minimal drainage to the base and skull. Potential inhibitors (evaluated in groups of 4–12 compounds) were similarly applied as solutions in acetone (2 mg/0.02 mL per total dose), 60 min prior to AA application. Appropriate positive and negative controls (AA or acetone alone) were run in each experiment. Edema development in each animal group (n = 8) was determined on the basis of ear plug weight. One hour postchallenge, animals were killed and the ears were quickly removed at the base. Plugs were then obtained from the tip of each ear with a biopsy punch (8 mm disk), and these were weighed to the nearest 0.1 mg as soon as possible. Results for tetraoxygenated naphthalene analogues are detailed in Table II, indicating the percent inhibition (±3% SE) observed and the statistical significance (p value) within each test group of compounds vs. positive and negative controls for n = 8 animals per group per compound. Four repeat evaluations of **11a** showed a variability of ±4% between test runs.

Test for Proinflammatory Activity. Test compounds were applied as acetone solutions, 1 mg of compound/0.02 mL per ear, once daily Monday through Friday. Ears were observed daily for signs of redness, scaling, or swelling, and ear thickness measurements were made three times per week. For ear thickness determinations, an Oditest dial gauge caliper (0–0.8 in. with graduations of 0.001 in.) was modified to increase the contact area and reduce the tension. The calipers were applied near the tip of the ear just distal to the cartilaginous ridges, and thickness was recorded in units of 0.001 in. To minimize variation due to technique, measurements throughout any one experiment were performed by a single investigator. This method allowed edema development in animal groups (n = 8) to be followed through time (typically, 28 days). Of the representative compounds tested, only diacetate **5** exhibited proinflammatory activity significantly different from negative (acetone) controls.

Registry No. **2**, 74237-20-0; **3**, 56961-92-3; **4**, 102631-97-0; **5**, 74237-22-2; **6**, 102631-98-1; **7**, 91037-32-0; **8**, 78237-04-4; **9a**, 91037-30-8; **9b**, 91037-31-9; **10**, 102631-99-2; **11a**, 91431-42-4; **11b**, 91814-23-2; **12** (X = 5-Cl), 89227-37-2; **12** (X = 6-F), 89226-84-6; **12** (X = 6-Br), 89226-85-7; **12** (X = 5-CN), 89227-39-4; **12** (X = 6-CN), 89226-86-8; **13** (X = H), 130-15-4; **13** (X = 5-NO_2), 22360-86-7; **13** (X = 5-SPh), 102632-23-5; **13** (X = 6-NO_2), 29284-76-2; **13a**, 571-63-1; **13b**, 605-93-6; **13c**, 2202-79-1; **14a**, 18690-92-1; **14b**, 102632-00-8; **14c**, 61103-10-4; **15a**, 6956-96-3; **15b**, 89226-90-4; **15c**, 102632-01-9; **15d**, 102632-02-0; **15e**, 102632-03-1; **15f**, 102632-04-2; **15g**, 102632-05-3; **15h**, 62345-18-0; **15i**, 62345-17-9; **15j**, 89226-91-5; **15k**, 102632-06-4; **15l**, 60892-43-5; **15m**, 102632-07-5; **15n**, 102632-08-6; **15o**, 18691-23-1; **15p**, 36626-24-1; **15q**, 102632-09-7; **15r**, 89226-93-7; **15s**, 97988-25-5; **16**, 61601-23-8; **17**, 91814-56-1; **18**, 91814-57-2; **19**, 91814-58-3; **20**, 91814-09-4; **21**, 102632-10-0; **22**, 102632-11-1; **23**, 102632-12-2; **24**, 102632-13-3; **25**, 91814-10-7; **26**, 91814-11-8; **27**, 91814-12-9; **28**, 91814-13-0; **29**, 91814-14-1; **30**, 91814-15-2; **31**, 91814-16-3; **32**, 91814-17-4; **33**, 97988-22-2; **34**, 91814-18-5; **35**, 91814-19-6; **36**, 91814-20-9; **37**, 91826-92-5; **38**, 91814-32-3; **39**, 91814-33-4; **40**, 91814-34-5; **41**, 91814-21-0; **42**, 91814-22-1; **43**, 91814-35-6; **44**, 91814-36-7; **45**, 91814-39-0; **46**, 97988-24-4; **47**, 102632-14-4; **48**, 102632-15-5; **49**, 102632-16-6; **50**, 102632-17-7; **51**, 102632-18-8; **52**, 91814-24-3; **53**, 91814-25-4; **54**, 91814-26-5; **55**, 91814-40-3; **56**, 91814-27-6; **57**, 91814-28-7; **58**, 91814-29-8; **59**, 91814-30-1; **60**, 91814-31-2; **61**, 91814-41-4; **62**, 102632-19-9; **63**, 102632-20-2; **64**, 102632-21-3; **65**, 91814-42-5; **66**, 91814-43-6; **67**, 91814-45-8; **68**, 102632-22-4; **69**, 97988-18-6; **70**, 97988-19-7; **71**, 97988-20-0; **72**, 97988-21-1; **73**, 91814-53-8; **74**, 97988-23-3; **75a**, 102632-25-7; **75b**, 102632-26-8; **76**, 102632-27-9; 2-chlorobutadiene, 126-99-8; p-benzoquinone, 106-51-4; propionic anhydride, 123-62-6; butyric anhydride, 106-31-0; 2-methylpropionic anhydride, 97-72-3; 2,2-dimethylpropionic anhydride, 1538-75-6; benzoic anhydride, 93-97-0; 5-acetamido-2,3-dimethoxy-1,4-naphthoquinone, 102632-24-6.

(40) We thank Dr. Michael Maddox and Lilia Kurz, Analytical Research, Syntex, for performing the nuclear Overhauser enhanced difference NMR spectra necessary to assign structures **75a** and **75b** unambiguously.