EtOH/H_2O to provide 710 mg (60.5%) of **21**: mp 201-203 °C; IR (Nujol mull) 1685, 1640 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.88 (s, 6 H, 3- and 5-Me), 4.46 (s, 2 H, $COCH_2$), 7.5-8.15 (m, 5 H, Ph). Anal. ($C_{15}H_{14}O_4$) C, H.

Measurement of log *P*. Values of log *P*, the *n*-octanol/water partition coefficient, were estimated from the reversed-phase HPLC retention times[18] in 7:3 or 9:1 (v/v) MeOH/water, using *p*-hydroquinone, benzyl alcohol, phenol, acetophenone, nitrobenzene, benzene, toluene, and *o*-xylene as standards.[19]

Preparation of Human Leukocyte Elastase. Fresh human leukocytes were obtained by leukapheresis from a healthy donor, frozen, and kept at -75 °C until use. Enzyme preparation followed published methods[20,21] with slight modifications: cells were washed in 0.14 M NaCl and homogenized in the presence of 1 M NaCl and 0.1% (w/v) Brij 35 (Sigma Chemical Co.). After centrifugation and concentration by dialysis against polyethylene glycol (mol wt 20 000), the material was chromatographed on Sephacryl S-300 (Pharmacia). Active fractions were combined, concentrated as before, and chromatographed on an affinity gel of bovine lung trypsin inhibitor attached to Sepharose CL-6B (Pharmacia). Active fractions were combined, concentrated to approximately 0.3 μM in active elastase, and frozen in 1-mL aliquots at -75 °C until use.

Assay of Human Leukocyte Elastase. The assay buffer was 25 mM potassium *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid, 1 M NaCl, 0.1% (w/v) Brij 35, pH 7.8, 25 °C. To 1.90 mL of this was added the pyrone (20 mM in Me_2SO) and Me_2SO to a total of 0.1 mL. The substrate (methoxysuccinyl-L-alanyl-L-alanyl-L-prolyl-L-valyl-*N*-(4-methylcoumarinamide); Peninsula Laboratories) was added as 1 μL of a 4.2 mM solution in Me_2SO. The enzyme was added as 20 μL of the above 0.3 μM stock. Fluorescence increase was assayed by excitation at 370 nm and emission at 460 nm. Five to seven different concentrations of pyrone were assayed and the rate data fit by the method of Cleland[22] to the equation for reversible inhibition when [substrate] $\ll K_m$.

Assay of Bovine Chymotrypsin and Porcine Pancreatic Elastase. Bovine α-chymotrypsin (Sigma Chemical) was assayed as above in a buffer of 25 mM potassium *N*-(2-hydroxyethyl)-piperazine-*N*-2-ethanesulfonic acid, 0.1 M KCl, pH 7.8, 25 °C, containing pyrone as above and the substrate 7-(glutaryl-L-phenylalanamido)-4-methylcoumarin (Sigma) at 25 μM. Porcine pancreatic elastase (Sigma) was assayed in 1.0 mL of the same buffer containing pyrone as above and 12.5-100 μM of the substrate CBZ-L-alanyl-*p*-nitrophenol ester. The reaction was followed by absorbance at 400 nm.

Registry No. 1, 68112-21-0; **2**, 74583-84-9; **3**, 74583-82-7; **4**, 98393-85-2; **5**, 96610-59-2; **6**, 98393-86-3; **7**, 98393-87-4; **8**, 98393-88-5; **9**, 98393-89-6; **10**, 98420-36-1; **11**, 98393-90-9; **12**, 98393-91-0; **13**, 98393-92-1; **14**, 98393-93-2; **15**, 98393-94-3; **16**, 65837-08-3; **17**, 98393-95-4; **18**, 5192-62-1; **19**, 50405-44-2; **20**, 24607-33-8; **21**, 98393-96-5; **22**, 20851-38-1; **23**, 675-10-5; MeCH=C(Ph)CHO, 4411-89-6; MeCH=CHCHO, 4170-30-3; $MeCO_2Me$, 79-20-9; $C_5H_{11}CO_2Me$, 106-70-7; PhCOOMe, 93-58-3; elastase, 9004-06-2; chymotrypsin, 9004-07-3.

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New Antiallergic Pyrano[3,2-*g*]quinoline-2,8-dicarboxylic Acids with Potential for the Topical Treatment of Asthma

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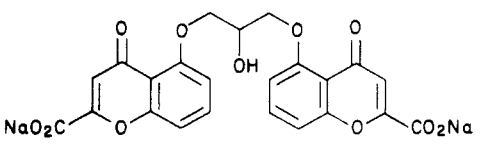
A number of antiallergic pyranoquinolinedicarboxylic acid derivatives with potential for the topical treatment of asthma have been synthesized. All the compounds have been evaluated against rat passive cutaneous anaphylaxis and in a dog hypotension screen. This is the first detailed description of the application of the latter screen for the identification of antiallergic agents. Two compounds, disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (**86**) and disodium 6-(methylamino)-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]-quinoline-2,8-dicarboxylate (**72**), were selected and further evaluated for their ability to induce phosphorylation of a 78 000 molecular weight protein associated with the rat peritoneal mast cell. Their ability to inhibit histamine release from these cells and from a mucosal mast cell preparation has also been evaluated. These compounds, nedocromil sodium (TILADE **86**) and minocromil (the free acid of **72**), are at present undergoing therapeutic evaluation. The rationale for the screening procedure and the relevance of the second carboxylic acid function of these dibasic acids to receptor binding are discussed.

It is now more than 16 years since the introduction of sodium cromoglycate (cromolyn sodium)[1] (1) into clinical



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practice for the prophylactic treatment of allergic diseases, especially asthma, rhinitis, and conjunctivitis. Despite considerable effort in this field by more than 50 drug companies[2] it remains the only prophylactic antiallergic compound clinically available. There are clearly many reasons for the failure to identify a follow-up drug, but the main one appears to be that the biological screens that have been used up to now have proved to be poor predictors of therapeutic efficacy. Conventionally, antiallergic activity has been measured by the ability of compounds to stabilize rat skin connective tissue mast cells (PCA test)[3] or to inhibit antigen-induced mediator release from passively sensitized human lung fragments.[4,5] However, it

(1) Cox, J. S. G.; Beach, J. E.; Blair, A. M. J. N.; Clarke, A. J.; King, J.; Lee, T. B.; Loveday, D. E. E.; Moss, G. F.; Orr, T. S. C.; Ritchie, J. T.; Sheard, P. *Adv. Drug. Res.* **1970**, *5*, 115.
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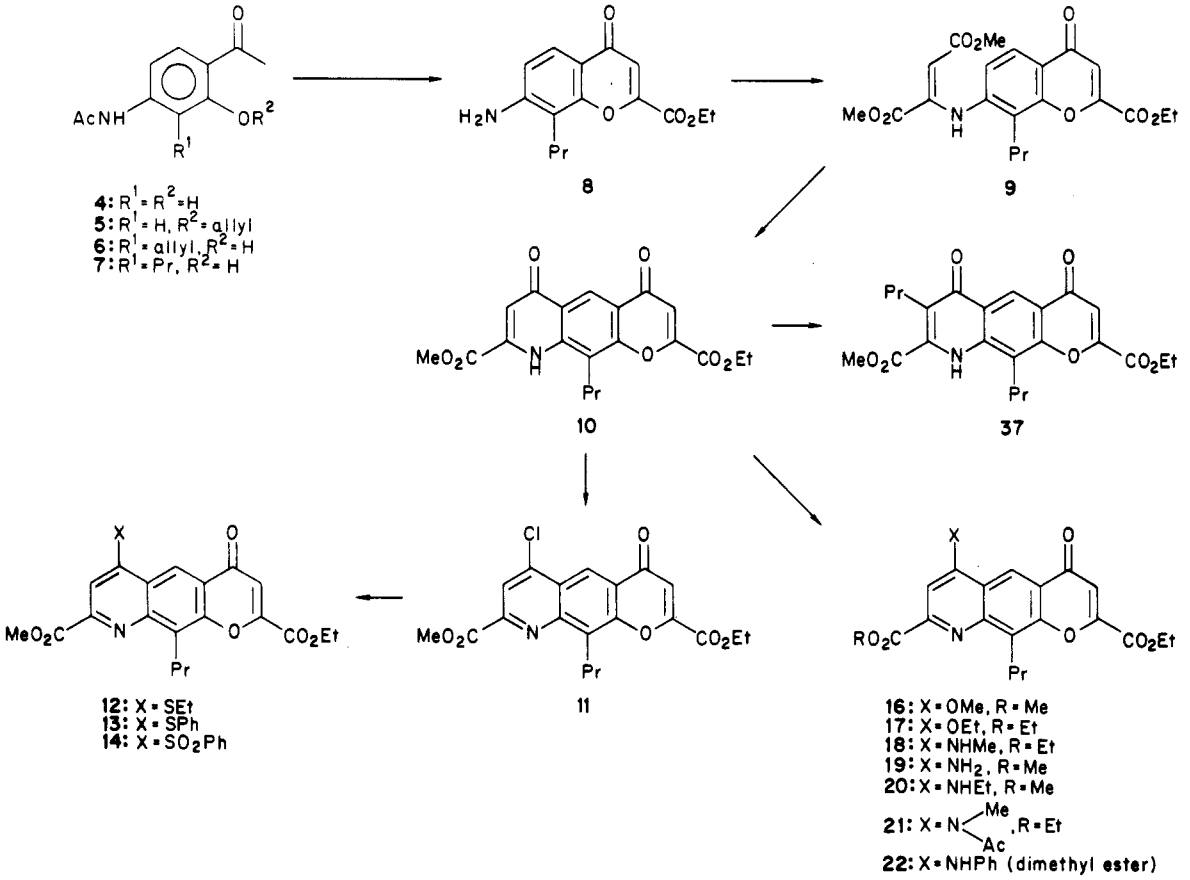
Scheme I



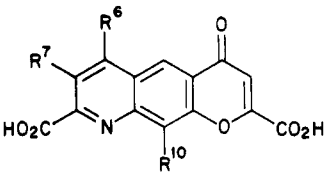
is now recognized that asthma is a multicomponent disease and the therapeutic usefulness of sodium cromoglycate is probably a reflection of more than one mode of action.[2] We have therefore employed a range of biological screens in our drug evaluation process and have developed compounds that conform to a particular biological profile (see below).

Sodium cromoglycate is poorly absorbed from the gastrointestinal tract and is rapidly eliminated from the body.[6] Consequently, the drug is only effective in asthma when given by inhalation. Generally, the structural requirements for activity and for good absorption have proven to be mutually exclusive.[2] The vast majority of antiallergic compounds so far described have been strongly acidic, hydrophilic molecules, the physicochemical properties of which result in pharmacokinetics that are unsuitable for systemic activity. Lack of success in producing an *orally* effective antiallergic agent is, therefore, perhaps, not surprising and may be another reason for the failure to produce a successor to sodium cromoglycate. In recent years the benefits of inhalation therapy have been recognized and, quite apart from the inherent difficulties of producing an antiallergic agent that can be taken orally, inhaled drugs for the treatment of asthma are now widely therapeutically preferred.[7]

We have, therefore, directed our efforts toward the discovery of topically effective agents for the treatment of asthma, with an improved profile of activity (duration of action, efficacy, potency) over sodium cromoglycate. This paper describes the synthesis and biological evaluation or pyranoquinolinedicarboxylic acid derivatives that appear to have fulfilled these criteria.

Chemistry

Two series of pyranoquinolines were synthesized: 6-substituted 4-oxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylic acids (**2**) and 9-substituted 4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylic acids (**3**). These were prepared by one of three conceptually different routes.



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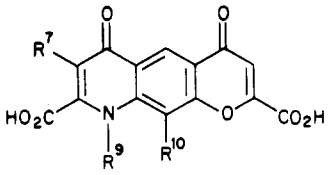

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Method I: construction of the fused pyridine ring upon a preformed substituted chromone nucleus. Various substituents were introduced via the pivotal intermediates **10** and **11** (Scheme I).

The chromone-2-carboxylic ester moiety was prepared by the Claisen condensation of diethyl oxalate and an appropriately substituted *o*-hydroxyacetophenone (e.g., **7**) followed by acid-catalyzed ring closure.[8] This ring closure

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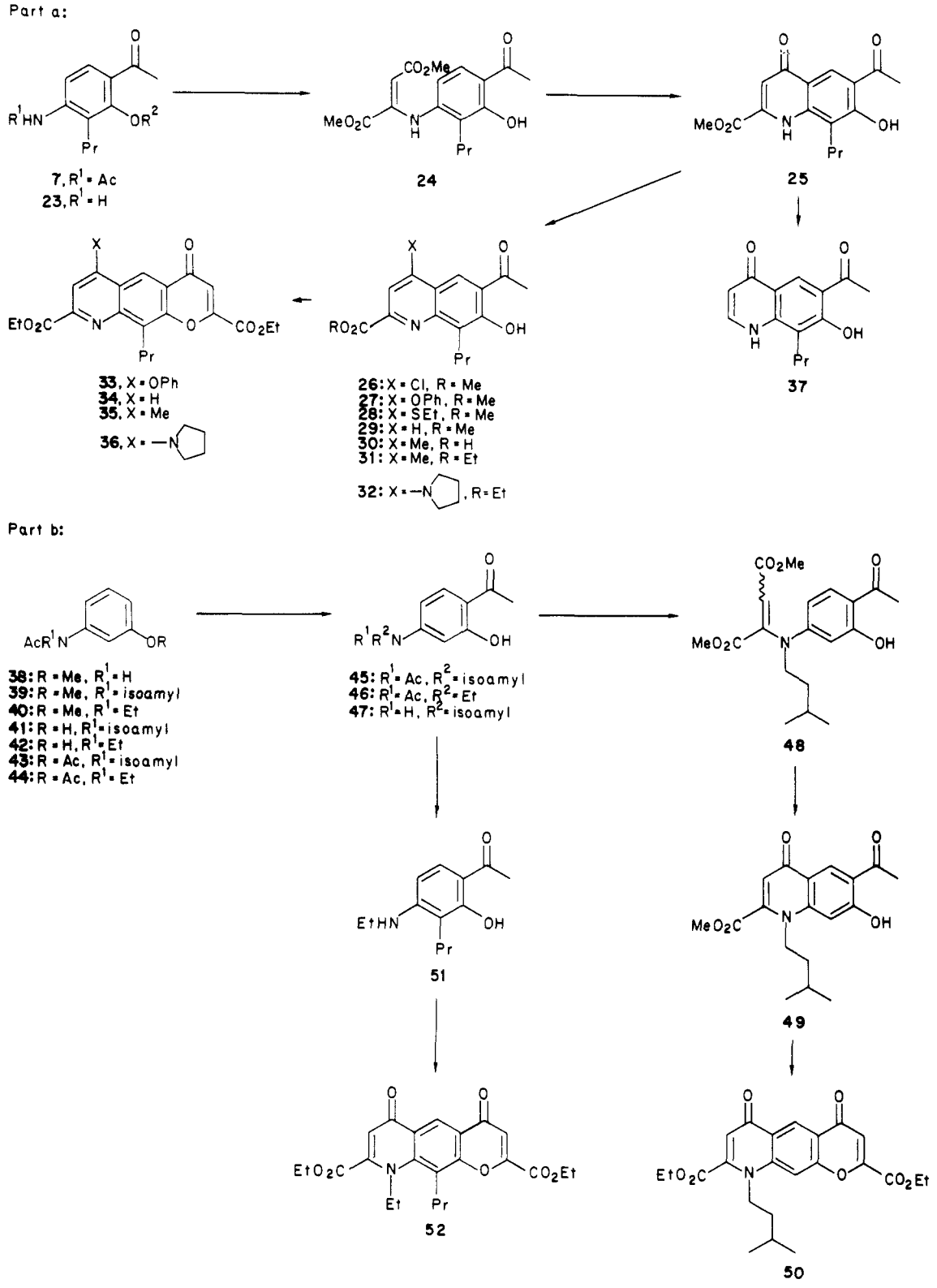
Scheme II



was accompanied by cleavage of the amide function to the free amine **8** and subsequent Michael addition to dimethyl acetylenedicarboxylate gave the adduct **9**, which was thermally cyclized to the pyranoquinoline **10**.[9]

Method II: construction of the fused pyranone ring upon a preformed quinoline nucleus (Scheme II). This method was developed in order to prepare a number of derivatives not available by direct substitution in compounds **10** and **11** (due to chemical sensitivity of those intermediates) (Scheme II, part a) and, more importantly, in order to prepare the N-alkylated pyranoquinolines (Scheme II, part b). The latter were not readily accessible via the route of Scheme I as the cyclization step to form the quinoline (acid catalyzed in this instance[9]) afforded mainly the products of retro-Michael fragmentation—viz. (alkylamino)chromone esters. Attempts to N-alkylate the pyranoquinoline **10** were also unsuccessful and only afforded O-alkylated compounds related to; e.g., **66** (Table I).

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Table I

compd no.	R^{2a}	R^6	R^7	R^{8a}	R^{10}	scheme	key intermed	further reactn[i]	PCA test: ID_{50},[h] mg/kg	PCA test: potency ratio[d]	dog hypotension screen[k]: dose[e]	dog hypotension screen[k]: % inhibn[f]	dog hypotension screen[k]: act. index[g]
57	α	H	H	α	Pr	IIa	**26**	B, C, D, A	7.11	0.35	10	38	3.8
58	a	Me	H	a	Pr	IIa	**23**	E, F, D, A	0.71	2.97	10	66	6.6
59	α	Cl	H	α	Pr	I	**11**	A	1.38	*c*	10	72	7.2
60	α	Cl	Pr	α	Pr	I	**15**	G, A	2.45	1.14	10	inact	0
61	α	Cl	H	α	H	III	**56**	G, A	4.23	0.72	10	inact	0
62	α	Cl	H	H	Pr	IIa	**37**	G, D, A	5.38	0.20	10	48	4.8
63	α	Cl	H	α	allyl	I	**6**	*b*	2.40	1.09	10	21	2.1
64	α	Br	H	α	Pr	I	**10**	H, A	0.55	4.62	10	48	4.8
65	α	MeO	H	α	Pr	I	**10**	J, A	0.30	8.35	10	43	4.3
66	α	EtO	H	α	Pr	I	**10**	F, A	0.30	8.47	10	32	3.2
67	α	PhO	H	α	Pr	IIa	**26**	I, D, A	1.12	1.75	10	68	6.8
68	α	EtS	H	α	Pr	I	**11**	K, A	0.14	11.1	10	59	5.9
69	α	PhS	H	α	Pr	I	**11**	L, A	1.87	1.78	10	66	6.6
70	α	$PhSO_2$	H	α	Pr	I	**11**	L, M, A	0.30	9.90	10	39	3.9
71	α	NH_2	H	α	Pr	I	**10**	*j*	0.31	8.25	20	82	4.1
72	α	MeNH	H	α	Pr	I	**10**	*j*	0.22	7.13	20	80	4.0
73	α	EtNH	H	α	Pr	I	**10**	*j*	0.18	13.8	10	42	4.2
74	α	PhNH	H	α	Pr	I	**10**	N, O, P, A	0.57	3.47	20	60	3.0
75	α	1-pyrrolidino	H	α	Pr	IIa	**26**	Q, F, D, A	0.86	4.13	10	57	5.7
76	α	Ac(Me)N	H	α	Pr	I	**18**	O, A	4.02	0.88	20	20	1.0

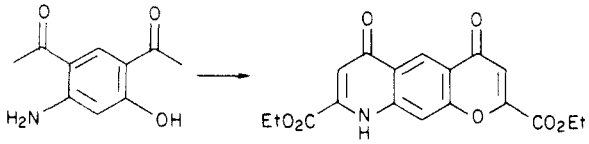
[a] α = CO_2Na; a = CO_2H. [b] See the Experimental Section. [c] Sodium cromoglycate not tested. [d] Sodium cromoglycate = 1.0. [e] In μg/kg per min for 15 min. [f] Area under curve over 3 h. [g] Percent inhibition/dose. [h] Each ID_{50} value was estimated from a regression line (significant at the 5% level in all cases). [i] Further reactions (see the Experimental Section): A. ester hydrolysis; B. EtSH/NaH, DMF; C. Raney Ni, EtOH; D. (i) diethyl oxalate/NaOEt, (ii) EtOH–HCl; E. (i) $CH_3COCH = CHCO_2H$, Δ, (ii) PPA, 100 °C; F. HCl(g)–EtOH, Δ; G. $POCl_3$; H. $POBr_3$; I. PhOH/KOH; J. MeI/NaH; K. EtSH/K_2CO_3; L. PhSH; M, *m*-CPBA; N. TsNCO; O. Ac_2O; P. (i) PhBr/Cu bronze, (ii) MeOH–HCl; Q. (i) pyrrolidine, glyme, Δ, (ii) 3 M H_2SO_4. [j] See ref 13. [k] The results of single experiments (see the Experimental Section).

Table II

compd no.	R^{2a}	R^7	R^{8a}	R^9	R^{10}	scheme	key intermed.	further reactn[i]	PCA test: ID_{50},[h] mg/kg	PCA test: potency ratio[d]	dog hypotension screen[j]: dose[e]	dog hypotension screen[j]: % inhibn[f]	dog hypotension screen[j]: act. index[g]
77	α	H	α	H	Pr	I	**10**	A	0.03	80	10	65	6.5
78	α	H	α	H	Me	III	*b*		0.11	12.7	10	66	6.6
79	α	H	α	H	H	III	**56**	A	0.22	11.6	10	78	7.8
80	α	Pr	α	H	Pr	I	**10**	B, A	0.14	10.9	10	6	0.6
81	α	H	H	H	Pr	IIa	**25**	A, C, D, A	6.03	0.35	20	72	3.6
82	H	H	*a*	H	Pr	IIa	**25**	E	0.78	6.5	100	19	0.19
83	α	H	α	Me	Pr	IIb	**38**	*c*	0.21	6.6	10	55	5.5
84	α	H	α	Et	H	IIb	**38**	*c*	10	0.16	10	76	7.6
85	α	H	α	Et	1-propenyl	IIb	**46**	*c*	2.96	0.77	10	84	8.4
86	α	H	α	Et	Pr	IIb	**51**	*c*	0.95	1.1	10	60	6.0
87	α	H	α	Pr	Pr	IIb	**38**	*c*	3.71	0.43	10	28	2.8
88	a	H	a	isoamyl	H	IIb	**50**	A	2.05	0.54	50	68	1.4

[a] α = CO_2Na; a = CO_2H. [b] Made by an analogous method to **79** (see the Experimental Section). [c] See the Experimental Section. [d] Sodium cromoglycate = 1.0. [e] In μg/kg per min for 15 min. [f] Area under curve over 3 h. [g] Percent inhibition/dose. [h] Each ID_{50} value was estimated from a regression line (significant at the 5% level in all cases). [i] Further reactions (see the Experimental Section): A. ester hydrolysis; B. (i) allyl bromide/K_2CO_3, DMF, (ii) Δ, (iii) Pd/C–H_2; C. Δ; D. (i) diethyl oxalate/base, (ii) H^+; E. DMF–dimethyl acetal·H_2SO_4. [j] The results of single experiments (see the Experiments Section).

Scheme III



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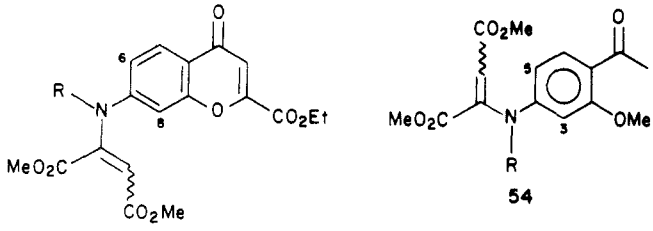
It was our observation that the regiochemistry of the thermal- or acid-catalyzed cyclizations to form the pyrido-ring in Schemes I and II could be predicted quite successfully by a consideration of the predominating Kekulé resonance forms of the aromatic nucleus.[10]



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Thus, structures **48** and **53** cyclize to afford the "angular" systems predominantly. For example, the isoamyloxy compound (**48**, Scheme II) gave only 5% yield of **49** whereas its regioisomer was obtained in 40% yield. In contrast, methoxyacetophenones **54**, which contain neither a fused heteroaromatic ring nor an intramolecular H bond capable of strongly influencing the distribution of orbitals around the benzene nucleus, cyclize cleanly to an approximately 1:1 mixture of the two possible isomeric quinolines. Analogous results have been observed by us and others[11] in the Claisen rearrangement of allyl ethers of closely related molecules. The presence of a substituent (i.e., methyl or propyl) at C-3 in **54** enables cyclization to take place at C-5 to afford high yields of the desired "linear" pyranoquinolines. However, similar substitution in the preferred site of cyclization (C-8) in **53** did not give rise to significant quantities of "linear" tricyclic compounds. In all cases complex mixtures were formed, but interestingly, if the blocking group was propyl, but not methyl, small yields of the "angular" tricycle could be isolated. This is believed to be due to loss of the blocking propyl group as propene; the analogous loss of a methyl substituent is not possible.

Compound **58** (Table I) was prepared by a modification of the above scheme. Michael addition of **23** to 4-oxo-2-pentenoic acid, followed by polyphosphoric acid cyclization of the adduct, gave the methylquinoline **30**, which was converted to **58** in the usual way, via the esters **31** and **35** (see Scheme II part a).

Method III: the simultaneous construction of the pyrano and pyrido rings upon an appropriately substituted benzene nucleus (Scheme III). This route has recently been described.[12]

6-Amino derivatives (**71–73, 76**) (see Table I) were prepared via a recently described amination reaction;[13] other 6- and 7-substituted pyranoquinolines were synthesized by well-known methods that require no further comment (see the Experimental Section for details). Methods I–III, and subsequent substitutent interconversions, all provided the compounds as diesters. As the pyran rings were found to be extremely susceptible to base cleavage, final ester hydrolysis was best carried out by titrating the theoretical amount of dilute aqueous sodium hydroxide solution into the reaction mixture at such a rate that the solution never became significantly alkaline. The products were isolated either as disodium salts or, after acidification, as diacids. In all cases the products were tested pharmacologically as solutions of sodium salts prepared, where necessary in situ by treatment of the diacid with sodium bicarbonate.

Table I shows the range of 6-substituted pyranoquinolines investigated, the general synthetic schemes employed, and the reaction sequence from the relevant intermediate. Similar data for the 4,6-dioxopyranoquinolines are given in Table II.

Discussion

Biological Results. Sodium cromoglycate and related antiallergic compounds are believed to exert their activity in (at least) two ways.[2]

First, they inhibit the release of mediators (histamine, leukotriene D_4, etc.) from mast cells and thereby prevent the onset of asthmatic symptoms due to the bronchoconstrictor and inflammatory effects of these mediators. We have used the rat PCA test as a semiquantitative primary screen to identify compounds that are at least as potent as sodium cromoglycate in their ability to stabilize *connective tissue* mast cells. Antiallergic compounds can induce the phosphorylation of a 78000 molecular weight protein of the rat peritoneal mast cell,[14] and this has been used by us as a further qualitative test, which is diagnostic of a cromoglycate-like mode of action on these mast cells. Other classes of compounds, e.g. β_2-adrenergic agonists and xanthine bronchodilators, which inhibit stimulated release of histamine from mast cells, do not induce this biochemical event. Recent attention has been directed toward the role of *mucosal* mast cells in chronic asthma, and we have identified a biological system that contains a large proportion of these cells.[15] The monkey lung lavage screen is an in vitro model, which has been developed in our laboratories, and measures the ability of compounds to inhibit histamine release from the lavage fluid of primates that have been reinfected with *Ascaris suum*.[15]

Second, antiallergic compounds also modulate bronchoconstriction that is purely reflex in nature, e.g. SO_2-induced bronchoconstriction in asthmatic patients.[16] We have also evaluated our compounds in the dog hypotension screen, which is thought to be predictive of this type of activity.[17] The dog hypotension screen gives a direct measure of the ability of compounds to occupy a cromoglycate receptor site, and it is on this basis, at least, that we believe compounds that are potent in this test may be useful antiasthmatic agents.

The compounds listed in Tables I and II were initially tested in the rat PCA assay and the dog hypotension screen.

In the 6-substituted pyranoquinolines **2** most of the compounds were more potent than sodium cromoglycate in the rat PCA test. This activity showed marked de-

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(11) Harwood, L. M. *J. Chem. Soc., Chem. Commun.* **1983**, 530.
(12) Coltman, S. C. W.; Eyley, S. C.; Raphael, R. A. *Synthesis* **1984**, 150.
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(16) Harries, M. G.; Parkes, P. E. G.; Lessof, M. H.; Orr, T. S. C. *Lancet* **1981**, 5.
(17) Cairns, H. In "Drugs Affecting the Respiratory System"; Temple, D. L., Jr., Ed. American Chemical Society: Washington, DC, 1980; p 99.

Table III

compd	PCA (potency)[a]	phosphoryln of 78 000 mol wt protein[b] (potency)	histamine rel from rat peritoneal mast cells[b] (potency)	histamine rel from monkey lung lavage cells;[c] IC_{30}, μM (rel. pot.)	dog hypotension[d] screen (rel act.)
sodium cromoglycate (1)	1	+	1	990 (1)	1
nedocromil sodium (86)	1.1	+	1	5.2 (190)	1.9
minocromil (sodium salt) (72)	7.1	++	6	28.5 (35)	1.25

[a] See the Experimental Section. [b] See ref 14. [c] See ref 15. [d] The results of single experiments (see the Experimental Section).

pendence on the nature of this 6-substituent and was highest for amino, halo, alkoxy, phenylsulfonyl, and alkylthio groups (**64–66, 68, 70–75**). The unsubstituted (**57**) and methylacetamido (**76**) compounds were less potent than sodium cromoglycate. In a series of 6-chloro derivatives, PCA activity was very sensitive to the nature of further substitution at C-7 and C-10 (**59–61, 63**). Removal of a carboxylate group at C-8 dramatically reduced potency in this screen (**62**). In the dog hypotension screen similar structure–activity relationships are seen. Activity is dependent on the nature of the substituent at C-6 and is lowest for the tertiary amide (**76**). The 6-chloro-10-propyl compound (**59**) showed the highest activity, and variations at C-7 and C-10 resulted in major losses of activity (**59–61, 63**). Again, removal of the acid group at C-8 reduces activity (**62**). In this 6-substituted series, all attempts to show statistical correlations between activity in either test with physicochemical substituent constants have been unsuccessful.

In the 4,6-dioxo series (**3**) about half of the compounds were more potent than sodium cromoglycate in the rat PCA test. Compounds unsubstituted at N-9 generally showed the highest activity, and compound **77** was 80 times as potent as sodium cromoglycate. In this series activity was enhanced by increasing alkyl substitution at C-10 (**77–79**) but reduced by both substitution at C-7 (**80**) and by increasing steric bulk at N-9 (**79, 84, 88/77, 83, 86, 87**). The monobasic compounds also showed reduced activity, particularly on removal of the acid group at C-8 (**81**). In the dog hypotension screen, compounds unsubstituted at N-9 again showed good activity, although this was now independent of the nature of the C-10 substituent (**77–79**). Similarly, high activity was seen for compounds substituted by methyl or ethyl groups at N-9 (**83–86**), although bulkier substituents reduced activity (**88**). Potency was also lower for the monobasic compounds and showed that the presence of the pyran-2-carboxylate group is essential for activity in this screen. Substitution at C-7 also dramatically reduced activity (**80**).

It may be noted that, although similar trends were sometimes observed in the rat PCA and dog hypotension screens, no statistical correlation between these two tests was found.

On the basis of the above screening results we selected compounds **86** (nedocromil sodium) and **72** (minocromil, sodium salt) for further evaluation, and their biological profiles are summarized in Table III. Both compounds induce phosphorylation of the 78 000 molecular weight mast cell protein, and this may be an important step in the biochemical mechanism by which they stabilize these cells. We have also evaluated these compounds for their ability to inhibit histamine release from monkey lung lavage cells.[15] Nedocromil sodium (**86**) was approximately 190 times and minocromil (sodium salt, **72**) approximately 35 times more potent than sodium cromoglycate in this screen. This is in sharp contrast to their activities in the rat PCA test when all three compounds had potencies within the same order of magnitude. The ability of these compounds to inhibit histamine release from rat peritoneal mast cells[14] has also been measured and closely reflects PCA activity (see Table III). Nedocromil sodium (TILADE, **86**) and minocromil (the free acid of **72**) are at present undergoing therapeutic evaluation.[18–20]

Receptor Role of the Second Carboxylate Function. Many of the *potent* antiallergy compounds that have previously been reported are dicarboxylic acids. The majority of these structures (notably benzodipyrans,[21] pyridoquinolines,[10] bis(oxamic acids),[22] and quinolinyloxamic acids[23]) share a common geometry with the pyranoquinolines described here, and this suggests a stereochemical role for both carboxylate groups. We have seen above that a second acid function is not essential for activity; however, it may serve to promote receptor affinity by binding at an auxiliary site on the cromoglycate receptor. It is interesting to note that in several series of monobasic antiallergic compounds activity is often optimal when suitable substituents have been introduced into a region of the molecule that would allow electron donation to this putative binding site, e.g. 7-substituted xanthines,[24] pyrimidoquinolines,[25] and pyridoquinazolines.[26] We have also found that the second acid function in bischromones related to sodium cromoglycate seems important. For example, the descarboxy analogue **89** is about 16 times less potent than sodium cromoglycate in the rat PCA test.[27]

We therefore have studied the ability of sodium cromoglycate to adopt a conformation that may allow it to

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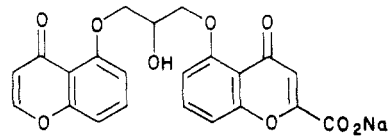

89

occupy the same receptor binding sites as the sterically constrained pyranoquinolines described above (e.g., nedocromil sodium 86, Figure 1b[28]). From molecular graphics[29] the benzopyran-2-carboxylate moieties common to both structures were superimposed. Simultaneously, the second carboxylate groups were superimposed and, with these restraints, the linking glyceryl chain of sodium cromoglycate was allowed to adopt its minimum energy conformation (Figure 1a). The resulting staggered conformation of sodium cromoglycate (Figure 1c), although different from that found in the crystal structure, has a very similar van der Waals steric repulsion energy. This low-energy conformation may be further stabilized by an intramolecular hydrogen bond between the hydroxy group and the carbonyl oxygen on the second chromone nucleus (perhaps via a molecule of water, which is not inconsistent with the X-ray crystal lattice[30]).

The above analysis suggests that sodium cromoglycate may readily adopt a conformation in which the two acid functions are disposed in a spacial relationship similar to that found in the pyranoquinolines and that this may be an important conformation at the cromoglycate receptor site.

Experimental Section

Melting points were determined with a Büchi melting point apparatus and are uncorrected. The structures of all compounds were consistent with their ^{1}H NMR spectra, which are determined on a Bruker WP 80-MHz spectrometer. Where represented by elemental symbols, the analyses of these elements are within ±0.4% of the theoretical values. Petrol refers to petroleum ether (bp 40–60 °C).

***N*-[4-Acetyl-3-(2-propenyloxy)phenyl]acetamide (5).** *N*-[4-Acetyl-3-hydroxyphenyl)acetamide (4; 78 g, 404 mmol), potassium carbonate (87 g, 63 mmol), and allyl bromide (68 g, 49 mL, 565 mmol) were stirred together in DMF (800 mL) for 5 h and then poured into a large volume of water. The aqueous mixture was extracted with ethyl acetate and the extract washed well with water, 10% NaOH solution, and water. Drying and evaporation gave 5: 76.0 g (81%); mp 101.5–102 °C; NMR ($CDCl_3$) δ 2.2 (3 H, s, CH_3CON), 2.6 (3 H, s, CH_3COAr), 4.6 (2 H, br d, OCH_2), 5.2–5.5 (2 H, m, $=CH_2$), 5.9–6.3 (1 H, m, CH=), 6.8 (1 H, dd, ArH), 7.7 (2 H, m, 2 ArH), 8.0 (1 H, br s, NH). Anal. ($C_{13}H_{15}NO_3 \cdot 2.5\%$ H_2O) C, H, N.

***N*-[4-Acetyl-3-hydroxy-2-(2-propenyl)phenyl]acetamide (6).** Compound 5 (1.0 g, 4.29 mmol) was heated under reflux in dimethylaniline (5 mL) for 4 h. The solution was cooled to ambient temperature; the precipitate was collected and washed with petrol. Drying gave 6: 0.7 g (70%); mp 183–185.5 °C; NMR ($CDCl_3$) δ 2.2 (3 H, s, CH_3CONH), 2.6 (3 H, s, CH_3COAr), 3.5 (2 H, m, $ArCH_2$), 5.0 (1 H, br d, =CH), 5.2 (1 H, br, =CH), 5.8–6.2 (1 H, m, CH=), 7.4 (1 H, br, NH), 7.6–7.9 (2 H, AB q, 2 ArH), 13.0 (1 H, s, OH). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

***N*-(4-Acetyl-3-hydroxy-2-propylphenyl)acetamide (7).** Compound 6 (49.2 g, 211 mmol) was dissolved in glacial acetic acid (600 mL) and hydrogenated at 3 atm over PtO_2 catalyst. When H_2 uptake was complete, the reaction was warmed to redissolve the precipitated products, and the catalyst was removed by filtration through a glass-fiber paper. The filtrate was evaporated, and the residue was washed with petrol and dried in vacuo to give 7: 48.8 g (98%); mp 197–198 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t, CH_3CH_2), 1.5 (2 H, m, $CH_2CH_2CH_3$), 2.2 (3 H, s, CH_3CON), 2.6 (3 H, s, CH_3COAr), 2.65 (2 H, t, $ArCH_2CH_2$), 7.2 (1 H, br s, NH), 7.6 (2 H, br s, ArH), 12.6 (1 H, s, OH). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

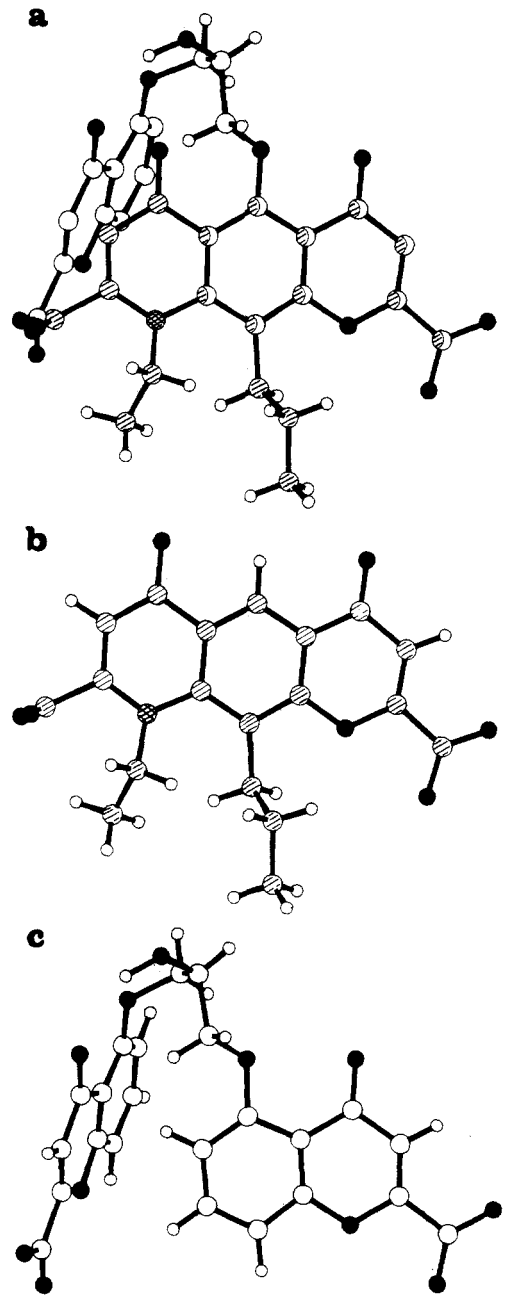

Figure 1. (a) Structures of nedocromil sodium (86) and sodium cromoglycate (1), superimposed by computer graphics. (b) X-ray-determined structure of nedocromil sodium (86). (c) Minimum energy conformation of sodium cromoglycate (1), after superimposition and energy minimization procedures (see text): O, carbon (sodium cromoglycate (1)); ◎, carbon (nedocromilsodium (86)); ◎, carbon atoms used for superimposition; ⊗, nitrogen; ●, oxygen; ○, hydrogen (some omitted for clarity).

Ethyl 7-Amino-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate (8). Sodium (12 g, 522 mmol) was dissolved in dry ethanol (300 mL), and a mixture of 7 (25 g, 106 mmol) and diethyl oxalate (38.2 g, 261 mmol, 35.5 mL) in dry ethanol (500 mL) was added. After heating under reflux for 2 h the reaction mixture was left for 1 h at room temperature, then poured into water, and made slightly acidic with dilute HCl. The solution was extracted into chloroform and was washed with brine. The organic layer

(28) The crystallographic coordinates used for nedocromil sodium in parts a and b of Figure are based on preliminary reults supplied by Dr. A. Freer, University of Glasgow (to be submitted for publication).

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was dried and evaporated, and the residue was dissolved in dry ethanol (400 mL) containing concentrated HCl (4 mL) and heated under reflux for 15 h. This mixture was poured into water, extracted with ethyl acetate, washed with water, dried, and evaporated to leave a sticky gum that, on trituration with ether–petrol, gave **8**: 28.6 g (98%); mp 107–108 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t, CH_2CH_3), 1.4 (3 H, t, CH_2CH_3), 1.6 (2 H, m, $CH_2CH_2CH_3$), 2.8 (2 H, t, $ArCH_2$), 4.4 (2 H, q, OCH_2), 6.6 (1 H, d, ArH), 7.0 (1 H, s, ArH), 7.9 (1 H, d, ArH), NH_2 not well resolved. Anal. ($C_{15}H_{17}NO_4 \cdot 4.2\%\ H_2O$) C, H, N.

(*Z*)-Dimethyl *N*-[2-(Ethoxycarbonyl)-4-oxo-8-propyl-4*H*-1-benzopyran-7-yl]-2-amino-2-butene-1,4-dioate (9). Amine **8** (28.6 g, 106 mmol) and dimethyl acetylenedicarboxylate (17.8 g, 15.4 mL, 125 mmol) were heated under reflux in ethanol (220 mL) for 17 h. On refrigeration **9** precipitated, was collected, and was dried: 24.7 g (57%); mp 148–149.5 °C; NMR ($CDCl_3$) δ 1.05 (3 H, t, CH_3CH_2), 1.44 (3 H, t, CH_2CH_3), 1.78 (2 H, m, $CH_2CH_2CH_3$), 3.0 (2 H, t, $ArCH_2$) 3.69 (3 H, s, CO_2CH_3), 3.78 (3 H, s, Co_2CH_3), 4.43 (2 H, q, OCH_2), 5.65 (1 H, s, =CH), 6.72 (1 H, d, ArH), 7.06 (1 H, s, ArH), 7.94 (1 H, d, ArH), 9.87 (1 H, br s, NH). Anal. ($C_{21}H_{23}NO_8$) C, H, N.

2-Ethyl 8-Methyl 6,9-Dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (10). Triester **9** (4.0 g, 9.59 mmol) was added in one portion to vigorously refluxing diphenyl ether (100 mL) with stirring. After 5 min the heating was stopped, and the cooled solution was poured into a large volume of petroleum ether (60–80 °C). The precipitated product was collected and dried and recrystallization from ethyl acetate afforded **10**: 3.0 g (81%); mp 187–188 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.4 (3 H, t, $CH_2CH_2CH_3$), 1.6 (2 H, m, $CH_2CH_2CH_3$), 3.2 (2 H, t, $ArCH_2$), 4.0 (3 H, s), 4.4 (2 H, q), 6.8 (2 H, s, 2 ArH), 7.1 (1 H, br s, NH), 8.5 (1 H, s, ArH). Anal. ($C_{20}H_{19}NO_7$) C, H, N.

2-Ethyl 8-Methyl 6,9-Dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (10). Triester **9** (4.0 g, 9.59 mmol) was added in one portion to vigorously refluxing diphenyl ether (100 mL) with stirring. After 5 min the heating was stopped, and the cooled solution was poured into a large volume of petroleum ether (60–80 °C). The precipitated product was collected and dried, and recrystallization from ethyl acetate afforded **10**: 3.0 g (81%); mp 187–188 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.4 (3 H, t, $CH_2CH_2CH_3$), 1.6 (2 H, m, $CH_2CH_2CH_3$), 3.2 (2 H, t, $ArCH_2$), 4.0 (3 H, s), 4.4 (2 H, g), 6.8 (2 H, s, 2 ArH), 7.1 (1 H, br s, NH), 8.5 (1 H, s, ArH). Anal. ($C_{20}H_{19}NO_7$) C, H, N.

2-Ethyl 8-Methyl 6-Chloro-4-oxo-10-propyl-4*H*-pyrano-[3,2-*g*]quinoline-2,8-dicarboxylate (11). Diester **10** (1 g, 2.59 mmol) in dry benzene (20 mL) was treated with $POCl_3$ (0.66 mL, 1.09 g, 7.1 mmol) at room temperature for 7 h. A further portion of $POCl_3$ (0.66 mL) was added. After an additional 18 h the mixture was poured into water and extracted with ethyl acetate. After washing with water and drying, evaporation gave a brown solid that was purified by chromatography on silica gel, eluting with 4:1 ether–petrol to afford **11**: 0.62 g (59%); mp 176–178 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.5 (3 H, t), 1.8 (2 H, m), 3.6 (2 H, t), 4.1 (3 H, s), 4.5 (2 H, q), 7.1 (1 H, s), 8.2 (1 H, s), 9.0 (1 H, s). Anal. ($C_{20}H_{18}ClNO_6$) C, H, Cl, N.

1-(4-Amino-2-hydroxy-3-propylphenyl)ethanone (23). Acetamide **7** (32 g, 136 mmol) in 1:1 concentrated HCl–water (800 mL) was heated at 100 °C with stirring until a clear solution was obtained. The mixture was cooled, diluted with ethanol (100 mL), and made basic with solid sodium carbonate. Ether extraction, washing with water, drying, and evaporation gave **23** as an oil that solidified on trituration with ether-petrol: 24.2 g (93%); mp 47–49 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.6 (2 H, m), 2.5 (3 H, s), 2.6 (2 H, m), 4.2 (2 H, br), 6.2 (1 H, d), 7.5 (1 H, d), 13.2 (1 H, s). Anal. ($C_{11}H_{15}NO_2$) C, H, N.

(*Z*)-Dimethyl 2-[(4-Acetyl-3-hydroxy-2-propylphenyl)-amino]butene-1,4-dioate (24). Amine **23** was converted to **24** (*Z* isomer), by the procedure used for the preparation of **9** but was not fully characterized and was used directly in the preparation of **25**.

Methyl 6-Acetyl-1,4-dihydro-7-hydroxy-4-oxo-8-propylquinoline-2-carboxylate (25). Compound **24** was converted to **25**, by the procedure used for the preparation of **10**. Recrystallization from cyclohexane gave **25**: 20 g (74%); mp 169–170 °C; NMR ($CDCl_3$) δ 1.03 (3 H, t), 1.67 (2 H, m, $CH_2CH_2CH_3$), 2.76 (3 H, s), 2.8 (2 H, t, $ArCH_2$), 4.07 (3 H, s), 6.83 (1 H, d, ArH), 8.7 (1 H, s, ArH), 8.83 (1 H, br, NH). Anal. ($C_{16}H_{17}NO_5$) C, H, N.

Methyl 6-Acetyl-4-chloro-7-hydroxy-8-propylquinoline-2-carboxylate (26). Quinoline **25** was chlorinated with $POCl_3$, by the procedure used for the preparation of **11**, to give **26**: 2.8 g (88%); mp 163–164 °C (cyclohexane); NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.7 (2 H, m), 2.87 (3 H, s), 8.3 (2 H, t), 4.06 (3 H, s), 8.03 (1 H, s), 8.63 (1 H, s), 12.03 (1 H, s, OH). Anal. ($C_{16}H_{16}ClNO_4$) C, H, Cl, N.

Methyl 6-Acetyl-7-hydroxy-4-phenoxy-8-propylquinoline-2-carboxylate (27). Compound **26** (1.0 g, 3.1 mmol) was added in small portions to a mixture of phenol (10.0 g, 106 mmol) and powdered potassium hydroxide (0.36 g, 6.2 mmol) at 60–65 °C with stirring. When addition was complete, the reaction was stirred for 1.5 h at 60–65 °C. The phenol was removed by steam distillation, and the residual yellow crystalline solid was filtered off and dried to give **27**: 0.81 g (69%); mp 195–196 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.75 (2 H, m, $CH_2CH_2CH_3$), 2.8 (3 H, s), 3.35 (2 H, t, $ArCH_2$), 3.95 (3 H, s), 7.1 (1 H, s), 7.4 (5 H, m), 8.85 (1 H, s), 12.0 (1 H, s). Anal. ($C_{22}H_{21}NO_5$) C, H, N.

Diethyl 4-Oxo-6-phenoxy-10-propyl-4*H*-pyrano[3,2-*g*]-quinoline-2,8-dicarboxylate (33). Quinoline **27** (1.0 g, 2.6 mmol) was added to a solution of sodium (0.25 g, 11 mmol) in dry ethanol (50 mL). Diethyl oxalate (2.9 mL, 16 mmol) was added. After 1 h at room temperature, the reaction was heated under reflux for 1.5 h, cooled, poured into water, and acidified with glacial acetic acid. The mixture was extracted with ethyl acetate, washed with water, and dried. The solvent was evaporated, the residue was taken into dry dioxane (50 mL) and HCl gas was passed in for 15 min. This solution was diluted with ethyl acetate, washed with water, aqueous bicarbonate solution, and water, and dried. On evaporation the residue was triturated with petrol to afford **33**: 0.6 g (50%); mp 173–178 °C; NMR ($CDCl_3$) δ 1.07 (3 H, t), 1.43 (6 H, m, 2 $CO_2CH_2CH_3$), 1.87 (2 H, m), 3.67 (2 H, t), 4.47 (4 H, q, 2 $CO_2CH_2CH_3$), 7.13 (1 H, s), 7.26 (1 H, s), 7.4 (5 H, m), 9.23 (1 H, s). Anal. ($C_{27}H_{25}NO_7$) C, N; H: calcd, 5.3; found, 6.0.

Methyl 6-Acetyl-1,4-dihydro-7-hydroxy-1-(3-methylbutyl)-4-oxoquinoline-2-carboxylate (49). Acetamide **38** (74.3 g, 45 mmol) was dissolved in dry DMF (400 mL) and added to a suspension of washed sodium hydride (28.5 g, 50% dispersion in oil, 600 mmol) in dry DMF (100 mL) under N_2. The mixture was stirred and cooled in ice, and isoamyl bromide (85 g, 600 mmol) was added dropwise. After stirring for 2 h at room temperature, the reaction was cautiously poured into aqueous ethanol. Extraction with ether, washing with water, drying, and evaporation gave an oil that was distilled to give **39**: 95.5 g (90%); bp (0.5 mm) 126–146 °C; NMR ($CDCl_3$) δ 0.9 (6 H, d), 1.4 (3 H, m), 1.9 (3 H, s), 3.7 (2 H, m), 3.8 (3 H, s), 6.5–7.3 (4 H, m). To **39** (95.5 g, 410 mmol) in dry CH_2Cl_2 (1 L) at −70 °C was added BBr_3 (163 g, 63 mL, 650 mmol). The mixture was allowed to warm to ambient temperature over 1.5 h, when it was poured into a large volume of water. Extraction with chloroform, drying, and evaporation afforded **41**, 92.2 g (100%), which was not further purified: NMR ($CDCl_3$) δ 0.8 (6 H, d), 1.4 (3 H, m), 1.9 (3 H, s), 3.7 (2 H, t), 6.5–7.3 (4 H, m), 9.0 (1 H, br s).

Acetamide **41** (92.2 g, 420 mmol), acetyl chloride (49.1 g, 625 mmol), and dry pyridine (22 mL) were heated under reflux in dry toluene (500 mL) for 5 h and then poured into water (2 L). The organic layer was separated, washed, dried, and evaporated to give the acetate **43**, 102 g (92%), which was not further purified: NMR ($CDCl_3$) δ 0.8 (6 H, d), 1.4 (3 H, m), 1.8 (3 H, s), 2.3 (3 H, s), 3.6 (2 H, t), 6.8–7.5 (4 H, m).

Compound **43** (102 g, 388 mmol), $AlCl_3$ (162 g, 1.22 mol), and NaCl (22.6 g) were heated at 160 °C for 2 h and then cooled. Ice water was added, the mixture was extracted with ether, and the organics were washed with water and reextracted into 10% aqueous NaOH. The aqueous layer was washed with ether, acidified, and finally reextracted into ether. Drying and evaporation gave **45**: 49.9 g (49%), which was not further purified: NMR ($CDCl_3$) δ 0.9 (6 H, d), 1.4 (3 H, m), 2.0 (3 H, s), 2.7 (3 H, s), 3.8 (2 H, t), 6.5–6.9 (2 H, m), 7.8 (1 H, d), 12.0 (1 H, s).

Compound **45** (49.9 g,190 mmol), glacial acetic acid (550 mL), and 48% aqueous HBr (110 mL) were heated under reflux for 2 h, poured into water, and extracted into ether. The extract was washed with water and aqueous bicarbonate solution, dried, and

evaporated to give **47**: 38.0 g (90%), which was not further purified: NMR ($CDCl_3$) δ 0.9 (6 H, d) 1.4 (3 H, m), 2.6 (3 H, s), 3.2 (2 H, t), 4.3 (1 H, br s), 6.0 (2 H, m), 7.4 (1 H, d), 13.0 (1 H, s).

Amine **47** (38.0 g, 170 mmol), dimethyl acetylenedicarboxylate (31.8 g, 220 mmol), and ethanol (300 mL) were heated under reflux for 7 h. The solvent was evaporated and the residue chromatographed on silica gel, eluting with 1:1 ether–petrol to give **48**: 41.4 g (67%), as a mixture of two isomers, which were not separated: NMR ($CDCl_3$) δ 0.9 (6 H, d), 1.4 (3 H, m), 2.6 (3 H, s), 3.5–3.9 (8 H, m), 4.6 and 5.0 (2 s, total 1 H), 6.8 (2 H, m), 7.8 (1 H, d), 12.0 (1 H, s).

To polyphosphoric acid (50 mL) stirred and heated on a steam bath was added **48** (10.9 g, 30 mmol) in one portion. After 10 min the hot mixture was poured into ice water–ethyl acetate and stirred during 1 h. Extraction with ethyl acetate, drying, and evaporation afforded a mixture of two isomers, which was chromatographed (Waters Preparative HPLC) to give **49**: 0.5 g (5%); mp 108–110 °C; NMR ($CDCl_3$) δ 1.0 (6 H, d), 1.6 (3 H, m), 2.7 (3 H, s), 3.9 (3 H, s), 4.2 (2 H, m), 6.5 (1 H, s), 6.8 (1 H, s), 8.9 (1 H, s), 12.2 (1 H, s). Anal. ($C_{18}H_{21}NO_5$) C, H, N. (The regioisomer was isolated in 40% yield).

Diethyl 6,9-Dihydro-9-(3-methylbutyl)-4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (50). Quinoline **49** (2.3 g, 6.95 mmol) and diethyl oxalate (7.5 mL, 60 mmol) in dry DMF (60 mL) were added to a suspension of washed sodium hydride (1.3 g, 50% dispersion in oil, 26.8 mmol) in DMF (75 mL) under N_2. After 24 h at room temerpature the mixture was poured into ice water and acidified with ice-cold dilute HCl. This solutin was extracted with ethyl acetate, dried, and evaporated. The residue was heated under reflux with saturated ethanolic HCl (300 mL) for 1 h, poured into ice water, and extracted with ethyl acetate. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel, eluting with ether, to give **50**: 1.2 g (40%); mp 149–150 °C; NMR (Me_2SO-d_6) δ 0.8 (6 H, d), 1.3 (6 H, t), 1.7 (3 H, m), 4.4 (6 H, m), 6.4 (1 H, s), 7.0 (1 H, s), 8.0 (1 H, s), 8.8 (1 H, s). Anal. ($C_{23}H_{22}NO_7$) C, H, N.

Diethyl 6,9-Dihydro-4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (56). Diester **56** was prepared by the method of Coltman et al.[12]

General Procedure for Hydrolyses. Analytically pure diester (1 equiv) was suspended in AR methanol (50 mL/mmol) maintained under reflux, and 0.1 M NaOH solution (2 equiv) was added dropwise over ca. 1 h. After the addition was completed, heating was continued for 15 min. To isolate the acid, the reaction solution was cooled, poured into water, and made slightly acid with dilute HCl. The precipitate was collected either by filtration or, if not practicable, by ethyl acetate extraction, drying, and evaporation. Alternatively, the disodium salt was isolated directly from the hydrolysis reaction by evaporation to a small volume and dilution with AR acetone. The resulting precipitate was collected, redissolved in deionized water, filtered through a Millipore filter, and freeze-dried.

The free acids were normally converted into the corresponding disodium salts by treatment with the theoretical amount of AR sodium bicarbonate in deionized water, Millipore filtration, and freeze-drying.

Disodium 6,9-Dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (77). Diester **10** was hydrolyzed in the standard manner to afford **77** (48%): NMR (Me_2SO-d_6) δ 1.0 (3 H, t), 1.6 (2 H, m), 3.1 (2 H, t), 6.08 (1 H, s), 6.6 (1 H, s), 8.6 (1 H, s). Anal. ($C_{17}H_{11}NNa_2O_7$·12.5% H_2O) C, H, N.

Disodium 6,9-Dihydro-4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (79). Diester **56** was hydrolyzed in the standard manner to **79** (60%): NMR (D_2O) δ 6.55 (1 H, s), 6.7 (1 H, s), 7.5 (1 H, s), 8.25 (1 H, s). Anal. ($C_{14}H_5NNa_2O_7$·10.8% H_2O) C, H, N.

Disodium 6,9-Dihydro-10-methyl-4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (78). Compound **78** was prepared analogously to **79**: NMR (D_2O) δ 2.2 (3 H, s), 6.4 (1 H, s), 6.6 (1 H, s), 8.0 (1 H, s). Anal. ($C_{15}H_{17}NNa_2O_7$·3.6% H_2O) C, H, N.

Disodium 6,9-Dihydro-4,6-dioxo-7,10-dipropyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (80). Compound **10** was allylated and subjected to Claisen rearrangement and catalytic reduction as for the conversion of **4** to **7**, affording the diester **15** (58% overall). Hydrolysis in the standard manner afforded **80**: NMR (Me_2SO-d_6) δ 0.97 (6 H, m), 1.6 (4 H, m), 3.1 (4 H, m), 6.7 (1 H, s), 8.7 (1 H, s), 10.6 (1 H, br s). Anal. ($C_{23}H_{17}NNa_2O_7$·11.2% H_2O) C, H, N.

Sodium 6,9-Dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2-carboxylate (81). Ester **25** was hydrolyzed by the standard method, and the resulting acid was heated under reflux in diphenyl ether until gas evolution ceased, whereupon the reaction was cooled and diluted with petroleum ether (60–80 °C) and the precipitate collected. The solid was dissolved in chloroform at reflux and removed by filtration and the filtrate evaporated to leave quinolinone **37** (75%): mp 250–252 °C; NMR (Me_2SO-d_6) δ 0.93 (3 H, t), 1.5 (2 H, m), 2.73 (3 H, s), 2.75 (2 H, t), 6.0 (1 H, d), 7.76 (1 H, m), 8.57 (1 H, s), 12.0 (1 H, br s), 12.7 (1 H, s). Anal. ($C_{14}H_{15}NO_3$) C, H, N: calcd, 5.7; found, 5.1.

Compound **37** was reacted with diethyl oxalate, using the experimental procedure employed in the preparation of **50**, and the resulting monoester was hydrolyzed to **81** by the standard method: NMR (Me_2SO-d_6) δ 0.97 (3 H, t), 1.73 (2 H, m), 3.2 (2 H, t), 6.1 (1 H, d), 6.07 (1 H, s), 8.1 (1 H, d), 8.7 (1 H, s). Anal. ($C_{16}H_{12}NNaO_5$·10.1% H_2O) C, H, N.

6,9-Dihydro-4,6-dioxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-8-carboxylic Acid (82). Compound **25** (10 g, 33 mmol) and dimethylformamide dimethyl acetal (7 g, 59 mmol) were heated in dry xylene (200 mL) on a steam bath for 3.5 h and then were left at room temperature overnight. More acetal (30 mmol) was added, and heating was continued for 2 h. The mixture was cooled, decanted from tar, and evaporated. The residue was heated on a steam bath for 2 h with dilute H_2SO_4 (300 mL), cooled, diluted with water, and filtered to afford **82** as a brown solid: 7.8 g (71%); mp 287 °C dec; NMR (CF_3CO_2D) δ 1.2 (3 H, t), 2.0 (2 H, m), 3.5 (2 H, t), 6.9 (1 H, d), 8.1 (1 H, s), 8.4 (1 H, d), 9.6 (1 H, s). Anal. ($C_{16}H_{13}O_5$) H; C: calcd, 64.2; found, 63.7; calcd, 4.7; found, 4.2.

Disodium 4-Oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (57). Compound **26** (1.0 g, 3.11 mmol) in dry DMF (50 mL) was added dropwise to NaSEt (from ethanethiol (12.4 mmol) and NAH (12.4 mmol)) in dry DMF (30 mL). After 2 h the solution was diluted with ethyl acetate and acidified with dilute HCl. The organic layer was separated, washed with water, and aqueous bicarbonate solution, and dried. After evaporation the residue was crystallized from cyclohexane to afford **28**: 0.52 g (48%); mp 193–195 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.5 (3 H, t, SCH_2CH_3), 1.67 (2 H, m), 2.83 (3 H, s), 3.26 (4 H, m), 4.03 (3 H, s), 7.76 (1 H, s), 8.57 (1 H, s), 12.0 (1 H, s). Anal. ($C_{18}H_{21}NO_4S$) C, H, N.

The thioether **28** (1.0 g, 2.88 mmol) was added to active Raney Nickel (16 g wet weight) in dry ethanol (100 mL) and heated under reflux for 1.5 h. The catalyst was removed, and the filtrate was evaporated. The residue was triturated with petrol and crystallized from ethanol to give **29**: 0.2 g (24%); mp 110–111 °C; NMR ($CDCl_3$) δ 1.01 (3 H, t), 1.73 (2 H, m), 2.83 (3 H, s), 3.33 (2 H, t), 4.07 (3 H, s), 7.93 (1 H, d), 8.2 (1 H, d), 8.26 (1 H, s), 11.9 (1 H, s). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

Quinoline **29** was treated with diethyl oxalate and sodium ethoxide as for **8** to give the diester **34**: 1.25 g (54%); mp 168–171 °C; NMR ($CDCl_3$) δ 1.03 (3 H, t), 1.5 (6 H, m, 2 CH_2CH_3), 1.86 (2 H, m), 3.66 (2 H, t), 4.5 (4 H, m), 7.1 (1 H, s), 8.12 (1 H, d), 8.45 (1 H, d), 8.63 (1 H, d). Anal. ($C_{21}H_{21}NO_6$) C, H, N.

The diester **34** was hydrolyzed by the general method to give **57**: (95%); NMR (Me_2SO-d_6) δ 0.92 (3 H, t), 1.76 (2 H, m), 3.53 (2 H, t), 6.66 (1 H, s), 7.86 (1 H, d), 8.45 (1 H, d), 8.5 (1 H, s). Anal. ($C_{17}H_{11}NNa_2O_6$·12.7% H_2O) C, H, N.

6-Methyl-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylic Acid (58). Amine **23** (37.2 g, 193 mmol) and 4-oxo-2-pentenoic acid (20 g, 175 mmol) were fused on a steam bath. After 10 min the melt solidified to afford the crude adduct, which was added with stirring to polyphosphoric acid (500 mL) heated on a steam bath. After 20 min the reaction mixture was poured into ice water and stirred for 1 h. The aqueous solution was shaken with ethyl acetate and the product extracted with aqueous bicarbonate solution. Acidification and ethyl acetate extraction, drying, and evaporation afforded the acid **30**: 16.5 g (35%); mp 125–127 °C (ethanol); NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.8 (2 H, m), 2.8 (6 H, s), 7.3 (2 H, t), 8.0 (1 H, s), 8.5 (1 H, s), 12.0 (1 H, s).

Anal. ($C_{16}H_{17}NO_4$·5.1% EtOH) C, H, N.

Compound **30** (6.5 g, 23 mmol) was esterified with ethanol (500 mL), saturated with HCl gas, by heating under reflux, for 1.5 h. The mixture was poured into water and extracted into ether, which was washed with aqueous bicarbonate solution. Drying and evaporation afforded **31**: 6.0 g (84%); mp 150–151 °C; NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.5 (3 H, t), 1.6 (2 H, m), 2.7 (3 H, s), 2.8 (3 H, s), 3.4 (2 H, t), 4.4 (2 H, q), 7.8 (1 H, s), 8.4 (1 H, s), 11.9 (1 H, s). Anal. ($C_{18}H_{21}NO_4$) C, H, N.

Quinoline **31** was converted to the diester **35** by the method used to prepare **8** (24%): mp 165–168 °C (petroleum ether 80–100 °C); NMR ($CDCl_3$) δ 1.1 (3 H, t), 1.5 (6 H, t), 1.9 (2 H, m), 2.9 (3 H, s), 3.6 (2 H, t), 4.6 (4 H, q), 7.1 (1 H, s), 8.0 (1 H, s), 8.8 (1 H, s). Anal. ($C_{22}H_{23}NO_6$) H, N; C: calcd, 66.5; found, 67.3.

Diester **35** was hydrolyzed to **58** by the standard method: (37%); mp 252–254 °C; NMR (Me_2SO-d_6) δ 0.9 (3 H, t), 1.6 (2 H, m), 2.8 (3 H, s), 3.3 (2 H, m), 6.8 (1 H, s), 7.8 (1 H, s), 8.3 (1 H, s). Anal. ($C_{18}H_{15}NO_6$·3.4% H_2O) C, H, N.

Disodium 6-Chloro-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (59). Compound **11** was hydrolyzed in the standard manner to afford **59**: (76%); NMR (Me_2SO-d_6) δ 1.0 (3 H, t), 1.8 (2 H, m), 3.6 (2 H, t), 6.7 (1 H, s), 8.1 (1 H, s), 8.7 (1 H, s). Anal. ($C_{17}H_{10}ClNNa_2O_6$·7.4H_2O) C, H, Cl, N.

Other halopyranoquinolines **60–64** were made as follows:

Compounds **60** and **61** were prepared, from **15** and **56** respectively, by the method described for the synthesis of **59** ($POCl_3$ followed by ester hydrolysis). **63** was prepared similarly from the 10-allyl analogue of **10** (prepared exactly as described for **10** but omitting the hydrogenation step **6** to **7**). Compound **62** was prepared by similar chlorination of **37** followed by Claisen ester condensation and hydrolysis in the usual way. **64** was synthesized by bromination of **10** with $POBr_3$, followed by ester cleavage. (See also reference 31).

Disodium 6-Methoxy-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (65). Compound **10** was alkylated under the same conditions as for **39**, and the resulting diester **16** was hydrolyzed to give **65** (35% overall): NMR (Me_2SO-d_6) δ 0.94 (3 H, t), 1.74 (2 H, m), 3.52 (2 H, t), 4.1 (3 H, s), 6.64 (1 H, s), 7.36 (1 H, s), 8.66 (1 H, s). Anal. ($C_{18}H_{13}NNa_2O_7$·14.6% H_2O) C, H, N.

Disodium 6-Ethoxy-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (66). Compound **77** was treated with dilute HCl and extracted into ethyl acetate. Drying and evaporation afforded the diacid (16.4 g, 47.8 mmol), which was heated under reflux in saturated ethanolic HCl (750 mL) for 72 h. More ethanol (500 mL) was added during passage of HCl gas while heating for a further 7 h. The reaction was cooled and filtered, and the filtrate was evaporated. The residue was chromatographed on silica gel, eluting with ether, to give the diester **17**, which was hydrolyzed in the usual manner to give **66** (9% overall): NMR (Me_2SO-d_6) δ 0.97 (3 H, t), 1.57 (3 H, t), 1.85 (2 H, m), 3.63 (2 H, t), 4.37 (2 H, q), 6.67 (1 H, s), 7.35 (1 H, s), 8.71 (1 H, s). Anal. ($C_{19}H_{15}NNa_2O_7$·6.1% H_2O) C, H, N.

Disodium 4-Oxo-6-phenoxy-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (67). Diester **33** was hydrolyzed in the usual manner to afford **67** (54%): NMR (Me_2SO-d_6), δ 0.95 (3 H, t), 1.8 (2 H, m), 3.7 (2 H, t), 6.69 (1 H, s), 6.98 (1 H, s), 7.46 (5 H, m), 8.86 (1 H, s). Anal. ($C_{13}H_{15}NNa_2O_7$·6.3% H_2O) C, H, N.

Disodium 6-(Ethylthio)-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (68). Compound **11** (1.0 g, 2.48 mmol), ethanethiol (0.37 mL, 5 mmol), potassium carbonate (0.35 g, 2.54 mmol), acetone (100 mL), and dry DMF (10 mL) were stirred together for 24 h and poured into water, and the precipitate was collected, washed well with acetone, and dried to give the ethylthio diester **12**: mp 159–160 °C; (94%). This ester was hydrolyzed in the usual maner to give **68** (54%): NMR (Me_2SO-d_6) δ 1.0 (3 H, t), 1.5 (3 H, t), 1.7 (2 H, m), 3.2 (2 H, q), 3.6 (2 H, t), 6.6 (1 H, s), 7.7 (1 H, s), 8.6 (1 H, s). Anal. ($C_{19}H_{15}NNa_2O_6S$·4.0% H_2O) C, H, N, S.

Disodium 4-Oxo-6-(phenylthio)-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (69). Compound **11** (4.04 g, 10 mmol) in 1,2-dichloroethane (30 mL) was treated at reflux with thiophenol (1.1 mL, 11 mmol) for 24 h. The solvent evaporated, and the residue was taken into chloroform, washed with sodium carbonate solution, and dried. Evaporation gave the phenylthio diester which was hydrolyzed in the normal manner to give **69** (51% overall): NMR (D_2O) δ 0.93 (3 H, t), 1.53 (2 H, m), 3.37 (2 H, t), 6.75 (1 H, s), 7.21 (1 H, s), 7.5 (5 H, br s), 8.13 (1 H, s). Anal. ($C_{23}H_{15}NNa_2O_6S$·12.0% H_2O) C, H, N.

Disodium 4-Oxo-6-(phenylsulfonyl)-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (70). The diester **13** (5 g, 10.5 mmol) in methylene chloride (80 mL) was treated with *m*-chloroperbenzoic acid (4.7 g, 23 mmol) at room temperature for 24 h. The reaction mixture was washed with aqueous sodium bicarbonate solution, water, and brine. Drying and evaporation gave the sulfonyl diester **14**, which was crystallized from 2-propanol and then hydrolyzed in the normal manner to give **70** (74% overall): NMR (Me_2SO-d_6) δ 0.9 (3 H, t), 1.73 (2 H, m), 3.57 (2 H, m), 6.73 (1 H, s), 7.7 (3 H, m), 8.03 (2 H, m), 8.6 (1 H, s), 9.16 (1 H, s). Anal. ($C_{23}H_{15}NNa_2O_8S$·9.6% H_2O) C, H, N, S.

Disodium 6-(Methylamino)-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (72). Quinoline **10** was converted to **18** by the published procedure[13] and hydrolyzed by the standard method to afford **72**: NMR (Me_2SO-d_6) δ 0.91 (3 H, t), 1.76 (2 H, m), 2.88 (3 H, br s), 3.46 (2 H, t), 6.61 (1 H, s), 6.8 (1 H, s), 7.61 (1 H, m), 8.8 (1, H, s). Anal. ($C_{18}H_{14}N_2Na_2O_6$·8.0% H_2O) C, H, N.

Compounds **71** and **73** were prepared similarly, via **19**[13] and **20**, respectively. (See also ref 31.)

Disodium 6-(*N*-Acetyl-*N*-methylamino)-4-oxo-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (76). Amine **18** (1.25 g, 3 mmol) in glacial acetic acid (25 mL) and acetic anhydride (25 mL) was heated under reflux for 12 h, cooled, and poured into water (1.5 L). This was extracted with ethyl acetate, washed with saturated bicarbonate solution and water, and dried. On concentration the acetamide **21** crystallized and was hydrolyzed in the normal manner to afford **76** (38% overall): NMR (Me_2SO-d_6) δ 0.97 (3 H, t), 1.7 (3 H, br s), 1.8 (2 H, m), 3.33 (3 H, s), 3.6 (2 H, t), 6.7 (1 H, s), 7.87 (1 H, s), 8.3 (1 H, s). Anal. ($C_{20}H_{16}N_2Na_2O_7$·11.8% H_2O) C, H, N.

Disodium 4-Oxo-6-(phenylamino)-10-propyl-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (74). Amine **19** was *N*-acetylated as in the synthesis of **21**; and the resulting acetamide (1.0 g, 2.35 mmol) was heated under reflux with copper bronze (0.75 g), potassium carbonate (0.35 g, 2.54 mmol), and bromobenzene (10 mL) for 24 h. The mixture was cooled, filtered, and evaporated. The residue was chromatographed to give 0.2 g (17%) of the *N*-phenylacetamide, which was heated under reflux in saturated methanolic HCl (40 mL) for 3 h to give **22** (87%). Ester hydrolysis in the normal manner gave **74** (51%): NMR (Me_2SO-d_6) δ 1.0 (3 H, t), 1.8 (2 H, m), 3.55 (2 H, t), 6.63 (1 H, s), 7.32 (5 H, m), 9.0 (1 H, s). Anal. ($C_{23}H_{16}N_2Na_2O_6$·14.6% H_2O) C, H, N.

Disodium 4-Oxo-10-propyl-6-(1-pyrrolidinyl)-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylate (75). Compound **26** (5.5 g, 15.5 mmol) was heated under reflux in glyme (100 mL) with pyrrolidine (3 mL, 34 mmol) for 72 h. Evaporation and chromatography on silica gel, eluting with ethyl acetate, gave the pyrrolidinoquinolinecarboxamide (21%), which was hydrolyzed by heating in 3 M H_2SO4 (15 mL) for 18 h. The reaction mixture was neutralized with ice-cold 0.880 ammonia and extracted with chloroform. The dried organic solution was evaporated, and the residue was esterified with ethanolic HCl (1 h) under reflux. This ester **32** was condensed with diethyl oxalate, as for **27**, and the resulting pyranoquinoline diester **36** was hydrolyzed in the standard manner to give **75**: NMR (Me_2SO-d_6) δ 0.9 (3 H, t), 1.6 (2 H, m), 2.0 (4 H, br) 3.7 (6 H, br), 6.6 (1 H, s), 7.0 (1 H, s), 8.8 (1 H, s). Anal. ($C_{21}H_{18}N_2Na_2O_6$·19.5% H_2O) C, H, N.

6,9-Dihydro-9-(3-methylbutyl)-4,6-dioxo-4*H*-pyrano[3,2-*g*]quinoline-2,8-dicarboxylic Acid (88). Diester **50** was hydrolyzed in the standard manner to afford **88** (81%): mp 302–304 °C dec; NMR (Me_2SO-d_6) δ 0.9 (6 H, d), 1.7 (3 H, m), 4.2 (2 H, br), 4.4 (2 H, m), 6.2 (1 H, s), 6.8 (1 H, s), 7.9 (1 H, s), 8.8 (1 H, s). Anal. ($C_{19}H_{17}NO_7$·2.9% H_2O) C, H, N.

Nedocromil sodium **86** was prepared from 3-acetamidoanisole (**38**) in 12 steps[32] (see Scheme II, part b), all of which are analogous

(31) Cox, D.; Cairns, H.; Chadwick, N.; Suschitzky, J. L. G. B. Patent 2035312, 1979.

to reactions already described: **38** was N-ethylated with ethyl iodide to yield **40** (cf. preparation of **39**). Demethylation (BBr_3) gave **42**, which was O-acetylated (acetic anhydride) to yield **44** and subjected to Fries rearrangement ($AlCl_3$) to give **46**. C-Propylation (three steps using the Claisen rearrangement as for the conversion of **4** to **7**) and deacetylation gave **51**, which was converted to the diester **52** in three steps (cf. conversion of **47** to **50**). Hydrolysis in the usual way gave the disodium salt **86**: NMR (Me_2SO-d_6) δ 1.0 (6 H, 2 t), 1.9 (2 H, m), 3.2 (2 H, t), 3.4 (H_2O), 4.5 (2 H, q), 6.1 (1 H, s), 6.7 (1 H, s), 8.7 (1 H, s).

The *N*-methyl **83** and analogues *N*-propyl **87** were prepared in an analogous manner. NMR (Me_2SO-d_6): **83** δ 0.98 (3 H, t), 1.8 (2 H, m), 3.24 (2 H, t), 3.8 (3 H, s), 6.03 (1 H, s), 6.68 (1 H, s), 8.64 (1 H, s); **87** δ 0.6 (3 H, t), 0.92 (3 H, t), 1.3 (2 H, m), 1.9 (2 H, m), 3.1 (2 H, m), 3.3 (H_2O), 4.14 (2 H, br t), 6.08 (1 H, s), 6.60 (1 H, s), 8.6 (1 H, s). The synthesis of the 10-propenyl compound **85** employed a similar reaction sequence but involved a palladium-catalyzed isomerization[33] instead of the hydrogenation step. NMR (Me_2SO-d_6) δ 0.9 (3 H, t), 2.0 (3 H, d), 4.5 (2 H, q), 6.1 (1 H, s), 6.5 (1 H, m), 6.5 (1 H, d), 6.65 (1 H, s), 8.6 (1 H, s).

Compound **84** was prepared by an analogous route to that used for the preparation of **88** from **38**. NMR (Me_2SO-d_6) δ 1.4 (3 H, t), 3.45 (H_2O), 4.4 (2 H, q), 5.9 (1 H, s), 6.85 (1 H, s), 7.9 (1 H, s), 8.85 (1 H, s).

Rat Passive Cutaneous Anaphylaxis. The method was essentially that used by Goose and Blair.[3] Female rats (100 g) were sensitized by the intradermal injection of 0.1 mL of rat antiegg albumin reaginic serum, diluted with saline, to give a control reaction size of just over 20-mm mean diameter. Antigen challenge was at 24 h, 0.25 mL of a 10 mg/mL egg albumin solution, 0.25 mL of Evans Blue dye (approximately 1 g % in saline), and 0.5 mL of saline being injected intravenously. The rats were killed and reaction sizes measured after 20 min.

Rats were used in groups of five and the percentage inhibition (%*I*) of PCA by each dose of a compound calculated by comparing the group mean reaction size with that of the control group.

Using decreasing doses from 10 mg/kg, two dose levels were sited, the higher dose giving between 50% and 80% and the lower dose between 50% and 20% inhibition. The ID_{50} (dose giving 50% inhibition) was read off the straight line log graph between the two points.

The test compound was dissolved in saline and the appropriate dose included in the 0.5 mL of diluent injected with the antigen. The ID_{50} of sodium cromoglycate was checked during each experiment and compared with the test compound to give a potency ratio (PR) for that compound, where

$$PR = ID_{50}(\text{sodium cromoglycate})/ID_{50}(\text{compd})$$

The method of statistical analysis was to construct a linear regression of response (percent inhibition of PCA between 20% and 80%) against log dose for each compound. The ID_{50} for each compound and sodium cromoglycate tested on same day were calculated from the regression lines, all of which were significant at the 5% level.

Dog Hypotension Screen. The use of this screen has previously been described.[2,17] Beagle dogs were anaesthetized with pentobarbitone sodium (30 mg/kg) i.v. and a saphenous vein was cannulated. A branch of the femoral artery was also cannulated and connected to a Devices blood pressure (bp) transducer and recorder, allowing measurement of phasic and mean arterial blood pressure and heart rate to be made. Pentobarbitone sodium (0.1 mg/kg per min) was infused intravenously for the remainder of the experiment to maintain a constant level of anesthesia.

A reproducible hypotensive response to a bolus injection of sodium cromoglycate was then obtained. (A starting dose of 10 μg/kg is recommended, which is then adjusted to give a submaximal fall in bp of about 25 mmHg). An interval of 15 min as was allowed between doses to ensure that there was no tachyphylaxis to the response.

When a consistent response to sodium cromoglycate had been obtained, the compound under test was infused intravenously at a dose of 10 μg/kg per min for a 15 min period. At the end of the drug-infusion period, and at 30-min intervals thereafter over the next 3 h, the standardized dose of sodium cromoglycate was administered. The inhibition of the cromoglycate response (afforded by the infusion of the test compound) was calculated and plotted against time (see ref 17 for an example of the graphical representation).

If the cromoglycate response was not blocked, the experiment was repeated on a subsequent occasion with an increased infusion dose of test compound (20 or 50 μg/kg per min). The activity index of the compound is determined by the dose infused (*d*) and the area under the percent inhibition vs. time curve (*I*).

$$\text{activity index} = I/d$$

The nature of this large-animal test is such that it was impracticable to perform duplicate experiments to enable statistical significance to be achieved. However, our experience of this screen has shown that when repeat experiments are performed, in related series of compounds, a high degree of consistency has been demonstrated.

Acknowledgment. The authors thank P. A. Cage, N. Chadwick, S. C. W. Coltman, I. D. Dicker, D. N. Hardern, and J. Shipman for assistance with chemical syntheses, D. M. Jackson, I. Richards, N. S. Clennett, K. Vendy, E. E. Wells, S. T. Harper, and M. E. Mather for the biological evaluations, and B. Patel for statistical analyses.

Registry No. **1**, 15826-37-6; **4**, 40547-58-8; **5**, 7298-72-8; **6**, 69069-03-0; **7**, 69049-54-3; **8**, 69049-56-5; **9**, 98331-34-1; **10**, 69049-59-8; **11**, 75452-48-1; **12**, 75452-64-1; **13**, 75453-61-1; **14**, 98331-35-2; **15**, 98331-36-3; **16**, 75452-51-6; **17**, 75453-69-9; **18**, 75452-61-8; **19**, 86694-93-1; **19** (N-acetylated), 98331-67-0; **20**, 98331-37-4; **21**, 98331-38-5; **22**, 98331-39-6; **23**, 75452-54-9; **24**, 98331-40-9; **25**, 75452-56-1; **26**, 75452-57-2; **27**, 75453-26-8; **28**, 75452-63-0; **29**, 75453-22-4; **30**, 75452-72-1; **31**, 75452-73-2; **32**, 75453-35-9; **33**, 75453-27-9; **34**, 75453-23-5; **35**, 75452-74-3; **36**, 75453-36-0; **37**, 98331-41-0; **38**, 588-16-9; **39**, 98331-42-1; **40**, 98331-43-2; **41**, 98331-44-3; **42**, 98331-45-4; **43**, 98331-46-5; **44**, 98331-47-6; **45**, 98331-48-7; **46**, 69049-62-3; **47**, 98331-49-8; **48**, 98331-50-1; **49**, 98331-51-2; **50**, 98331-52-3; **51**, 69049-68-9; **52**, 69049-72-5; **55**, 79324-45-1; **56**, 75498-16-7; **57**, 75453-25-7; **58**, 75452-75-4; **59**, 75452-50-5; **60**, 98331-53-4; **61**, 98331-54-5; **62**, 98331-55-6; **63**, 98331-56-7; **64**, 98331-57-8; **65**, 75452-53-8; **66**, 75453-70-2; **67**, 75453-28-0; **68**, 75452-65-2; **69**, 75453-62-2; **70**, 98331-58-9; **71**, 98331-59-0; **72**, 75452-62-9; **73**, 75453-49-5; **74**, 75453-59-7; **75**, 75453-37-1; **76**, 98331-60-3; **77**, 69049-61-2; **77** (free acid), 69049-60-1; **78**, 98360-92-0; **79**, 75498-15-6; **80**, 98331-61-4; **81**, 98331-62-5; **82**, 98331-63-6; **83**, 98331-64-7; **84**, 98360-93-1; **85**, 98331-65-8; **86**, 69049-74-7; **87**, 98331-66-9; **88**, 77941-06-1; allyl bromide, 106-95-6; diethyl oxalate, 95-92-1; dimethyl acetylenedicarboxylate, 762-42-5; phenol, 108-95-2; isoamyl bromide, 107-82-4; dimethylformamide dimethyl acetal, 4637-24-5; 4-oxo-2-pentenoic acid, 4743-82-2; thiophenol, 108-98-5; bromobenzene, 108-86-1; *N*-phenylacetamide, 103-84-4; pyrrolidine, 123-75-1; 6-acetyl-7-hydroxy-8-propyl-4-pyrrolidin-1-yl-2-quinolinecarboxamide, 98331-68-1.

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