The salt was recrystallized four times from ethanol and finally from MeOH/ethyl acetate to give 3.5 g of the L-(+)-tartaric acid salt of **45**: mp 159–162 °C; $[\alpha]^{22}_D$ +33.5° (*c* 3, MeOH). Anal. ($C_{18}H_{18}Cl_2NO_3$) C, H, N.

For optical purity determinations the bases (ca. 30 mg) were mixed with an equivalent amount of (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol in 0.5 mL of $CDCl_3$, and the ^1H NMR spectra was recorded. Under these conditions δ (CH_3NH) was 2.23 for **45** and 2.31 ppm for (+)-**45**.

Pharmacology. Inhibition of DA, NE, and 5-HT uptake in vitro, tetrabenazine ptosis, and 5-HTP potentiation was measured as earlier described.[21,47]

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Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase, and Chymotrypsin by Elasnin and Other 4-Hydroxy-2-pyrones[1]

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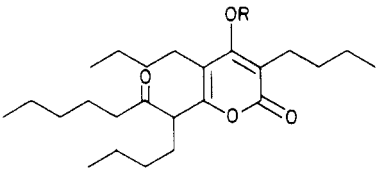
Elasnin and 15 related 4-hydroxy-2-pyrones have been assayed for in vitro inhibition of human leukocyte elastase, porcine pancreatic elastase, and bovine chymotrypsin. Inhibition constants for HL elastase range from 0.1 to 10 mM. The principal determinant of potency against the elastases is probably the substituent at position 3, which may account for the observed strong homology between the elastases in their inhibition by these compounds. Acetylation of the 4-hydroxy group has no effect on inhibition. The inhibition is noncovalent; there is no evidence of enzyme acylation by these pyrones.

Since leukocyte elastase has been implicated in a number of inflammatory and degradative disease states,[2] we have been seeking specific synthetic inhibitors of this enzyme. After Omura et al. reported the isolation and structure of elasnin (**1**),[3] J.R.P. published a synthesis of this naturally occurring compound.[4] We report here the in vitro enzyme inhibitory properties of elasnin and a number of its analogues.

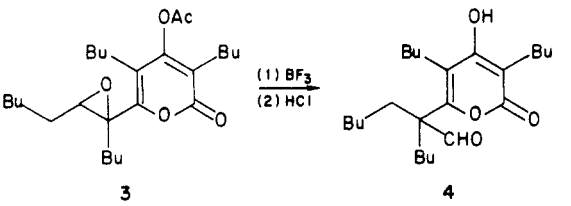


1, R = H
2, R = Ac

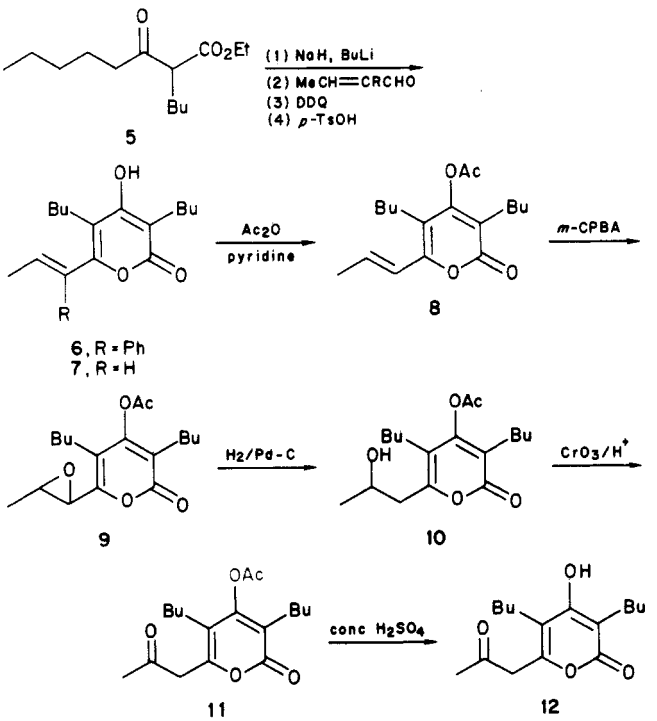
Chemistry. Boron trifluoride induced rearrangement of epoxide **3**,[4] followed by acid-catalyzed deacetylation, produced the tertiary aldehyde **4** exclusively (Scheme I). This transformation could also be achieved by employing proton acids such as concentrated H_2SO_4, HCOOH, or $HClO_4$/dioxane. The vinyl derivatives **6** and **7** were obtained as described previously[4] by reaction of the dianion of keto ester **5** with the requisite aldehyde, oxidation of the resulting δ-hydroxy keto ester to the diketo ester, and enol lactonization. Whereas the *O*-acetate derived from the phenyl compound **6** proved to be completely inert under forced epoxidation conditions,[5] the less hindered propenyl derivative **8** gave the expected epoxide **9** in good yield. The latter was transformed into the elasnin analogue **12** as described previously[4] (Scheme II).

Acylation of the dianion of **5** with methyl acetate and methyl hexanoate gave the diketo esters **13** and **14**, which were cyclized to the corresponding enol lactones **15** and **16**. The latter was again deprotonated (NaH, *n*-BuLi), and the resulting dianion was reacted with methyl benzoate to provide the benzoyl derivative **17** (Scheme III). The

Scheme I



Scheme II



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‡ Institute of Organic Chemistry.

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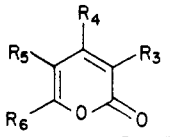
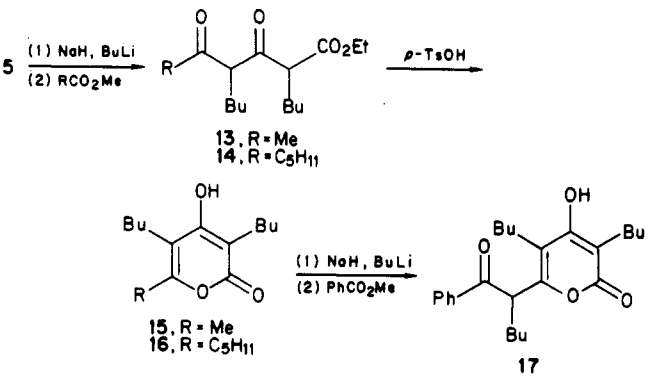
Table I. Molecular Properties and Enzyme Inhibition Constants



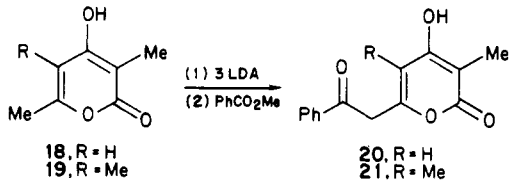
	molar refractivity[a]					enzyme inhibn[b]					
						HL elastase		PP elastase		chymotrypsin	
compd	R_3	R_4	R_5	R_6	log P	obsd	calcd[c]	obsd	calcd[c]	obsd	calcd[c]
1	4.0	0.4	4.0	10.8	2.52	4.03	3.92	4.16*	4.54	3.65	3.81
2	4.0	2.5	4.0	10.8	3.53	*d*		5.09**	4.54	3.72	3.81
4	4.0	0.4	4.0	9.0	1.52	3.82	3.81	4.82*	4.28	3.87	3.70
6	4.0	0.4	4.0	5.7	1.39	3.44*	3.60	3.85**	3.82	3.49	3.19
9	4.0	2.5	4.0	3.0	2.17	3.47	3.42	3.50**	3.45	3.30	2.86
10	4.0	0.4	4.0	3.3	1.92	3.46	3.44	2.97**	3.49	3.15	2.89
11	4.0	2.5	4.0	3.0	2.82	3.54	3.42	3.60**	3.45	3.18	2.86
12	4.0	0.4	4.0	3.0	1.74	3.31	3.42	3.13	3.45	2.09	2.86
15	4.0	0.4	4.0	1.0	0.79	3.19	3.30	3.25**	3.17	2.87	2.61
17	4.0	0.4	4.0	11.4	1.21	4.02	3.96	4.67*	4.62	4.10	3.89
18	1.0	0.4	0.0	1.0	0.74	2.20*	2.25	2.41**	2.12	2.46	2.61
19	1.0	0.4	1.0	1.0	0.36	2.31*	2.31	1.66*	2.12	2.74	2.61
20	1.0	0.4	0.0	7.3	0.78	3.01*	2.65	2.67*	3.00	3.29*	3.38
21	1.0	0.4	1.0	7.3	0.73	2.33*	2.65	2.49	3.00	3.16	3.38
22	0.0	0.4	0.0	7.3	0.43	2.06**	2.30	*d*		3.20	3.38
23	0.0	0.4	0.0	1.0	0.50	2.09**	1.90	2.51**	1.76	2.20	2.61

[a] Units (from ref 19) normalized such that MR(H) = 0 and MR(CH_3) = 1. [b] As $-\log(K_i)$, with K_i in mol/L. Standard error: if not noted, <0.05;* <0.10; **, <0.20. [c] Calculated from the equations of Table III. [d] Not determined.

Scheme III



Scheme IV



yield of acylation products **20** and **21** could be improved by generating the dianions of **18** and **19** employing 3 equiv of lithium diisopropylamide, 1 equiv of base serving to deprotonate the product as soon as formed in order to prevent reprotonation of the remaining dianion at its more basic site (Scheme IV).

Table II. Correlation Matrix for the Compounds of Table I

	MR_3	MR_4	MR_5	MR_6	log P
MR_3	1.00				
MR_4	0.44	1.00			
MR_5	0.99	0.44	1.00		
MR_6	0.26	−0.06	0.26	1.00	
log P	0.73	0.74	0.72	0.36	1.00

Results and Discussion

The 16 4-hydroxy-2-pyrones of Table I are a set of elasnin analogues with varying substituents at all available positions of the ring. The widest variation is at position 6, to assess the importance of the 5-(undecan-6-one) substituent of elasnin.

Chromatography by HPLC gave estimates of log P (the octanol/saline partition coefficient), which have been used to assess whether the inhibition by these compounds is principally hydrophobic.

Table I presents the enzyme inhibition data, as well as the hydrophobicity and molar refractivity values used in regression. For human leukocyte elastase (HLE) and chymotrypsin, the compounds have a range of inhibition constants from 0.1 to 10 mM. For porcine pancreatic elastase (PPE) the inhibition extends into the 10 μM range.

We find that elasnin is neither as specific nor as potent toward leukocyte elastase as previously reported:[3a,b] from three enzyme preparations we measured $K_i = 140 \pm 40$ μM, compared to the reported 3.3 μM. For elasnin inhibition of porcine pancreatic elastase and chymotrypsin we obtained inhibition constants of 70 ± 10 and 230 ± 15 μM, respectively, in excellent agreement with Ohno et al.[3b]

The factors that determine inhibition and specificity can be inferred from the data of Table I. First, the homology between the enzymes can be explored by expressing the inhibition of one enzyme as a function of the others:

$$pK_{i,HLE} = (0.49 \pm 0.13)pK_{i,PPE} + (1.45 \pm 0.43)$$

$$n = 16,\ r = 0.72,\ s = 0.50$$

(1) Contribution No. 691 from the Institute of Organic Chemistry and No. 209 from the Institute of Bio-Organic Chemistry.
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Table III. Structure–Activity Relationships for Compounds of Table I

enzyme	coeff of molar refract[a]				const (β_0)	statistics[b]			
	R_3	R_4	R_5	R_6		n	F	r^2	s
HL elastase	0.35 ± 0.03	ns[c]	ns	0.06 ± 0.01	1.83	15	95.6	0.94	0.19
PP elastase	0.35 ± 0.08	ns	ns	0.14 ± 0.03	1.63	15	29.1	0.83	0.44
chymotrypsin	ns	ns	ns	0.12 ± 0.02	2.49	16	31.1	0.69	0.33

[a] These are the β_{1-4} of the regression equation, for MR in the units of Table I. [b] See ref 23. [c] ns = not significantly different from zero, with $p > 0.05$.

No terms involving chymotrypsin were significant ($p > 0.1$). Thus, for these pyrones there is a common determinant of binding that is not shared by chymotrypsin, and PP elastase is approximately twice as sensitive to this factor as HL elastase. What the determinants of binding are is suggested by regression on the properties of the compounds.

The five parameters chosen are the molar refractivity of substituents at positions 3–6 and the overall log P. As shown in Table II, several of these are highly cross-correlated in this series. In addition, factor analysis on these data shows that no more than two parameters can be kept in regression. In simple linear regression of pK_i on log P, the slopes for HLE, PPE, and chymotrypsin are respectively 0.39 ± 0.17, 0.77 ± 0.20, and 0.24 ± 0.15, with correlation coefficients (r^2) of 0.28, 0.51, and 0.16. While hydrophobicity is a positive determinant in inhibition, especially for PP elastase, it cannot account for most of the variance in pK_i.

Table III gives the results of regression of pK_i on molar refractivities, i.e. fit to the equation

$$pK_i = \beta_0 + \beta_1 MR_3 + \beta_2 MR_4 + \beta_3 MR_5 + \beta_4 MR_6$$

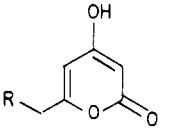
Nonsignificant parameters (β_i) were stepped out to leave the values shown. Here, the results suggest that specific interactions are becoming apparent. First, note that the term for R_4 is not significant for any of the enzymes. Since R_4 consists of OH and $OCOCH_3$ in this series, this does not support the suggestion of Groutas et al.[6] that H-bond donation or acceptance by R_4 may be important in binding. Second, the substituent at position 3 or 5 (but not both)[7] is a major determinant for both elastases, such that an increase of one methylene unit reduces K_i by a factor of 2. The fact that this term is not significant for chymotrypsin suggests that the observed overall homology between the elastases may be largely due to similar interactions at this position. It is worth noting that if the pyrone carbonyl were bound in the "oxyanion hole",[8] the R_3 substituent could be placed in the P_1 site, where both PP and HL elastase prefer alkyl substitution.[9] Third, terms for substituent size at position 6 are small but significant for all the enzymes. When an additional term was included in regression to indicate whether or not R_6 contained an aryl moiety, it was not significant, which suggests that the interaction at R_6 may be hydrophobic and not specific for any particular group in this series.

Abeles and Powers have recently shown that chloropyrones and chloroisocoumarins can be potent, even irreversible inhibitors of serine proteinases,[10,11] and that enzyme acylation by attack of serine 195 on the pyrone carbonyl is the necessary first step in the process. Compound **17**, the best inhibitor of chymotrypsin, was therefore added to excess chymotrypsin and UV difference spectra (vs. an equal concentration of chymotrypsin) recorded. No differences were seen, which suggests that chymotrypsin is not being acylated by **17**. This is not surprising, considering the electron donation of R_3–R_6 and the alkaline stability of these compounds (unlike those of Abeles and Powers). A noncovalent mode of binding is also suggested by the fact that inhibition in all cases is immediate and not time dependent. The mechanism of inhibition by elasnin and its analogues is probably more similar to the hydrophobic inhibition of HL elastase by cis-unsaturated fatty acids[12] than to that of the chloropyrones.

Experimental Section

Melting points (uncorrected) were obtained on a Mel-Temp apparatus. Infrared spectra were recorded on a Perkin-Elmer grating instrument. ^{13}C and ^1H NMR spectra were obtained with Bruker WH 90 and Varian T-60 instruments. Mass spectra were recorded on either Atlaswerke CH-4 or CH-7 spectrometers. Combustion analyses were performed by Syntex Analytical Research or Atlantic Microlab. High-pressure liquid chromatography was done with a Spectra Physics 8000 system and a Brownlee 10-μm RP-18 column. Ultraviolet spectra were recorded on a Perkin-Elmer 559A, and enzyme inhibition assays were performed on a Perkin-Elmer 650-40 fluorimeter with temperature control from a Haake circulating-water bath.

Elasnin (**1**) and *O*-acetylelasnin (**2**) were synthesized as described;[4] the sample of elasnin used for inhibition studies contained 7% of the acetate. Literature procedures were used for the preparation of 3,6-dimethyl-4-hydroxy-2-pyrone[13] (**18**), 4-hydroxy-3,5,6-trimethyl-2-pyrone[14] (**19**), and the phenacyl derivative **22**.[15] 4-Hydroxy-6-methyl-2-pyrone (**23**) was purchased from the Aldrich Chemical Co.



22, R = PhCO
23, R = H

6-(1-Butyl-1-formylhexyl)-3,5-dibutyl-4-hydroxy-2-pyrone (4). Boron trifluoride etherate (0.44 mL, 3.58 mmol) was added to an ice-cold solution of epoxide **3** (440 mg, 1.01 mmol) in dry dichloromethane (10 mL). After 90 min at ambient temperature, the reaction mixture was quenched with dilute $NaHCO_3$ solution (5 mL) and extracted with Et_2O. The organic phase was washed once with brine, dried over $MgSO_4$, and evaporated to dryness. The residue thus obtained was dissolved in EtOH (10 mL) containing 2 N HCl (1.0 mL) and stirred at 80 °C for 2 days. The reaction mixture was concentrated and extracted with Et_2O. After washing with brine, the organic phase was dried ($MgSO_4$) and

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(7) Since MR for positions 3 and 5 are highly correlated (Table II), which position is the determinant of binding cannot be absolutely determined. However, on the basis of data for compounds **20** and **22**, we suggest that it is most likely position 3.
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evaporated. The residue was purifed by preparative TLC (silica gel, 10% EtOAc/hexane) to give aldehyde **4** (175 mg, 44.6%) as a viscous oil: IR (film) 3100, 2720, 1720, 1660, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.63 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 200.6 (CHO), 165.8, 165.4, 155.6, 115.6, 104.7 (pyrone carbons). Anal. ($C_{24}H_{40}O_4$) C, H.

3,5-Dibutyl-4-hydroxy-6-(1-phenyl-1-propenyl)-2-pyrone (6). To a solution of ethyl 2-butyl-3-oxooctanoate[16] (5.3 g, 21.9 mmol) in dry THF (100 mL) was added sodium hydride (50% oil emulsion, 1.1 g, 22.9 mmol) in one portion. The resulting suspension was stirred at 50 °C for 30 min and then cooled to 0 °C. Dropwise addition of *n*-BuLi (1.6 M hexane solution, 14.4 mL, 23 mmol) was followed 15 min later by a solution of α-phenylcrotonaldehyde[17] (3.2 g, 21.9 mmol) in THF (30 mL). After stirring at 0 °C for 1 h, the reaction was quenched by dropwise addition of H_2O (50 mL) containing AcOH (5 mL). The resulting mixture was concentrated on a rotary evaporator at 30 °C. The residue was extracted with Et_2O/pentane (1:1). The organic phase was washed twice with water and dried over $MgSO_4$. Evaporation left 9.6 g of an oil that was refluxed with DDQ (6.5 g, 28.6 mmol) in dioxane (200 mL) for 18 h. Removal of the solvent by rotary evaporation left a dark oil, which was partially purified by chromatography on silica gel (hexane/CH_2Cl_2/EtOAc 7:2:1). The resulting oil (4.7 g) was refluxed in toluene (100 mL) containing *p*-TsOH (200 mg) through 4A molecular sieves for 20 h. Solvent removal, followed by chromatography on silica gel (hexane/EtOAc 3:1), furnished 770 mg (10.4% overall) of **6** as a crystalline mass. Data for an analytical sample (from *i*-Pr_2O/hexane): mp 124–125 °C; IR (Nujol mull) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.82 (d, J = 7 Hz, 3 H, vinyl Me), 6.21 (q, J = 7 Hz, 1 H, vinyl H), 7.26 (s, 5 H, phenyl); mass spectrum m/e 340 (M^+), 325, 298, 297, 269. Anal. ($C_{22}H_{28}O_3$) C, H.

3,5-Dibutyl-4-hydroxy-6-(1-propenyl)-2-pyrone (7). Reaction of the dianion of **5** (16.3 g, 67.35 mmol) with crotonaldehyde (6.6 mL, 79.66 mmol), followed by oxidation of the crude ketol with DDQ in dioxane and cyclization of the resulting diketo ester with *p*-TsOH in toluene (reflux through 4A sieves) as described for the preparation of **6**, furnished 5.65 g (31.7%) crystalline **7**: mp 151–152 °C (MeOH/H_2O); IR (Nujol mull) 1670, 1640 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.89 (d, J = 5 Hz, 3 H, vinyl Me); mass spectrum m/e 264 (M^+), 249, 235, 221, 193. Anal. ($C_{16}H_{24}O_3$) C, H.

4-Acetoxy-3,5-dibutyl-6-(1-propenyl)-2-pyrone (8). A solution of **7** (4.5 g, 17.0 mmol) in pyridine (20 mL) was treated with acetic anhydride (20 mL). After standing at room temperature for 2 h, the reaction mixture was concentrated to dryness and the residue was evaporated several times with toluene. Crystallization of the crude product from hexane gave 4.8 g (92.3%) of **8**: mp 74–75 °C; IR (Nujol mull) 1770, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.92 (d, J = 6 Hz, 3 H, vinyl Me), 2.32 (s, 3 H, OAc); mass spectrum m/e 306 (M^+), 264, 249, 221, 193. Anal. ($C_{18}H_{26}O_4$) C, H.

4-Acetoxy-3,5-dibutyl-6-(1,2-epoxypropyl)-2-pyrone (9). A solution of **8** (4.33 g, 14.15 mmol), *m*-chloroperoxybenzoic acid (85%, 3.73 g, 18.4 mmol), and 4,4'-thiobis(6-*tert*-butyl-3-methyl)phenol (50 mg) in 1,2-dichloroethane (100 mL) was gently refluxed for 1 h. The cooled solution was transferred to a separatory funnel with the aid of dichloromethane (200 mL). After it was washed twice with dilute $NaHCO_3$ solution and water, the organic phase was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc 3:1) to give the epoxide **9** (4.0 g, 87.9%) as an oil: IR (film) 1770, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (d, J = 5 Hz, 3 H, oxirane Me), 2.32 (s, 3 H, OAc), 3.4–3.7 (m, 2 H, oxirane H); ^{13}C NMR ($CDCl_3$) δ 167.4, 162.7, 158.3, 152.1, 120.4, 117.2, 54.9, 53.3. Anal. ($C_{18}H_{26}O_5$) C, H.

4-Acetoxy-3,5-dibutyl-6-(2-hydroxypropyl)-2-pyrone (10). A solution of epoxide **9** (3.8 g, 11.8 mmol) in EtOAc (120 mL) was hydrogenated over 10% Pd–C (240 mg) at atmospheric pressure. After hydrogen uptake had ceased, the catalyst was removed by filtration through Celite. The filtrate was evaporated, and the residue was passed through a column of silica gel (hexane/EtOAc 1:1) to give **10** as an oil: 3.67 g (96%); IR (film) 3420, 1765, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (d, J = 6 Hz, 3 H, *Me*CHOH), 2.32 (s, 3 H, OAc), 4.27 (m, 1 H, MeC*H*OH); ^{13}C NMR ($CDCl_3$) δ 167.6, 164.2, 159.2, 157.2, 118.1, 114.7, 65.96, 40.344. Anal. ($C_{18}H_{28}O_5$) C, H.

4-Acetoxy-3,5-dibutyl-6-(2-oxopropyl)-2-pyrone (11). To a stirred solution of alcohol **10** (240 mg, 0.74 mmol) in acetone (10 mL) was added dropwise an excess of Jones reagent. After 30 min, excess oxidizing agent was destroyed with a few drops of *i*-PrOH. The reaction mixture was concentrated under reduced pressure. Extractive workup and chromatography on silica gel (hexane/EtOAc 3:2) provided the ketone **11**: 180 mg (75.5%); mp 46–48 °C (Et_2O/pentane); IR (Nujol mull) 1760, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.25 (s, 3 H, MeCO), 2.32 (s, 3 H, OAc), 3.63 (s, 2 H, $COCH_2$); ^{13}C NMR ($CDCl_3$) δ 201.7, 167.3, 163.6, 158.8, 152.3, 119.2, 115.7. Anal. ($C_{18}H_{26}O_5$) C, H.

3,5-Dibutyl-4-hydroxy-6-(2-oxopropyl)-2-pyrone (12). A solution of **11** (530 mg, 1.65 mmol) in concentrated H_2SO_4 (10 mL) was stirred for 30 min at 0 °C. After it was quenched with ice and extracted with Et_2O, the crude product was recrystallized from ether/hexane: 360 mg (78%) of **12**; mp 145–147 °C; IR (Nujol mull) 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3 H, MeCO), 3.60 ($COCH_2$); mass spectrum m/e 280 (M^+), 238, 223, 209, 196. Anal. ($C_{16}H_{24}O_4$) C, H.

3,5-Dibutyl-4-hydroxy-6-methyl-2-pyrone (15). To a solution of the dianion of **5** (13.1 g, 54.13 mmol) in THF (200 mL), prepared as previously described, was added methyl acetate (6.35 mL, 80 mmol) at 0 °C with stirring. After 30 min, the reaction mixture was acidified with dilute HCl, concentrated in vacuo, and extracted with Et_2O. The dried ($MgSO_4$) solution was evaporated to dryness, and the residue, dissolved in 150 mL of toluene containing 300 mg of *p*-TsOH, was refluxed through 4A molecular sieves for 18 h. The cooled solution was extracted three times with dilute $NaHCO_3$. The acidified (dilute HCl) aqueous extracts were extracted with ether to give a solid, which was recrystallized from acetone/cyclohexane: 480 mg (3.7%); mp 141–143 °C. Data for an analytical sample of **15** (from *i*-Pr_2O): mp 146 °C; mass spectrum m/e 238 (M^+), 223, 209, 196. Anal. ($C_{14}H_{22}O_3$) C, H.

3,5-Dibutyl-4-hydroxy-6-pentyl-2-pyrone (16). Reaction of the dianion of **5** (8.2 g, 33.88 mmol), prepared as described previously, with methyl hexanoate (5.4 g, 41.5 mmol) and cyclization with *p*-TsOH/toluene/4A sieves (as for **15**) provided 2.3 g (23%) of **16** as an oil that solidified on standing: IR (film) 3150, 1650, 1630 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 166.9, 165.7, 159.5, 112.4, 103.5. Anal. ($C_{18}H_{30}O_3$) C, H.

6-(1-Benzoylpentyl)-3,5-dibutyl-4-hydroxy-2-pyrone (17). Successive deprotonation of **16** (2.05 g, 6.97 mmol), dissolved in THF (100 mL) and HMPA (20 mL), with NaH (335 mg, 6.98 mmol, 50 °C, 1 h) and BuLi (1.33 M, 5.2 mL, 6.92 mmol, 0 °C, 15 min) resulted in a green solution of the dianion. After the addition of methyl benzoate (1.36 g, 10 mmol) and stirring at 0 °C for 30 min, the reaction mixture was acidified (dilute HCl) and concentrated under reduced pressure. Extractive workup with Et_2O and chromatography (silica gel, hexane/EtOAc 7:3) furnished **17** (578 mg (21%)) as an oil: IR (film) 1630 cm^{-1} (br); ^1H NMR ($CDCl_3$) δ 4.53 (t, J = 7 Hz, 1 H, COCH), 5.9 (m, 1 H, OH), 7.25–8.1 (m, 5 H, Ph). Anal. ($C_{25}H_{34}O_4$) C, H.

4-Hydroxy-3-methyl-6-phenacetyl-2-pyrone (20). To a solution of LDA in THF (30 mL), prepared at −40 °C from *i*-Pr_2NH (2.77 mL, 19.8 mmol) and BuLi (1.33 M, 14.4 mL, 19.15 mmol), was added a solution of **18**[13] (840 mg, 6.0 mmol) in HMPA (10 mL) at −20 °C. After 15 min, methyl benzoate (1.36 g, 10 mmol) was added. The yellow suspension was stirred at −20 °C for 30 min and at 0 °C for another 30 min, acidified (dilute HCl), concentrated, and extracted with EtOAc. After two washes with water, the organic phase was extracted twice with dilute $NaHCO_3$ solution. The aqueous extracts were acidified (dilute HCl), and the solid that separated was filtered off and washed with water. Recrystallization from EtOH provided 660 mg (45%) of **20**: mp 221–223 °C (lit.[15] mp 217–219 °C); ^1H NMR (Me_2SO-d_6) δ 1.77 (s, 3 H, Me), 4.40 (s, 2 H, $COCH_2$), 6.23 (s, 1 H, vinyl H), 7.5–8.2 (m, 5 H, Ph).

3,5-Dimethyl-4-hydroxy-6-phenacetyl-2-pyrone (21). Deprotonation of **19**[14] (700 mg, 4.54 mmol) followed by acylation of the dianion with methyl benzoate was carried out as for the conversion of **18** to **20**. The crude product crystallized from

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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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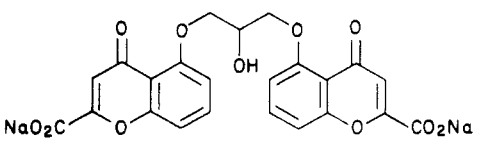
A number of antiallergic pyranoquinolinedicarboxylic acid derivatives with potential for the topical treatment of asthma have been synthesized. All the compounds have been evaluated against rat passive cutaneous anaphylaxis and in a dog hypotension screen. This is the first detailed description of the 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

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