rotenone oxime (5), acetylrotenone oxime (6), carbethoxyrotenone oxime (7), dehydrorotenone (9), acetylrotenolone (10), and derrisic acid (11) were prepared using the procedures documented under "Chemistry Section". Verification and purity of these compounds were established by combustion analysis and spectral data, using the instrumentation described above.

6a,12a-Dihydro-8,9-dimethoxy-2-(1-methylethyl)[1]benzopyrano[3,4-*b*]furo[2,3-*h*][1]benzopyran-6(12*H*)-one (Isorotenone; 3). A slurry of 1 (25.0 g, 63.4 mmol) in 300 mL of concentrated HCl was heated at reflux for 20 min. Gas evolution (HCl) was copius. The mixture was cooled and the precipitate was collected. TLC (silica gel; 9:1 $CHCl_3$–CH_3OH) indicated mostly 1, with a minor amount of 3 which displayed an R_f slightly higher than 1. The solid was suspended in 400 mL of concentrated HCl and 100 mL of water and heated at reflux. After 60 h, TLC indicated mainly 3, with some 1 present. After an additional week at reflux, TLC indicated the absence of 1. The mixture was cooled and the solid was collected and recrystallized from methanol (600 mL) to yield 8.50 g (34%) of 3 as white prisms: mp 174–176 °C, lit.[29] mp 177–178 °C; IR (Nujol) 1780 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.27 [d, J = 7 Hz, 6 H, $CH(CH_3)_2$]. Anal. ($C_{23}H_{22}O_6$) C, H, N.

Isolation of Dequelin (12) and Tephrosin (13). The powdered root of *Lonchocarpus nicou*[30] (384 g) was exhaustively extracted with 500 mL of ether using a Soxhlet apparatus. On cooling, the solution deposited crystals of rotenone which were collected (20 g). The filtrate was concentrated and the dark residue was reconstituted in 200 mL of CCl_4, whereupon another crop of rotenone (12 g) was deposited. The CCl_4 filtrate was concentrated to leave 58 g of syrup, which was dissolved in 200 mL of ethanol. Sodium acetate (8 g) was added and reflux was maintained for 2 h. The ethanol was removed by evaporation, and the residue was suspended in CH_2Cl_2 and filtered. The filtrate was concentrated and the residue was dissolved in 100 mL of acetone. The resulting yellow crystals which formed were collected (6.7 g) and successively crystallized from ether, ethyl acetate, and acetonitrile. The resulting white crystals were shown by high-performance LC to be a mixture of 12 and 13. A Varian Model 8500 high-performance LC system was used for this and subsequent composition determinations. A two-column system was employed; a 4 × $^1/_8$ in. o.d. precolumn packed with C_{18} Porasil (Waters Associates; 37–50 μm) was followed by a 30 cm × 4 mm i.d. column of μBondapak C_{18} (Waters Associates). Elution was accomplished with 3:2 CH_3CN–H_2O.

A portion of the mixture of 12 and 13 (1.4 g) was chromatographed on a silicic acid column. Elution with 9:1 CH_2Cl_2–$CHCl_3$ yielded 12 (457 mg), mp 169–173 °C, lit.[27] mp 171 °C. Subsequent elution with 4:1 CH_2Cl_2–$CHCl_3$ initially afforded mixtures of 12 and 13 and then pure 13 (446 mg), mp 197–198 °C, lit.[27] mp 197–198 °C.

Pharmacological Methods. Measurement of SRS-A Antagonism. The SRS-A used in the present study was obtained by collection of peritoneal shock fluid from rats subjected to passive peritoneal anaphylaxis as described previously.[31] The material was assayed on a strip of guinea pig ileum suspended in a tissue bath containing Tyrode's solution, maintained at 37 °C, and continuously aerated with 95% O_2–5% CO_2. Responses were checked in the presence of atropine (5×10^{-7} M) and mepyramine maleate (10^{-6} M),[32] as well as the selective SRS-A antagonist FPL 55712, to assure pharmacological activity and selectivity.

A dose–response study allowed us to select a dose of SRS-A which produced a contraction which was ca. 60% of the maximum. This dose was employed in the evaluation of all antagonists. All antagonists were added to the tissue bath 2 min prior to challenge with SRS-A. Activity was recorded by calculating the percent inhibition induced by the antagonist.

In vivo measurement of inhibition of allergic reactions was done using the method of Greig and Griffin.[33] Guinea pigs were immunized to ovalbumin with a 1-mL subcutaneous and a 1-mL intraperitoneal administration of a 10% solution of ovalbumin. After sensitization developed (ca. 4 weeks), the animals were challenged with aerosolized antigen (ovalbumin). Time of collapse was the end point. Failure to collapse within a 6-min exposure to the antigen was considered maximal protection. The collapse times for animals receiving the antagonists were compared with collapse times of control animals. Bartlett's test was employed to delineate the homogeneity of variances among the treatment groups. Since the variances were homogeneous, a one-way analysis of variance and a Dunnett's test were utilized to determine any significant differences between experimental and control groups.

Acknowledgment. The authors are grateful to Fisons Pharmaceutical Ltd., Loughborough, Leics., for supplying a sample of FPL 55712. We also thank Ben J. Cerimele for statistical analyses.

(29) S. Takei, *Biochem. Z.*, **157**, 1 (1925).
(30) The powdered *Lonchocarpus nicou* root was obtained from S. B. Penick, Lyndhurst, N. J.
(31) H. J. Rapp, *J. Physiol. (London)*, **158**, 35 (1961).
(32) J. W. Ross, H. Smith, and B. A. Spicer, *Int. Arch. Allergy Appl. Immunol.*, **51**, 226 (1976).
(33) M. E. Greig and R. L. Griffin, *J. Med. Chem.*, 18, 112 (1975).

Bronchodilator and Antiulcer Phenoxypyrimidinones[1]

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Series of 5-phenoxy-2(1*H*)-pyrimidinones, 5-phenoxy-4(3*H*)-pyrimidinones, and related compounds were prepared in a follow-up of a lead prepared as a potential cyclic nucleotide regulating agent. Compounds were evaluated for bronchodilator activity in histamine-challenged guinea pigs and for antiulcer activity in a cold-restraint, stressed rat ulcer model. Bronchodilator activity comparable to, or greater than, that of theophylline was found in both the 2(1*H*)- and 4(3*H*)-pyrimidinone series and was most prominent in analogues containing either an electron-withdrawing or -donating substituent in the para position of the phenoxy ring. Significant antiulcer activity was observed only in the 2(1*H*)-pyrimidinone series among three closely related analogues. One of these, 5-(*m*-methylphenoxy)-2(1*H*)-pyrimidinone (3), exhibited more potent antiulcer effects than the clinically useful antiulcer agent carbenoxolone, without demonstrating bronchodilator activity.

Series of chemical prototypes having certain structural features in common with the clinically useful bronchodilator theophylline were prepared as potential cyclic nucleotide regulating agents. One of these, 5-phenoxy-2-

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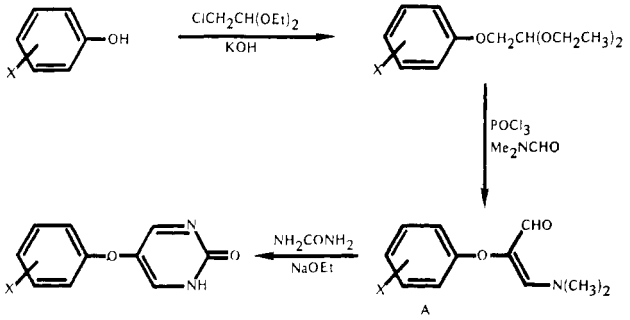
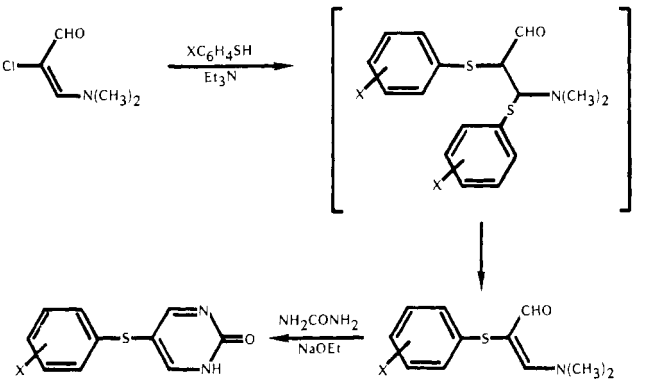
Scheme I. Synthesis of 5-Phenoxy-2(1*H*)-pyrimidinones



Scheme II. Synthesis of 5-Thiophenoxy-2(1*H*)-pyrimidinones

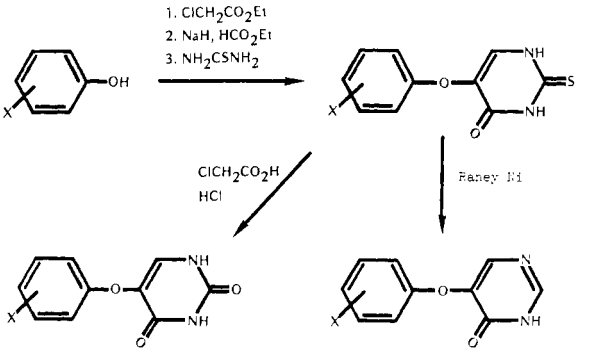


(1*H*)-pyrimidinone (compound 1, Table I), prevented bronchoconstriction in guinea pigs challenged with histamine and protected cold-restraint, stressed rats from gastric ulceration. This compound provided the basis for a structure–activity study reported here, which was aimed at optimizing antiulcer and bronchodilator activity.

Chemistry. The 5-phenoxy-2(1*H*)-pyrimidinones (**1–21**, Table I) were synthesized as illustrated in Scheme I by cyclization of an acrolein precursor with urea in the presence of sodium ethoxide. The 2-substituted 3-(dimethylamino)acrolein precursors (Table II) were obtained by Vilsmeier reaction of phenoxyacetaldehyde diethyl acetals using a modification of the method of Arnold.[3] The required acetals, in turn, were prepared by reaction of phenolate anion with chloroacetaldehyde diethyl acetal.[4] Phenoxyacetic acids were inferior substrates to phenoxyacetaldehyde diethyl acetals in the Vilsmeier reaction. Thus, while phenoxyacetic acid gave acrolein **1a** in only 11% yield, an 81% yield of **1a** was obtained using phenoxyacetaldehyde diethyl acetal as the substrate.

The 5-(thiophenoxy)-2(1*H*)-pyrimidinones (**32–35**, Table I) were prepared according to Scheme II by cyclization of the appropriate 2-(thiophenoxy)-3-(dimethylamino)-acroleins with urea in the presence of sodium ethoxide. Attempted preparation of the latter intermediates by Vilsmeier reaction with (thiophenoxy)acetaldehyde diethyl acetals was unsuccessful. The only products isolated from this reaction were disulfides. The 2-(thiophenoxy)-3-(dimethylamino)acroleins were prepared instead by reaction

Scheme III. Synthesis of 5-Phenoxy-4(3*H*)-pyrimidinones



of the appropriate thiophenols with 2-chloro-3-(dimethylamino)acrolein[5] in the presence of triethylamine. As shown in Scheme II, it seems likely that the mechanism of this reaction involves initial Michael addition of thiophenol to the acrolein double bond, followed by displacement of the activated α-chloro group by a second equivalent of thiophenol; the bis(thiophenol) adduct then loses thiophenol to generate the acrolein.

The 5-phenoxy-4(3*H*)-pyrimidinones (**22–31**, Table I) were prepared as illustrated in Scheme III by Raney nickel desulfurization[4] of the corresponding 5-phenoxypyrimidine-2(1*H*)-thiones, most of which were readily available by known procedures.[6,7] Hydrolysis of these pyrimidine-2(1*H*)-thiones with chloroacetic acid in concentrated HCl afforded the 5-phenoxyuracils (**36–39**, Table I).[6,8]

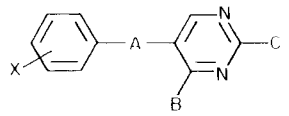
Biological Results and Discussion

Bronchodilator Activity. Theophylline, a clinically useful bronchodilator, served as a positive standard in the guinea pig bronchodilator test, giving 20 to 30% protection against histamine-induced bronchoconstriction. Both series of pyrimidinones exhibited potent activity, with some compounds producing protection equal to theophylline at ten times lower doses, while at high doses producing greater protection. The degree of protection elicited by the parent compounds (**1** and **22**, Table I) was comparable to that of theophylline; however, bronchodilator activity was markedly enhanced by para substitution of the phenoxy moiety with either electron-withdrawing or electron-donating groups. Thus, *p*-methyl (**4**), ethyl (**9**), chloro (**12**), and bromo (**16**) derivatives in the 2(1*H*)-pyrimidinone series and *p*-methyl (**24**) and *p*-chloro (**26**) derivatives in the 4(3*H*)-pyrimidinone series were all highly potent, nearly completely blocking the histamine effect. By comparison, meta-substituted derivatives generally had significantly reduced activity, in the range of theophylline (**7**, **11**, **21**, and **23**), or were inactive (**3**, **8**, **13**, and **25**). Ortho-substituted derivatives (**2** and **27**) lacked significant bronchodilator activity, as did the 4-isopropyl derivative **10**. Replacement of the phenolic ether bridge (**1**, **4**, and **12**) by a thioether bridge (**32**, **34**, and **35**) led to a diminution of bronchodilator activity.

Bronchodilator activity was also observed with the uracils **36**, **38**, and **39**. Substituent effects among these compounds paralleled those found in the 2(1*H*)- and 4(3*H*)-pyrimidinone series. Although uracils were generally

(1) Presented in part by C. A. Lipinski, J. G. Stam, J. N. Pereira, N. R. Ackerman, and H.-J. Hess at the 176th National Meeting of the American Chemical Society; see "Abstracts of Papers", 176th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 10–15, 1978, American Chemical Society, Washington, D.C., 1978, Abstr. MEDI 030.
(2) Syntex Research, Palo Alto, CA 94304.
(3) Z. Arnold and F. Sorm, *Collect. Czech. Chem. Commun.*, **23**, 452 (1958).
(4) C. A. Lipinski, J. G. Stam, G. G. DeAngelis, and H.-J. Hess, U.S. Patent 3 922 345 (1975); *Chem. Abstr.*, **84P**, 59552*u*.
(5) Z. Arnold, *Collect. Czech. Chem. Commun.*, **26**, 3051 (1961).
(6) C.-N. Chang, S.-A. Yang, T.-T. Wang, and Y. Hu, *Yao Hsueh Hsueh Pao*, **10**, 600 (1963); *Chem. Abstr.*, **60**, 14504*b*.
(7) E. A. Falco, P. B. Russell, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 4466 (1951).
(8) C. A. Lipinski, U. S. Patent 4 080 454 (1978).

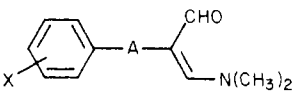
Table I. Bronchodilator and Antiulcer Pyrimidinones[a]



no.	X	A	B	C	mp, °C	solvent	formula	anal.	% yield	broncho-dilator act.: % protec-tion[b]	antiulcer act.: % decrease in total no. of lesions (p value)[c] 10 mg/kg po	100 mg/kg ip
1	H	O	H	OH	180–181	CH_3COCH_3	$C_{10}H_8N_2O_2$	C, H, N	49	28	37 (+)	81 (+)
2	2-CH_3	O	H	OH	208–209	CH_3CO_2H	$C_{11}H_{10}N_2O_2$	C, H, N	67	6	0 (−)	25 (−)
3	3-CH_3	O	H	OH	163–164	C_2H_5OH	$C_{11}H_{10}N_2O_2$	C, H, N	46	3	38 (+)	70 (+)
4	4-CH_3	O	H	OH	186–187	CH_3COCH_3	$C_{11}H_{10}N_2O_2$	C, H, N	15	86	57 (+)	0 (−)
5	2-CH_3, 4-CH_3	O	H	OH	137–138	$CH_3CO_2C_2H_5$	$C_{12}H_{12}N_2O_2$	C, H, N	69		6 (−)	0 (−)
6	3-CH_3, 4-CH_3	O	H	OH	124–126	$(i\text{-}C_3H_7)_2O$	$C_{12}H_{12}N_2O_2$	C, H, N	48	14	54 (−)	53 (−)
7	3-CH_3, 5-CH_3	O	H	OH	209–210	$CH_3CO_2C_2H_5$	$C_{12}H_{12}N_2O_2$	C, H, N	35	38	50 (−)	53 (+)[d]
8	3-C_2H_5	O	H	OH	152–153	$CH_3CO_2C_2H_5$	$C_{12}H_{12}N_2O_2$	C, H, N	37	3	28 (−)	53 (+)[d]
9	4-C_2H_5	O	H	OH	158–159	CH_3COCH_3	$C_{12}H_{12}N_2O_2$	C, H, N	37	98	0 (−)	27 (−)
10	4-i-C_3H_7	O	H	OH	175–177	C_2H_5OH	$C_{13}H_{14}N_2O_2$	C, H, N	11	0	0 (−)	9 (−)
11	3-Cl	O	H	OH	177–178	CH_3COCH_3	$C_{10}H_7ClN_2O_2$	C, H, N, Cl	29	22	22 (−)	0 (−)
12	4-Cl	O	H	OH	218–220	C_6H_6–CH_3CO_2H	$C_{10}H_7ClN_2O_2$	C, H, N	84	92	7[e] (−)	0 (−)
13	3-OCH_3	O	H	OH	163–165	CH_3CO_2H–H_2O	$C_{11}H_{10}N_2O_3$	C, H, N	3	3	55 (−)	82 (+)[d]
14	4-OCH_3	O	H	OH	169–170	CH_3CO_2H–H_2O	$C_{11}H_{10}N_2O_3$	C, H, N	42	45	0 (−)	0 (−)
15	4-F	O	H	OH	220–221	$CHCl_3$–CH_3OH	$C_{10}H_7FN_2O_2$	C, H, N, F	6	66	0[f] (−)	0 (−)
16	4-Br	O	H	OH	211–213	C_2H_5OH	$C_{10}H_7BrN_2O_2$	C, H, N, Br	65	90	0[f] (−)	0 (−)
17	4-SCH_3	O	H	OH	197–199	CH_3OH	$C_{11}H_{10}N_2O_2S$	C, H, N	40	55	0 (−)	0 (−)
18	4-$SOCH_3$	O	H	OH	259–260	CH_3OH	$C_{11}H_{10}N_2O_3S$	C, H, N	73	10	50 (−)	0 (−)
19	2-Cl, 4-Cl	O	H	OH	210–211	CH_3COCH_3–H_2O	$C_{10}H_6Cl_2N_2O_2$	C, H, N, Cl	50	47	22 (−)	19[g] (−)
20	3-CH_3, 4-Cl	O	H	OH	224–225	$CH_3CO_2C_2H_5$–CH_3OH	$C_{11}H_9ClN_2O_2$	C, H, N	40	6	0 (−)	14 (−)
21	3-CF_3	O	H	OH	191–192	CH_3CO_2H–H_2O	$C_{11}H_7F_3N_2O_2$	C, H, N[h]	16	26	39 (−)	
22	H	O	OH	H	189–191	CH_3CO_2H–H_2O	$C_{10}H_8N_2O$	C, H, N	82	23	0 (−)	
23	3-CH_3	O	OH	H	143–145	CH_3COCH_3–hexane	$C_{11}H_{10}N_2O_2$	C, H, N	54	28	0 (−)	
24	4-CH_3	O	OH	H	122–124	CH_3COCH_3–$CH_3COC_2H_5$	$C_{11}H_{10}N_2O_2$	C, H, N	49	92	27 (−)	
25	3-Cl	O	OH	H	159–161	CH_3COCH_3–hexane	$C_{10}H_7ClN_2O_2$	H, N[i]	50	13		18[g] (−)
26	4-Cl	O	OH	H	163–164	pptd from base	$C_{10}H_7ClN_2O_2$	C, H, N	49	92		0 (−)
27	2-Cl	O	OH	H	163–165	CH_3COCH_3	$C_{10}H_7ClN_2O_2$	C, H, N, Cl	50	13		
28	4-F	O	OH	H	146–147	$CH_3CO_2C_2H_5$–hexane	$C_{10}H_7FN_2O_2$	C, H, N	27	80	28 (−)	
29	3-Cl, 4-Cl	O	OH	H	165–166	$CHCl_3$–hexane	$C_{10}H_6Cl_2N_2O_2$	C, H, N[j]	74	45		
30	3-OCH_3	O	OH	H	150–152	$CH_3CO_2C_2H_5$–hexane	$C_{11}H_{10}N_2O_3$	C, H, N	57	7	0 (−)	
31	4-OCH_3	O	OH	H	172–174	$CH_3CO_2C_2H_5$–$CHCl_3$–CH_3OH	$C_{11}H_{10}N_2O_3$	C, H, N	39	26	0 (−)	
32	H	S	H	OH	213–215	C_2H_5OH	$C_{10}H_8N_2OS$	C, H, N	38	23	45 (−)	47 (−)
33	3-CH_3	S	H	OH	208–210	$CH_3CO_2C_2H_5$	$C_{11}H_{10}N_2OS$	C, H, N	25		0 (−)	78 (+)
34	4-CH_3	S	H	OH	222–224	CH_3CO_2H–H_2O	$C_{11}H_{10}N_2OS$	C, H, N	30	26	18 (−)	64 (+)
35	4-Cl	S	H	OH	218–220	CH_3CO_2H–H_2O	$C_{10}H_7ClN_2OS$	C, H, N, S, Cl	53	63		
36	H	O	OH	OH	297–298	C_2H_5OH–H_2O	$C_{10}H_8N_2O_3$	*k*	60	25	22 (−)	
37	3-CH_3	O	OH	OH	291–295	pptd from reaction	$C_{11}H_{10}N_2O_3$	C, H, N	92	7	0 (−)	67[g] (+)
38	4-CH_3	O	OH	OH	>300	C_2H_5OH	$C_{11}H_{10}N_2O_3$	*k*	87	35	0 (−)	
39	4-Cl	O	OH	OH	>300	CH_3CO_2H	$C_{10}H_7ClN_2O_3$	C, H, N	40	50	35 (−)	
carbenoxolone												88 (+)
theophylline										20–30		

[a] Structures drawn in enolic form for clearness of presentation. [b] Respiratory status of guinea pigs exposed to histamine aerosol expressed as percent protection. Control = 0; complete protection = 100. Drugs given 60 mg/kg po 1 h prior to histamine challenge. [c] Ulcer status expressed as percent decrease in total number of lesions in cold-restraint, stressed rats. Active: $p \leq 0.05$ (+); inactive: $p > 0.10$ (−). Drugs 10 mg/kg po or 100 mg/kg ip given 3 h before stress. [d] $p > 0.05$, $p < 0.10$. [e] Tested 60 mg/kg po. [f] Tested 32 mg/kg po. [g] Tested 32 mg/kg ip. [h] F: calcd, 22.25; found, 22.83. [i] C: calcd, 53.95; found 54.99. *m/e* ^{35}Cl isotope: calcd, 222.0196; found, 222.0198. [j] Cl: calcd, 27.59; found, 27.15. [k] Reference 6.

Table II. 2-Phenoxy- and 2-Thiophenoxy-3-(dimethylamino)acroleins



no.	X	A	mp, °C	solvent	formula	anal.	% yield
1a	H	O	115-117	C_6H_6-hexane	$C_{11}H_{13}NO_2$	C, H, N	81
2a	2-CH_3	O	141-143	subl 125 °C (0.1 mm)	$C_{12}H_{15}NO_2$	C, H, N	33
3a	3-CH_3	O	103-105	$CH_3CO_2C_2H_5$-hexane	$C_{12}H_{15}NO_2$	C, H, N	61
4a	4-CH_3	O	87-89	subl 125 °C (0.1 mm)	$C_{12}H_{15}NO_2$	C, H, N	22
5a	2-CH_3, 4-CH_3	O	137-138	$CH_3CO_2C_2H_5$	$C_{13}H_{17}NO_2$	C, H, N	69
6a	3-CH_3, 4-CH_3	O	124-126	$(i\text{-}C_3H_7)_2O$	$C_{13}H_{17}NO_2$	C, H, N	48
7a	3-CH_3, 5-CH_3	O	135-136	C_2H_5OH-hexane	$C_{13}H_{17}NO_2$	C, H, N	34
8a	3-C_2H_5	O	65-66	$(C_2H_5)_2O$-cyclohexane	$C_{13}H_{17}NO_2$	C, H, N	44
9a	4-C_2H_5	O	45-48	$(C_2H_5)_2O$-pet. ether	$C_{13}H_{17}NO_2$	C, H, N	15
10a	4-i-C_3H_7	O	79-80	$(C_2H_5)_2O$-hexane	$C_{14}H_{19}NO_2$	C, H, N	36
11a	3-Cl	O	125-126	C_6H_6-hexane	$C_{11}H_{12}ClNO_2$	C, H, N, Cl	42
12a	4-Cl	O	125-126	CH_3COCH_3-hexane	$C_{11}H_{12}ClNO_2$	C, H, N, Cl	48
13a	3-OCH_3	O	78-79	$CH_3CO_2C_2H_5$-hexane	$C_{12}H_{15}NO_3$	C, H, N	4
14a	4-OCH_3	O	103-104	$(C_2H_5)_2O$	$C_{12}H_{15}NO_3$	C, H, N	26
15a	4-F	O	98-100	hexane-$CH_3CO_2C_2H_5$	$C_{11}H_{12}FNO_2$	C, H, N, F	59
16a	4-Br	O	131-133	hexane-$CH_3CO_2C_2H_5$	$C_{11}H_{12}BrNO_2$	C, H, N, Br	70
17a	4-SCH_3	O	75-77	hexane-$CH_3CO_2C_2H_5$	$C_{12}H_{15}NO_2S$	C, H, N, S	15
19a	2-Cl, 4-Cl	O	114-116	hexane-$CH_3CO_2C_2H_5$	$C_{11}H_{11}Cl_2NO_2$	C, H, N, Cl	3[a]
20a	3-CH_3, 4-Cl	O	142-143	$CH_3CO_2C_2H_5$	$C_{12}H_{14}ClNO_2$	C, H, N, Cl	53
21a	3-CF_3	O	108-110	hexane-$CH_3CO_2C_2H_5$	$C_{12}H_{12}F_3NO_2$	C, H, N, F	44
32a	H	S	90-92	$(i\text{-}C_3H_7)_2O$	$C_{11}H_{13}NOS$	C, H, N	36
34a	4-CH_3	S	88-90	$(i\text{-}C_3H_7)_2O$	$C_{12}H_{15}NOS$	C, H, N	29
35a	4-Cl	S	96-97	$(C_2H_5)_2O$	$C_{11}H_{12}ClNOS$	C, H, N, S[b]	23

[a] Prepared from 2,4-dichlorophenoxyacetic acid. See Experimental Section. [b] Cl: calcd, 14.67; found, 15.09.

Table III. Activity in Histamine Aerosol Test. Percent Protection and Duration of Action[a]

	dose, mg/kg po				duration, h, at 60 mg/kg po			
no.	3	10	30	60	1	2	4	8
4	2	18	81	86	86	86	8	10
12	22	37	71	92	92	94	94	88

[a] Respiratory status of guinea pigs exposed to histamine aerosol expressed as percent protection. Control = 0; complete protection = 100. Duration (h) refers to the time between dosing and initiation of histamine challenge.

less active than the corresponding 2(1*H*)- and 4(3*H*)-pyrimidinones, their activity is believed to contribute to that of the pyrimidinones, since both 2(1*H*)-pyrimidinones and 4(3*H*)-pyrimidinones are metabolized to uracils.[9]

Marked differences, which may be related to rates of metabolism, were observed in duration of action of the pyrimidinones. Thus, pyrimidinones **4** and **12** were equiefficacious when given 1 h before histamine challenge (Table I). However, pyrimidinone **12** was still highly active when given 8 h before challenge, in contrast to **4** which was inactive (Table III). A quantitative structure–activity study was not attempted, since dose–response curves of members of the pyrimidinones were not parallel (compounds **4** and **12**, Table III).

Although the pyrimidinones and especially the uracils bear a structural resemblance to theophylline, it is uncertain whether bronchodilator activity is related to elevation of cyclic AMP levels.

Antiulcer Activity. Antiulcer activity was assessed in rats using carbenoxolone, a clinically useful ulcer-healing agent, as a positive standard. This drug, which is not active orally in rats because of hydrolysis of the hemisuccinate ester moiety by the intestinal microflora in this species,[10] demonstrated significant antiulcer activity by intraperitoneal administration at doses of 30 mg/kg.

Some members of the 2(1*H*)-pyrimidinones, but not the 4(3*H*)-pyrimidinones, exhibited significant antiulcer activity. Within the 2(1*H*)-pyrimidinone series, significant oral activity was confined to three closely related analogues, the parent compound (**1**) and the *m*- and *p*-methyl derivatives (**3** and **4**). These compounds provided significant protection from stress-induced ulceration at doses of 10 mg/kg, po. Several other meta-substituted derivatives, **7**, **8**, and **13**, also demonstrated antiulcer activity but only by intraperitoneal administration at higher doses (100 mg/kg). Replacement of the phenolic ether bridge in **1**, **3**, and **4** by a thioether bridge (**32**, **33**, and **34**) resulted in a decrease or loss of antiulcer activity. The uracils **36** and **38**, which are related to pyrimidinones **1** and **4**, were inactive. However, uracil **37**, which is a metabolite of pyrimidinone **3** in rat, rhesus monkey, and man,[8,9] exhibited activity at 32 mg/kg, po.

In more detailed studies, compound **3** was shown to stimulate gastric mucus secretion in vivo over the same dose range that prevents experimentally induced gastric ulceration in rats and to display mucotropic effects in vitro in a system employing segments of rat gastric glandular mucosa.[11] A direct correlation between the mucotropic and antiulcer effects suggests that stimulation of mucus secretion, presumably mediated by increased cyclic AMP production, underlies the antiulcer activity of this agent. Following administration of **3** (50 mg/kg, ip) to rats, cyclic AMP levels increased by 50% in the glandular region of the stomach and, although compound **3** had no significant effects on rat gastric mucosal cyclic AMP phosphodiesterase, its metabolite **37** had the same inhibitory effect as theophylline (I_{50} = 100 μM).[12] Compound **3** is rapidly orally absorbed in the rat,[9] and the greater oral activity of 2(1*H*)-pyrimidinone (**3**) over uracil **37** may be due to less favorable absorption of the very insoluble uracil. Inhibition

(9) H. D. Doshan, Pfizer Inc., unpublished work.
(10) P. Iveson, W. E. Lindup, D. V. Parke, and R. T. Williams, *Xenobiotica*, 1, 79 (1971).
(11) J. N. Pereira, G. A. Mears, J. P. Leader, and L. A. Hohnke, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 37(2), A484 (1978).
(12) J. P. Leader, Pfizer Inc., unpublished work.

of gastric acid secretion does not appear to contribute significantly to the antiulcer activity of **3**.[11]

Dose–response comparisons established that **3** administered orally is approximately three times as potent as carbenoxolone administered intraperitoneally in protecting rats from gastric ulceration. Futhermore, in contrast to carbenoxolone, **3** elicits its protective effects in rats without fluid retention or altering urinary electrolyte output. Compound **3** has been assigned the nonproprietary (USAN) name tolimidone.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Analytical Department of Pfizer Inc. Where analyses are indicated only by symbols of the elements, analytical values were within ±0.4% of theoretical values. NMR and/or mass spectra were obtained on all compounds and were consistent with structures and assignments. NMR spectra were recorded on either a Varian A-60 or T-60 spectrometer, and the mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. Representative procedures for the preparation of compounds **1–21** (Scheme I), **32–35** (Scheme II), and **22–31** (Scheme III) are illustrated below.

Phenoxyacetaldehyde Diethyl Acetals. The crude intermediates were prepared by literature procedures[4,14] and were used without extensive purification for the preparation of the 2-(substituted phenoxy)-3-(dimethylamino)acroleins. A typical procedure is illustrated below.

3-Methylphenoxyacetaldehyde Diethyl Acetal. Into a 3-L three-neck flask equipped with a pressure-compensating dropping funnel, overhead stirrer, and condenser with solvent takeoff was added 99.1 g (1.5 mol) of 85% KOH pellets. The dry flask was heated, and to the stirred hot KOH pellets was added 170.0 g (1.5 mol) of *m*-cresol. The flask was maintained at such a temperature that the contents were at 90–100 °C so that the phenolate anion did not precipitate. A clear solution of molten potassium *m*-cresolate and H_2O resulted. To the stirred molten solution, 375 g (2.46 mol) of chloroacetaldehyde diethyl acetal (bp 152–156 °C) was added at such a rate that the mixture remained at 90 to 100 °C. The mixture (two phases) was stirred rapidly and heated, and an azeotrope of the acetal and H_2O was removed via the takeoff on the condenser. Distillation was continued until the distilling vapor was at 140 to 150 °C. On cooling, the azeotrope separated into an aqueous and acetal layer and the acetal could be readded to the reaction mixture. The mixture was stirred vigorously and heated for 6 h, during which time KCl precipitated. The crude cooled mixture was poured into a stirred solution of ice–H_2O and ether. The layers were separated and the aqueous layer was washed with two more portions of ether. The combined ether solutions were washed with cold 2 N NaOH solution and then dried over anhydrous Na_2SO_4, treated with activated charcoal and concentrated on a steam bath to a mobile amber oil weighing 424 g. The residual traces of ether were removed by distillation at atmospheric pressure, and then excess chloroacetaldehyde diethyl acetal was removed by distillation at H_2O aspirator pressure (20 mm). Final distillation under high vacuum at 0.2 to 0.3 mmHg at 90–95 °C gave 291.7 g (85%) of 3-methylphenoxyacetaldehyde diethyl acetal as a clear colorless oil: NMR ($CDCl_3$) δ 7.35–6.7 (4 H, m), 4.8 (1 H, t), 3.96 (2 H, d), 3.68 (4 H, m), 2.3 (3 H, s), 1.21 (6 H, t). Anal. ($C_{13}H_{20}O_3$) C, H.

2-(3-Methylphenoxy)-3-(dimethylamino)acrolein (3a). To 175 g (2.4 mol) of DMF was added 358 g (2.4 mol) of $POCl_3$, maintaining the temperature at 25 °C by an external ice bath. Upon completion of the addition, the resultant amber heavy oil was warmed to 50 °C for 45 min and then cooled to 25 °C. $CHCl_3$ (700 mL) was added and the resultant solution was brought to reflux. While reflux continued, 190 g (0.8 mol) of 3-methylphenoxyacetaldehyde diethyl acetal was added over a 30-min period. Provision was made to trap the large amount of HCl that evolved during the addition. After a 3-h reflux following the addition, the cooled $CHCl_3$ solution was cautiously added to a stirred mixture of 2.3 kg of K_2CO_3, 2 L of ice–H_2O, and 2 L of a 9:1 benzene–ethanol solution. The organic layer was separated, concentrated to a dark oil, and taken up in $CHCl_3$ to remove inorganic salts. The $CHCl_3$ was removed in vacuo to give a dark red oil. Isopropyl ether was added to precipitate 87 g (61%) of a granular light yellow solid, mp 99–101 °C. Recrystalization from ethyl acetate–hexane gave an analytical sample: mp 103–105 °C; NMR ($CDCl_3$) δ 9.23 (1 H, s), 7.33–6.6 (4 H, m), 6.60 (1 H, s), 3.05 (6 H, s), 2.32 (3 H, s). Anal. ($C_{12}H_{15}NO_2$) C, H, N.

2-(2,4-Dichlorophenoxy)-3-(dimethylamino)acrolein (19a). To the reagent prepared from 640 g (8.75 mol) of DMF and 908 g (5.93 mol) of $POCl_3$ was added 442 g (2.0 mol) of 2,4-dichlorophenoxyacetic acid using the procedure of Arnold[5] to give 13.7 g (3%) of needle-shaped crystals. Recrystallization from hexane–ethyl acetate (1:2) gave needles, mp 114–116 °C. Anal. ($C_{11}H_{11}Cl_2NO_2$) C, H, N, Cl.

2-(4-Methylthiophenoxy)-3-(dimethylamino)acrolein (34a). A solution of 4.08 g (0.03 mol) of 2-chloro-3-(dimethylamino)-acrolein, 6.06 g (0.06 mol) of triethylamine, and 3.72 g (0.03 mol) of *p*-toluenethiol in 50 mL of ethanol was heated at reflux for 2.5 h. The cooled reaction solution was poured over H_2O and extracted with ether. The ether extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo to a 100-mL volume. Isopropyl ether (50 mL) was added and the total volume was reduced to 100 mL on a steam bath. Cooling resulted in crystallization of 1.9 g (29%) of **34a**: mp 88–90 °C; NMR ($CDCl_3$) δ 9.06 (1 H, s), 7.53 (1 H, s), 7.05 (4 H, s), 3.28 (6 H, s), 2.28 (3 H, s). Anal. ($C_{12}H_{15}NOS$) C, H, N.

2-(3-Methylthiophenoxy)-3-(dimethylamino)acrolein (33a). The crude intermediate was prepared from a commercially available mixture (K&K Labs) of ~70% *m*-toluenethiol and 30% *p*-toluenethiol, utilizing the procedure for the preparation of compound **34a**.

5-(3-Methylphenoxy)-2(1*H*)-pyrimidinone (3). To a solution of 39.2 g (0.576 mol) of NaOEt in 150 mL of EtOH was added 59.3 g (0.288 mol) of 2-(3-methylphenoxy)-3-(dimethylamino)-acrolein (**3a**) and 34.6 g (0.576 mol) of urea. The mixture was heated at reflux for 4 h, 10 mL of H_2O was added, and refluxed was continued for another 2 h. When the mixture was cooled, 30.3 g of a crude tan solid (mp >300 °C) precipitated and was collected by filtration and dried. The crude sodium salt was dissolved in 1200 mL of hot H_2O and filtered from a small quantity of insoluble material. Glacial acetic acid was added to pH 6.0, resulting in crystallization of 21.4 g (46%) of a tan solid, mp 162–163 °C. Recrystallization from ethanol gave an analytical sample: mp 163–164 °C; NMR (Me_2SO) δ 12.0 (N-H, exchange), 8.25 (2 H, s), 7.4–6.5 (4 H, m), 2.26 (3 H, s). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

5-(Thiophenoxy)-2(1*H*)-pyrimidinones 32–35. These compounds were prepared from the (thiophenoxy)(dimethylamino)acroleins **32a–35a** using the procedure for compound **3**.

5-(3-Methylthiophenoxy)-2(1*H*)-pyrimidinone (33). Crude product containing **34** was prepared from **33a** using the procedure described for **3**. Fractional crystallization of the crude product from ethyl acetate resulted in isolation of the 3-methylthiophenoxy isomer (**33**), mp 208–210 °C, uncontaminated by the 4-methylthiophenoxy isomer (**34**), mp 222–224 °C. The isomeric purity of **33** could be readily determined by the position of the NMR absorption of the tolylthio methyl group: NMR (Me_2SO) for **33**, δ 2.4 (3 H, s); for **34**, 2.27 (3 H, s). Anal. ($C_{11}H_{10}N_2OS$) C, H, N.

5-Phenoxy-4-hydroxypyrimidine (22). To a solution of 22.0 g (0.55 mol) of NaOH in 700 mL of H_2O was dissolved 121 g (0.55 mol) of 2-thio-4-hydroxy-5-phenoxypyrimidine. To the stirred solution was added slowly 1250 g of wet Raney nickel at such a rate that the resultant foaming remained under control. The mixture was stirred well and heated at reflux for 2 h. The suspension was filtered while still hot, and the Raney nickel residue was washed with 1.5 L of boiling water, with care being taken that the catalyst remained wet. The combined aqueous solutions were concentrated to 700 mL and acidified with acetic acid to precipitate a white solid. This was collected by filtration and dried to give 85.5 g (82.5%) of **22**, mp 186–188 °C. Recrystallization

(13) C. G. Van Arman, L. M. Miller, and M. P. O'Malley, *J. Pharmacol. Exp. Ther.*, **133**, 90 (1961).
(14) M. Crauland, French Patent 1 026 008 (1953); *Chem. Abstr.*, **52P**, 5466*g*.

from aqueous acetic acid gave **22**: mp 189–191 °C; NMR (Me_2SO) δ 8.13 (1 H, s), 7.88 (1 H, s), 7.58–6.38 (5 H, m). Anal. ($C_{10}H_8N_2O_2$) C, H, N.

5-[4-(Methylsulfinyl)phenoxy]-2(1*H*)-pyrimidinone (18). To a slurry of 1.17 g (0.005 mol) of **17** in 20 mL of methanol at 0 °C was added 1.175 g (0.0055 mol) of $NaIO_4$ in 10 mL of H_2O, and the slurry was stirred at 0 °C for 8 h and then for 20 h at 25 °C. The resultant slurry was diluted with H_2O and the methanol was removed in vacuo. Filtration gave 0.917 g (73%) of a white solid, mp 259–260 °C. Anal. ($C_{11}H_{10}N_2O_3S$) C, H, N.

Pharmacological Procedures. Guinea Pig Histamine Aerosol. Bronchodilator activity was evaluated according to the method of Van Arman, Miller, and O'Malley[13] in conscious female Reed–Willet guinea pigs (200–250 g) fasted overnight. One hour following po administration of vehicle, or the test drug dissolved in 1 N HCl or suspended in vehicle, each animal was challenged with histamine aerosol as follows: a 0.4% aqueous solution of histamine was placed in a Vaponephrine standard nebulizer (Vaponephrine Co., Edison, N.J.) and sprayed under an air pressure of 6 psi into a closed 8 × 8 × 12 in. transparent plastic container for 1 min. Immeditely thereafter, the guinea pig was placed in the container. The respiratory status (a reflection of bronchoconstriction) of the guinea pig after 1 min in the container was scored as follows: 0, normal breathing; 1, slightly deepened breathing; 2, labored breathing; 3, severely labored breathing and ataxia; 4, unconsciousness.

The percent inhibition values were derived by comparing the total score of a group of eight treated animals with that of a similar control group to generate a single value with no standard deviation. In order to assess the significance of a result, data on 11 groups of eight animals were recalculated by assigning a percent inhibition value to each animal (based on the score 0 = 100% inhibition, 1 = 75%, 2 = 50%, 3 = 25%, and 4 = 0) and where more than 90% of control scores were 4. Based on this calculation, the standard deviation of the mean value for eight animals was 5.33. As a result, it is likely that any two values differing by more than 11% would be significantly different based on the premise that values differing by more than 2 standard deviations represent different populations.

Cold-Restraint, Stress-Induced Gastric Ulceration. Ulcer protective effects were determined in rats using a modification of the method of Perkins.[15] Cold-restraint, stress-induced gastric ulceration was produced by immobilizing nonfasted, female, 90–120 g Sprague–Dawley rats in a supine position and placing them in a refrigerator at 12 °C for 3 h. Experimental drugs suspended in saline containing 10 g/L carboxymethylcellulose and 1 g/L Tween 80 were administered 3 h before the initiation of the stress period. At the conclusion of the ulcerogenic stress, the rats were sacrificed by cervical dislocation, their stomachs were removed, and the degree of gastric ulceration was determined. Differences in number of ulcers per animal were compared with Wilcoxon rank sum tests in order to calculate a p value. Compounds were considered active (+) if the decrease in number of ulcers per animal compared to control were significant at the $p \leq 0.05$ level.

(15) W. E. Perkins and L. Vars, *Br. J. Pharmacol.*, **47**, 847 (1973).

Synthesis of α-Methylene-γ-butyrolactones: A Structure–Activity Relationship Study of Their Allergenic Power

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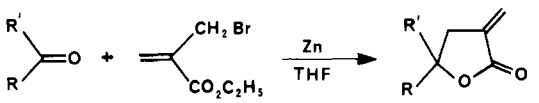
Thirty-five α-methylene-γ-butyrolactones have been prepared and their allergenic properties tested on the skin of guinea pigs experimentally sensitized to (a) alantolactone (**1**), (b) isoalantolactone (**2**), and (c) α-methylene-γ-butyrolactone (**3**). The two first groups of animals cross-react to lactones containing 9 to 18 carbon atoms but not to smaller α-methylene-γ-butyrolactones. Conversely, animals sensitized to α-methylene-γ-butyrolactone react only with α-methylene-γ-butyrolactones containing 6 and 7 carbon atoms. These results are discussed in relation with the allergic contact dermatitis mechanism.

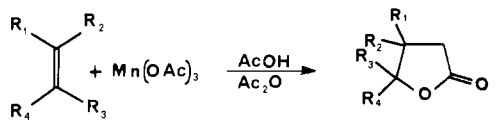
The biological properties of compounds containing the α-methylene-γ-butyrolactone moiety have been the focus of a considerable amount of work. Research has been focussed mainly on their cytotoxic, antitumoral,[1,2] antibacterial,[3] and plant growth inhibition activities.[4] These compounds, which occur naturally in many plants[5] (such as compositae, frullanaceae, magnoliaceae, etc.), can also cause severe allergic contact dermatitis (ACD) in man.[6]

We have been interested in this last property for some time. The mechanism of ACD has not been completely elucidated.[7] It is generally believed that an allergen (hapten), in order to become a true antigen, must bind to a protein (or another carrier). Why are some compounds allergenic while other are not? To approach the answer to this fundamental question, we have prepared a series of γ-monosubstituted and β,γ- and γ,γ-disubstituted α-methylene-γ-butyrolactones and tested their allergenic

Scheme I. Reformatsky Synthesis of γ-Substituted α-Methylene-γ-butyrolactone



Scheme II. Synthesis of Butyrolactones from Manganese Triacetate and Olefins



(1) C. H. Smith, J. Larner, A. M. Thomas, and S. M. Kupchan, *Biochim. Biophys. Acta*, **276**, 94 (1972).
(2) J. L. Hartwell and B. J. Abbott, *Adv. Pharmacol. Chemother.*, **7**, 117 (1969).
(3) K. H. Lee, T. Ibuka, R. Y. Wu, and T. A. Geissman, *Phytochemistry*, **16**, 1177 (1977).
(4) M. R. Garciduenas, X. A. Dominguez, J. Fernandez, and G. Alaniz, *Rev. Latinoam. Quim.*, **3**, 52 (1972).
(5) H. Yoshioka, T. J. Mabry, and B. N. Timmermann, "Sesquiterpene Lactones, Chemistry, NMR and Plant Distribution", University of Tokyo Press, Tokyo, 1973.
(6) J. C. Mitchell and G. Dupuis, *Br. J. Dermatol.*, **84**, 139 (1971).
(7) P. G. H. Gell and B. Benacerraf, *J. Exp. Med.*, **113**, 571 (1961).

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