1.5 g of off-white solid. Crystallization from MeOH gave crystals of **6a**: mp 285 °C dec; yield 1.02 g (45%); $[\alpha]^{20}_D$ −72.6° (*c* 1, MeOH).

By a similiar procedure, **6b** was prepared from **5b**: $[\alpha]^{20}_D$ +74.7° (*c* 1, MeOH).

Attempts to isolate the other diastereoisomer from the mother liquor were not successful. The combined residues from the mother liquors were dissolved in 100 mL of CH_2Cl_2 and added dropwise over 15 min to an ice-cold solution of 50 mL of anisole in 200 mL of trifluoroacetic acid. After the addition was complete, the solution was stirred at room temperature for 1 h and then evaporated under vacuum to a dark brown oil. This was added dropwise to a solution of 500 mL of ether saturated with HCl. The resulting precipate was removed by filtration, stirred with 300 mL of Et_2O, collected, and sucked dry to give 26.4 g (93%) of pale tan powder. This was acylated with *d*-isobornyl chloroformate as described above and purified by crystallization to give 47% of **3b**, mp 163–166 °C.

Measurement of [^{3}H]Norepinephrine Release in the Rabbit Ear Artery. The rabbit ear artery was isolated and cannulated as described in ref 29; before mounting in the perfusion chamber, the artery was labeled by equilibration with [^{3}H]norepinephrine (150 µCi in 10 mL of Krebs solution) at 37 °C for 45 min with constant gassing with 95% O_2/5% CO_2. The artery was then mounted and allowed to slowly be perfused with Krebs solution for 7–8 h before the experiment was begun; the artery was usually stimulated periodically (5 Hz for 10 s at 12-min intervals) during this washout period.

Basal and stimulated norepinephrine release were determined in the following manner. Extraluminal and intraluminal outflow tubes were joined with a "T" connector; flow rate was adjusted to exactly 2 mL/min for the combined flow. A basal sample was collected for 2 min, directly into a glass scintillation vial (4-mL volume). The artery was then stimulated at 1.5–2 Hz for 2 min, and a second sample was collected. Collection of this sample was delayed 30 s to allow for dead space in the perfusion chamber; i.e., collection was begun 30 s after initiation of stimulation and continued for 30 s following termination of stimulation. Basal and stimulation samples were then mixed with 10 mL of scintillation fluid (Aquasol-II, New England Nuclear Corp.) for counting. This procedure was repeated at 20-min intervals.

Registry No. (±)-1·HCl, 86238-66-6; **2a**, 34771-95-4; **3a**, 86238-67-7; **3b**, 86287-10-7; **4a**, 86238-68-8; **4b**, 86287-11-8; **5a**, 86238-69-9; **5b**, 86287-12-9; **6a**, 86238-70-2; **6a** (free base), 86238-71-3; **6b**, 86238-73-5; **6b** (free base), 86238-72-4; (±)-**6**, 86287-13-0.

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Gastric Antisecretory 9*H*-Xanthen-9-amines

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A series of 9*H*-xanthen-9-amines possessing a wide variety of nitrogen substituents at C-9 was prepared for evaluation of gastric antisecretory activity. These substituents included the acetamidine, imidate, pyrimidine, thiazoline, quinuclidine, 2-hydrazinopyridine, aminopiperidine, aminoalkylimidazole, and aminoalkylpyridine moieties. The majority of compounds in this series inhibited gastric acid secretion when tested orally in the pylorus-ligated rat. Potency was increased by intraduodenal administration and diminished by incubation with gastric juice, suggesting partial degradation of the compounds in the gastric environment. A representative example, 3-(9*H*-xanthen-9-ylamino)-1-ethylpiperidine, exhibited similar activity in dogs, although no free compound could be detected in the blood. It is therefore hypothesized that this compound is either rapidly bound to tissue and/or metabolized to an active species.

Numerous and diverse examples of N-substituted 9*H*-xanthen-9-amines have been reported in the patent literature along with descriptions of antisecretory and antiulcer activity.[1] These claims are particularly interesting in light of the reported chemical instability of these compounds, a finding that apparently discouraged several investigators[2,3] from pursuing further studies. Thus, despite earlier interest in xanthene chemistry, a more detailed description of biological activity and its relation to molecular structure has only recently appeared for a series of 9-substituted (alkylthio)-9*H*-xanthenes.[4] In our own efforts to identify a promising antisecretory agent, we investigated a series of N-substituted 9*H*-xanthen-9-amines and describe our results at this time.

Chemistry. A variety of synthetic methods was employed to prepare the compounds described in this paper (see Scheme I). The classical reaction of 9*H*-xanthen-9-ol (I) with nucleophiles (Nu-H) in mildly acidic solution was used to synthesize a number of 9*H*-xanthen-9-yl amides (**2**, **3**, **5**, and **7**), as well as the allylamine (**14**), the formic acid hydrazide (**18**), and the piperazine carbamate (**21**).[5-8] Alkylation of less reactive or less stable nucleophiles by 9-hydroxy-9*H*-xanthenyl *N*-methylcarbamate (II) was selected for the preparation of sulfonamide **1**, hydrazines **19** and **20**, and aminothiazoline **27**.[9,10] Alkaline hydrolysis

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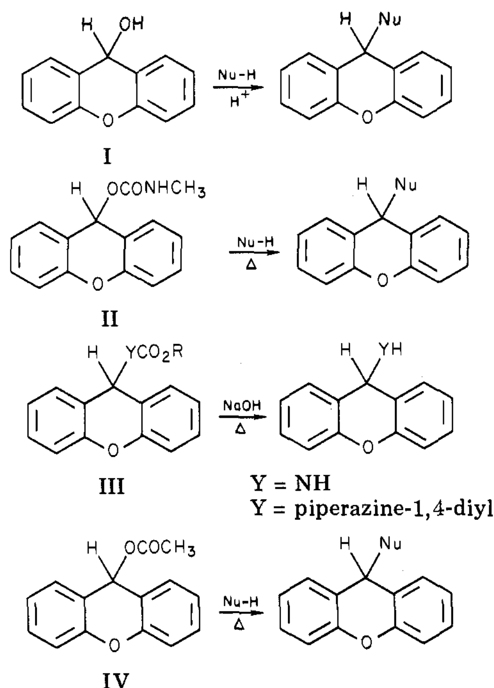
Scheme I



of substituted *N*-carbamates (III) as previously described gave the parent amine **13** and piperazine **31**.[10,11] Attempts to prepare 9-bromo-9*H*-xanthene by the peroxide-catalyzed reaction of xanthene with *N*-bromosuccinimide resulted in the isolation of succinimide **4**. Reaction of the formamidine transfer reagent, 3,5-dimethyl-1-pyrazolecarboximidamide nitrate,[12] with **13** yielded pyrazole **8** rather than 9*H*-xanthen-9-ylguanidine. Treatment of **13** with ethyl acetimidate fluoroborate in a nonpolar solvent afforded a transimidation product (**16**) rather than amidine **17**. Similar transimidation reactions have been described for the reaction of amino ester hydrochlorides with imidate esters.[13] The desired acetamidine (**17**) was obtained by treatment of **16** with alcoholic ammonia. The majority of xanthene derivatives described in this paper were synthesized by reflux of the appropriate amine with 9-hydroxy-9*H*-xanthenyl acetate (IV) in an inert solvent.[1c] This permited the preparation of the more basic derivatives without their ensuing acid-catalyzed decomposition (vide infra).

Results and Discussion

Table I depicts the gastic antisecretory activity of the compounds shown as an increase in pH and/or a decrease in titratable acid output in the pylorus-ligated rat.[18] The majority of the compounds in this series produced a significant increase in gastric pH following oral administration. Most of these showed nearly maximal gastric antisecretory activity at a dose of 50 mg/kg and minimal activity at 10 mg/kg. Several compounds, including **29**, **32**, **35**, **38**, **44**, and **46**, were also effective at a lower dose (see Table II). This inhibition of gastric acid secretion was characterized by both a dose-related reduction in gastric acid titer and a dose-independent reduction in gastric juice volume (rarely more than 50%). The reduction of titratable acidity and elevation of gastric pH is believed to be an advantageous feature for an antiulcer agent.

Virtually all compounds active in the rat (**13–19** and **21–49**) possess basic amino substituents. Conversely, most of the compounds lacking statistically significant activity (**1–5**, **8**, and **10–12**) possess nonbasic 9-amino substituents. This suggests a correlation between the antisecretory activity of N-substituted 9*H*-xanthen-9-amines and the basicity of their 9-nitrogen atom.

Table III illustrates the antisecretory activity of a typical 9*H*-xanthen-9-amine (**29**) in a second species, the pentagastrin-stimulated dog. Administration of 1 mg/kg iv resulted in a suppression of acid output, peaking at 1 to 2 h and lasting at least 3 h.

The oral activity exhibited by these compounds in rats contrasts with the reported chemical instability of 9*H*-xanthen-9-amines in acidic solution.[2,3] In order to circumvent this potentially deleterious effect of gastric juice, several of the more potent compounds were dosed by intraduodenal and intravenous routes (see Table II). Administration by these routes resulted in greater activity than the oral route. This activity is primarily a result of enhanced depression of secretion volume. The lower oral potency is consistent with partial degradation in the acidic (pH 1.5) environment of the fasting rat stomach. In order to test this point, several active compounds were exposed to neutralized or untreated rat gastric juice (pH 1.0) and administered intraduodenally. The results illustrated with **29** in Table IV showed that a 60-min prior incubation of compound with neutralized gastric juice at 25 °C produced the expected antisecretory effect, while a similar incubation with untreated juice resulted in a time-dependent loss of the antisecretory effect.

The lifetime of a 9*H*-xanthen-9-amine (**29**) was examined in vivo in the dog and in vitro in dog blood to compare the kinetics of compound disappearance with that of inhibition of acid secretion (see Experimental Section for details). Compound **29** was stable for several days in dog blood adjusted to pH 10.2, and even after 20 min at pH 7.5, two-thirds of the compound still remained intact. However, after administration of 2 mg/kg, iv, of **29**, no compound could be detected in samples taken from 0 to 2 h. In contrast, administration of **29** at half this dose resulted in a strong antisecretory effect, peaking from 1 to 2 h after administration. We therefore suggest that this compound, and presumably active antisecretory 9*H*-xanthen-9-amines in general, are most probably either rapidly tissue bound and/or metabolized to the active species.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR (Perkin-Elmer Infracord) and NMR (Perkin-Elmer R-24 or Varian T-60) spectra of all compounds were evaluated as being completely consistent with the assigned structures. Elemental analyses were obtained by the Analytical and Physical Chemistry Section of Smith Kline & French Laboratories. Satisfactory elemental analyses were obtained for all final products, except as noted in Table I. The majority of the required precursor amines were obtained commercially; 4-amino-1-methylpiperidine,[19] 2-(3-aminopropyl)-pyridine,[20] 1-methylhomopiperazine,[21] 4-(dimethylamino)-

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Table I. 9*H*-Xanthen-9-amines: Physical Properties and Gastric Antisecretory Activity

H NR$_1$R$_2$ O

no.	R_1	R_2	method[a,b]	purification[a]	mp, °C	formula[c]	gastric antisecretory act. (PLR)[d] ΔpH[e]	ΔTAO[e]
1	H	SO_2CH_3	C(5)[f]	III(1:8), I(7:11)	168–170	$C_{14}H_{13}NO_3S$[g]	*h*	*h*
2	H	$SO_2C_6H_5$	*i*	I(5:12)	200–202 (200–200.5)[i]		*h*	*h*
3	H	2-(OH)C_6H_4CO	B(2)[j]	I(5:2)	210–212	$C_{20}H_{15}NO_3$	*h*	*h*
4	$COCH_2CH_2CO$		*a*	I(7)	246–248	$C_{17}H_{13}NO_3$	*h*	*h*
5	H	$CO_2CH_2C_6H_5$	*k*	*k*	164–166 (165–166)[k]		*h*	*h*
6	H	SO_2NH_2	A(5)	III(1:8), I(8)	186–187	$C_{13}H_{12}N_2O_3S$	2.0	87
7	OH	$CONH_2$	*l*	*l*	185 dec (185)[l]		1.9	88
8	$C(CH_3)=CHC(CH_3)=N$		*a*	I(6)	140–141	$C_{18}H_{16}N_2O$	*h*	*h*
9	CH_3	9-xanthenyl	*m*	I(1:6)	169–171 (170)[m]		*h*	72
10	H	3-pyridyl	A(2)	I(1:6)	155–158	$C_{18}H_{14}N_2O$[n]	*h*	*h*
11	H	2-pyrimidyl	A(2)	I(1)	182–184	$C_{17}H_{13}N_3O$	*h*	48
12	H	2-thiazolyl	A(2)	I(1:6)	181–182 dec	$C_{16}H_{12}N_2OS$	*h*	
13	H	H	*k*	I(6)	154–155[o] (153–154)[k]		4.5	93
14	H	$CH_2CH=CH_2$	B(2)	II	130–133[p]	$C_{16}H_{15}NO$	4.4	*h*
15	$CH_2CH=CH_2$	$CH_2CH=CH_2$	A[q]	I(6)	50–52	$C_{19}H_{19}NO$	1.3	
16	$=C(OEt)CH_3$		*a*	I(6)	74–76.5	$C_{17}H_{17}NO_2$	4.4	97
17	$=C(NH_2)CH_3$		*a*	I(1:6)	149–151.5	$C_{14}H_{12}N_2O$	1.1	88
18	H	NHCHO	*a*	I(4)	155–156	$C_{15}H_{14}N_2O_2$	2.0	80
19	H	NH(2-pyridyl)	C(1)	I(7)	137–138	$C_{18}H_{15}N_3O$	1.4	73
20	H	NHC_6H_5	C(1)[m]	I(9)	108–111 dec (100)[m]	$C_{19}H_{16}N_2O$	*h*	
21	$(CH_2)_2N(CO_2Et)(CH_2)_2$		*r*	*r*	144–146 (145–146)[r]		2.2	
22	H	CH_2– O	A(1)	I(6)	90–92	$C_{19}H_{21}NO_2$	3.6	87
23	H	SO_2	A(2)	I(8)	135	$C_{17}H_{17}NO_3S$	1.5	57
24	H	CH_2CH_2(1-morpholino)	A(1)	IV(8)	oil	$C_{19}H_{22}N_2O_2$[s]	2.6	47
25	H	3-quinuclidinyl	A(1)	I(6)	105–107	$C_{20}H_{22}N_2O$	2.4	65
26	H	CH_2CH_2 N N CH_3	A(1)	IV(9)	85–88	$C_{19}H_{21}N_3O$[t]	3.0	88
27	H	2-(2-thiazolinyl)	C(1)	I(1:6)	222–223	$C_{16}H_{14}N_2OS$	3.6	95
28	H	N—CH_3	A(1)	I(6)	96.5–98	$C_{19}H_{22}N_2O$	6.4	99
29	H	N CH_2CH_3	A(1)	III(7)	44–47	$C_{20}H_{24}N_2O$	5.6	98
30	CH_3	N CH_2CH_3	A(1)	III(8)	oil	$C_{21}H_{26}N_2O$[u]	5.1	98

31	$(CH_2)_2NH(CH_2)_2$		v	v	113-115 (118-119)[v]		5.3	98
32	$(CH_2)_2N(CH_3)(CH_2)_2$		A(2)	I(1:6)	114-116	$C_{18}H_{20}N_2O$	5.4	97
33	$(CH_2)_2N(CH_3)(CH_2)_3$		A(1)	IV(8)	oil	$C_{19}H_{22}N_2O$	4.6	92
34	$(CH_2)_2CH(NMe_2)(CH_2)_2$		A(1)	I(6)	92-93	$C_{20}H_{24}N_2O$	5.8	97
35	$(CH_2)_3CH(NMe_2)CH_2$		A(1)	IV(10)	oil	$C_{20}H_{24}N_2O$[w]	3.1	80
36	$(CH_2)_4CH(CH_2NMe_2)$		A(1)	I(6)	81-83	$C_{21}H_{26}N_2O$	5.2	95
37	H	$CH_2C_6H_5$	A(2)[x]	III(6:8)	oil	$C_{20}H_{17}NO$[y]	1.9	69
38	H	CH_2CH_2(1-imidazolyl)	A(1)	I(1:6)	90-92	$C_{18}H_{17}N_3O$	5.8	98
39	H	CH_2(2-pyridyl)	A(1)	I(6)	83-84	$C_{19}H_{16}N_2O$	4.8	96
40	H	CH_2(3-pyridyl)	A(1)	I(1:6)	103-104	$C_{19}H_{16}N_2O$	4.2	94
41	CH_3	CH_2(3-pyridyl)	A(1)	I(6)	105-107	$C_{20}H_{18}N_2O$	3.6	92
42	H	CH_2(4-pyridyl)	A(1)	IV(6:8)	83-85	$C_{19}H_{16}N_2O$	3.6	77
43	H	CH_2CH_2(2-pyridyl)	A(1)[z]	III(7)	oil	$C_{20}H_{18}N_2O$	5.9	99
44	CH_3	CH_2CH_2(2-pyridyl)	A(1)	III(8)	oil	$C_{21}H_{20}N_2O$	5.6	99
45	CH_3CH_2	CH_2CH_2(2-pyridyl)	A(1)[aa]	III(8)	oil	$C_{22}H_{22}N_2O$	4.3	86
46	H	$CH(CH_3)CH_2$(2-pyridyl)	A(1)	III(8)	oil	$C_{21}H_{20}N_2O$	3.8	89
47	H	$(CH_2)_3$(2-pyridyl)	A(1)	III(8)	oil	$C_{21}H_{20}N_2O$[bb]	5.7	99
48	H	$CH(CH_3)(CH_2)_3$(2-pyridyl)	A(1)	III(8)	oil	$C_{23}H_{24}N_2O$	6.1	100
49	$(CH_2)_3CH$(3-pyridyl)		A(1)	III(8)	oil	$C_{22}H_{20}N_2O$	1.8	65
50	$(CH_2)_2N$(2-pyridyl)$(CH_2)_2$		A(1)	I(1:6)	162-163	$C_{22}H_{21}N_3O$	h	h

[a] See Experimental Section for general methods, reported as method (solvent); specific compound synthesis; list of solvent codes; and purification procedures, reported as procedure (solvent). [b] Yields were 30-80% unless noted otherwise. [c] All compounds gave C, H, and N analyses within 0.4% of the theoretical values unless noted otherwise. [d] Pylorus-ligated rat (50 mg/kg, po); see Experimental Section for method and statistical analyses. [e] Increase in mean pH or percent decrease in mean titratable acid output of gastric juice in treated vs. control groups. [f] Reaction at room temperature for 70 h, yield 9%. [g] C: calcd, 61.07; found, 60.31. [h] Change not statistically significant at this dose by Student's t test at the 0.01 level. [i] Reference 14. [j] Yield 6%. [k] Reference 11. [l] Reference 1c. [m] Reference 10. [n] N: calcd, 10.21; found, 9.76. [o] Melting point of acetate salt. [p] Boiling point, °C (0.05 mm). [q] Solvent was $NH(CH_2CH{=}CH_2)_2$. [r] Reference 15. [s] C: calcd, 73.52; found, 74.17. [t] C: calcd, 74.24; found, 72.80; N: calcd, 13.67; found, 13.10. [u] C: calcd, 78.22; found, 77.11; N: calcd, 8.69; found, 8.25. [v] Reference 16. [w] C: calcd, 77.89; found, 78.34; N: calcd, 9.08; found, 8.56. [x] Reference 17. [y] C: calcd, 83.60; found, 82.70. [z] Yield 22%. [aa] Yield 21%. [bb] C: calcd, 79.72; found, 78.31; N: calcd, 8.85; found, 8.35.

Table II. Effect of Different Routes of Administration on Acid Secretion Inhibition in the PLR

no.	vol[b]	titr acid[c]	acid output[d]
		ED_{50}, po[a]	
32	e	9.8 (7.6-12.6)	6.8 (3.6-12.2)
44	e	7.4 (5.7-9.5)	4.8 (2.6-8.5)
46	e	11.4 (8.8-14.7)	7.3 (4.1-12.1)
35	e	11.6 (6.6-15.9)	8.9 (4.3-16.3)
38	e	14.8 (12.9-16.6)	7.7 (4.0-13.6)
29	e	12.2 (7.5-16.0)	8.9 (4.7-14.9)
		ED_{50}, id[a]	
46	2.2 (0.9-4.7)	6.3 (4.7-9.0)	1.3 (0.5-2.7)
35	1.2 (0.3-2.9)	6.9 (4.6-11.3)	0.9 (0.2-2.1)
38	2.4 (0.6-6.7)	8.4 (6.5-13.7)	1.7 (0.5-4.4)
29	3.8 (1.4-7.8)	10.4 (8.4-12.8)	2.3 (0.8-4.8)
		ED_{50}, iv[a]	
35	6.3 (4.0-19.3)	4.9 (3.5-8.0)	3.2 (1.8-5.9)
38	3.3 (1.5-9.5)	4.1 (2.4-8.3)	2.0 (0.7-5.0)
29	2.3 (0.9-5.3)	2.3 (1.3-4.0)	1.2 (0.3-2.7)

[a] ED_{50} values with 95% confidence limits in parentheses were calculated as described under Experimental Section. [b] Volume, mL/2 h. [c] Titratable acidity, mequiv of H^+/L. [d] μequiv of H^+/2 h. [e] No statistically significant ($p >$ 0.01) dose response.

piperidine,[22] 3-(dimethylamino)piperidine,[22] and 1-(2-aminoethyl)imidazole[23] were prepared by the cited procedures; *N*-ethyl-3-(methylamino)piperidine was synthesized as described below. Chromatography was performed by the "dry column" method as previously described.[24] General methods A-C described below were used with the indicated solvents to synthesize the majority of the compounds listed in Table I.

List of solvents and purification procedures: 1, benzene; 2, toluene; 3, AcOH; 4, EtOH; 5, dioxane; 6, hexane; 7, acetone; 8, Et_2O; 9, MeOH; 10, $CHCl_3$; 11, isopropyl ether; 12, H_2O; I, recrystallization; II, distillation; III, chromatography on silica; IV, chromatography on Al_2O_3.

***N*-Ethyl-3-(methylamino)piperidine.** A solution of *N*-ethyl-3-piperidinone hydrochloride in 75 mL of H_2O was brought to pH 12 with 40% NaOH and extracted with 4 × 100 mL of Et_2O. The extract was dried (K_2CO_3) and evaporated, leaving 7.6 g of the free base. It was dissolved in 20 mL of CH_3OH, mixed with a solution of methylamine (3.1 g, 100 mmol) in 20 mL of CH_3OH, and then added to prereduced PtO_2 (0.25 g) in 20 mL of CH_3OH. After hydrogenation at ca. 3 atm for 1.5 h, H_2 uptake ceased, and the mixture was filtered. The solvent was removed under N_2, and the residue was distilled in vacuo, yielding a colorless liquid (4.4 g, 52%), bp 88-90.5 °C (28 mm).

General Method A(1). *N*-Methyl-*N*-(3-pyridinylmethyl)-9*H*-xanthen-9-amine (41). A solution of 9-hydroxy-9*H*-xanthenyl acetate[10] (3.4 g, 14.2 mmol) and 3-[(methylamino)methyl]pyridine (1.73 g, 14.2 mmol) in 70 mL of benzene was refluxed for 24 h. The cooled solution was washed twice with 50 mL of 5% aqueous $NaHCO_3$ and dried over K_2CO_3. Evaporation of the solvent and recrystallization of the residue from hexane afforded white crystals (2.86 g, 67%), mp 105-107 °C.

General Method B(3:4). 2-(9*H*-Xanthen-9-yl)hydrazinecarboxaldehyde (18). To a solution of formic acid hydrazide (3.0 g, 50.0 mmol) in 25 mL of EtOH-AcOH (1:1) was added 9*H*-xanthe-9-ol[25] (5.0 g, 25.2 mmol). The solution was stirred for 24 h, chilled in an ice bath, and filtered. The solid was washed and recrystallized from EtOH to give white crystals (3.31 g, 52%), mp 155-156 °C.

General Method C(1). 2-[2-(9*H*-xanthen-9-yl)hydrazino]pyridine (19). A solution of 9-hydroxy-9*H*-xanthenyl *N*-methylcarbamate[9] (3.0 g, 11.8 mmol) in 30 mL of dry benzene

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Table III. Effect of 29[a] on Pentagastrin-Induced Acid Secretion in Gastric Fistula Dogs

	1 h predrug: control	hours postdrug 1 h	2 h	3 h
acid output[b]	12.15 ± 1.87	3.37 ± 0.75	2.46 ± 0.96	4.60 ± 0.32
% change		−72	−80	−62

[a] Administered 1 mg/kg iv. [b] Mean titratable acid output plus or minus standard error (equivalents per hour).

Table IV. Antisecretory Activity of 29 in PLR After Preincubation in Gastric Juice

dose of 29, mg/kg	preincubation medium, time	vol, mL	acid titer, mequiv/L	acid output, μequiv/2 h
15	neutralized juice, 60 min	2.5[a]	26[a]	70[a]
15	juice at pH 1.0, 2 min	4.6[b]	43[a]	191[a]
15	juice at pH 1.0, 30 min	6.0	62	367
15	juice at pH 1.0, 60 min	5.6	59	324[b]
vehicle control	neutralized juice	6.2	69	426

[a] Statistically significant at the 0.01 level compared to vehicle controls. [b] Statistically significant at the 0.05 level compared to vehicle controls.

was refluxed with 2-pyridylhydrazine (6.0 g, 55 mmol) until CO_2 evolution ceased. Evaporation of the solvent, trituration with cold acetone, and recrystallization from the same solvent gave cream-colored crystals (2.4 g, 70%), mp 137–138 °C.

3,5-Dimethyl-1-(9*H*-xanthen-9-yl)pyrazole (8). A solution of 3,5-dimethyl-1-pyrazolecarboximidamide nitrate[12] (1.5 g, 7.31 mmol) in 50 mL of absolute EtOH containing the free base of **13** (1.5 g, 5.83 mmol) was refluxed for 2 h. The solution was concentrated in vacuo. The precipitate was recrystallized from EtOH, treated with cold 10% NaOH, and extracted into ether. Evaporation of the solvent and recrystallization of the residue from hexane afforded white crystals (0.45 g, 28%), mp 140–141 °C.

Ethyl *N*-(9*H*-Xanthen-9-yl)ethanimidate (16). A stirred suspension of powdered acetamide (0.46 g, 7.8 mmol) in 15 mL of dry methylene chloride was treated dropwise under N_2 at −10 °C with a solution of triethyloxonium fluoroborate (1.46 g, 7.7 mmol) in 10 mL of dry CH_2Cl_2 and stirred at room temperature for 18 h. A solution of **13** (1.23 g, 6.22 mmol) in 5 mL of dry CH_2Cl_2 was added. The mixture was stirred for 3 h and filtered, and the filtrate was concentrated. Recrystallization of the residue from hexane afforded crystals (0.6 g, 36%), mp 74–76.5 °C.

***N*-(9*H*-Xanthen-9-yl)ethanimidamide (17).** A solution of **16** (1.8 g, 9.0 mmol) in 50 mL of absolute EtOH was treated at 0 °C with a three fold excess of anhydrous ammonia in EtOH and stirred at 25 °C for 48 h. Evaporation of the solvent and recrystallization of the residue from benzene–hexane gave white needles (0.66 g, 41%), mp 149–151.5 °C.

1-(9*H*-Xanthen-9-yl)-2,5-pyrrolidinedione (4). A stirred solution of xanthene (9.1 g, 50 mmol) in 300 mL of CCl_4 was treated with *N*-bromosuccinimide (8.9 g, 50 mmol) and benzoyl peroxide (0.15 g, 0.7 mmol) and refluxed for 24 h. Filtration of the solid from the cooled mixture and recrystallization from acetone afforded white crystals (7.1 g, 51%), mp 246–248 °C.

Statistical Methods. The Student's t test was used to determine statistical significance in comparing group responses.[26] Significance at the $p < 0.01$ level was chosen as the criterion for activity in the PLR assay. The ED_{50}s with 95% confidence limits were calculated by using parallel line bioassay methods and Fieller's theorum.[27] For the oral and intraduodenal titratable acid parameters, the dose–response lines deviated slightly but statistically significantly from parallelism; therefore, the individual lines were employed for the ED_{50} calculations in these two cases.

Biological Test Methods. Pylorus-Ligated Rat.[18] Male Carworth Farms rats (270–360 g) were food deprived with full access to water for 18 h. While the rat was under ether anesthesia, the abdomen was opened, and the pylorus was securely ligated. Test compounds in PEG 200 were administered to six rats either by gavage 1 h before ligation, intraduodenally immediately following ligation, or intravenously at the time of ligation. The animals were sacrificed 2 h after ligation, and the gastric contents were collected and centrifuged. The gastric juice volumes and pHs were measured, and an aliquot of the supernatant was titrated with standardized aqueous NaOH to pH 7.4 to determine the titratable acid concentration. The titratable acid output was calculated as the product of volume and the titratable acid concentration. All data are presented as the mean of the experimental group of six to seven animals. Propantheline bromide (0.5 mg/kg) administered intravenously resulted in an insignificant change in gastric pH and a 95% reduction in titratable acid output. Experiments involving preincubation of compound in gastric juice had the following modifications: gastric juice was obtained from separate 4-h ligated rats and stored frozen; each compound was dissolved in PEG 200 (80 mg/mL) and added to gastric juice to give a concentration of 7.5 mg/mL; the gastric juice was neutralized with 1.0 N NaOH either before or after addition of test compound; the compound solutions were administered intraduodenally immediately following ligation.

Gastric Fistula Dog. Four chronic fistula dogs (11.2–16.2 kg) with a gastric cannula implanted in the fundus of the stomach were fasted 24 h. The lateral saphenous vein was catheterized, and a saline infusion was maintained until the pH of gastric juice samples (collected at 15-min intervals) remained above 5.0 for 30 min. Infusion of 8 (μg/kg)/h of pentagastrin produced maximal stimulation. After a plateau of acid secretion had been established for 1 h, **29** (1 mg/kg iv) was administered as a solution in PEG 200. Sample volume and titratable acid concentration were determined on the centrifuged gastric juice, and the titratable acid output was calculated. The values in Table III are given as the mean plus or minus standard error.

Dog Blood Level Studies. Samples of blood and standard solutions of compound, 1 mL each, were diluted with 1 mL of water and adjusted to pH 10.2 with 10% aqueous NaOH. Hexane (4 mL) was added, and the mixture was shaken for 20 min. Following centrifugation, 3 mL of the hexane layer was removed, and an internal standard (Tofranil) was added. This solution was evaporated under nitrogen, and the residue was analyzed by flame-ionization GC on a 0.25 in. × 6 ft glass column containing 0.1% OV-1 on GLC-110. This method gave a sensitivity of 0.5 μg of compound per milliter of blood.

When this assay procedure was employed, a solution containing 3 μg/mL of **29** in dog blood at pH 10.2 was stable for 4 days. Similar incubation of **29** at pH 7.5 resulted in detection of two thirds of the compound unchanged after 20 min. However, no **29** was detected in blood samples taken from a dog at 0, 15, 30, 60, and 120 min after a 2 mg/kg iv dose. If a typical blood volume of 60–70 mL/kg in the dog is assumed, the maximum blood level of **29** might reach 28–33 μg/mL immediately after iv dosing, which is over 50 times greater than the detection limit of this method.

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Structure–Activity Relationships of Convulsant and Anticonvulsant Barbiturates: A Computer-Graphic-Based Pattern-Recognition Analysis[1]

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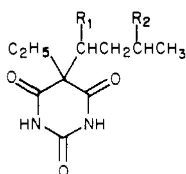
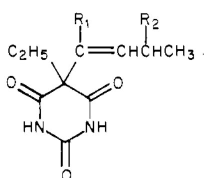
A computer-graphic-based pattern-recognition study of two series of 5-ethyl-5-substituted barbiturates has been undertaken in an attempt to find a correlation between molecular conformation and convulsant and anticonvulsant activity. Studies of a first (trial) set of barbiturates related to pentobarbital revealed a region of space in which at least one low-energy conformation of the hydrocarbon side chain of each of the anticonvulsant barbiturates resides. Another region was occupied by a low-energy conformation of each of the convulsant barbiturates. These regions of space are, thus, possible pharmacophores for convulsant and anticonvulsant activity. Analysis of a second (test) set of barbiturates related to phenobarbital has shown that the activities and structures of these molecules are consistent with the above model. These pharmacophores thus provide a basis for the design of rigid, new analogues with potent convulsant or anticonvulsant activities.

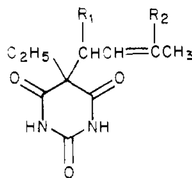
Studies on barbiturates[2,3] and related drugs[4-6] indicate that variations in molecular conformation rather than electronic or other physicochemical properties are responsible for the qualitative activity differences between structurally related convulsant and anticonvulsant drugs. In the series of barbiturates 1–3, for example, compounds



1a, $R_1 = R_2 = H$ (butethal)
b, $R_1 = CH_3$; $R_2 = H$ (pentobarbital)
c, $R_1 = H$; $R_2 = CH_3$ (amytal)
d, $R_1 = R_2 = CH_3$



2a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$ (vinbarbital)
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$



3a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$

1a, **2d**, **3c**, and **3d** are convulsant, while the remainder, although closely related, are anticonvulsant.[2,3] The importance of conformation in determining the qualitative activity differences is particularly evident in **1d**, for which the *S*(−) isomer is anticonvulsant, while the *R*(+) isomer and the racemic compound are potent convulsants.[7] In an effort to explain the structure–activity relationships of these barbiturates, we have studied their molecular conformations using classical[8] and molecular orbital[9] potential energy calculations, ^1H and ^{13}C NMR,[10] and X-ray crystallography.[11] However, while the preceding data provide details of the solution, crystal, and gas phase conformations, they do not directly identify the convulsant and anticonvulsant pharmacophores responsible for biological activity. In the present paper, we report a computer-graphic-based pattern-recognition study of barbiturates **1–3** that has enabled us to identify the distinct conformational regions likely to be responsible for convulsant and anticonvulsant activity in this series. We also report potential energy calculations on two more series of barbiturates in which small structural changes convert the anticonvulsant **4**[12] to the convulsants **5** and **6**[13] and the anticonvulsants **7** and **8** to the convulsants **9** and **10**.[12,14]

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