70, 72). The investigations on the isolated guinea pig right atrium and ileum were performed according to the experimental conditions previously described.[10] Intrinsic activity, pD_2, and pA_2 were determined from cumulative dose-response curves[31] with histamine dihydrochloride as standard agonist. The incubation time (usually 25 min) after dosage of each single concentration of the guanidines was adapted to the time required for achieving an equilibrium. H_2-receptor selectivity was verified by experiments in presence of cimetidine (10 μM and 1 μM) and metroprolol (1 μM). The positive chronotropic effect in the atrium was not blocked by metoprolol but was competitively antagonized by cimetidine, e.g., the pA_2 value found for cimetidine versus **63** was 6.6 and was not significantly different from the pA_2 found versus histamine (pA_2 = 6.4). The time required for washing out **63** from the tissue after submaximal (ca. 90%) stimulation was over 150 min in comparison with about 25 min for washing out histamine.

The evaluation of the positive inotropic activity on the isolated electrically stimulated (1 Hz, duration 1 ms) guinea pig papillary muscle was performed analogously to a method reported[24] using guinea pig papillary muscles of the right ventricle (Krebs-Henseleit solution, gassed with O_2/CO_2 95:5, bath temperature 32.5 °C). The pD_2 values were calculated from increase in contractile force in cumulative concentration-response curves.

The investigations at isolated perfused, guinea pig hearts (Langendorff technique) followed a procedure described[32] using impromidine as reference compound. The compounds were injected as a bolus directly into the perfusion stream.

Acknowledgment. I am grateful to Monika Ewald-Feldt, Berlin (West), and Dr. H. Engler, Heumann Pharma, Nuremberg (FRG), who performed the pharmacological tests on the isolated atrium and ileum of the guinea pig, and to Dr. G. Baumann, Munich (FRG), for the investigations on isolated, working guinea pig hearts and for excellent cooperation. Heumann Pharma, Nuremberg (FRG), is thanked for support of this work; especially, the valuable collaboration with Dr. H. Schickaneder is greatly acknowledged.

(31) van Rossum, J. M. *Arch. Int. Pharmacodyn. Ther.* **1963**, *143*, 299.

(32) Schrader, J.; Baumann, G.; Gerlach, E. *Pflügers Arch.* **1977**, *372*, 29.

2-[[(4-Amino-2-pyridyl)methyl]sulfinyl]benzimidazole H^+/K^+-ATPase Inhibitors. The Relationship between Pyridine Basicity, Stability, and Activity

Robert J. Ife,* Catherine A. Dyke, David J. Keeling, Eugene Meenan, Malcolm L. Meeson, Michael E. Parsons, Carolyn A. Price, Colin J. Theobald, and Anthony H. Underwood

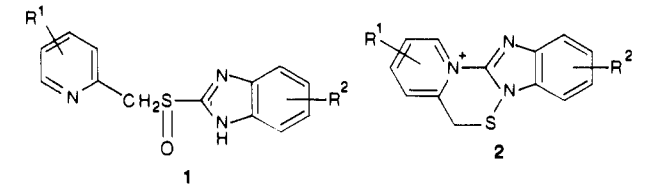
Smith Kline & French Research Ltd., The Frythe, Welwyn, Hertfordshire, England. *Received December 12, 1988*

The benzimidazole sulfoxide class of antisecretory H^+/K^+-ATPase inhibitors need to possess high stability under neutral physiological conditions yet rearrange rapidly at low pH to the active sulfenamide **2**. Since the initial reaction involves internal nucleophilic attack by the pyridine nitrogen, control of the pyridine pK_a is critical. In this paper we show that by utilizing the powerful electron-donating effect of a 4-amino substituent on the pyridine, moderated by the electron-withdrawing effect of a 3- or 5-halogen substituent, a combination of high potency (as inhibitors of histamine-stimulated gastric acid secretion) and good stability under physiological conditions can be obtained. Furthermore, the role of the steric interaction between the 3/5-substituents and the 4-substituent in modifying the electron-donating ability of the 4-amino group is exemplified, and additional factors affecting stability are identified. One compound, in particular, 2-[[(3-chloro-4-morpholino-2-pyridyl)methyl]sulfinyl]-5-methoxy-(1*H*)-benzimidazole (**3a**, SK&F 95601), was chosen for further development and evaluation in man.

Inhibition of the proton-pumping H^+/K^+-ATPase as a means of controlling gastric pH has attracted considerable attention in recent years with the discovery of the benzimidazole sulfoxide class of antisecretory agents.[1-3] Such compounds, in particular omeprazole (**1a**), have proved to be effective in the clinic for the treatment of acid-related gastrointestinal disorders. It has been demonstrated that irreversible inhibition of the H^+/K^+-ATPase occurs following acid activation of these compounds within the acidic compartments of the parietal cell and covalent binding of a reactive intermediate to one or more critical thiol groups on the enzyme present in the apical membrane.[5-7]

The chemical transformations that take place at low pH have recently been established and shown to involve an internal rearrangement, initiated by attack of the pyridine nitrogen on the benzimidazole 2-position, followed by a complex cascade of reactions, the cyclic sulfenamide (**2**) being postulated as the principal thiophilic species responsible for activity.[8-11] The direct relationship between the basicity of the pyridine and biological activity previously reported[15,16] can therefore, in part, be accounted for in terms of the increasing reactivity of the parent sulfoxide.

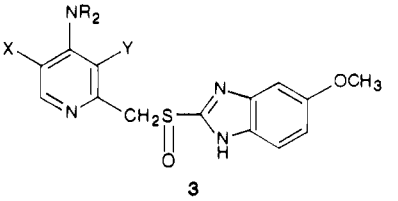
With the aim of identifying compounds combining high potency as inhibitors of histamine-stimulated gastric acid secretion with good stability under physiological conditions, during the course of our work to elucidate the



1

2

1a (omeprazole): R^2 = 5-OCH_3; R^1 = 3-CH_3, 4-OCH_3, 5-CH_3



3

* Author to whom correspondence should be addressed.

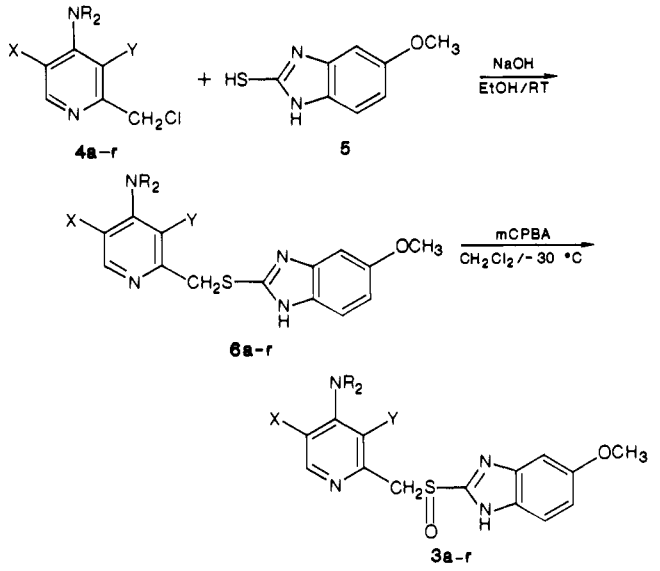
(1) Olbe, L.; Berglindh, T.; Elander, B.; Helander, H.; Fellenius, E.; Sjostrand, S. E.; Sundell, G.; Wallmark, B. *Scand. J. Gastroenterol.* **1979**, *14*, (*Suppl. 55*), 131.

(2) Fellenius, E.; Berglindh, T.; Sachs, G.; Olbe, L.; Elander, B.; Sjostrand, S. E.; Wallmark, B. *Nature (London)* **1981**, *290*, 159.

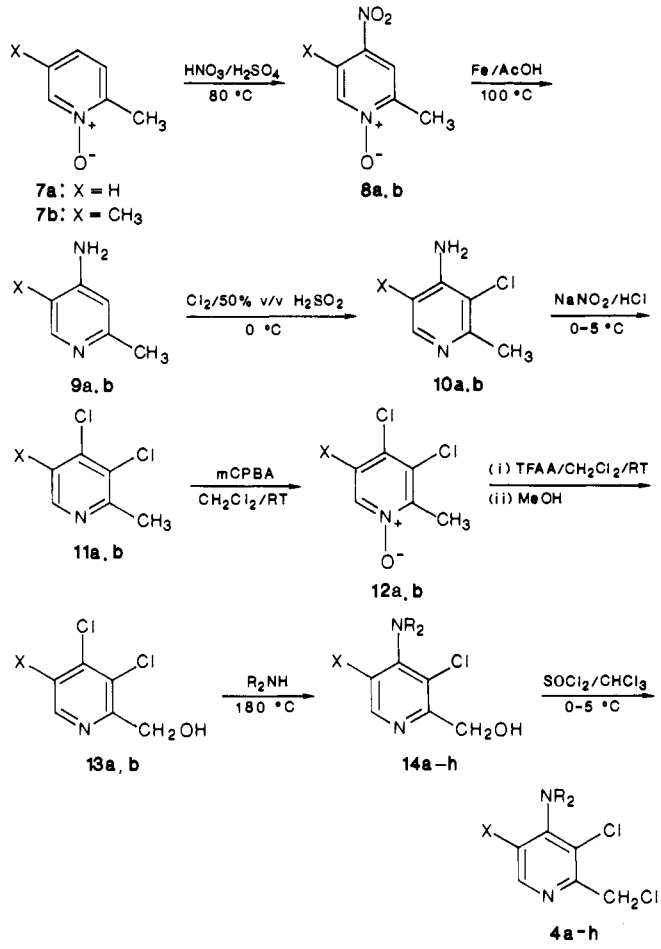
(3) Brandstrom, A.; Lindberg, P.; Junggren, U.; Wallmark, B. *Scand. J. Gastroenterol.* **1986**, *21* (*Suppl. 118*), 54.

0022-2623/89/1832-1970$01.50/0

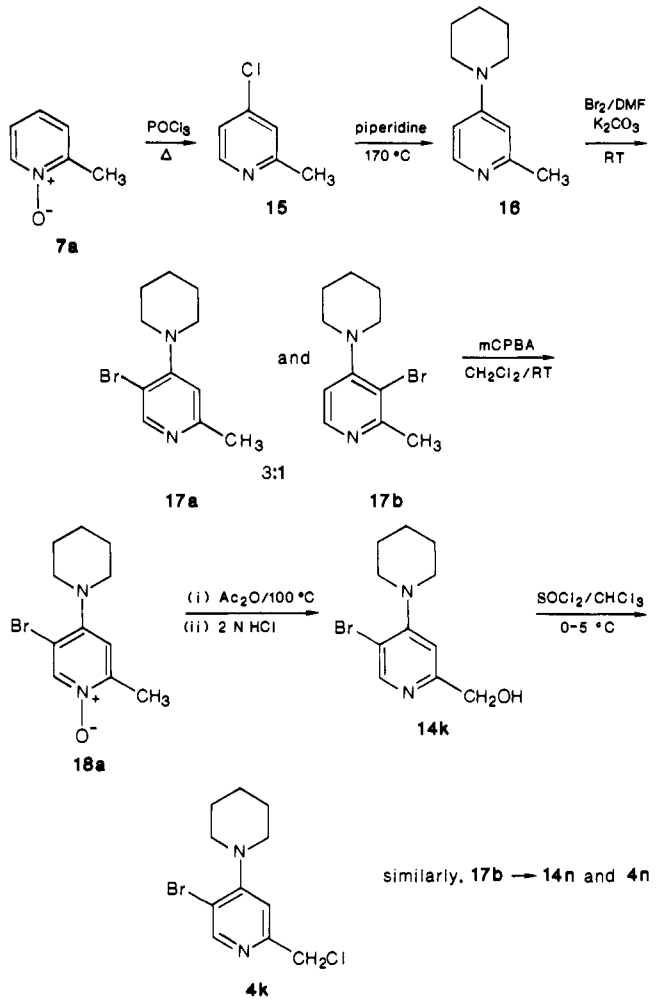
Scheme I



Scheme II



Scheme III



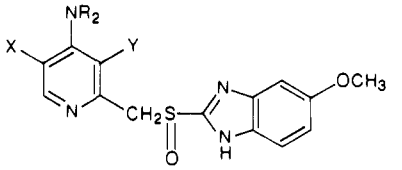
mechanism of action of these compounds, the relationship between the pyridine pK_a, stability, and biological activity in the series of compounds of general structure **3** was investigated. In this paper we show that by utilizing the opposing electronic characteristics of amino and halo substituents on the pyridine ring, compounds with the desired combination of properties can be obtained.

Chemistry

All compounds in Table I were synthesized by the reaction of the appropriately substituted 2-(chloromethyl)-

(4) Clisold, S. P.; Campoli-Richards, D. M. *Drugs* **1986**, *32*, 15.
(5) Lorentzon, P.; Eklundh, B.; Brandstrom, A.; Wallmark, B. *Biochim. Biophys. Acta* **1985**, *817*, 25.
(6) Keeling, D. J.; Fallowfield, C.; Milliner, K. J.; Tingley, S. K.; Ife, R. J.; Underwood, A. H. *Biochem. Pharmacol.* **1985**, *34*, 2967.
(7) Keeling, D. J.; Fallowfield, C.; Underwood, A. H. *Biochem. Pharmacol.* **1987**, *36*, 339.
(8) Figala, V.; Klemm, K.; Kohl, B.; Kruger, U.; Rainer, G.; Schaefer, H.; Senn-Bilfinger, J.; Sturm, E. *J. Chem. Soc., Chem. Commun.* **1986**, 125.
(9) Lindberg, P.; Nordberg, P.; Alminger, T.; Brandstrom, A.; Wallmark, B. *J. Med. Chem.* **1986**, *29*, 1329.
(10) Sturm, E.; Kruger, U.; Senn-Bilfinger, J.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. *J. Org. Chem.* **1987**, *52*, 4573.
(11) Senn-Bilfinger, J.; Kruger, U.; Sturm, E.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. *J. Org. Chem.* **1987**, *52*, 4582.
(12) Brandstrom, A.; Lindberg, P.; Junggrens, U. *Scand. J. Gastroenterol.* **1985**, *20* (*Suppl. 108*), 15.
(13) Lindberg, P.; Brandstrom, A.; Wallmark, B. In *Proceedings of the IXth International Symposium on Medicinal Chemistry*; Mutschler, E., Winterfeldt, E., Eds.; VCH: Berlin, 1987; p 457.
(14) Van Allan, J. A.; Deacon, B. D. *Org. Synth., Collect. Vol. IV*, **1963**, 569.
(15) Talik, Z.; Brekiesz, B. *Rocz. Chem.* **1967**, *41*, 279.
(16) Blanz, E. J., Jr.; French, F. A.; DoAmarol, J. R.; French, D. A. *J. Med. Chem.* **1970**, *13*, 1124.

Table I. 2-[[(3/5-Halo-4-amino-2-pyridyl)methyl]sulfinyl]-5-methoxybenzimidazoles—Synthesis



compd	NR_2	X	Y	route of synthesis	crystn solvent	yield, %[a]	mp, °C[b]	formula[c]
3a		H	Cl	A	EtOH	68	indeterminate	$C_{18}H_{19}ClN_4O_3S$
3b		H	Cl	A	$EtOH/CH_3CN$	35	indeterminate	$C_{19}H_{21}ClN_4O_2S$
3c		H	Cl	A	$EtOH/CH_3CN$	45	indeterminate	$C_{18}H_{19}ClN_4O_2S$
3d	$N(CH_3)_2$	H	Cl	A	EtOAc	23	113–4	$C_{16}H_{17}ClN_4O_2S$
3e		CH_3	Cl	A	CH_3CN	30	147–8	$C_{19}H_{21}ClN_4O_3S$
3f		CH_3	Cl	A	CH_3CN	33	151–2	$C_{20}H_{23}ClN_4O_2S$
3g		CH_3	Cl	A	CH_3CN	62	145–6	$C_{19}H_{21}ClN_4O_2S$
3h	$N(CH_3)_2$	CH_3	Cl	A	CH_3CN	64	147–9	$C_{17}H_{19}ClN_4O_2S$
3i	$N(CH_3)_2$	Cl	H	C	EtOAc	50	155–7	$C_{16}H_{17}ClN_4O_2S$
3j		Br	H	C	CH_3CN	79	indeterminate	$C_{18}H_{19}BrN_4O_3S$
3k		Br	H	B	ether	79	114–8	$C_{19}H_{21}BrN_4O_2S$
3l		Br	H	C	CH_3CN	62	indeterminate	$C_{18}H_{19}BrN_4O_2S$[d]
3m	$N(CH_3)_2$	Br	H	C	CH_3CN	73	indeterminate	$C_{16}H_{17}BrN_4O_2S$
3n		H	Br	B	ether	43	indeterminate	$C_{19}H_{21}BrN_4O_2S \cdot H_2O$
3o		H	F	C	EtOAc	65	142–6	$C_{18}H_{19}FN_4O_3S$
3p	$N(CH_3)_2$	H	F	C	EtOAc	63	145–7	$C_{16}H_{17}FN_4O_2S$
3q		F	H	C	EtOAc	71	109–11	$C_{18}H_{19}FN_4O_3S \cdot 0.15H_2O$
3r	$N(CH_3)_2$	F	H	C	EtOAc	49	99–101	$C_{16}H_{17}FN_4O_2S$

[a] Yields from sulfide have not been optimized. [b] ^1H NMR and IR were consistent with the assigned structures. Unless indicated, all values for C, H, N, S, Br, or Cl were within ±0.4% of calculated values. [c] Compounds decompose on melting; clearly defined melting points are not always obtainable. [d] C, H, N, S, Br requires 1.7% w/w CH_3CN and 1.2% w/w $CHCl_3$.

pyridine **4** with 5-methoxy-2-mercaptobenzimidazole (**5**),[14] followed by low-temperature oxidation of the sulfide **6** with *m*-chloroperbenzoic acid (Scheme I). The (chloromethyl)pyridines were prepared by a number of alternative routes.

The 3-chloropyridines for compounds **3a–h** were synthesized according to method A as indicated in Scheme II. This route allowed maximum flexibility by introducing the 4-amino substituent at a late stage. In the case of the 5-unsubstituted compounds **3a–d**, advantage was taken of the regioselective chlorination of 4-amino-2-picoline (**9a**). This reaction is of interest since chlorination appears to take place exclusively in the more hindered 3-position. Furthermore, while chlorination of 4-amino-2,5-lutidine (**9b**) occurred readily to give the desired 3-chloro derivative (**10b**), all attempts to carry out a similar reaction with 4-amino-2,3-lutidine failed to give any of the required 5-chloro derivative.

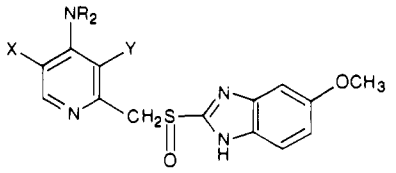
Intermediates for the 4-piperidino bromo derivatives **3k** and **3n** were prepared using method B (Scheme III). Interestingly, despite the larger bulk of both the halogen an the 4-amino substituent, significant bromination still occurs in the 3-position. All other compounds were prepared according to method C (Scheme IV), from halo 2-picolines prepared by known synthetic methods.[15–17]

Results and Discussion

All compounds were initially assessed for their ability to inhibit histamine-stimulated gastric acid secretion in the lumen perfused rat model after intravenous or intraduodenal administration, with the more interesting compounds being evaluated further in the Heidenhain pouch dog. The available data are summarized in Table II. Due to the high lability of the benzimidazoles at low pH we chose not to measure routinely the pyridine pK_a of the final products but rather the pK_a of the corresponding hydroxymethyl intermediates **14**. These are approximately

(17) Abblard, J.; Decoret, C.; Cronenberger, L.; Pacheco, H. *Bull. Soc. Chim. Fr.* **1972**, *6*, 2466.

Table II. 2-[[(3/5-Halo-4-amino-2-pyridyl)methyl]sulfinyl]-5-methoxybenzimidazoles—Biological Activity and Stability



compd	NR_2	X	Y	$PyCH_2OH$ pK_a[a]	$t_{1/2}$, h, pH 7.4[b]	inhibn of histamine-stimulated gastric acid secretion: rat[c] ED_{50}, μmol/kg	inhibn of histamine-stimulated gastric acid secretion: HP dog[d] ED_{50}, μmol/kg iv
3a		H	Cl	5.28	1296	1.17 (iv) [0.59–2.18] 1.14 (id) [0.79–1.53]	1.01 [0.79–1.30]
3b		H	Cl	5.95	175	7.72 (id) [4.64–15.9]	1.16 [0.87–1.50]
3c		H	Cl	7.58	36	poor solubility	
3d	$N(CH_3)_2$	H	Cl		17	0.44 (iv) [0.35–0.55] 0.38 (id) [0.16–0.65]	0.26 [0.16–0.46]
3e		CH_3	Cl	5.32	>3000	1.94 (id) [1.29–1.73]	2.20 [wide limits]
3f		CH_3	Cl		444	2.38 (iv) [1.44–3.17] 2.51 (id) [0.91–5.60]	
3g		CH_3	Cl		60	0.82 (iv) [0.36–1.78]	0.57 [0.47–0.64]
3h	$N(CH_3)_2$	CH_3	Cl	5.92	432	0.34 (iv) [0.24–0.44] 1.48 (id) [0.98–2.14]	0.29 [0.20–0.37]
3i	$N(CH_3)_2$	Cl	H		96	1.32 (iv) [0.50–2.13]	0.77 [0.41–0.99]
3j		Br	H		nonlinear kinetics	14% inhibn at 1 μmol/kg iv	
3k		Br	H	5.40	864	0.85 (iv) [0.36–1.90] 1.05 (id) [0.62–1.53]	2.94 [2.64–3.97]
3l		Br	H	6.92	49	not dose related	
3m	$N(CH_3)_2$	Br	H	5.80	101	0.26 (iv) [0.04–0.61] 1.75 (id) [0.87–2.72]	0.91 [0.41–1.61]
3n		H	Br		300	0.85 (iv) [0.27–2.40]	
3o		H	F	5.89	144	0.7 (iv) [0.46–1.00] 3.2 (id) [2.22–4.01]	2.75 [2.02–5.97]
3p	$N(CH_3)_2$	H	F	7.12	11	not dose related	
3q		F	H	5.72	418	0.65 (id) [wide limits]	5.07 [3.39–11.3]
3r	$N(CH_3)_2$	F	H	6.96	26	37% inhibn at 1 μmol/kg iv	

[a] pK_a at 37 °C of the corresponding 2-(hydroxymethyl)pyridine intermediates **14**. [b] Half-life in acetonitrile/buffer (1:3) at ambient temperature. [c] Lumen perfused anaesthetized rat [ED_{50}, 95% confidence limits, $n = 9$]. [d] Conscious Heidenhain pouch dog [ED_{50}, 95% confidence limits, $n = 9$].

2 log units higher than the corresponding sulfoxide[18] and, where available, are also listed in Table II. Low aqueous solubility precluded stability measurements in water alone. Half-lives were therefore assessed in an acetonitrile/buffer mixture, and again, these data are listed in Table II.

Although a 4-amino substituent would be the most effective way to raise the pyridine pK_a, there is, however, a limitation. For a compound to be useful it must possess relatively high stability around neutral pH yet rearrange rapidly at low pH, thereby optimizing selectivity. With very high pK_a's the compounds become too unstable to be of any interest. Furthermore, such compounds become increasingly difficult to isolate and handle following the final-stage oxidation of the sulfide.[19,20] However, by modulating the powerful electron-donating effect of the 4-amino substituent with an electron-withdrawing halogen

(18) As an illustration, the pK_a of omeprazole is reported to be 3.98 (ref 13) and the pK_a of the corresponding hydroxymethyl intermediate is 6.29 (this paper).

(19) Certain simple amino analogues have been prepared, e.g., the deschloro analogue of **3a** ($t_{1/2}$ 5.2 h compared with 1296 h for **3a**); L. P. Crawford and C. A. Dyke, unpublished SK&F results. See also: Sih, J. C. US Pat. US 4,619,997, and King, F. D.; Joiner, K. R. Eur. Pat. Appl. EP 178,438.

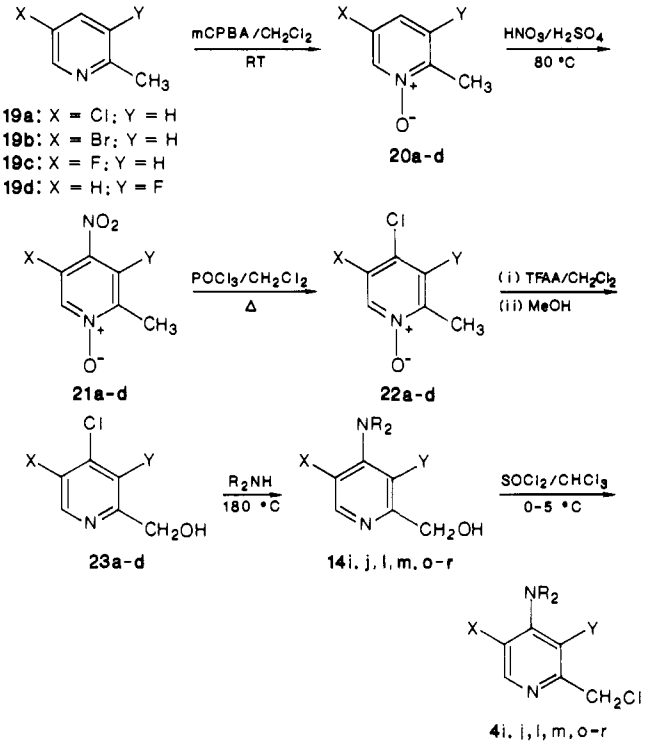
(20) In the present series we generally found that compounds with $t_{1/2}$'s of less than ca. 50 h became increasingly difficult to handle in a routine way.

Table III. Comparison of Compounds 3a and 3h with Omeprazole

compd	logP^a	PyCH$_2$OH pK_a[b]	$t_{1/2}$ pH 7.4[c]	$t_{1/2}$ pH 1.0[c]	Heidenhain pouch dog ED$_{50}$, μmol/kg iv[d]	Heidenhain pouch dog % inhibn (±SEM) id[e]
3a	1.81	5.28	1296 h	1 min	1.01 [0.79–1.30]	48 ± 9 at 2 μmol/kg
3h		5.92	432 h	1 min	0.29 [0.20–0.37]	67 ± 7 at 1 μmol/kg
omeprazole (**1a**)	2.18	6.29	456 h	2 min	0.20 [0.07–0.54]	53 ± 8 at 1 μmol/kg

[a] Determined at 37 °C at pH 7.4 for compound **3a** and 7.55 for **1a**. [b] pK_a at 37 °C of the corresponding 2-(hydroxymethyl)pyridine intermediates **14**. [c] Half-life in acetonitrile/buffer (1:3) at ambient temperature. [d] [ED$_{50}$, 95% confidence limits, n = 9]. [e] Due to the time course difference (Figure 1), peak inhibition is given for compound **3a** and mean plateau inhibition from 1 to $2^{1/2}$ h for compounds **3h** and **1a**. The same group of four dogs was used for each compound.

Scheme IV



substituent, general structure **3**, reactivity can be controlled.

In addition to the electronic effects of the pyridine substituents, as in the case of omeprazole,[12] the pK_a and stability of the compounds are also dependent on the steric interaction between the substituents which gives rise to a loss of conjugation between the 4-substituent and the pyridine ring. Unlike the situation in omeprazole-related compounds, however, the electronic effect of the 3/5-halogen and steric interaction with the 4-substituent act in concert to affect the pK_a.

Since the electronic effect of the three halogen substituents on the pyridine pK_a is the same in each case,[21] differences in pK_a and stability of **3** with similar 4-substituents can be related directly to the size of the halogen substituent. Thus, when a pair of 4-(dimethylamino) analogues are compared, for example, compounds **3m** and **3r**, the larger 5-bromo substituent can be seen to lead to a lower pK_a (pK_a's 5.80 and 6.96 for **14m** and **14r**, respectively) and increased stability compared to the corresponding 5-fluoro analogue ($t_{1/2}$'s 101 and 26 h, respectively).

The effect of varying the nature of the 4-amino substituent on pK_a and stability is more complex since both steric and electronic factors are involved. The observed order of stability and basicity for the series of cyclic amino 3-chloro analogues **3a–c** and corresponding hydroxymethyl intermediates **14a–c**, however, does correlate with reported σ_p values which incorporate both an electronic and an appropriate steric component (morpholino –0.5, piperidino –0.57, and pyrrolidino –0.9).[22] The dimethyl analogue **3d** ($t_{1/2}$ 17 h), however, does appear to be more unstable than would have been predicted simply on the basis of the corresponding σ_p value (–0.7).

Interestingly, the introduction of an electron-releasing 5-methyl substituent has little effect on the pyridine pK_a; compare compounds **14a** and **14e**. In this case, it would appear that the electron-donating effect of the substituent is essentially canceled by the increased twisting about N–C$_{py}$ bond. In addition, despite the similar pyridine pK_a's, the 5-methyl substituent appears to confer increased stability; compare **3a** and **3e**. Clearly, there are effects on reactivity over and above those arising simply from the differences in pyridine pK_a's. This is further illustrated by the higher reactivity of 3-substituted compounds over the corresponding 5-substituted analogues; compare compounds **3i** and **3d**, compounds **3r** and **3p**, compounds **3q** and **3o**, and compounds **3k** and **3n**.

The compounds described in this paper cover a wide range of stabilities. Compound **3d** [X = H, Y = Cl, NR$_2$ = N(CH$_3$)$_2$], for example, is a highly potent inhibitor of histamine-stimulated gastric acid secretion in both the rat and dog. However, with a half-life of only 17 h, this compound was particularly difficult to handle and was considered too unstable for further evaluation. Compound **3h** [X = CH$_3$, Y = Cl, NR$_2$ = N(CH$_3$)$_2$], on the other hand, is of similar activity to compound **3d** after intravenous administration but with greatly improved stability ($t_{1/2}$ 432 h), clearly demonstrating the potential for separating in vivo potency and stability.

In selecting compounds for further evaluation, additional criteria were also taken into consideration. Thus, compound **3a** (X = H, Y = Cl, NR$_2$ = morpholino) combined a satisfactory level of potency in the rat, after both intravenous and intraduodenal administration, with very high stability and good water solubility for this class of compound (ca. 15 mg mL^{-1} at pH 10). Table III compares compounds **3a** and **3h** with omeprazole. All three compounds display a high level of acid activation but, when the stabilities at high and low pH are compared, compound **3a** is the most pH selective.

Figure 1 shows the time course of inhibition of histamine-stimulated acid secretion after intraduodenal administration in the Heidenhain pouch dog. Whereas compound **3h** and omeprazole attain maximum inhibition after 1 h, compound **3a** takes some $2^1/_2$ h to peak. On the basis of the peak effect, compound **3a** was found to be

(21) The pK_a's of 3-bromo-, 3-chloro-, and 3-fluoropyridine are 2.84, 2.84, and 2.97 at 25 °C, respectively, compared with 5.2 for pyridine. From: Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.

(22) Gum, W. F., Jr.; Joullie, M. M. *J. Org. Chem.* **1967**, *32*, 53.

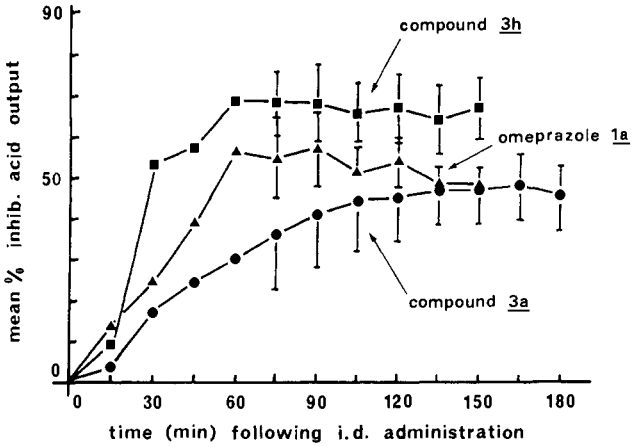


Figure 1. Time course of mean (n = 4) inhibition of histamine-stimulated gastric acid secretion in the Heidenhain pouch dog following id administration of omeprazole (**1a**; 1 μmol/kg) and compounds **3a** (2 μmol/kg) and **3h** (1 μmol/kg).

around half as potent of omeprazole whereas compound **3h** appears to be marginally more potent after intraduodenal administration, although statistically, this was not significant.[23]

From the above discussion, it is apparent that there is not a simple relationship between the pyridine basicity, stability, and in vivo biological activity. However, by utilizing the electron-withdrawing effect of a halogen substituent and the steric interaction between substituents on the pyridine ring, the reactivity of 4-aminopyridyl benzimidazole sulfoxides can be modulated. Compounds combining high potency as inhibitors of histamine-stimulated gastric acid secretion with good stability under physiological conditions can be identified. Despite the higher potency of **3h**, however, compound **3a** SK&F 95601, was chosen for further development and evaluation in man on the basis of its greater stability and solubility.

Experimental Section

Chemistry. Melting points were determined with a Buchi 510 melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 580B spectrometer. ^1H NMR spectra were recorded at 250 MHz on a Bruker AM250 spectrometer or at 60 MHz on a Jeol PMX60 spectrometer, and chemical shifts are reported in parts per million (δ) downfield from the internal standard Me_4Si. Mass spectra were obtained on a VG70-70F (Altrincham, U.K.) spectrometer. Elemental analyses (C, H, N) were performed on a Perkin-Elmer PE240 instrument. Analytical figures were all within ±0.4% of theoretical unless otherwise indicated. Preparative column chromatography was conducted by using silica gel 60 (70–230 mesh ASTM) from Merck.

Synthesis of Benzimidazole Sulfoxides—General Procedures.[24] **Method A. 4-Amino-3-chloro-2-methylpyridine (10a).** Previously condensed chlorine (20 mL) was allowed to evaporate into a stirred, ice-cooled solution of 4-amino-2-methylpyridine (**9a**)[25] (44 g, 0.407 mol) in sulfuric acid (1 L, 50% v/v) in a flask fitted with a dry ice–acetone condenser. After stirring for a further 2 h, the solution was treated with 40% sodium hydroxide to pH 13, while not allowing the temperature to rise above 50 °C. The mixture was extracted thoroughly with ether, and the combined extracts were dried (K_2CO_3), treated with charcoal, filtered, and evaporated to low volume in vacuo. Addition of petroleum ether (40/60) gave a white solid which was recrystallized from chloroform/petroleum ether (40/60) to yield **10a**, 37.23 g (64%), mp 108–10 °C. Anal. ($C_6H_7ClN_2$) C, H, N. NMR ($CDCl_3$) δ 2.54 (3 H, s, CH_3), 4.60 (2 H, br s, NH_2), 6.49 (1 H, d, J = 5.5 Hz, py-5H), 7.98 (1 H, d, J = 5.5 Hz, py-6H).

3,4-Dichloro-2-methylpyridine (11a). Sodium nitrite (23.05 g, 0.485 mol) was added in portions to a stirred solution of **10a** (33.46 g, 0.162 mol) in concentrated hydrochloric acid (700 mL) at 0–5 °C. After 1 h at 0–5 °C and 2 h at 25 °C, 40% sodium hydroxide was added dropwise, keeping the temperature below 50 °C by means of an ice bath. The mixture was extracted three times with ether, and the combined extracts were dried (K_2CO_3) and evaporated in vacuo to give **11a** as a low-melting solid, 25.25 g (96%). NMR ($CDCl_3$) δ 2.67 (3 H, s, CH_3), 7.25 (1 H, d, J = 5 Hz, py-5H), 8.26 (1 H, d, J = 5 Hz, py-6H); MS (EI) m/z 163 (base), 161, 126.

3,4-Dichloro-2-(hydroxymethyl)pyridine (13a). *m*-Chloroperbenzoic acid (32.63 g, 0.189 mol) in dichloromethane (400 mL) was added dropwise to a solution of **11a** (25.53 g, 0.158 mol) in dichloromethane (100 mL), while maintaining the temperature at 20–25 °C. After 16 h, the solution was washed with 1 N sodium hydroxide (2 × 200 mL), dried (K_2CO_3), and filtered to give a pale yellow solution of **12a**. Trifluoroacetic anhydride (44.61 g, 0.212 mol) was added dropwise over 30 min, while maintaining the temperature at 15–20 °C. After the reaction mixture was allowed to stand for 2 days, methanol (100 mL) was added with cooling and the solution was evaporated in vacuo. The residue was treated with water (200 mL) and saturated sodium carbonate (200 mL), and the product was extracted into dichloromethane, dried (K_2CO_3), and evaporated in vacuo to a yellow solid. Recrystallization from petroleum ether (60/80) afforded **13a**, 15.76 g (56%), mp 66–8 °C. Anal. ($C_6H_5Cl_2NO$) C, H, N. NMR ($CDCl_3$) δ 4.26 (1 H, br s, OH), 4.81 (2 H, s, CH_2), 7.38 (1 H, d, J = 5 Hz, py-5H), 8.38 (1 H, d, J = 5 Hz, py-6H).

3-Chloro-2-(hydroxymethyl)-4-morpholinopyridine (14a). Morpholine (14.68 g, 0.169 mol) and **13a** (6 g, 0.034 mol) were heated in a sealed vessel at 180 °C for 4 h. After cooling, the mixture was treated with water and extracted three times with chloroform. The extracts were dried (K_2CO_3) and evaporated in vacuo, and the residue was chromatographed (silica, $CHCl_3$/MeOH) to give an oil which, on crystallization from petroleum ether (60/80), gave **14a**, 5.16 g (67%), mp 80–2 °C. Anal. ($C_{10}H_{13}ClN_2O_2$) C, H, N. NMR ($CDCl_3$) δ 3.21 (4 H, t, J = 5 Hz, 2 × CH_2N), 3.89 (4 H, t, J = 5 Hz, 2 × CH_2O), 4.74 (2 H, s, CH_2OH), 6.83 (1 H, d, J = 5.5 Hz, py-5H), 8.33 (1 H, d, J = 5.5 Hz, py-6H).

3-Chloro-2-(chloromethyl)-4-morpholinopyridine Hydrochloride (4a). Thionyl chloride (7.96 g, 0.067 mol) in chloroform (35 mL, dried over alumina) was added dropwise to a stirred solution of **14a** (5.1 g, 0.0223 mol) in chloroform (60 mL, dried over alumina) cooled with an ice bath. The mixture was allowed to warm to room temperature and, after 2 h, evaporated to low volume in vacuo. Addition of ether afforded **4a** as a white solid, 6.19 g (98%), mp 199–201 °C. Anal. ($C_{10}H_{12}Cl_2N_2O$·HCl) C, H, N. NMR (DMSO-d_6) δ 3.45 (4 H, t, J = 4.5 Hz, 2 × CH_2N), 3.76 (4 H, t, J = 4.5 Hz, 2 × CH_2O), 4.98 (2 H, s, CH_2Cl), 7.31 (1 H, d, J = 6.4 Hz, py-5H), 8.45 (1 H, d, J = 6.4 Hz, py-6H).

2-[[(3-Chloro-4-morpholino-2-pyridyl)methyl]thio]-5-methoxy-(1*H*)-benzimidazole Monohydrate (6a). Sodium hydroxide (5 N, 5.68 mL, 0.0284 mol) was added over 5 min to a stirred mixture of **4a** (3.66 g, 0.013 mol) and 5-methoxy-2-mercaptobenzimidazole (**5**) (2.33 g, 0.013 mol) in ethanol (60 mL). After 16 h, the solvents were evaporated in vacuo and the residue was treated with water to give a solid, which was recrystallized from ethanol to give **6a**, 4.38 g (83%), mp 124–5 °C. Anal. ($C_{18}H_{19}ClN_4O_2S$·H_2O) C, H, N. NMR ($CDCl_3$) δ 3.22 (4 H, t, J = 4.5 Hz, 2 × CH_2N), 3.85 (3 H, s, CH_3), 3.89 (4 H, t, J = 4.5 Hz, 2 × CH_2O), 4.53 (2 H, s, CH_2S), 6.83 (1 H, m, benzim-6H), 6.86 (1 H, d, J = 5.5 Hz, py-5H), 7.05 (1 H, br m, benzim-4H), 7.40 (1 H, br m, benzim-7H), 8.37 (1 H, d, py-6H).

2-[[(3-Chloro-4-morpholino-2-pyridyl)methyl]sulfinyl]-5-methoxy-(1*H*)-benzimidazole (3a). A solution of *m*-chloroperbenzoic acid (1.61 g, 9.3 mmol) in dichloromethane (75 mL) was added over 30 min to a stirred solution of **6a** (3.35 g, 8.6 mmol) in dichloromethane (150 mL), while maintaining the temperature between −30 and −35 °C. After 1 h, ammonia gas was passed through the solution for 5 min and the precipitrate was filtered

(23) In three out of four dogs compound **3h** was significantly more potent than **1a** (paired two-tailed t test, $p < 0.05$, mean plateau inhib 73 ± 5% and 47 ± 8%, respectively).

(24) For further details, see: Ife, R. J. *Eur. Pat. Appl.'s* EP 184,322 and EP 246,774.

(25) den Hertog, H. J.; Overhoff, J. *Recl. Trav. Chim.* **1950**, *69*, 468.

off. The solution was evaporated in vacuo, and the residue was chromatographed (silica, MeOH–NH_3/$CHCl_3$) to give an oil which, on crystallization from ethanol, gave **3a**, 2.6 g (68%), mp indeterminate. Anal. ($C_{18}H_{19}ClN_4O_3S$) C, H, N, Cl, S. NMR (DMSO-d_6) δ 3.09 (4 H, t, J = 4.5 Hz, 2 × CH_2N), 3.72 (4 H, t, J = 4.5 Hz, 2 × CH_2O), 3.81 (3 H, s, OCH_3), 4.86 (2 H, s, CH_2SO), 6.92 (1 H, d of d, J = 9 Hz, 2.4 Hz, benzim-6H), 7.03 (1 H, d, J = 5.4 Hz, py-5H), 7.09 (1 H, d, J = 2.4 Hz, benzim-4H), 7.52 (1 H, d, J = 9 Hz, benzim-7H), 8.28 (1 H, d, J = 5.4 Hz, py-6H); IR (Nujol) ν_{max} 3300–2500 (NH), 1623, 1578 (C=N + ring modes), 1007, 985 (S=O and C–O) cm^{-1}.

Method B. 2-Methyl-4-piperidinopyridine (16). Piperidine (27.27 g, 0.32 mol) and 4-chloro-2-methylpyridine (**15**)[26] (13.65 g, 0.107 mol) were heated in a sealed vessel at 170 °C for 4.5 h. After cooling, the mixture was treated with water and extracted thoroughly with ether. The combined extracts were dried (K_2CO_3) and evaporated in vacuo, and the residue was distilled at reduced pressure to give **16**, 15.45 g (82%), bp 105–10 °C (1 mmHg): NMR ($CDCl_3$) δ 1.64 [6 H, br s, $(CH_2)_3$], 2.43 (3 H, s, CH_3), 3.31 (4 H, br s, 2 × CH_2N), 6.50 (2 H, m, py-3H and 5H), 8.13 (1 H, d, J = 6 Hz, py-6H); MS (EI) m/z 175 (base), 92.

5-Bromo-2-methyl-4-piperidinopyridine (17a) and 3-Bromo-2-methyl-4-piperidinopyridine (17b). A solution of bromine (27.2 g, 0.17 mol) in DMF (50 mL) was added over 20 min to a stirred mixture of **16** (15 g, 0.085 mol) and potassium carbonate (23.5 g, 0.17 mol) in DMF (50 mL) at 25–30 °C. After 3.5 h, the mixture was evaporated in vacuo and the residue was treated with water, adjusted to pH 13 with 40% sodium hydroxide, and extracted with ether. The combined extracts were dried (K_2CO_3) and evaporated in vacuo, and the residue was chromatographed (silica, n-hexane/ether) to give **17a** as an oil, 11.18 g (52%) [NMR ($CDCl_3$) δ 1.64 [6 H, br m, $(CH_2)_3$], 2.40 (3 H, s, CH_3), 3.04 (4 H, br m, 2 × CH_2N), 6.59 (1 H, s, py-3H), 8.28 (1 H, s, py-6H)], and **17b** as an oil, 3.5 g (16%) [NMR ($CDCl_3$) δ 1.66 [6 H, br, m, $(CH_2)_3$], 2.66 (3 H, s, CH_3), 3.00 (4 H, br m, 2 × CH_2N), 6.66 (1 H, d, J = 5 Hz, py-5H), 8.18 (1 H, d, J = 5 Hz, py-6H)].

5-Bromo-2-methyl-4-piperidinopyridine *N*-Oxide (18a). m-Chloroperbenzoic acid (7.3 g, 0.042 mol) in dichloromethane (100 mL) was added over 1 h to a stirred solution of **17a** (9 g, 0.035 mol) in dichloromethane (50 mL) at 20–25 °C. After 16 h, the solution was washed with 10% aqueous sodium carbonate, dried (K_2CO_3), and evaporated in vacuo. The residue was chromatographed (silica, $CHCl_3$/MeOH) and crystallized from ether to give **18a**, 6.79 g (71%), mp 115–6 °C. Anal. ($C_{11}H_{15}BrN_2O$) C, H, N. NMR ($CDCl_3$) δ 1.64 [6 H, br m, $(CH_2)_3$], 2.44 (3 H, s, CH_3), 3.00 (4 H, br m, 2 × CH_2N), 6.72 (1 H, s, py-3H), 8.30 (1 H, s, py-6H).

5-Bromo-2-(hydroxymethyl)-4-piperidinopyridine (14k). A solution of **18a** (0.77 g, 2.84 mmol) in acetic anhydride (4 mL) was heated at 100 °C for 1 h. The mixture was evaported in vacuo, and the black residue was treated with toluene (15 mL) and evaporated again. Dilute hydrochloric acid (6 mL) was added, and the solution was heated at 100 °C for 2 h. After cooling and diluting with water (20 mL), the pH was adjusted to 13 with 40% sodium hydroxide. The product was extracted into chloroform, dried (K_2CO_3), and evaporated in vacuo to an oil. Purification by chromatography (silica, $CHCl_3$/MeOH) and crystallization from ether gave **14k**, 0.3 g (39%), mp 116–8 °C. Anal. ($C_{11}H_{15}BrN_2O$) C, H, N. NMR ($CDCl_3$) δ 1.70 [6 H, m, $(CH_2)_3$], 2.80 (1 H, br s, OH), 3.13 (4 H, m, 2 × CH_2N), 4.64 (2 H, s, CH_2OH), 6.81 (1 H, s, py-3H), 8.46 (1 H, s, py-6H).

2-[[(5-Bromo-4-piperidino-2-pyridyl)methyl]sulfinyl]-5-methoxy-(1*H*)-benzimidazole (3k). Compound **14k** was converted to **3k** via the corresponding 2-(chloromethyl)pyridine **4k** and sulfide **6k** by analogy with method A, overall yield 74%, mp 114–8 °C. Anal. ($C_{19}H_{21}BrN_4O_2S$) C, H, N, Br, S. NMR (DMSO-d_6) δ 1.48 [6 H, m, $(CH_2)_3$], 2.76 (4 H, m, 2 × CH_2N), 3.82 (3 H, s, OCH_3), 4.57 + 4.68 (2 H, d of d, J = 13 Hz, CH_2SO), 6.50 (1 H, s, py-3H), 6.94 (1 H, br d, J = 8 Hz, benzim-6H), 7.09 (1 H, br s, benzim-4H), 7.55 (1 H, d, benzim-7H), 8.41 (1 H, s, py-6H); IR (Nujol) ν_{max} 3320–2200 (NH), 1620, 1580 (C=N + ring modes), 1051, 1021 (S=O and C–O) cm^{-1}.

Method C. 5-Fluoro-2-methylpyridine *N*-Oxide (20c). 5-Amino-2-methylpyridine[27] (34.21 g, 0.316 mol) in water (220 mL) was treated with concentrated hydrochloric acid (158 mL), and the solution was cooled to between −5 and 0 °C. Sodium nitrite (43.65 g, 0.63 mol) was added in portions with stirring over 30 min at −5 to 0 °C, and after a further 5 min, 60% w/w hexafluorophosphoric acid (169 mL) was added dropwise with cooling. The orange precipitate was filtered, washed with ice-cold water, ethanol, and ether, and dried under vacuum. This solid was added cautiously to petroleum ether (800 mL, bp 120–160 °C) at 90–95 °C over 30 min, with rapid stirring. After a further 5 min, the mixture was cooled, and the petrol decanted from a dark oil and extracted with 2 N hydrochloric acid. The extracts were combined with the oil, extracted with ether, and then basified with cooling by using 40% sodium hydroxide. The crude 5-fluoro-2-methylpyridine (**19c**) was extracted into dichloromethane and dried (K_2CO_3). The filtered solution was treated with m-chloroperbenzoic acid (65.5 g, 0.38 mol) and allowed to stand for 16 h. Ammonia gas was bubbled through with cooling for 10 min, and the precipitated solids were filtered off. After evaporation in vacuo, the residue was dissolved in chloroform (500 mL), washed with 2 N sodium hydroxide (2 × 250 mL), dried (K_2CO_3), and evaporated to give **20c** as a low-melting solid, 31.52 g (78%): NMR ($CDCl_3$) δ 2.48 (3 H, s, CH_3), 7.00 (1 H, m, py-4H), 7.23 (1 H, t, J = 5 Hz, py-3H), 8.22 (1 H, m, py-6H); MS (EI) m/z 127 ($M^{\cdot+}$), 110 (base), 96, 90, 83, 57.

5-Fluoro-2-methyl-4-nitropyridine *N*-Oxide (21c). A nitrating mixture of 30% oleum (110 mL) and fuming nitric acid (187 mL) was added dropwise over 20 min with cooling and stirring to a solution of **20c** (33.89 g, 0.267 mol) in concentrated sulfuric acid (120 mL) at 5–8 °C. The mixture was allowed to warm to room temperature over 1 h and then heated on a steam bath for 2 h. After cooling, the solution was poured onto ice (1 kg) and neutralized by addition of solid ammonium carbonate. The mixture was extracted with chloroform, dried (K_2CO_3), and evaporated in vacuo to a solid which was triturated with petroleum ether (60/80) to give **21c**, 33.09 g (72%), mp 100–5 °C. Anal. ($C_6H_5FN_2O_3$) C, H, N. NMR ($CDCl_3$) δ 2.51 (3 H, s, CH_3), 8.05 (1 H, d, J = 9 Hz, py-3H), 8.32 (1 H, d, J = 6 Hz, py-6H).

4-Chloro-5-fluoro-2-methylpyridine *N*-Oxide (22c). Phosphoryl chloride (52.7 mL, 0.57 mol) in dichloromethane (250 mL) was added dropwise under a stream of nitrogen to a stirred solution of **21c** (32.94 g, 0.19 mol) in dichloromethane (250 mL) at 5–10 °C. After standing for 16 h at room temperature, the solution was refluxed for 4 h, cooled, and poured onto ice (400 g). The mixture was stirred for 10 min and then adjusted to pH 9 with cooling, by using 40% sodium hydroxide. The aqueous phase was separated and further extracted with dichloromethane. The combined extracts were dried (K_2CO_3) and evaporated in vacuo to a solid, which was triturated with petroleum ether (60/80) to give **22c**, 27.39 g (89%), mp 100–2 °C. Anal. (C_6H_5ClFNO) C, H, N. NMR ($CDCl_3$) δ 2.46 (3 H, s, CH_3), 7.30 (1 H, d, J = 9 Hz, py-3H), 8.27 (1 H, d, J = 4.5 Hz, py-6H).

4-Chloro-5-fluoro-2-(hydroxymethyl)pyridine (23c). Trifluoroacetic anhydride (50.72 g, 0.242 mol) was added dropwise over 30 min to a stirred and cooled solution of **22c** (13.0 g, 80.5 mmol) in dichloromethane (180 mL), while maintaining the temperature at 10–15 °C. The solution was allowed to warm to room temperature and stood for 7 days. After pouring onto ice, the pH was adjusted to 13 by addition of saturated potassium carbonate followed by 40% sodium hydroxide. The aqueous layer was separated and further extracted with dichloromethane (150 mL), and the combined organic layers were dried (K_2CO_3) and evaporated to a red oil. Purification by chromatography (silica, $CHCl_3$/MeOH) followed by trituration with petroleum ether (40/60) afforded **23c**, 6.83 g (53%), mp 50–2 °C. Anal. ($C_6H_5ClNFO \cdot 0.1H_2O$) C, H, N. NMR ($CDCl_3$) δ 4.74 (2 H, s, CH_2OH), 7.44 (1 H, d, J = 6 Hz, py-3H), 8.43 (1 H, s, py-6H).

5-Fluoro-2-(hydroxymethyl)-4-morpholinopyridine (14g). Morpholine (6.74 g, 77.4 mmol) and **23c** (2.5 g, 15.5 mmol) were heated for 4 h at 180 °C in a sealed vessel. After cooling, the mixture was taken up in ethanol and evaporated in vacuo, and the residue was treated with water and extracted three times with chloroform. The extracts were dried (K_2CO_3) and evaporated in vacuo, and the residue was triturated with ether to give a brown

(26) Kato, T. *J. Pharm. Soc. Jpn.* **1955**, *75*, 1239.

(27) Graf, R. *J. Prakt. Chem.* **1933**, *133*, 19.

solid. Recrystallization from acetonitrile afforded **14q**, 2.49 g (76%), mp 134–6 °C. Anal. ($C_{10}H_{13}FN_2O_2$) C, H, N. NMR ($CDCl_3$) δ 3.28 (4 H, m, 2 × CH_2N), 3.85 (4 H, m, 2 × CH_2O), 4.65 (2 H, s, CH_2OH), 6.73 (1 H, d, J = 7 Hz, py-3H), 8.16 (1 H, d, J = 5 Hz, py-6H).

2-[[(5-Fluoro-4-morpholino-2-pyridyl)methyl]sulfinyl]-5-methoxy-(1*H*)-benzimidazole (3q). Compound **14q** was similarly converted to **3q** via the corresponding 2-(chloromethyl)-pyridine **4q** and sulfide **6q** by analogy with method A, overall yield 59%, mp 109–11 °C. Anal. ($C_{18}H_{19}FN_4O_3S·0.15H_2O$) C, H, N, S. NMR (DMSO-$d_6$) δ 2.95 (4 H, m, 2 × CH_2N), 3.61 (4 H, m, 2 × CH_2O), 3.80 (3 H, s, OCH_3), 4.58 (2 H, d of d, J = 13 Hz, CH_2SO), 6.59 (1 H, d, J = 8 Hz, py-3H), 6.92 (1 H, d of d, J = 9 Hz, 2.5 Hz, benzim-6H), 7.08 (1 H, d, J = 2.5 Hz, benzim-4H), 7.54 (1 H, d, J = 9 Hz, benzim-7H), 8.20 (1 H, d, J = 6 Hz, py-6H); IR (Nujol) ν_{max} 3400–2450 (NH), 1600, 1590 (C=N + ring modes), 1100, 1060 (S=O and C–O) cm^{-1}.

Stability and pK_a Measurements. Stability at ambient temperature (20 °C) was determined in acetonitrile/phosphate buffer (1:3) which had been adjusted to the appropriate pH. The initial concentration of compounds was between 1 × 10^{-3} and 3 × 10^{-4} M, and the decrease in concentration was monitored by HPLC (μBondapak C_{18}, CH_3CN/KH_2PO_4, pH 7.4, 20–80% CH_3CN in 25 min, 1 mL min^{-1}, 40 °C, detector UV 280 nm). Half-lives ($t_{1/2}$) were determined from the linear regression of ln of the concentration vs time (h).

The pK_a's of the substituted 2-(hydroxymethyl)pyridines were measured spectrophotometrically at 37 °C.

In Vivo Biological Assays. Initial studies were carried out by using the stomach lumen perfusion technique in the anaesthetized rat with continuous monitoring of the H^+ activity of the effluent perfusate using a microflow glass electrode. Acid secretion was stimulated by an intravenous infusion of histamine at 0.25 μmol/(kg·min), and the inhibitors were given either intravenously or intraduodenally when a submaximal plateau of secretion had been achieved. Percentage inhibition was calculated from a comparison of the acid secretion at peak inhibition compared to the secretory plateau immediately prior to administration. ED_{50} values were obtained by using three dose levels with three animals at each dose level.

Studies were also carried out in the conscious dog surgically prepared with a Heidenhain pouch 1–3 years previously. Acid secretion was stimulated by an intravenous infusion of histamine at 0.5 μmol/(kg·h) and gastric juice collected by gravity drainage at 15-min intervals. An aliquot of the juice was titrated to pH 7.4 with 0.1 N sodium hydroxide and the acid output calculated from the product of the acid concentration and volume of gastric juice secreted. Inhibitors were given either intravenously or intraduodenally, through a chronically implanted duodenal cannula, when a submaximal plateau of secretion had been established. Percentage inhibition was calculated from a comparison of the acid output in the 15-min sample at peak inhibition with the acid output in the sample immediately prior to compound administration. ED_{50} values were calculated by using three dose levels with three animals at each dose level.

Acknowledgment. We acknowledge the contributions made by members of the Physical Organic Chemistry Department at Smith Kline & French Research Ltd., who provided analytical data, and members of the Department of Pharmacology, who provided biological data.

Cardiac Glycoside-like Structure and Function of 5β,14β-Pregnanes

J. F. Templeton,*[†] V. P. Sashi Kumar,[†] D. Bose,[‡,§] and F. S. LaBella*[‡]

Faculty of Pharmacy and Department of Pharmacology and Therapeutics and Department of Internal Medicine, Faculty of Medicine, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E OW3. *Received December 7, 1988*

5β-Reduction and 14β-substitution convert the planar progesterone molecule to the cardiac glycoside configuration—A and D rings of the steroid moiety are bent toward the α-face relative to the B and C rings. Potency of the 5β,14β-derivative in a [^{3}H]ouabain binding assay or its ability to inhibit the sodium pump in red blood cells is enhanced by 3β-hydroxylation, 20β-hydroxylation, and 3β-glycosidation. Synthesis of 14,20β-dihydroxy-3β-(β-D-glucopyranosyloxy)-5β,14β-pregnane from digitoxin is described. The glucoside is $^1/_{20}$ as potent as ouabain and elicits prominent, sustained, positive inotropy in isolated cardiac muscle.

The high-affinity binding of the cardiac glycosides to its biological receptor, i.e. Na^+,K^+-ATPase, is noted also for its high degree of structural specificity.[1] In previous studies from our laboratory,[2-6] it was demonstrated that certain derivatives of progesterone are inhibitors of [^{3}H]ouabain binding to cell-membrane preparations and inhibit the sodium pump in isolated tissues. The most active congeners previously identified are represented by chlormadinone acetate (17α-acetoxy-6-chloropregna-4,6-diene-3,20-dione; CMA). CMA and related compounds, however, in contrast to the cardiostimulant cardiac glycosides, produce only transient positive inotropy associated with progressively developing cardiodepression. Important structural distinctions between CMA and the cardiac glycosides may account for this major important difference in myocardial response. For example, whereas the steroid nucleus of the mammalian hormones is essentially planar, in the cardiac glycosides the cis configuration of the A/B and C/D ring junctions orientate the A and D rings almost perpendicular to the B and C rings. We proposed that the apparent paradoxical cardiodepression occurring in association with the binding to and inhibition of Na^+,K^+-ATPase by CMA reflects an additional action, i.e. inhibition by the steroid of a metabolic process. The latter effect may account for depression of myocardial cell function which counteracts positive inotropy resulting from inhibition of the enzyme.

We have pointed out that 5β-reduction and 14β-substitution of the planar progesterone molecule convert the

[†] Faculty of Pharmacy.
[‡] Department of Pharmacology and Therapeutics.
[§] Department of Internal Medicine.

(1) Guntert, T. W.; Linde, H. H.A. In *Cardiac Glycosides*; Freef, K., Ed.; Springer-Verlag: Berlin, 1981; Part 1, Chapter 2, pp 13–24.
(2) Chow, E.; Kim, R. S.; LaBella, F. S.; Queen, G. *Br. J. Pharmacol.* **1979**, *67*, 345.
(3) Kim, R. S.; LaBella, F. S.; Zunza, H.; Zunza, F.; Templeton, J. *Mol. Pharmacol.* **1980**, *18*, 402.
(4) LaBella, F. S.; Bihler, I.; Kim, R. S. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1057.
(5) LaBella, F. S.; Bihler, I.; Templeton, J.; Kim, R. S.; Hnatowich, M.; Rohrer, D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 2806.
(6) LaBella, F. S.; Bihler, I.; Kim, R. S. *Nature (London)* **1979**, *28*, 571.

0022-2623/89/1832-1977$01.50/0