Synthesis and Antiinflammatory Activity of Metabolites of Piroxicam

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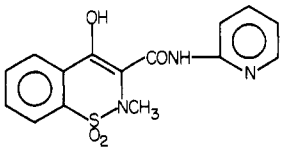
Four possible pyridine monohydroxylated metabolites of the antiinflammatory agent piroxicam have been synthesized for comparison with a natural pyridine-hydroxylated metabolite of this compound. In addition, another metabolite of piroxicam, derived from dehydration of the parent drug, has been made and characterized. The antiinflammatory activity of these compounds and four other known metabolites of piroxicam has been measured in the carrageenan-induced rat paw edema model and all are found to be less active than piroxicam itself.

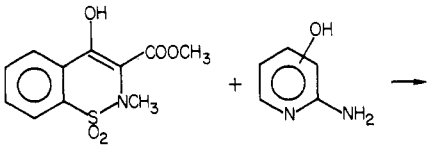
Previous publications[1-3] from these laboratories have reported on the preparation and potent antiinflammatory activity of the clinically effective antiarthritic[4,5] agent piroxicam (1). Pharmacokinetic[3,6] and metabolism studies



piroxicam (1)

in animals[7] indicate 1 to have a long serum half-life and that among several metabolites derived from 1 is a pyridine-hydroxylated derivative, as well as a dehydration product. The purpose of the present study was to prepare and characterize the dehydration product and all of the four possible pyridine-hydroxylated derivatives of 1. These compounds, as well as four other available metabolites of piroxicam, were then examined for their antiinflammatory properties in the carrageenan-induced rat paw edema test.

Chemistry. In order to prepare the pyridine-hydroxylated derivatives of 1, all four of the possible monohydroxylated derivatives of 2-aminopyridine were required for reaction with the known[8] ester 2. 2-Amino-3-



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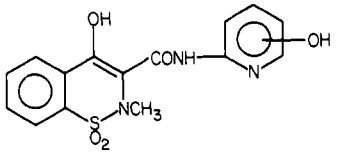
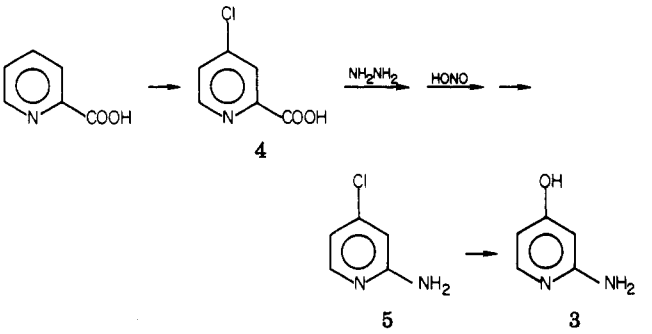


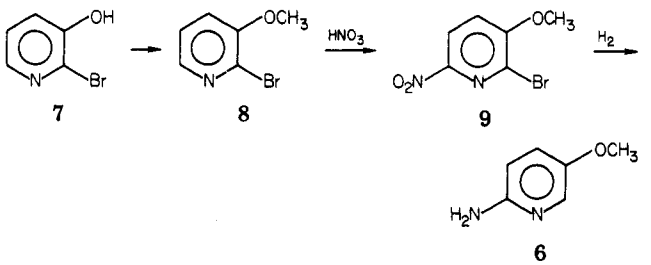
hydroxypyridine and 2-amino-6-hydroxypyridine were purchased, while 2-amino-4-hydroxypyridine (3) was synthesized by a multistep sequence. Preparation of compound 3 began with picolinic acid which was chlorinated



according to Graf.[9] The product, 4-chloro-2-pyridinecarboxylic acid (4), was found (mass spectrum) to be heavily contaminated with a dichloro impurity, which was removed by a final MeOH recrystallization. The chloro acid 4 was then converted to 2-amino-4-chloropyridine (5) via the corresponding hydrazide and azide.[9] Finally, treatment of 5 with KOH yielded the desired 2-amino-4-hydroxypyridine (3) whose picrate salt was identical by melting point with that reported.[10]

Combination of the appropriate 2-aminohydroxypyridine with the ester 2 in hot xylene solution produced the desired amides 11, 12, and 14 (Table I). An example of the preparative method for compound 11 is given under Experimental Section.

Several attempts to prepare 2-amino-5-hydroxypyridine by known methods[11,12] proved cumbersome and, in our hands, produced only impractical yields of the unstable product. Apparently, the para relationship of the amine and hydroxyl functions leads to an unstable compound which is readily converted to a highly colored, intractable material. The stable, known[13] 2-amino-5-methoxypyridine (6), on the other hand, was readily prepared in three steps from 2-bromo-3-hydroxypyridine (7). Combination of



amine 6 with ester 2 produced the desired amide, *N*-(5′-methoxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (10). This compound, however, was demethylated only in poor yields by HBr/

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(6) E. H. Wiseman, Y.-H. Chang, and J. G. Lombardino, *Pharmacologist*, 18, 219 (1976).
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(8) J. G. Lombardino, E. H. Wiseman, and W. M. McLamore, *J. Med. Chem.*, 14, 1171 (1971).
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(10) G. B. Barlin and W. Pfleider, *J. Chem. Soc. B*, 1425 (1971).
(11) J. A. Moore and F. J. Marascia, *J. Am. Chem. Soc.*, 81, 6049 (1959).
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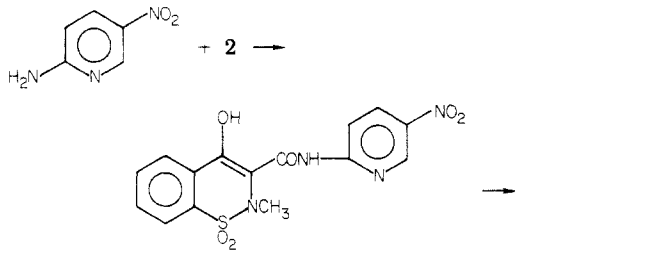
HOAc to give small amounts of the desired product **13** (Table I). Other procedures employing demethylating agents, such as BBr_3/CH_2Cl_2, $NaOCH_3/Me_2SO$, or pyridine hydrochloride, on compound **10** gave low yields of demethylated product **13** or destroyed the benzothiazine nucleus to a complex mixture of products.

We then turned to a synthetic sequence which required diazotization of the corresponding amine to produce **13**.



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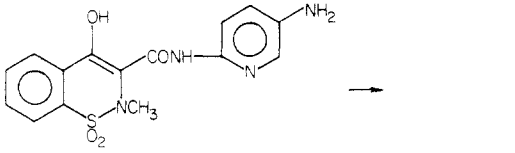

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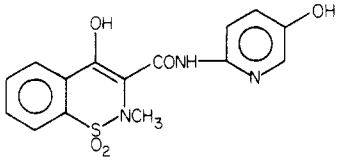

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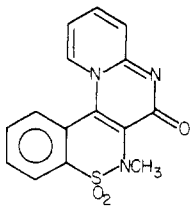
The 5′-nitro compound **15**, prepared from ester **2** and 2-amino-5-nitropyridine, was reduced to the 5′-amino compound **16**. Diazotization of **16** and warming the diazonium salt in acid solution produced good yields of the desired **13**.

Cyclodehydration of piroxicam was carried out in pyridine solution in the presence of acetic anhydride. Fractional crystallization from EtOAc produced a high-melting compound, which was characterized as the cyclodehydrated compound **17**. The identity of **17** was confirmed by IR,



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high-resolution MS, NMR, and combustion analytical data. Other metabolites of piroxicam were prepared by previously described procedures (see Table II).

Pharmacology. Assessment of antiinflammatory activity depended upon inhibition of edema formation in the rat paw in response to subplantar injection of carrageenan. The experimental procedure followed that of Winter et al.[14] The response of drug-treated animals (six per group) was compared with that of animals (eight per group) concurrently receiving vehicle alone or phenylbutazone. Drugs were administered by the oral route, dissolved in a solution containing 4% polyethylene glycol and 0.2% Tween 80 in water. Dilute sodium hydroxide was added as necessary to ensure solution. Control animals received vehicle only.

Results of this testing are presented in Tables I and II. Some of these compounds had moderate to weak antiinflammatory activity in the rat paw edema test when examined in the 10–33 mg/kg dose range. Certain compounds (i.e., **14** and **17**) approach the potency of phenylbutazone (Table I), but they are less potent than piroxicam which has an ED_{50} of 2 mg/kg in this test.[3]

Discussion

Metabolic studies[7] with piroxicam have identified several metabolites, including a pyridine-hydroxylated derivative and a cyclodehydration product derived from **1**. The present effort was aimed at preparing all four possible pyridine-hydroxylated derivatives of piroxicam for comparison with a hydroxylated metabolite isolated from piroxicam-treated animals. Results of these comparisons, indicating the 5′-hydroxy compound **13** to be formed in vivo, will be reported elsewhere by Hobbs and Twomey.[15] In addition, piroxicam was subjected to dehydrating conditions and a product, characterized as **17**, was shown[15] to be identical with the cyclodehydration product isolated from dogs and monkeys dosed with piroxicam. These metabolites, as well as several others (**18–21**) previously described in the literature, are all much less active than piroxicam as antiinflammatory agents. Apparently, piroxicam embodies a unique combination of functional groups which are all required to produce highly potent antiinflammatory activity in vivo. This observation is in line with the very restrictive structure–activity relationships previously observed for piroxicam and related analogues as in vitro inhibitors of prostaglandin synthesis.[16]

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. A Varian A-60 spectrometer (Me_4Si standard) was used to measure NMR spectra, and mass spectra were determined on a Hitachi Perkin-Elmer Model RMU-6E or on an AEI-MS-30 mass spectrometer. IR spectra were determined in KBr pellets. Analyses were carried out by the Physical Measurements Laboratory of Pfizer Inc. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.40% of the theoretical values.

2-Aminohydroxypyridines. 2-Amino-3-hydroxypyridine and 2-amino-6-hydroxypyridine were purchased from Aldrich Chemical Co. and used as received. 2-Amino-4-hydroxypyridine (**3**), picrate mp 223–227 °C (lit.[10] mp 224–227 °C), was made in five synthetic steps as outlined under Discussion. The first four steps to produce compound **5** were carried out essentially according to Graf,[9] with the only complication coming in the chlorination of picolinic acid to produce **4**. Contrary to literature[9] reports, significant amounts of a dichloropicolinic acid were found in the reaction mixture. Purification of **4** was finally achieved by recrystallization from MeOH to yield 17% of **4**: mp 180–181 °C. Anal. ($C_6H_4NO_2Cl$) C, H, N. The substitution pattern on this compound was further substantiated by NMR (Me_2SO-d_6) τ 2.25 (d, 1, J = 2.5 Hz, 5 H), 2.15 (s, 1, 3 H), 0.85 (d, 1, J = 2.5 Hz, 6 H). 2-Amino-4-chloropyridine (**5**) was then smoothly converted to **3** (69% yield) by the method of Barlin and Pfleider[10] using KOH in a steel pressure vessel.

2-Amino-5-methoxypyridine (6) was prepared in three steps as follows: (1) 2-Bromo-3-hydroxypyridine (**7**; Pfaltz & Bauer Chemical Co.) was converted to **2-bromo-3-methoxypyridine** (**8**) by the method of Nedenskov et al.,[17] mp 40–43 °C (lit.[16] mp 45 °C). (2) Using the method of Den Hertog et al.,[18] 10.5 g (0.056

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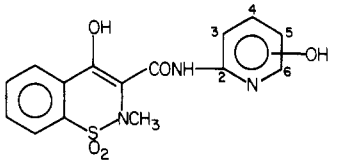
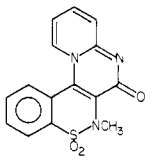
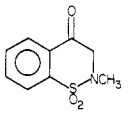
Table I. Possible Hydroxypyridyl Metabolites of Piroxicam



compd	position of OH group	yield, %	mp, °C	crystn solvent[a]	formula[b]	antiinflammatory act.,[c] % inhibn of edema dose, mg/kg: 10	33
11	3	37	264–265	M	$C_{15}H_{13}N_3O_5S$	13	41
12	4	9	246–248	M	$C_{15}H_{13}N_3O_5S$	10	24
13	5	60	260–263 dec		$C_{15}H_{13}N_3O_5S \cdot HCl$	15	17
14	6	38	229–230	M	$C_{15}H_{13}N_3O_5S$	31	40
1 (piroxicam)[d]						67	75
phenylbutazone						35	50

[a] M = methanol; EA = ethyl acetate; E = ethanol. [b] Satisfactory analyses for C, H, and N were obtained for all of these compounds. [c] Antiinflammatory activity is reported as a mean inhibition of carrageenan-induced edema 4 h after an oral dose of test compound. [d] See ref 3.

Table II. Some Metabolites of Piroxicam

no.	structure	yield, %	mp, °C	crystn solvent[a]	formula[b]	antiinflammatory act.,[c] % inhibn of edema dose, mg/kg: 10	33
17		6	286–287	EA	$C_{15}H_{11}N_3O_3S$	38	46
18			106–108[d]	E	$C_9H_9NO_3S$		8
19	2-methyl-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide[e]		131–132[e]	M	$C_8H_7NO_3S$		12
20	1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide		228[f]		$C_7H_5NO_3S$		0
21	4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylic acid 1,1-dioxide		144 dec[g]		$C_{10}H_9NO_5S$		0

[a-c] See corresponding footnotes in Table I. [d] Lit.[19] mp 107–108.5 °C. [e] Lit.[20] mp 131–132 °C. [f] Eastman Organic Chemicals. [g] Lit.[21] mp 144–156 °C dec. Decomposition of this compound takes place rapidly in solution and thus the biological assay may actually be measuring the antiinflammatory activity of the decarboxylated compound 18.

mol) of 8 was added in 10 min with vigorous stirring to a mixture of concentrated H_2SO_4 (42 mL) and fuming HNO_3 (42 mL) at 0 °C. The clear yellow reaction was then heated to 55 °C for 1 h, cooled, and added dropwise to 1100 mL of stirred ice–H_2O to produce **2-bromo-3-methoxy-6-nitropyridine** (**9**) which, after filtration and drying, weighed 9.2 g (71%): mp 133–136 °C; IR 6.54 and 7.39 cm^{-1} (NO_2). Anal. ($C_6H_5N_2O_3Br$) C, H, N. (3) To 1.0 g (4.3 mmol) of **9** in 200 mL of EtOH containing 100 mg of 10% Pd/C was added 0.197 g (4.9 mmol) of NaOH. This mixture was stirred under a H_2 atmosphere until 4 equiv of H_2 was absorbed. The suspension was filtered and the filtrate was acidified with 6 N HCl. All solvent was removed in vacuo, the residue was basified with 10 N NaOH and extracted several times with Et_2O, and the extracts were dried (Na_2SO_4). Removal of solvent yielded 0.31 g (59%) of **6** as a red oil.

A **picrate salt of 6** was prepared by adding aqueous picric acid to **6** dissolved in H_2O. Filtration of the resulting yellow solids yielded, after an EtOH recrystallization, **2-amino-5-methoxypyridine picrate**, mp 235 °C dec. Anal. ($C_{12}H_{11}N_5O_8$) C, H, N.

An ***N*-acetyl derivative of 6** was prepared by combination of **6** with acetic anhydride in pyridine solution. Filtration of the colorless solids and benzene–petroleum ether recrystallization yielded **2-acetamido-5-methoxypyridine**, mp 99–101 °C (lit.[13] mp 102–103 °C).

6-Methyl-6*H*-7-oxopyrido[1,2-*a*]pyrimido[5,4-*c*]-1,2-benzothiazine 5,5-Dioxide (17). To 9.9 g (0.030 mol) of piroxicam (**1**) in 200 mL of dry pyridine was added 40 mL of acetic anhydride, and the solution was refluxed for 18 h. After all solvents were removed under high vacuum at 80 °C, the residual mixture of products (TLC) was partitioned between 200 mL of $CHCl_3$ and 100 mL of 0.5 NaOH. The organic layer was separated and dried (Na_2SO_4). Evaporation of all solvent left a dark viscous oil, which was thoroughly triturated with hot ether. The resulting dark residue was crystallized from 2-propanol (50 mL) to yield crude product. Final purification to analytical purity was achieved by recrystallization from a large volume of EtOAc: yield 0.59 g (6%), mp 286–287 °C; IR 6.05, 6.20, 6.48, 6.77, 7.46, 8.48 μm; mass spectrum, *m/e* 313.0463 ($C_{15}H_{11}N_3O_3S$ calcd 313.0520), 271.0570 (CNO), 249.0853 (SO_2), 248.0416 (SO_2H, base peak), 233.0580 (SO_2, CH_3), 220.0855; NMR (CF_3COOD) τ 6.53 (s, 3 H, CH_3), a multiplet at 2.4–0.7 (8 H, aromatic protons) in which a pair of doublets at 0.76 and 1.50 (J = 3.5 Hz) could be distinguished. Anal. ($C_{15}H_{11}N_3O_3S$) C, H, N.

***N*-(3′-Hydroxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (11).** A solution of

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(19) H. Zinnes, R. A. Comes, and J. Shavel, Jr., *J. Org. Chem.*, **31**, 162 (1966).
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4.0 g (0.015 mol) of methyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide[8] (**2**) and 1.8 g (0.0165 mol) of 2-amino-3-hydroxypyridine in 300 mL of dry xylene under a N_2 atmosphere was refluxed for 18 h. Periodically, some solvent was distilled off and replaced with dry xylene. Thin-layer chromatography ($CHCl_3$/MeOH, 4:1) was an efficient monitoring tool for determining complete conversion to the more polar product. Cooling and filtration of the suspension yielded, after recrystallization from MeOH, 1.9 g (37%) of **11**, mp 264–265 °C. This method was applied to the preparation of the other pyridine-hydroxylated amides **12** and **14** shown in Table I.

***N*-(5′-Hydroxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (13) via Cleavage of the Corresponding Methyl Ether.** (a) A suspension of 0.66 g (2.45 mmol) of 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide[8] (**2**) in 40 mL of *m*-xylene was placed in a reaction flask fitted with a Soxhlet extractor (thimble containing Linde 4A molecular sieves). The reaction was heated to reflux and a solution of 0.35 g (2.8 mmol) of **6** dissolved in 23 mL of *m*-xylene was added slowly. After 24 h at reflux, the reaction was cooled and the solids were filtered and dried to yield 0.52 g (59%) of ***N*-(5′-methoxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (10)**: mp 298 °C dec; IR 6.11 (CO), 7.36 (SO_2), 7.9 (OCH_3), 8.41 cm^{-1} (SO_2). Anal. ($C_{16}H_{15}N_3O_5S$) C, H, N.

(b) To 0.25 g (0.69 mmol) of the above 5′-methoxy compound **10** was added 25 mL of 30% HBr/HOAc. Heating the solution at 100 °C and monitoring by TLC ($CHCl_3$/MeOH eluant, 4:1) indicated no reaction within 2.5 h, but a small amount of a more polar material developed in 18 h. Significant amounts of starting material remained and was still present after 5 days. A sample of the reaction mixture taken at 5 days indicated by TLC and mass spectrum (*m/e* 347) that a small amount of **13** had formed.

Addition of more HBr gas to the HOAc solution, or carrying out the HBr/HOAc reaction in a sealed vessel at temperatures as low as 50 °C, produced complex mixtures of products. Other demethylating reagents gave either no reaction [e.g., $NaOCH_3$/Me_2SO or BBr_3 or $C_6H_5Si(CH_3)_3/I_2$] or extensive decomposition of the starting material (e.g., pyridine/HCl/210 °C, H_2SO_4/HOAc/90 °C, or HBr/H_2O/100 °C).

Since conversion of the methyl ether **10** to the desired **13** proved inefficient, and purification of larger amounts of **13** would be prohibitive by this route, another synthetic approach to **13** was utilized.

***N*-(5′-Hydroxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (13) via Diazotization of the Corresponding Amine.** (a) In a flask fitted with a Soxhlet extractor (containing 4A molecular sieves) was placed 13.5 g (0.05 mol) of the known[8] ester **2**, 1300 mL of *m*-xylene and 9.3 g of 2-amino-5-nitropyridine (mp 186–188 °C; Aldrich Chemical Co.). The mixture was placed under a N_2 atmosphere and refluxed for 24 h, at which time an additional 2.7 g of ester **2** was added. After an additional 24 h at reflux, the reaction was filtered while hot to yield, after collecting a second crop from the filtrate, a total of 18.3 g (81%) of ***N*-(5′-nitro-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (15)**: mp 258–260 °C dec; IR 6.64 (NO_2), 7.44 and 8.48 cm^{-1} (SO_2). Anal. ($C_{15}H_{12}N_4O_6S$) C, H, N.

(b) In a 50-mL flask was placed 0.5 g (0.0013 mol) of the nitro compound **15**, 10 mL of concentrated HCl, and 1.5 g (0.008 mol) of $SnCl_2 \cdot 2H_2O$. The mixture was heated to 90 °C and after 2.5 h had thickened to a white mass. The reaction was evaporated to dryness in vacuo, and the residue thoroughly washed with a 1:1 mixture of ether/acetone. Filtration of the suspended solids yielded 0.40 g (69%) of ***N*-(5′-amino-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide dihydrochloride (16)**: mp 215 °C dec; IR 3.35 (NH_3^+), 7.4 and 8.6 cm^{-1} (SO_2); mass spectrum, *m/e* 346 (calcd 346), 282 (SO_2), 109. Recrystallization from dilute MeOH gave analytically pure **16** as a yellow monohydrate. Anal. ($C_{15}H_{14}N_4O_4S \cdot 2HCl \cdot H_2O$) C, H, N.

(c) A suspension of 2.0 g (0.004 mol) of the amine **16** in 40 mL of H_2O was brought to pH 3 with $NaHCO_3$. After 10 mL of HOAc and 80 mL of 20% H_2SO_4 was added, the reaction was cooled to 0 °C and a solution of 0.36 g (0.005 mol) of $NaNO_2$ in 15 mL of H_2O was slowly added. After 1 h, a little urea was added to quench the excess HONO and the yellow suspension (behind a shield) was slowly added to 175 mL of 20% H_2SO_4 at 75 °C. The reaction temperature was then raised to 100 °C over 1 h, during which time approximately 120 mL of N_2 was collected. After the reaction was cooled to 0 °C, saturated $NaHCO_3$ solution was added to pH 3 to produce a pale orange solid. Filtration and drying yielded 1.2 g of crude ***N*-(5′-hydroxy-2′-pyridyl)-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide hydrochloride (13)**. Further purification was achieved by dissolving the crude solid in dilute NaOH, filtering, and then extracting the filtrate with $CHCl_3$. The aqueous layer was separated and acidified with 6 N HCl to pH 3, and the solid precipitate was filtered. Treatment of the solid in hot MeOH/$CHCl_3$ (1:4) with Darco gave, after filtration through filter aid, evaporation of the filtrate, and trituration with 2-propanol, analytically pure **13** in 60% yield as a pale yellow solid: mp 260–263 °C dec; IR 3.04, 7.39, 8.50 μm; mass spectrum, *m/e* 347 (calcd 347), 283 (SO_2), 110. Anal. ($C_{15}H_{14}N_3O_5SCl$) C, H, N.

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