section of these laboratories for elemental analyses and spectral measurements.

References and Notes

(1) K. Natsuka, H. Nakamura, T. Negoro, H. Uno, and H. Nishimura, Abstracts, 96th Annual Meeting of Pharmaceutical Association of Japan, Nagoya, April 1976, p 1, and 97th Annual Meeting of Pharmaceutical Association of Japan, Tokyo, April 1977, p 123.
(2) For part 1 of this series, see K. Natsuka, H. Nakamura, H. Uno, and S. Umemoto, *J. Med. Chem.*, **18**, 1240 (1975).
(3) H. Nakamura and M. Shimizu, *Arch. Int. Pharmacodyn. Ther.*, **221**, 105 (1976).
(4) B. E. Maryanoff and M. J. Zelesko, *J. Pharm. Sci.*, **67**, 590 (1978).
(5) (a) P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965); (b) A. S. Horn and J. R. Rodgers, *J. Pharm. Pharmacol.*, **29**, 257 (1977); (c) L. B. Mellett and L. A. Woods, *Fortschr. Arzneimittelforsch.*, **5**, 155 (1963); (d) O. J. Braenden, N. B. Eddy, and H. Halbach, *Bull. W.H.O.*, **13**, 937 (1955); (e) E. Ohki, S. Oida, Y. Ohashi, A. Yoshida, K. Kamoshita, and H. Takagi, *Chem. Pharm. Bull.*, **22**, 1014 (1974); (f) M. Takeda, H. Inoue, K. Noguchi, Y. Honma, M. Kawamori, G. Tsukamoto, Y. Yamawaki, S. Saito, K. Aoe, T. Date, S. Nurimoto, and G. Hayashi, *J. Med. Chem.*, **20**, 221 (1977); (g) K. Flick, E. Frankus, and E. Friderichs, *Arzneim.-Forsch.*, **28**, 107 (1978).
(6) H. Nishimura, H. Uno, K. Natsuka, N. Shimokawa, M. Shimizu, and H. Nakamura, German Offen. 2502729 (1975) [*Chem. Abstr.*, **83**, 193387 (1975)] and 2610433 (1976) [*Chem. Abstr.*, **86**, 29881 (1977)].
(7) (a) Y. Yamakawa, *Yakugaku Zasshi*, **80**, 292 (1960); (b) B. Reichert and W. Hoffmann, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **274**, 153 (1936); (c) E. C. Dodds, W. Lawson, and P. C. Williams, *Proc. R. Soc. London, Ser. B*, **132**, 119 (1944).
(8) L. H. Goodson and H. Christopher, *J. Am. Chem. Soc.*, **72**, 358 (1950).
(9) F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1941).
(10) E. Siegmund, R. Cadmus, and G. Lu, *Proc. Soc. Exp. Biol. Med.*, **95**, 729 (1957).
(11) F. Haffner, *Dtsch. Med. Wochenschr.*, **55**, 731 (1929).
(12) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(13) (a) E. J. Fellows and G. E. Ullyot in "Medicinal Chemistry", Vol. I, C. M. Suter, Ed. in Chief, Wiley, New York, N.Y., 1951, p 399; (b) Y. Yamakawa, *Yakugaku Zasshi*, **80**, 289 (1960).
(14) M. Yoshimoto, T. Kamioka, T. Miyadera, S. Kobayashi, H. Takagi, and R. Tachikawa, *Chem. Pharm. Bull.*, **25**, 1378 (1977).
(15) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(16) (a) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966); (b) A. F. Casy in "Medicinal Chemistry", Part I, A. Burger, Ed., Wiley-Interscience, New York and London, 1970, p 81.
(17) K. Fromherz, *Arch. Int. Pharmacodyn. Ther.*, **85**, 387 (1951).
(18) E. Mahacsi and W. Leimgruber, U.S. Patent 3914233 (1975) [*Chem. Abstr.*, **84**, 74487 (1976)].

Synthesis and Antihypertensive Activity of 5-Thio-2-pyridinecarboxylic Acid Derivatives

Neville Finch,* Thomas R. Campbell, Charles W. Gemenden, Michael J. Antonaccio, and Henry J. Povalski

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey 07901.

Received June 12, 1978

Synthesis of various substituted 5-thio-2-pyridinecarboxylic acids and their derivatives is described by three methods, i.e., displacement of nitrite from methyl 5-nitro-2-pyridinecarboxylate (**10**) by a thiol anion, alkylation of methyl 5-thio-2-pyridinecarboxylate derived from reaction of the diazotized methyl 5-amino-2-pyridinecarboxylate (**5**) with thiocyanate followed by borohydride reduction of the product, and alkylation of 5-thio-2-pyridinecarbonitrile followed by hydrolysis. 5-Thio-2-pyridinecarbonitrile was obtained from butyl 6-methyl-3-pyridyl sulfoxide (**2**) by nitrosation and dehydration of the oxime. Many of these 5-thio-2-pyridinecarboxylic acid derivatives were orally active antihypertensive agents in the spontaneously hypertensive rat. Optimization of the structural parameters for this activity yielded 5-[(*m*-trifluorobenzyl)thio]-2-pyridinecarboxylic acid (**41**) and its sulfoxide, **42**. Further biological studies with these compounds are described.

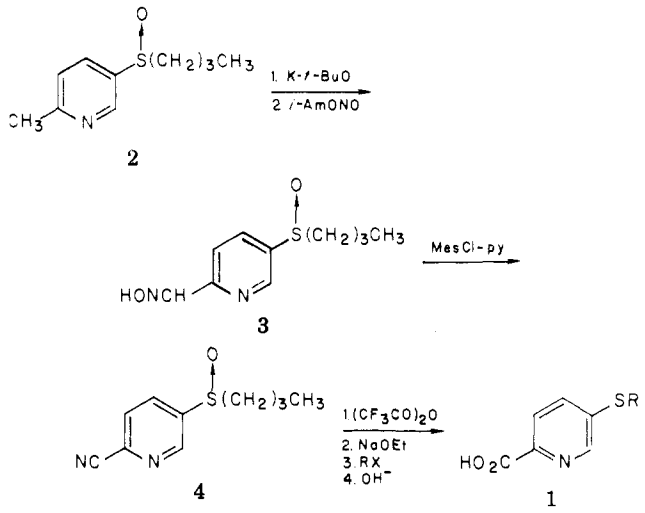
Vasodilators are of growing importance in the therapy of cardiovascular diseases. Their use as antihypertensive agents has increased since concomitant administration of β-adrenergic drugs has become recognized as an excellent regimen.[1] More recently, interest has developed in the use of vasodilators for cardiac failure.[2]

Fusaric acid, 5-butyl-2-pyridinecarboxylic acid (**11**), has been shown to be a dopamine β-hydroxylase inhibitor in man,[3] although the antihypertensive effect seen in man and animals from both fusaric acid (**11**) and its amide seems better explained by direct peripheral arteriolar relaxation.[4] Therefore we concluded that certain substituted 5-thio-2-pyridinecarboxylic acids, **1**, might also be useful peripheral vasodilators.

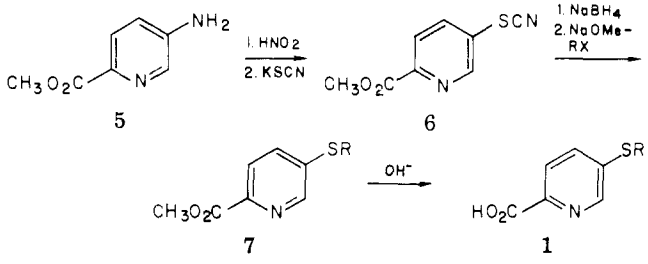
Chemistry. The synthesis of 5-thio-2-pyridinecarboxylic acid (**1**, R = H) has been described by Delarge,[5] but only one derivative, **1** [R = $-C(=S)N(CH_3)_2$], has since been reported.[6] Extensive work has been carried out on the isomeric 3-thio-2-pyridinecarboxylic acids because of their hypoglycemic activity.[7] The paucity of work on 5-substituted 2-pyridinecarboxylic acids reflects the synthetic difficulties inherent in the 2,5 orientation of substituents in the pyridine ring. We have recently described a new route to such compounds.[8] Butyl 6-methyl-3-pyridyl sulfoxide[8] (**2**) prepared by this route could be nitrosated to the oxime of 5-(butylsulfinyl)-2-pyridinecarboxaldehyde (**3**). The oxime **3** could be dehydrated to 5-(butylsulfinyl)-2-pyridinecarbonitrile (**4**). The Pummerer rearrangement of the sulfoxide **4** then provided an intermediate which could be alkylated directly to provide a wide range of 5-thio-2-pyridinecarbonitrile derivatives. Hydrolysis of these derivatives with base provided the acids **1**. This procedure (Scheme I) is illustrated in the Experimental Section for 5-(benzyl-

0022-2623/78/1821-1269$01.00/0

Scheme I



Scheme II

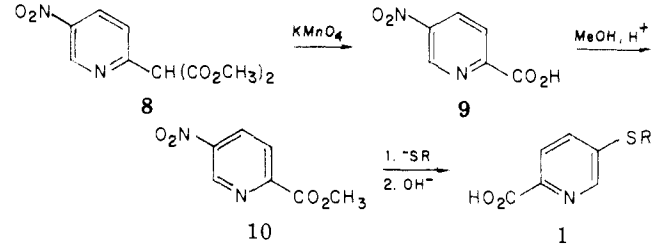


thio)-2-pyridinecarboxylic acid (18). The first compounds to be made were prepared by Scheme I. The compound most structurally related to fusaric acid (11), i.e., 5-(butylthio)-2-pyridinecarboxylic acid (12), showed a good antihypertensive effect in the spontaneously hypotensive rat (SHR). We, therefore, chose to explore other synthetic routes to such compounds.

The Delarge synthesis[5] failed in our hands, yielding only the products of addition of thiol to the diazonium cation, and no tractable products were obtained from reaction with sodium sulfide. However, a variant, the reaction of the diazonium salt with a mixture of cuprous and potassium thiocyanate, did give methyl 5-(thiocyanato)-2-pyridinecarboxylate (6) which could be reduced to methyl 5-thio-2-pyridinecarboxylate (7, R = H) by sodium borohydride. Alkylation of this ester, 7 (R = H), and hydrolysis gave the desired compound, 1 (Scheme II). The overall yields for both methods were not completely satisfactory for certain compounds.

In view of the growing interest in some of the compounds, a third scheme was developed. Syntheses of 5-nitro-2-pyridinecarboxylic acid (9) were reported by Cooper[9] and Deady,[10] but in our hands neither process was satisfactory. Instead we oxidized dimethyl 5-nitro-2-pyridinemalonate (8) with potassium permanganate. This malonate derivative was obtained from reaction of 2-chloro-5-nitropyridine with the sodio derivative of dimethyl malonate. Cooper[11] had previously reported on the synthesis of this type of product but had not described its oxidation. This process provided 55% overall yield of 5-nitro-2-pyridinecarboxylic acid (9) from 2-chloro-5-nitropyridine. Reaction of methyl 5-nitro-2-pyridinecarboxylate (10) in DMF with the mercaptide anion gave the 5-thio derivative directly by displacement of nitrite. The *p*-carbomethoxy group provides sufficient activation of the nitro group for a good nucleophile to readily give nucleophilic aromatic substitution. Hydrolysis of the

Scheme III



substitution products then gave the desired compounds, 1 (Scheme III).

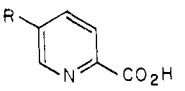
Oxidation of the substitution products in methylene chloride with 1 equiv of *m*-chloroperbenzoic acid gave sulfoxides which were hydrolyzed to the 5-sulfinyl-2-pyridinecarboxylic acids. In one case, oxidation of compound 44, this procedure yielded an approximately 1:1 diastereoisomeric mixture based on NMR evidence. This mixture could not be conveniently separated and was tested as if it were a single compound, 46. Oxidation with excess *m*-chloroperbenzoic acid gave the sulfones. However, as oxidation to this level virtually abolished the activity of one of the most active compounds, i.e., 41 → 43, synthesis of sulfones was not actively pursued.

Pharmacology. The preliminary pharmacological data were obtained from the effects on the blood pressure and heart rate of the spontaneously hypertensive rat (SHR) (see Table I). The closest analogue to fusaric acid (11), 5-(butylthio)-2-pyridinecarboxylic acid (12), was less active with regard to blood pressure reduction. A potentially important difference was that it caused little or no effect on heart rate. Oxidation of compound 12 to the sulfone 13 abolished the antihypertensive activity. This proved to be a consistent structure–activity relationship (SAR). 5-[[*m*-(Trifluoromethyl)benzyl]thio]-2-pyridinecarboxylic acid (41) was one of the most active antihypertensive agents, but oxidation to the sulfone 43 gave a compound with only a very slight antihypertensive effect.

Utilizing the limited knowledge of the SAR of fusaric acid, the 3-chloropropyl compound 14 was prepared in the hope that it would be more potent. This proved not to be the case. Nevertheless, as the antihypertensive activity was not lost, substitution at the end of the alkyl chain appeared to be a permitted change. The obvious, next choice of phenyl gave compound 15, which again was still active but no more so than the original compound, 12. Stepwise reduction of the length of the chain yielded a significant result. The one-carbon separation between the phenyl and the sulfur atom, e.g., compound 18, was significantly more antihypertensive than compound 12. Curiously, the two-carbon separation, e.g., compound 17, and the direct attachment, e.g., compound 22, gave compounds which could be dropped from consideration because they were poorly tolerated in the rat. The focus of further work was therefore on 5-(benzylthio)-2-pyridinecarboxylic acid (18).

The next objective was determination of the optimal nature and location of a substituent or substituents in the phenyl ring. While QSAR methods were rejected as being still too cumbersome, consideration was given to various operational schemes which have been published to address the problem of optimal aromatic substitution.[12] The outstanding compounds proved to be the *m*-chloro (19), *m*-bromo (32), and *m*-trifluoromethyl (41) compounds. Compound 41 especially attracted attention as it was well tolerated, and at 200 mg/kg po in the SHR it could cause blood pressure falls in excess of 80 mm with slight bradycardia. While oxidation to the sulfone 43 dramatically

Table I



compd[a]	R	salt	synth scheme	mp, °C	recrystn solvent	formula	dose, mg/kg po	antihypertensive effect in SHR[b]	microanalyses[c]
11	$CH_3(CH_2)_3$-						100	++++	
12	$CH_3(CH_2)_3S$-		I	100-102	EtOH	$C_{10}H_{13}NO_2S$	100	++	C, H, N
13	$CH_3(CH_2)_3SO_2$-		I	155-157	EtOH	$C_{10}H_{13}NO_4S$	100	inactive	C, H, N
14	$ClCH_2(CH_2)_2S$-		I	115-117	water, pH 9 → 4	$C_9H_{10}ClNO_2S$[d]	100	++	C, H, N
15	$Ph(CH_2)_3S$-		III	100-103	aq MeOH	$C_{15}H_{15}NO_2S$	100	++	C, H, N
16	$Ph(CH_2)_3S(\rightarrow O)$-	Na	III	290-300	aq 2-propanol	$C_{15}H_{14}NNaO_3S$	100	+	C, H, N
17	$Ph(CH_2)_2S$-		I	111-113	water, pH 9 → 4	$C_{14}H_{13}NO_2S$	100	poorly tolerated	C, H, N
18	$PhCH_2S$-		I	169-171	2-propanol	$C_{13}H_{11}NO_2S$	100	+++	C, H, N
19	3-$ClC_6H_4CH_2S$-		I	156-158	2-propanol	$C_{13}H_{10}ClNO_2S$	100	+++	C, H, N
20	4-$ClC_6H_4CH_2S$-		I	153-155	2-propanol	$C_{13}H_{10}ClNO_2S$	100	+++	C, H, N
21	3-ClC_6H_4S-		III	115-116	benzene-MeOH	$C_{12}H_8ClNO_2S$	100	++	C, H, N
22	4-ClC_6H_4S-	Na	III	290-295	aq EtOH	$C_{12}H_7ClNNaO_2S$	30	+ poorly tolerated at 100	C, H, N
23	4-$ClC_6H_4S(\rightarrow O)$-		III	182-185	ether-MeOH	$C_{12}H_8ClNO_3S$	100	+	C, H, N
24	2-$ClC_6H_4CH_2S$-		I	155-157	2-propanol	$C_{13}H_{10}ClNO_2S$	100	+	C, H, N
25	2,4-$Cl_2C_6H_3CH_2S(\rightarrow O)$-	Na	III	257-260	aq 2-propanol	$C_{13}H_8Cl_2NNaO_3S$	100	++	C, H, N
26	2,6-$Cl_2C_6H_3CH_2S$-	Na	III	305-310 dec	aq 2-propanol	$C_{13}H_8Cl_2NNaO_2S$	100	+	C, H, N
27	3,4-$Cl_2C_6H_3CH_2S$-	Na	II	256-258	MeOH	$C_{13}H_8Cl_2NNaO_2S$	100	+	C, H, N
28	3-$ClC_6H_4CH(CH_3)S$-		III	120-123	ether-EtOAc	$C_{14}H_{12}ClNO_2S$	100	+	C, H, N
29	$ClC_6H_4CH_2S(\rightarrow O)$-		III	168-170	aq MeOH	$C_{13}H_{10}ClNO_3S$	100	+++	C, H, N
30	4-$ClC_6H_4CH_2S(\rightarrow O)$-		I	197-199	aq EtOH	$C_{13}H_{10}ClNO_3S$	100	++	C, H, N
31	4-$BrC_6H_4CH_2S$-	Na	II	285-288	aq 2-propanol	$C_{13}H_9BrNNaO_2S$	100	+++	C, H, N
32	3-$BrC_6H_4CH_2S$-	Na	III	260-265	aq 2-propanol	$C_{13}H_9BrNNaO_2S$	100	++++	C, H, N
33	4-$FC_6H_4CH_2S$-	Na	II	280-286	aq 2-propanol	$C_{13}H_9FNNaO_2S$	100	++	C, H, N
34	3-$FC_6H_4CH_2S$-		I	155-157	EtOH	$C_{13}H_{10}FNO_2S$	100	++	C, H, N
35	4-$CH_3C_6H_4CH_2S$-	Na	III	286-289	aq 2-propanol	$C_{14}H_{12}NNaO_2S$	100	+	C, H, N
36	3-$CH_3C_6H_4CH_2S$-		I	143-145	water, pH 9 → 4	$C_{14}H_{13}NO_2S$	100	+	C, H, N
37	4-$OCH_3C_6H_4CH_2S$-	Na	III	276-280	aq 2-propanol	$C_{14}H_{12}NNaO_3S$	100	+	C, H, N
38	4-$OCH_3C_6H_4CH_2S(\rightarrow O)$-		III	184-187	aq HOAc	$C_{14}H_{13}NO_4S$	100	++	C, H, N
39	3-$OCH_3C_6H_4CH_2S(\rightarrow O)$-		III	106-107	aq MeOH	$C_{14}H_{13}NO_4S$	100	+	C, H, N
40	3-$CNC_6H_4CH_2S$-	Na	II	229-231	aq 2-propanol	$C_{14}H_9N_2NaO_2S$	100	++	C, H, N
41	3-$CF_3C_6H_4CH_2S$-		I, II	117-119	MeOH	$C_{14}H_{10}F_3NO_2S$	100 200	+++ +++++	C, H, N
42	3-$CF_3C_6H_4CH_2S(\rightarrow O)$-		II	170-175	aq MeOH	$C_{14}H_{10}F_3NO_3S$	100 200	+++ ++++	C, H, N
43	3-$CF_3C_6H_4CH_2SO_2$-	Na	II	315-320 dec	aq 2-propanol	$C_{14}H_9F_3NNaO_4S$	100	+	C, H, N
44	3-$CF_3C_6H_4CH(CH_3)S$-		II	114-116	aq MeOH	$C_{15}H_{12}F_3NO_2S$	100	+++	C, H, N
45	3-$CF_3C_6H_4CH[(CH_2)_2CH_3]S$-		III	124-127	EtOAc-Et_2O	$C_{17}H_{16}F_3NO_2S$	100	++	C, H, N
46	3-$CF_3C_6H_4CH(CH_3)S(\rightarrow O)$-		III	150-152	aq MeOH	$C_{15}H_{12}F_3NO_3S$	100	++	C, H, N
47	3-$BrC_6H_4CH_2S(\rightarrow O)$-	Na	III	270 dec	aq MeOH	$C_{13}H_9BrNNaO_3S$	100	++	C, H, N

[a] All compounds have compatible UV, IR, and NMR spectra. [b] +, <20 mm; ++, >20 mm; +++, >40 mm; ++++, >60 mm; +++++, >80 mm (maximum blood pressure fall). [c] C, H, and N microanalyses conform to 0.4% of theory. [d] C: calcd, 46.65; found, 47.36.

reduced the antihypertensive activity, oxidation to the sulfoxide **42** produced an only slightly less active compound. This sulfoxide **42** was somewhat better tolerated in the rat and caused a more marked bradycardia. Branching at the benzylic position, as in compounds **44** and **45**, did not improve activity.

Further biological studies in dogs and monkeys were therefore carried out with 5-[[*m*-(trifluoromethyl)-benzyl]thio]-2-pyridinecarboxylic acid (**41**) and its sulfoxide **42**. The activity of these compounds on the blood pressure and heart rate of unanesthetized dogs and monkeys is shown in Table II. Fusaric acid (**11**) was markedly effective in lowering blood pressure in both the renal hypertensive dog (RHD) and normotensive monkey (NM). Appreciable tachycardia and emesis were consistently observed with this agent in the dog but not the monkey. The high intrinsic heart rate of the monkey may account for absence of a presumably reflexogenic tachycardia noted in other species.

Compound **41** was inactive at 30 mg/kg po in the RHD; however, moderate hypotensive effects did occur in the monkey coupled with slight bradycardia. The compound became poorly tolerated by the third day of dosing, causing suspension of its evaluation. Compound **42**, the sulfoxide derivative of compound **41**, in contrast to the findings in the SHR, proved to be more effective than the latter compound, at least in the monkey where significant hypotension and bradycardia occurred. Compound **42**, however, was only slightly active in the RHD. Emesis, which frequently accompanied fusaric acid (**11**) therapy in the RHD, was not found with either compound **41** or **42**. In view of these modest effects in dog and monkey

Table II. Hypotensive Activity of Compounds in Unanesthetized Renal Hypertensive Dogs (RHD) and Normotensive Monkeys (NM)[a]

compd		max Δ ± SE of BP (mmHg) and HR (beats/min) act. in RHD day 1	day 2	day 3
11, 60 mg/kg po	MBP	−50 ± 7	−45 ± 12	−30 ± 7
	HR	+32 ± 18	+35 ± 17	+33 ± 5
41, 30 mg/kg po	MBP	+12	+22	+24
	HR	+5	+9	−12
42, 100 mg/kg po	MBP	−7 ± 1	−10	
	HR	+10 ± 2	+2	

compd		act. in NM day 1	day 2	day 3	day 4
11, 30 mg/kg po (day 1), 100 mg/kg po (day 2)	SBP	−28	−53		
	HR	−20	(+10) (−10)		
41, 100 mg/kg po	SBP	−27 ±10	−25 ± 16	−25 ± 16[b]	
	HR	−40 ± 12	−26 ± 24	−20 ± 20	
42, 100 mg/kg po	SBP	−30 ± 10	−29 ± 10	−19 ± 8	−19 ± 4[c]
	HR	−43 ± 30	−83 ± 12[c]	−73 ± 7[c]	−33 ± 24[c]

[a] MBP = mean blood pressure; SBP = systolic blood pressure; HR = heart rate; po = per os. [b] Toxicity noted with 41 (study suspended). [c] $p < 0.05$.

relative to fusaric acid (11), it was decided not to study these compounds further.

Experimental Section

Chemistry. IR spectral data were obtained as CH_2Cl_2 solution or Nujol mulls on a Perkin-Elmer 21 or 521. NMR spectra were obtained in $CDCl_3$ and $(CD_3)_2SO$ on a Varian A-60, using Me_4Si as an internal standard. Mass spectra were obtained on an AEI MS-902 instrument at 70 eV. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Spectra were obtained on all isolated intermediates and final products, and these were compatible with the structural assignments.

5-(Benzylthio)-2-pyridinecarboxylic Acid (18) (Scheme I). Butyl 6-methyl-3-pyridyl sulfoxide[8] (2) (7.5 g, 0.038 mol) in tetrahydrofuran (60 mL) was added with stirring to a solution of K-*t*-BuO (5.5 g, 0.049 mol) in tetrahydrofuran (60 mL) under nitrogen. The solution was stirred for 2 h at room temperature and cooled to 5 °C in an ice bath, and then isoamyl nitrite (14.6 g, 0.125 mol) in tetrahydrofuran (30 mL) was added in one portion. The ice bath was removed and the reaction mixture stirred overnight at room temperature. The tetrahydrofuran was removed in vacuo and the residue partitioned between water and ether. The aqueous part was made acid and washed with ether. It was then brought to pH 7, saturated with salt, and extracted with ether. The ether extracts were combined, washed (saturated NaCl), and dried ($MgSO_4$). Removal of the ether gave 5-(butylsulfinyl)-2-pyridinecarboxaldehyde oxime (3): 1.95 g; mp 90–91 °C. Anal. ($C_{10}H_{14}N_2O_2S$) C, H, N.

The oxime 3 (12 g, 0.053 mol) was dissolved in pyridine (80 mL) and cooled in an ice bath. Methanesulfonyl chloride (7.3 g, 0.064 mol) was added in one portion with stirring. The ice bath was removed after 2 h and the reaction mixture stirred overnight. The pyridine was removed in vacuo. The residue was partitioned between ether and water. The aqueous phase was extracted (ether). The etheral extracts were washed and dried ($MgSO_4$). Removal of the ether in vacuo gave 5-(butylsulfinyl)-2-pyridinecarbonitrile (4) (9.5 g) as an oil which crystallized. This material was recrystallized from 2-propanol to give the pure nitrile 4: mp 54–55 °C. Anal. ($C_{10}H_{12}N_2OS$) C, H, N.

5-(Butylsulfinyl)-2-pyridinecarbonitrile. 4 (5.9 g, 0.0283 mol) was refluxed in trifluoroacetic anhydride (40 mL) for 3 h. The anhydride was removed in vacuo and the residue dissolved in ether. The ethereal solution was washed (10% $KHCO_3$ and saturated NaCl), dried ($MgSO_4$), and concentrated in vacuo. Half of the crude residue of 5-[[1-(trifluoroacetoxy)butyl]thio]-2-pyridinecarbonitrile (3.4 g) was dissolved in ethanol. Under nitrogen a solution of sodium (0.310 g, 0.0135 mol) in ethanol was added. After the mixture was stirred for 1 h at room temperature, benzyl bromide (2.4 g, 0.0140 mol) was added and the mixture heated overnight at 65 °C. Removal of the ethanol in vacuo and partition of the residue between ether and water gave an ethereal extract which was washed, dried, ($MgSO_4$), and concentrated in vacuo. The residue of 5-(benzylthio)-2-pyridinecarbonitrile (2.6 g) was refluxed overnight in methanol (20 mL) containing 20% aqueous KOH (15 mL). The methanol was removed in vacuo. The aqueous residue was washed with ether and then adjusted to pH 4 with concentrated HCl. The precipitate of 5-(benzylthio)-2-pyridinecarboxylic acid (1, R = CH_2Ph) was collected, washed, and recrystallized from 2-propanol to give analytical pure 18: mp 169–171 °C; a 55% yield based on 5-(butylsulfinyl)-2-pyridinecarbonitrile (4). Anal. ($C_{13}H_{11}NO_2S$) C, H, N.

5-[(*p*-Bromobenzyl)thio]-2-pyridinecarboxylic Acid (31) (Scheme II). Methyl 5-(Thiocyanato)-2-pyridinecarboxylate (6). Methyl 5-amino-2-pyridinecarboxylate (5) (28.3 g, 0.186 mol), obtained by catalytic reduction of methyl 5-nitro-2-pyridinecarboxylate (10)[5,9] (vide infra), was dissolved in 20% H_2SO_4 (80 mL) and cooled to −4 °C. The solution was stirred while sodium nitrite (14.31 g, 0.207 mol) in water (30 mL) was added dropwise. The temperature was maintained at 0 °C for a further 15 min and then a well-blended mixture of potassium thiocyanate (28 g, 0.288 mol) and cuprous thiocyanate (8 g) was added portionwise with stirring. The solution was stirred for 3 h following addition and then extracted (CH_2Cl_2). The methylene chloride extracts were washed, dried, and concentrated in vacuo. The residue was crystallized from ethanol to give methyl 5-(thiocyanato)-2-pyridinecarboxylate (6): mp 150–155 °C; 27.9 g (77%). Anal. ($C_8H_6H_2O_2S$) C, H, N.

Methyl 5-Thio-2-pyridinecarboxylate (7, R = H). Methyl 5-(thiocyanate)-2-pyridinecarboxylate (6) (15 g, 0.0773 mol) was slurried in ice-cooled methanol (150 mL). Under N_2, $NaBH_4$ (2.93 g, 0.0775 mol) was added portionwise to the well-stirred reaction mixture at such a rate that the temperature did not exceed 18 °C. The mixture was stirred for a further 15 min. The resultant solution was concentrated to dryness in vacuo. The residue was slurried with CH_2Cl_2 and cold, diluted HCl under N_2. The CH_2Cl_2 layer was washed (water), dried ($MgSO_4$), and concentrated to dryness in vacuo. The crude thiol ester 7 (R = H) was used directly.

Alkylation. The thiol ester 7 (R = H) derived from 5 g (0.0295 mol) of the thiocyanate 6 by the above procedure was dissolved in methanol (50 mL). Under N_2, methanolic 1.24 N NaOMe (25 mL, 0.031 mol) was added. *p*-Bromobenzyl bromide (7.1 g, 0.0284 mol) was added and the mixture was refluxed for a few minutes under N_2. After allowing the mixture to stand overnight at room temperature, methanol was concentrated and cooled, and crystals of methyl 5-[(*p*-bromobenzyl)thio]-2-pyridinecarboxylate (7) (R = $CH_2C_6H_4$-4-Br) [2.6 g, 31% based on methyl 5-(thiocyanato)-2-pyridinecarboxylate (6); mp 108–111 °C] were collected. Anal. ($C_{14}H_{12}BrNO_2S$) C, H, N.

Hydrolysis. The crystalline methyl ester above was redissolved in the methanolic mother liquors. Aqueous 2 N NaOH (20 mL) was added. The mixture was heated on a stream bath for 30 min and then concentrated in vacuo. On cooling, the essentially aqueous medium deposited crystals of the sodium salt of 5-[(*p*-bromobenzyl)thio]-2-pyridinecarboxylic acid (31): mp 285–288 °C; 5.7 g [(63% based on methyl 5-(thiocyanato)-2-pyridinecarboxylate (6)]. Anal. ($C_{13}H_9BrNNaO_2S$) C, H, N.

5-[(*p*-Chlorophenyl)thio]-2-pyridinecarboxylic Acid (22) (Scheme III). 5-Nitro-2-pyridinecarboxylic Acid (9). Dimethyl 5-nitro-2-pyridinemalonate (8)[9] (152.5 g, 0.6 mol) was slurried in ice–water (1 L) and aqueous 20 N NaOH (30 mL) was added. A hot solution of $KMnO_4$ (133 g, 0.675 mol) in water (550 mL) and 20 N NaOH (15 mL) was added rapidly with vigorous mechanical stirring. Additional solid $KMnO_4$ (436 g, 2.21 mol) was added portionwise along with 20 N NaOH (70 mL). As the exothermic reaction subsided, the reaction mixture was heated with a hot-water bath for the remainder of the permanganate

addition. Upon completion of the addition (1–2 h), Celite was added to the mixture, which was then filtered while hot. The residue was well washed with hot 2 N NaOH. The filtrate was cooled and adjusted to pH 3–4 with 12 N HCl. Upon cooling, a cream-colored crystalline precipitate of 5-nitro-2-pyridinecarboxylic acid (**9**) developed: 60.15 g (60%); mp 208–210 °C dec (lit.[9] mp 210–212 °C).

Methyl 5-Nitro-2-pyridinecarboxylate (10). 5-Nitro-2-pyridinecarboxylic acid (**9**) (106.4 g, 0.633 mol) was slurried in methanol (1 L). Concentrated H_2SO_4 (57 mL) was added and the mixture was refluxed. A clear solution developed which was refluxed for 4 h and then cooled in ice. To the well-stirred, cooled solution ice-cold 2 N NaOH (420 mL) was added. The final adjustment to approximate neutrality was made with aqueous Na_2CO_3. Further ice–water (200 mL) was added with stirring. Crystals separated which were collected to give methyl 5-nitro-2-pyridinecarboxylate (**10**): 98 g (85%); mp 150–153 °C (lit.[9] mp 154–156 °C).

Methyl 5-[(*p*-Chlorophenyl)thio]-2-pyridinecarboxylate. Methyl 5-nitro-2-pyridinecarboxylate (**10**) (7 g, 0.0384 mol) was dissolved in warm DMF (75 mL) and added to a solution of *p*-chlorothiophenol (6 g, 0.0415 mol) in DMF (60 mL) containing 50% NaH in mineral oil (1.92 g, 0.04 mol) under N_2. The reaction mixture was heated for 6 h on a steam bath, then filtered, diluted with ice water, and adjusted to neutrality (5 N HCl), and ethyl acetate extracted. The combined extracts were washed (water), dried ($MgSO_4$), and concentrated to dryness in vacuo. The crystalline residue was crude methyl 5-[(*p*-chlorophenyl)thio]-2-pyridinecarboxylate (8.7 g, 81%). An analytical sample, mp 92–94 °C, was obtained by recrystallization from ethyl acetate. Anal. ($C_{13}H_{10}ClNO_2S$) C, H, N.

Hydrolysis. The crude methyl 5-[(*p*-chlorophenyl)thio]-2-pyridinecarboxylate (8.5 g, 0.0304 mol) was refluxed in aqueous ethanolic 2 N NaOH for 2 h. Some insoluble material was removed by filtration after addition of hot water (200 mL). The filtrate was cooled in an ice bath. The sodium salt of 5-[(*p*-chlorophenyl)thio]-2-pyridinecarboxylic acid crystallized out (7.15 g, 82%): mp 290–295 °C. Anal. ($C_{12}H_7ClNNaO_2S$) C, H, N. The free acid was obtained by solution of the sodium salt (4.1 g) in boiling water (150 mL) and reduction of the pH to 3 with 6 N HCl. On cooling, 5-[(*p*-chlorophenyl)thio]-2-pyridinecarboxylic acid (**22**) (3.65 g, 97%), mp 156–159 °C, crystallized. Anal. ($C_{12}H_8ClNO_2S$) C, H, N.

Oxidation to Sulfoxides. 5-[[*m*-(Trifluoromethyl)benzyl]sulfinyl]-2-pyridinecarboxylic Acid (42). Methyl 5-[[*m*-(trifluoromethyl)benzyl]thio]-2-pyridinecarboxylate (3.73 g, 0.0114 mol) was dissolved in CH_2Cl_2 (100 mL). *m*-Chloroperbenzoic acid (2.48 g of 87% purity, 0.0125 mol) was added as a solid with stirring. The solution was allowed to stand overnight. The CH_2Cl_2 was removed in vacuo. The residue was dissolved in ether. The ether was washed (aqueous $NaHCO_3$, water), dried ($MgSO_4$), and removed in vacuo. The crystalline residue (3.7 g) was recrystallized from methanol–ether to give methyl 5-[[*m*-(trifluoromethyl)benzyl]sulfinyl]-2-pyridinecarboxylate (2.4 g): mp 127–131 °C. Anal. ($C_{15}H_{12}F_3NO_3S$) C, H, N.

The sulfoxide ester (2.3 g, 0.0067 mol) was dissolved in methanol (10 mL) and 1 N NaOH (7.4 mL) added. After the mixture was allowed to stand overnight at room temperature, methanol was removed in vacuo. The aqueous residue was made acidic with a few drops of 6 N HCl. The precipitate was collected and recrystallized from aqueous methanol to give 5-[[*m*-(trifluoromethyl)benzyl]sulfinyl]-2-pyridinecarboxylic acid (**42**) (1.55 g, 70%): mp 170–175 °C. Anal. ($C_{14}H_{10}F_3NO_3S$) C, H, N.

Oxidation to Sulfones. 5-[[*m*-(Trifluoromethyl)benzyl]sulfonyl]-2-pyridinecarboxylic Acid (43). Methyl 5-[[*m*-(trifluoromethyl)benzyl]thio]-2-pyridinecarboxylate was reacted in an exactly analogous procedure, using 2 equiv of *m*-chloroperbenzoic acid. The ester sulfone was hydrolyzed to the acid in the same manner that was used for the sulfoxide, except that, on removal of the methanol in vacuo, the sodium salt of the acid **43** separated: mp 315–320 °C. Anal. ($C_{14}H_8F_3NNaO_4S$) C, H, N.

Pharmacology. Antihypertensive Assay in Spontaneous Hypertensive Rats. Male SHR approximately 16 weeks of age with systolic blood pressures greater than 150 mmHg were used for these studies.

Animals were placed in individual Lucite restraint cages to restrict excess movement. The cages were placed in a large plastic chamber and the animal tails passed through an inflatable occlusive cuff. A pneumatic, rubber bulb pulse sensor was placed distal to the cuff (width 0.5 cm) and taped circumferentially about the tail. The connecting tubing from the pulse sensor was attached to a pneumatic pulse transducer (Narco Bio Systems) and a solenoid-controlled manifold connected to a blood pressure cuff pump (Narco Bio Systems) was calibrated to deliver maximum air pressure of 250 mmHg. Upon completion of all connections, the chamber door was closed and a warm-air delivery system turned on. The system was electrically modified to heat upon demand of a thermistor probe within the chamber to maintain a temperature of 32.5 ± 0.5 °C. Air volume was such as to exchange three chamber volumes per minute. Animals were allowed to acclimate for 1 h to ensure adequate circulation in the tail. During this time, pressure calibration was checked and set on each of the electrosphygmographs (Narco Bio Systems).

After 1 h of acclimation at least three systolic blood pressure readings were taken on each group of animals. Pressure in the occlusion cuff was raised to 250 mmHg, so that arterial pulse displacements were no longer apparent, and then gradually lowered. The systolic pressure was identified by the location of the point that the pulse reemerged. Heart rates were determined by counting the pressure pulses.

All drugs were administered at a standard dose of 100 mg/kg (in some cases also at 200 mm/kg) by gavage in a mixture containing 3% cornstarch, 5% PEG-400, and 1 drop of Tween 80 per milliliter.

Animals were dosed daily for either 2 or 4 consecutive days with four to six rats used for each drug studied. Blood pressures were recorded at 1, 2, 3, and 24 h after each drug administration. Reported antihypertensive activity represented peak falls in pressure.

Antihypertensive Assay in Renal Hypertensive Dogs. Male mongrel dogs were made hypertensive by unilateral nephrectomy and either renal artery constriction[13] or kidney encapsulation[14] on the contralateral side.

Four to six weeks were allowed to elapse after experimental surgery for convalescence and the establishment of elevated blood pressure. Animals were trained to lie quietly in a supine position while their blood pressure was measured by direct femoral artery puncture with a 22-gauge, 1-in. hypodermic needle connected by polyethylene tubing to a Statham 23AA pressure transducer and displayed on a Sanborn recorder. Heart rate was counted manually. Drugs were given orally once daily in solid form by gelatin capsule. Blood pressures were determined at 1.5, 3.6, and 24 h after each drug administration with reported activity represented by maximum changes in blood pressure and heart rate over a daily monitored session.

Blood Pressure Assay in Normotensive Monkeys. Squirrel monkeys of either sex were placed in primate chairs for restraint purposes. Their tails were warmed with an infrared lamp to optimize circulation. Systolic blood pressure was measured indirectly by sphygmomanometry. An occlusion cuff was placed at the base of the tail and connected to an inflation system and pulse transducer (Narco Bio Systems). Pressure in the occlusion cuff was raised to 250 mmHg so that arterial pulse displacements were no longer apparent and then gradually lowered. The pressure decay curve was calibrated and displayed on a Beckman dynograph, the systolic pressure being identified by the location of the point where the pulse reemerged. Heart rates were counted manually from the pressure record. Drugs were administered by gavage as a suspension in 3% cornstarch containing 5% PEG-400 and 1 drop of Tween 80 per 10 milliliters. Animals were dosed daily and pressures were monitored at 1.5, 3, 5, and 24 h after each dose. Data were analyzed by using the paired *t* test.[15] Activity was presented as the peak change following each administration of a compound.

Acknowledgment. We wish to acknowledge the support and encouragement of Dr. Max Wilhelm and

helpful discussions with Professor Peter Yates. We thank G. Robertson for microanalyses, R. Behnke for NMR spectra, and N. Cahoon for IR and UV spectra.

References and Notes

(1) C. I. Johnston, *Med. J. Aust., Spec. Suppl.*, 3 (1975).
(2) J. N. Cohn and J. A. Franciosa, *N. Engl. J. Med.*, **297**, 27 (1977).
(3) R. J. Matta and G. F. Wooten, *Clin. Pharmacol. Ther.*, 14, 541 (1973).
(4) S. G. Chrysant, P. Adamopoulos, M. Tsuchiya, and E. D. Frohlich, *Am. Heart J.*, **92**, 335 (1976).
(5) J. Delarge, *Pharm. Acta Helv.*, **44**, 637 (1969).
(6) H. Hidaka, F. Hara, N. Harada, Y. Hashizume, and M. Yano, *J. Pharmacol. Exp. Ther.*, **191**, 384 (1974).
(7) B. Blank, N. W. DiTullio, L. Deviney, J. T. Roberts, A. Magnani, M. Billig, and H. L. Saunders, *J. Med. Chem.*, **20**, 1572 (1977), and references cited therein.
(8) N. Finch and C. W. Gemenden, *J. Org. Chem.*, **40**, 569 (1975).
(9) G. H. Cooper and R. L. Rickard, *J. Chem. Soc. C*, 3257 (1971).
(10) L. W. Deady, R. A. Shanks, A. D. Campbell, and S. Y. Chooi, *Aust. J. Chem.*, **24**, 385 (1971).
(11) G. H. Cooper and R. L. Rickard, *J. Chem. Soc. C*, 772 (1971).
(12) J. G. Topliss, *J. Med. Chem.*, **15**, 1006 (1972).
(13) H. Goldblatt, J. Lynch, R. F. Hanzal, and W. W. Summerville, *J. Exp. Med.*, **59**, 347 (1934).
(14) I. H. Page, *J. Am. Med. Assoc.*, **113**, 2046 (1939).
(15) G. W. Snedecor and W. G. Cochran in "Statistical Methods", Iowa State University Press, Ames, IA, 1967, pp 91–98.

2,5,5-Trimethylthiazolidine-4-carboxylic Acid, a D(−)-Penicillamine-Directed Pseudometabolite of Ethanol. Detoxication Mechanism for Acetaldehyde

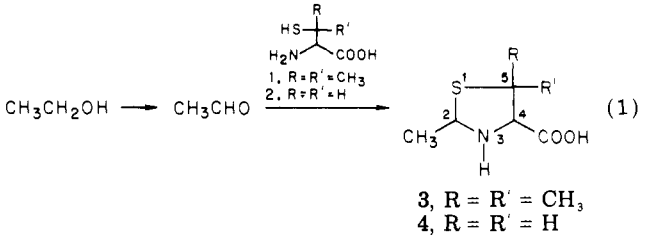
Herbert T. Nagasawa,* David J. W. Goon, and Eugene G. DeMaster

Medical Research Laboratories, Veterans Administration Hospital, and the Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55417. *Received July 24, 1978*

A directed detoxication mechanism for acetaldehyde (AcH) is described wherein ethanol-derived AcH, circulating in the blood of rats given ethanol-*1*-^{14}C and disulfiram or pargyline, was sequestered by condensation with administered D(−)-penicillamine (1). The product of this condensation, 2,5,5-trimethylthiazolidine-4-carboxylic acid (3), which was excreted in the urine without acetyl conjugation, was quantitatively determined by inverse isotope dilution measurements. Acetylation of the urine permitted the isolation of the corresponding *N*-acetyl derivative in crystalline form. The chirality of **3** was deduced by NMR analysis to be 72% 2*S*,4*S* and 28% 2*R*,4*S*. Although acetylation selectively acetylated the predominant isomer, this acetylated derivative was identical in all respects with a chemically synthesized product. This suggests that the in vivo condensation of AcH and 1 is not enzyme mediated.

The increasingly accepted hypothesis that acetaldehyde (AcH), the first metabolic product of ethanol, may be the causative agent in initiating the multitude of acute pharmacological and chronic pathophysiological effects of alcohol[1] suggested to us to seek methods to reduce its circulating blood levels. It was reasoned that the benefits of such lowered blood AcH might be protective for peripheral organs such as the heart and brain which contain only traces of alcohol dehydrogenase[2] and therefore are exposed only to *circulating* AcH—that which escaped hepatic metabolism.

We have succeeded in sequestering blood AcH generated from the metabolism of ethanol in rats by administration of the metabolically inert sulfhydrylamino acid, D(−)-penicillamine (1). This amino acid condensed with the ethanol-derived AcH in vivo to form the cyclic imino acid, 2,5,5-trimethylthiazolidine-4-carboxylic acid (**3**).[3] The elevation in blood AcH levels in disulfiram pretreated rats after ethanol administration, a consequence of aldehyde dehydrogenase inhibition by disulfiram, was sequestered by 1[4] concomitant to a corresponding increase in the excretion of **3** in the urine, thus relegating the overall process (see eq 1) to a detoxication mechanism for AcH.



A detailed proof of the structure of **3** and elucidation of the probable nonenzymatic nature of this in vivo condensation of AcH and 1 by reference to the chirality of synthetically produced **3** constitute the major thrust of this report. Additional in vivo experiments not previously reported are also described, showing the exclusivity of 1 in sequestering blood AcH compared to other sulfhydrylamino acid analogues and derivatives.

With respect to the stereochemical consideration alluded to, it is possible to infer a priori that since (a) a new chiral center at C-2 is generated in **3**, formed by the condensation reaction, and (b) the fixed chirality at the α position of D(−)-penicillamine (1)[5] should be unaffected during this process, any stereoselectivity imparted to the prochiral center, viz., to C-2, of the product isolated from rat urine (as in **3a** or **3b**, vide infra) could reflect an *enzymatic* mechanism for this condensation. On the other hand, lack of stereoselectivity at C-2 would relegate this to a nonenzymatic process. These considerations are somewhat nullified if steric constraints on 1 direct the incoming CH_3CHO to a sterically preferred orientation of the methyl group. Such asymmetric induction would impart to the C-2 carbon a preferred chirality which may not necessarily be enzyme directed.

Results

Detoxication Reaction for AcH. Our initial experiments were based on the expectation that administration of the natural sulfhydrylamino acid, L-cysteine (2), to ethanol-treated rats would direct its condensation with the ethanol metabolite, AcH, in vivo to the cyclic imino acid, 2-methylthiazolidine-4-carboxylic acid (**4**). Subsequent