solvent from the extract gave 10 g of a residue which was treated with 42 mL of 1 N HCl and 7.7 g (0.095 mol) of KCNO and maintained at 0 °C during the portionwise addition of 16.3 mL of concentrated HCl. The mixture was heated at 100 °C for 1.5 h, cooled, and extracted with EtOAc. Isolation of acidic material from the extract gave 3.1 g (25%) of the product. A sample from HOAc had mp 251–253 °C.

1,3-Dimethyl-5-(2,4,5-trichlorophenyl)hydantoin (4j, Table I). This was prepared from **4f** with Me_2SO_4, similar to the preparation of **4e** described above.

5-(2,4,5-Trichlorophenyl)hydantoin Sodium Salt. To a suspension of 13.9 g (0.05 mol) of 5-(2,4,5-trichlorophenyl)hydantoin in 150 mL of H_2O was added 50 mL of 1.0 N NaOH. After stirring for several hours at room temperature all but a trace of the solid had dissolved. The mixture was filtered and then concentrated to dryness in vacuo. The residue was recrystallized from EtOH to give 5.1 g of the product (34%) as a white solid which sinters at 288 °C, gradually shrinks, and melts with decomposition at 294–297 °C. Anal. ($C_9H_4Cl_3N_2O_2Na$) C, H, N. This procedure is not completely reproducible. From run to run salts containing varying degrees of hydration were obtained.

1-(5-Nitro-2-thiazolyl)-5-(2,4,5-trichlorophenyl)hydantoin (6). A solution of 3 g (0.0061 mol) of 1-[bromo(2,4,5-trichlorophenyl)acetyl]-3-(5-nitro-2-thiazolyl)urea in 5 mL of DMF containing 0.3 g of NaH (45% dispersion in mineral oil) was stirred at room temperature for 0.5 h and poured onto ice. Acidification with AcOH provided the product which was recrystallized from 2-PrOH to give 0.7 g (30%), mp 203–205 °C dec (after drying for 3 days at 100 °C under high vacuum). Anal. ($C_{12}H_5Cl_3N_4O_4S$) C, H, N.

2-Bromo-2-(2,4,5-trichlorophenyl)acetamide. A mixture of 29 g (0.12 mol) of (2,4,5-trichlorophenyl)acetic acid[13] in 24.6 mL of $SOCl_2$ was heated under reflux for 2 h, and the $SOCl_2$ was then removed in vacuo. The residue was treated with 22.4 g (0.14 mol) of Br_2 and a trace of red P and heated for 2 h at 130–150 °C under illumination. Excess Br_2 and HBr were removed by passing air through the reaction mixture for 1 h, and the contents of the flask was then dissolved in Me_2CO and added dropwise to concentrated NH_4OH. The solid that formed was recrystallized from C_6H_6 to give 24 g of the product (62%), mp 132–134 °C. Anal. ($C_8H_5BrCl_3NO$) C, H, N.

Bromo(2,4,5-trichlorophenyl)acetyl Isocyanate. A suspension of 6.3 g (0.0265 mol) of 2-bromo-2-(2,4,5-trichlorophenyl)acetamide in 30 mL of 1,2-dichloroethane containing 2.2 mL of oxalyl chloride was heated under reflux for 17 h. Distillation furnished the product as an oil (3.0 g, 43%), bp 130 °C (0.7 mm). The material absorbs moisture readily and was not analyzed but used as is in the next step.

1-[Bromo(2,4,5-trichlorophenyl)acetyl]-3-(5-nitro-2-thiazolyl)urea. The above isocyanate (3.0 g, 0.007 mol) in about 5 mL of THF was added dropwise to a solution of 1.0 g (0.0072 mol) of 2-amino-5-nitrothiazole in 25 mL of THF, and the mixture was allowed to remain at room temperature overnight. The mixture was filtered, and solvent was removed from the filtrate in vacuo. The residue was recrystallized from EtOH to give 1.3 g (38%) of the product, mp 176–177 °C. Anal. Calcd for $C_{12}H_6BrCl_3N_4O_4S$: C, 29.5; H, 1.2; N, 11.5. Found: C, 30.7; H, 1.4; N, 11.7. This material appears to lose HBr upon further crystallization and satisfactory analytical values could not be obtained. The product is adequate for use in the cyclization step.

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(2) Netherlands Patent 6 505 226 (Oct 25, 1965) (Ciba Ltd.).
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Synthesis and Hypoglycemic Activity of 4-Substituted 3-Mercaptopicolinic Acids[1]

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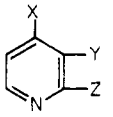
3-Mercapto-4-methylpicolinic acid was one of very few compounds derived from 3-mercaptopicolinic acid (3-MPA) to have hypoglycemic activity. In an effort to find compounds with greater potency than 3-MPA, several 4-substituted 3-mercaptopicolinic acids (4-OMe, OC_6H_5, SMe, SH, Cl, NH_2, Et; **1–7**) were prepared and tested in 48-h fasted rats. None was hypoglycemic in this test system after oral dosing of 150 mg/kg.

3-Mercaptopicolinic acid (3-MPA) is a potent inhibitor of gluconeogenesis[2] in several animal models primarily by virtue of its ability to inhibit the enzyme phosphoenolpyruvate carboxykinase (PEPCK).[3-6] Since this enzyme is one of the key regulatory enzymes in the de novo synthesis of glucose, inhibition of PEPCK should result in a lowering of blood glucose levels in fasted and diabetic animals. Although the concept of lowering blood sugar levels in diabetics by inhibiting their elevated rates of gluconeogenesis has been an idea of long standing,[7] 3-MPA has been the first agent potent enough to lower blood glucose levels in a number of animal models by this

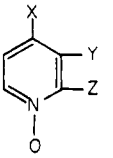
Table I. Pyridines



No.	X	Y	Z	Mp, °C	Recrystn solvent	% yield	Formula[a]
1	OMe	SH	CO_2H	186-187	MeCN	38	$C_7H_7NO_3S$
2	OC_6H_5	SH	CO_2H	174-175	MeOH	30	$C_{12}H_9NO_3S$[b]
3	SMe	SH	CO_2H	229-230[c]	HOAc-H_2O	33	$C_7H_7NO_2S_2$
4	SH	SH	CO_2H	199-201[c]	H_2O	31	$C_6H_5NO_2S_2$
5	Cl	SH	CO_2H	265-267[c]	MeCN	42	$C_6H_4ClNO_2S$
6	NH_2	SH	CO_2H	247-249[c]	H_2O	20	$C_6H_6N_2O_2S$[d]
7a	Et	SH	CO_2H	174-176	H_2O	58	$C_8H_9NO_2S$[e]
7b	Et	SH	$CONH_2$	151-153	MeCN	40	$C_8H_{10}N_2OS$
13a	OMe	SMB[f]	CO_2Me	60-61	Et_2O	92	$C_{16}H_{17}NO_4S$
13b	OC_6H_5	SMB	CO_2Me	92-93	Cyclohexane	63	$C_{21}H_{19}NO_4S$
13c	SMe	SMB	CO_2Me	85-86	Et_2O	60	$C_{16}H_{17}NO_3S_2$
13d	SMB	SMB	CO_2Me	120-122	MeOH		$C_{23}H_{23}NO_4S_2$
13e	Cl	SMB	CO_2Me	50-52	MeOH-H_2O	42	$C_{15}H_{14}ClNO_3S$
13f	NH_2	SMB	CO_2Me	159-161[c]	EtOH-Et_2O	80	$C_{15}H_{16}N_2O_3S\cdot HCl$[b]
13g	Et	SMB	CN	67-69	MeOH	85	$C_{16}H_{16}N_2OS$
13h	Et	SMB	$CONH_2$	95-96	MeOH-H_2O	81	$C_{16}H_{18}N_2O_2S$
13i	NHAc	SMB	CO_2Me	174-176[c]	MeOH-Et_2O	70	$C_{17}H_{18}N_2O_4S\cdot HCl$
14a	OMe	SMB	CO_2H	148-150	MeOH-H_2O	84	$C_{15}H_{15}NO_4S$[g]
14b	OC_6H_5	SMB	CO_2H	140-142	EtOH-H_2O	95	$C_{20}H_{17}NO_4S$
14c	SMe	SMB	CO_2H	150-152	MeOH	93	$C_{15}H_{15}NO_3S_2$
14d	SMB	SMB	CO_2H	150	MeCN	82	$C_{22}H_{21}NO_4S_2$
14e	Cl	SMB	CO_2H	169-170	MeOH-H_2O	73	$C_{14}H_{12}ClNO_3S$[h]
14f	NH_2	SMB	CO_2H	200-202	MeOH-H_2O	71	$C_{14}H_{14}N_2O_3S$
14g	Et	SMB	CO_2H	139-141	MeCN	81	$C_{16}H_{17}NO_3S$

[a] Compounds for which formulas are given were analyzed for C, H, and N and often also for Br, Cl, and/or S; analytical values were within ±0.4% of the calculated values unless otherwise noted. [b] Hydrate. [c] With decomposition. [d] S: calcd, 18.84; found, 18.22. [e] Contains 0.75 mol of H_2O. [f] *p*-$MeOC_6H_4CH_2S$-. [g] C: calcd, 59.00; found, 59.56. [h] Hemihydrate.

Table II. Pyridine *N*-Oxides



No.	X	Y	Z	Mp, °C	Recrystn solvent	% yield	Formula[a]
8	NO_2	Br	CO_2Me	175-177	C_6H_5Me-petr ether	47	$C_7H_5BrN_2O_5$
9	NO_2	SMB[b]	CO_2Me	115-116	EtOAc-petr ether	40	$C_{15}H_{14}N_2O_6S$
10	SMB	SMB	CO_2Me	147-148	EtOAc		$C_{23}H_{23}NO_5S_2$
11	SMB	Br	CO_2Me	132-133	EtOAc		$C_{15}H_{14}BrNO_4S$
12a	OMe	SMB	CO_2Me	99-100	CCl_4	90	$C_{16}H_{17}NO_5S$
12b	OC_6H_5	SMB	CO_2Me	116-117	EtOAc-petr ether	90	$C_{21}H_{19}NO_5S$
12c	Cl	SMB	CO_2Me	112	EtOAc-petr ether	60	$C_{15}H_{14}ClNO_4S$
12d	SOMB	Br	CO_2Me	140	$MeCl_2$-Et_2O	90	$C_{15}H_{14}BrNO_5S$
12e	SO_2MB	Br	CO_2Me	180-182	$MeCl_2$	80	$C_{15}H_{14}BrNO_6S$
16	NO_2	SMB	H	168-169	Me_2CO	30	$C_{13}H_{12}N_2O_4S$
17	OMe	SMB	H	145-146	Me_2CO	60	$C_{14}H_{15}NO_3S$
18	Et	Br	H	70-72	Methylcyclohexane	61	C_7H_8BrNO

[a] Compounds for which formulas are given were analyzed for C, H, and N and often also for Br, Cl, and/or S; analytical values were within ±0.4% of the calculated values unless otherwise noted. [b] *p*-$MeOC_6H_4CH_2S$-.

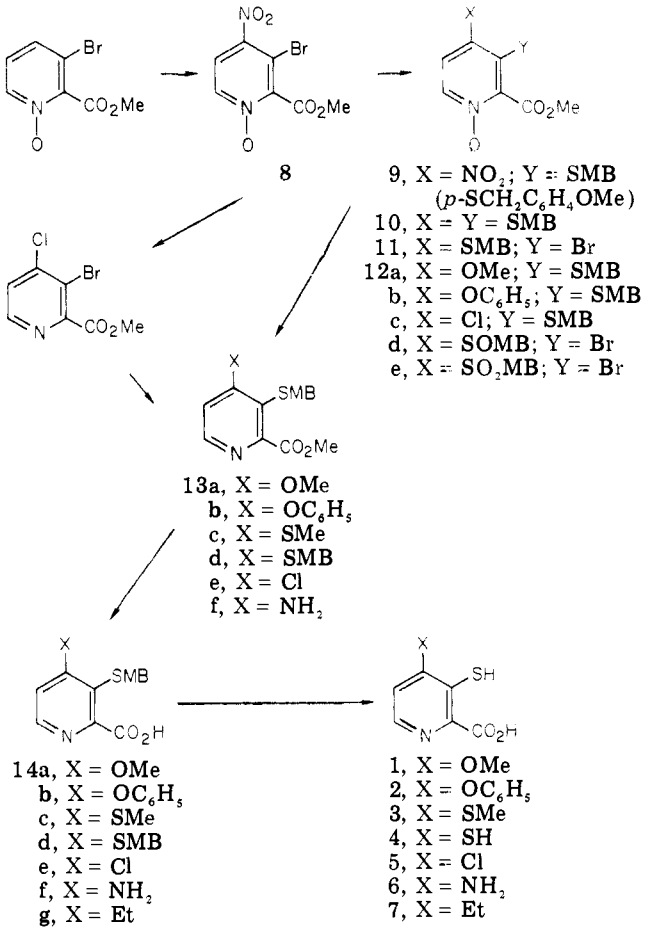
mechanism[2,8] without undesirable side effects.

Efforts to enhance the potency of 3-MPA led us to prepare and study its isomers,[8] a number of its derivatives,[8,9] and analogues in which pyridine was replaced with other heterocycles.[10] In addition, several examples were prepared in which an additional chloro,[8] mercapto,[10] methyl,[8] or carboxyl group[10] was introduced.

The biological findings are readily summarized. The only hypoglycemic compounds in these series were certain *S*-acyl derivatives,[8,9] 3-mercapto-4-methylpicolinic acid,[8] and 3-mercapto-2-indolecarboxylic acid.[10] The most potent acyl derivatives proved less potent than 3-MPA when compared in dose range studies.[9] The indole analogue, although reasonably hypoglycemic after an intraperitoneal dose of 200 mg/kg, was lethal by the 4-h sampling time.[10] The 4-methyl congener of 3-MPA has hypoglycemic activity when given to fasted rats at 300 mg/kg ip.[8] Although its activity was minimal, 3-mercapto-4-methylpicolinic acid was the only example of a 4-substituted 3-mercaptopicolinic acid available for study. This, together with the fact that it was one of very few compounds in a large series which had any measurable activity, led us to prepare other 4-substituted 3-mercaptopicolinic acids. The compounds prepared (1–7) are listed in Table I.

In general, the syntheses were based on the knowledge that the 4-nitro group in pyridine *N*-oxides undergoes facile

Scheme I

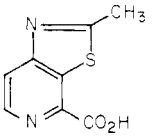


nucleophilic displacement.[11] Thus, an appropriate intermediate for several analogues (1, 2, and 4) was methyl 3-(*p*-methoxybenzylthio)-4-nitropicolinate *N*-oxide (9), derived from the reaction of methyl 3-bromo-4-nitropicolinate *N*-oxide (8) with *p*-methoxybenzyl mercaptan (MBM).[12] The sequences leading to 1–7 are shown in Scheme I.

The preparation of 9 was complicated by the concomitant formation of methyl 3,4-bis(*p*-methoxybenzylthio)picolinate *N*-oxide (10) and methyl 3-bromo-4-(*p*-methoxybenzylthio)picolinate *N*-oxide (11) as well as by the recovery of unreacted 8. After manipulation of several reaction variables, optimum yields of 9 were 35–40%. It was impossible to significantly alter the composition of the product mixture except in the relative amounts of 8 and 10. The optimum was a mixture containing 9, 10, and 11 in a ratio of about 35:15:45. Column chromatography on silica was required to separate and purify these compounds.

Nucleophilic displacement of the nitro group of 9 gave 10 and 12a,b, deoxygenation gave 13a,b,d, hydrolysis gave 14a,b,d, and removal of the methoxybenzyl (MB) group as shown in Scheme I gave 1, 2, and 4. In light of previous experience with the preparation of methyl 6-chloro-3-mercaptopicolinate,[8] attempts were made to remove the MB group with the substrates 13a,b,e, with the hope that isolation of products might be simplified. The reactions were carried out with mercuric acetate in trifluoroacetic acid and seemed to proceed straightforwardly. The products were soluble in organic solvents and behaved as expected in thin-layer chromatographic and spectral systems. However, elemental and mass spectral analyses disclosed that these materials contained mercury which could not be removed even after repeated exposures to hydrogen sulfide in several solvents. Accordingly this approach was not pursued further. Rather, the mercapto acids 1–7 were obtained from the corresponding methoxybenzylthio acids 14a–g.

Methyl 4-amino-3-(*p*-methoxybenzylthio)picolinate (13f) was prepared by reducing 9 with iron powder in acetic acid. Hydrolysis and removal of the MB group yielded 4-amino-3-mercaptopicolinic acid (6). Several unexpected findings were noted in an unsuccessful attempt to prepare the corresponding 4-*N*-acetyl derivative. Although 13f can be acetylated readily to give methyl 4-acetamido-3-(*p*-methoxybenzylthio)picolinate (13i) (characterized as the hydrochloride), this was not the case with 4-amino-3-(*p*-methoxybenzylthio)picolinic acid (14f) under a variety of acylating conditions. The acid 14f was recovered unchanged. When the ester 13i was hydrolyzed with dilute alkali, 14f rather than the desired *N*-acetyl acid was the product. Copper sulfate catalyzed ester cleavage of 13i was accompanied by loss of the MB group and subsequent cyclization to produce 2-methyl[5,4-*c*]thiazolopyridine-4-carboxylic acid (14h). The bicycle was obtained after removal of the copper with hydrogen sulfide. Its structure was consistent with all analytical data. This marked the first time that we had noted loss of the MB group other than in a mercuric ion catalyzed reaction.



14h

Efforts to prepare methyl 3-(*p*-methoxybenzylthio)-4-nitropicolinate (13, X = NO_2) by allowing 9 to react with phosphorus trichloride at reduced temperatures were unsuccessful. This technique had been employed successfully to prepare 4-nitropyridine from the corresponding *N*-oxide.[13] In our hands no reaction occurred with 9 below room temperature and at room temperature conversion was directly to methyl 4-chloro-3-(*p*-methoxybenzylthio)picolinate (13e) with no evidence for the intermediacy of either the chloro *N*-oxide 12c or 13 (X = NO_2), using thin-layer chromatography as a monitor.

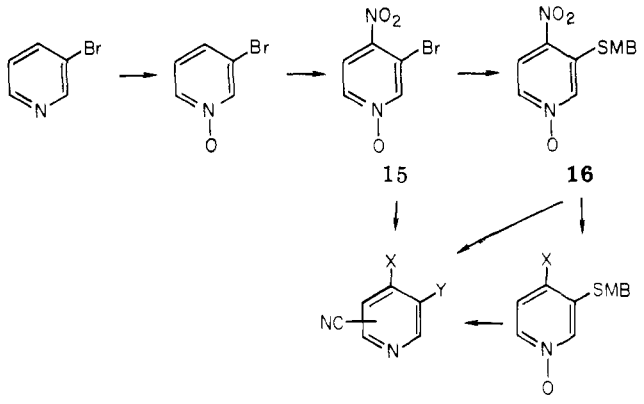
Since 13e was prepared in a single operation in this way, it served as the precursor to 4-chloro-3-mercaptopicolinic acid (5). 5 was also prepared by the longer sequence (9 → 12c → 13e → 14e → 5).

An alternative attempt to prepare 13 (X = NO_2) relied upon the possible deoxygenation of 8 with phosphorus trichloride to give methyl 3-bromo-4-nitropicolinate. However, as with 9, deoxygenation and displacement occurred simultaneously to produce methyl 3-bromo-4-chloropicolinate. Again there was no evidence for the intermediacy of deoxygenated nitro compound or of a chloro *N*-oxide.

Treatment of methyl 3-bromo-4-chloropicolinate with MBM yielded a mixture of products similar to those produced from 8 and MBM. The products 13d,e and methyl 3-bromo-4-(*p*-methoxybenzylthio)picolinate were separated on a silica column and were isolated respectively in a ratio of 2.5:5:1.

When the conversion of 9 to 12 (X = SMe) was attempted using slightly more than 1 equiv of sodium methylmercaptide, a complex mixture resulted. From this mixture two products were isolated and identified. One was methyl 3-(*p*-methoxybenzylthio)-4-methylthiopicolinate (13c), identical with material prepared by treatment of 13e with methyl mercaptide. The second, obtained in much smaller quantity, was methyl 3-(*p*-

Scheme II



methoxybenzylthio)-4,6-bismethylthiopicolinate.

There are reports of pyridine *N*-oxides being deoxygenated and substituted with mercaptans but usually in the presence of anhydrides or sulfonyl halides.[14] The latter reagents are postulated to react with the *N*-oxide oxygen and facilitate nucleophilic attack by the mercaptan onto the pyridine ring. Since there are unknown products from this reaction, it is unclear in what manner the *N*-oxide oxygen is lost.

Hydrolysis of **13c** to produce **14c** followed by deblocking of sulfur yielded 3-mercapto-4-methylthiopicolinic acid (**3**).

An attractive alternative to the routes employed above was to subject the substrates 3-bromo-4-nitropyridine *N*-oxide (**15**)[15] or 3-(*p*-methoxybenzylthio)-4-nitropyridine *N*-oxide (**16**) to the conditions of the Reissert–Kaufman reaction.[16] Previous success with such an approach had led to mercaptopicolinic acids with additional carboxyl or mercapto groups.[10] Other positive features of such an approach were that the starting material for this sequence, 3-bromopyridine, was commercially available and inexpensive, deoxygenation and introduction of the potential carboxylic acid were effected essentially simultaneously, and either **15** or **16** was a potentially suitable substrate for the Reissert–Kaufman reaction as well as a precursor for several analogues (Scheme II).

A moderate yield of **16** (35–50%) was obtained upon treatment of **15** with MBM. Subjecting either **15** or **16** to the conditions of the Reissert–Kaufman reaction resulted in each instance in recovery of starting material. When the substrate for the Reissert–Kaufman reaction was 4-methoxy-3-(*p*-methoxybenzylthio)pyridine *N*-oxide (**17**), a mixture of products was isolated, with 4-methoxy-5-(*p*-methoxybenzylthio)picolinonitrile the major component. This was determined initially by examination of the NMR spectrum of the reaction mixture and confirmed subsequently by partially purifying this component using preparative thin-layer chromatography and/or dry column chromatography.

Although this approach was unsuitable as a general route to 4-substituted mercaptopicolinic acids, it did lead to 4-ethyl-3-mercaptopicolinic acid (**7**). 3-Bromo-4-ethylpyridine[17] was converted to its *N*-oxide **18** with *m*-chloroperoxybenzoic acid. Treatment of **18** with dimethyl sulfate and then aqueous sodium cyanide gave 3-bromo-4-ethylpicolinonitrile as the only isolable product. Displacement of bromine with MBM yielded 4-ethyl-3-(*p*-methoxybenzylthio)picolinonitrile (**13g**) in high yield. Alkaline hydrolysis in alcohol for 18 h produced the amide **13h** while heating **13g** in aqueous KOH for 60 h gave the corresponding acid **14g**. In each case, mercuric ion catalyzed cleavage of the MB group yielded the corresponding mercaptan, 4-ethyl-3-mercaptopicolinamide (**7b**), and 4-ethyl-3-mercaptopicolinic acid (**7a**).

Attempts to prepare 4-fluoro-3-mercaptopicolinic acid by treating **9**, **12c**, or **13e** with KF in DMF[18] or in MeCN with crown ethers[19] were abortive. Starting materials were recovered.

Discussion

The 4-substituted analogues of 3-MPA (**1**–**7**) were tested for hypoglycemic activity in 48-h fasted rats after oral dosing of 150 mg/kg. The analogues were uniformly inactive in this system. The continuing demonstration that modifications of any nature decreased or destroyed the hypoglycemic activity of 3-MPA[8-10] forces one to propose a receptor site for 3-MPA with a high degree of structural specificity.

The juxtaposition of nitrogen, carboxyl group, and mercaptan in 3-MPA is similar to that of the same functions in cysteine. It is tempting to speculate that 3-MPA binds at a site normally occupied by a key cysteine (probably in a protein) that is involved in the functioning of PEPCK. 3-MPA may be effective in this role because it simulates a favored configuration of the protein-bound cysteine. If this is so it is easier to see why further elaboration of the 3-MPA structure leads to less active compounds (poorer fit).

PEPCK has a preferential requirement for divalent magnesium and GTP (or ITP). For the enzyme to have maximum activity in the direction of phosphoenolpyruvate synthesis, a second divalent cation is required and with the purified enzyme manganese is preferred.[20] Bentle and Lardy have reported that PEPCK from rat is activated by a ferrous ion containing protein.[21,22] This laboratory has also indicated that 3-MPA inhibits PEPCK and interacts with the metal-containing protein.[23] Presumably, the inhibitory activity of 3-MPA in this system results from its interaction with the "activator" protein. Conversely, 3-aminopicolinic acid, which is hyperglycemic,[8] enhances the activity of the PEPCK "activator" protein.[23] These findings suggest that 3-MPA might inhibit PEPCK by interacting with divalent cations.

However, the data presented below argue against such a mechanism for 3-MPA. In vitro studies were carried out with the purified enzyme and 3-MPA. Increasing concentrations of manganese ion were added to the system with no evidence that the inhibitory action of 3-MPA was being reversed.[6] A similar study using PEPCK from rat liver cytosol, which contains "activator" protein, produced comparable results. Neither added manganese nor ferrous ions had any effect on the inhibitory action of 3-MPA.[24] It is more difficult to explain the inactivity of closely related 3-MPA analogues using an argument which invokes binding or chelating of an essential metal ion since in some cases the analogues might be thought to bind or chelate as well as or better than 3-MPA.

PEPCK is an enzyme known to contain free mercapto groups. It is also recognized that on reaction with thiol reagents, the activity of the enzyme is reduced or abolished.[21,25] Thus, our current speculation is that 3-MPA acts by interfering, in some way, with one or more key thiol groups because 3-MPA resembles a particular preferred configuration of cysteine essential for the enzymatic activity of PEPCK.

Experimental Section

All melting points were taken in a Thomas-Hoover melting point apparatus and are uncorrected. Compounds for which formulas are given were analyzed for C, H, and N and often also for Br, Cl, and S; analytical values were within ±0.4% of the calculated values unless otherwise noted. Analyses were performed

by members of our Analytical and Physical Chemistry Section.

Methyl 3-Bromo-4-nitropicolinate *N*-Oxide (8). To a stirred solution of 125 mL of H_2SO_4, 13 mL of 30% oleum, and 50 mL of red fuming HNO_3 was added 35 g (0.15 mol) of methyl 3-bromopicolinate *N*-oxide.[8] The mixture was stirred and heated on a steam bath for 3 h in such a way that the nitrogen oxides were easily vented (one neck of flask left open). The resulting solution was poured carefully over ice with stirring. The precipitated solid was collected, washed with H_2O, dried, and recrystallized. Extraction of the aqueous filtrate with $CHCl_3$ removed starting material which was recovered when the $CHCl_3$ was evaporated.

Methyl 3-(*p*-Methoxybenzylthio)-4-nitropicolinate *N*-Oxide (9). A steady stream of a solution of 30.8 g (0.2 mol) of *p*-methoxybenzyl mercaptan (MBM)[12] in 200 mL of CH_2Cl_2 was added to a stirred solution of 50 g of Et_3N in 200 mL of CH_2Cl_2 under N_2. A slurry of 27.7 g (0.1 mol) of **8** in 300 mL of CH_2Cl_2 was added steadily and the solution was stirred overnight at room temperature under N_2. The solution was washed with H_2O, dried, and concentrated. The residue was dissolved in EtOAc–cyclohexane (1:2 by volume) and chromatographed on a silica column (Brinkmann, 70–230 mesh). The first fractions contained MBM and its disulfide. The next fractions contained unreacted **8** followed closely by **9**. Succeeding fractions contained methyl 3,4-bis(*p*-methoxybenzylthio)picolinate *N*-oxide (**10**) and methyl 3-bromo-4-(*p*-methoxybenzylthio)picolinate *N*-oxide (**11**), with **10** being removed from the column last.

Methyl 4-Methoxy-3-(*p*-methoxybenzylthio)picolinate *N*-Oxide (12a). To a stirred, cooled solution of 0.4 g (9.5 mmol) of a 57% mineral oil dispersion of NaH in 50 mL of MeOH was added a solution of 2.8 g (8 mmol) of **9**. After stirring for 1 h at room temperature the MeOH was removed and the residue was dissolved in a mixture of H_2O and $CHCl_3$. The $CHCl_3$ was washed with H_2O, dried, and evaporated. The residue was recrystallized.

Methyl 3-(*p*-Methoxybenzylthio)-4-phenoxypicolinate *N*-Oxide (12b). A solution of sodium phenoxide was prepared in 60 mL of 1,2-dimethoxyethane (DME) from 1.05 g of NaH (57% in mineral oil dispersion) and 1.9 g (0.025 mol) of phenol. To this cooled, stirred solution was added a solution of 7 g (0.02 mol) of **9** in 100 mL of DME. After 2 h at room temperature, the solvent was removed and the residue was partitioned between EtOAc and H_2O. The organic phase was washed with H_2O, dried, and evaporated.

Methyl 4-Chloro-3-(*p*-methoxybenzylthio)picolinate *N*-Oxide (12c). A solution of 2 g (6 mmol) of **9** in 100 mL of MeOH was stirred, cooled, and saturated with gaseous HCl. The solution was stirred 30 min after saturation was complete and the solvent was removed. The residue was evaporated with C_6H_6 and CH_2Cl_2 and recrystallized.

Sulfoxide and Sulfone of Methyl 3-Bromo-4-(*p*-methoxybenzylthio)picolinate *N*-Oxide (12d and 12e). The substrate **11** was oxidized with *m*-chloroperoxybenzoic acid using conditions described previously for the preparation of the methyl sulfoxide and sulfone of 3-MPA.[10]

Deoxygenation with PCl_3. To a stirred, cooled solution of 0.01 mol of **8** (or similar substrate) in 90 mL of $CHCl_3$ (dried over $CaCl_2$) was added dropwise 15 mL of PCl_3 at 25–30 °C. Stirring was continued for 2 h at room temperature and the solution was poured into a mixture of ice and $CHCl_3$. The mixture was adjusted to pH 7–8 with solid Na_2CO_3; layers were separated; the aqueous phase was extracted with $CHCl_3$; and the organic phase was washed with H_2O, dried, and evaporated. The residue contains a small amount of phosphorus-containing material. For most purposes the product was used as such, but the impurity could be removed readily by passing the crude reaction product through a silica column with EtOAc–cyclohexane (1:1 by volume). The impurity remains at the top of the column; the product trails the solvent front closely.

Methyl 3-bromo-4-chloropicolinate: mp 39–40 °C (hexane); 80%. Anal. ($C_7H_5BrClNO_2$) C, H, N, Br.

Methyl 3-bromo-4-(*p*-methoxybenzylsulfonyl)picolinate: mp 175 °C ($MeCl_2$); 95%. Anal. ($C_{15}H_{14}BrNO_5S$) C, H, N, Br, S.

The other examples **13a,d,e**, were prepared in similar fashion from **12a**, **10**, and **9**, respectively.

Deoxygenation with Fe–HOAc (13b and 13f). A mixture of 0.04 mol of **9** or **12b** and 24 g of powdered Fe in 235 mL of HOAc was stirred at 90–100 °C for 1 h. The mixture was cooled to room temperature, diluted with H_2O and EtOAc, and adjusted to pH 7–8 with solid Na_2CO_3. The layers were separated, the aqueous layer was extracted again with EtOAc, and the organic phases were washed with H_2O and dried. Evaporation of the solvent left the crude products. The amine **13f** was isolated and purified as its hydrochloride.

Methyl 4-Chloro-3-(*p*-methoxybenzylthio)picolinate (13e). To a cooled, stirred slurry of 1 g of a 57% mineral oil dispersion of NaH in 15 mL of dry THF was slowly added 4.6 g (0.03 mol) of MBM in 20 mL of THF. The mixture was stirred 15 min under N_2 and then a solution of 7.3 g (0.028 mol) of methyl 3-bromo-4-chloropicolinate in 25 mL of THF was added. The mixture was heated and stirred for 2.5 h under N_2. The THF was removed, the residue was distributed between H_2O and $CHCl_3$, and the $CHCl_3$ layer was washed, dried, and evaporated. The residual gum (11 g) was taken up in EtOAc–cyclohexane (1:2 by volume) and a solid precipitated. The solid was collected and washed with the same solvent mixture. The combined filtrates were placed on a column of silica and eluted with the EtOAc–cyclohexane mixture. Fractions of 50 mL were collected.

Fractions 5 and 6 contained **13e** and a little starting material. They were combined and rechromatographed using the same solvent system to give 3.4 g of **13e**.

Fractions 9 and 10 were combined and evaporated to give 0.6 g of methyl 3-bromo-4-(*p*-methoxybenzylthio)picolinate, mp 127–129 °C (CCl_4). Anal. ($C_{15}H_{14}BrNO_3S$) C, H, N, Br, S.

Fractions 12–19 were combined and evaporated to give 1.8 g of **13d**.

Methyl 3-(*p*-Methoxybenzylthio)-4-methylthiopicolinate (13c). A suspension of sodium methyl mercaptide was prepared by bubbling 1 g of methyl mercaptan into a stirred, cooled suspension of 350 mg of 57% NaH in a mineral oil dispersion in 25 mL of DME. A solution of 1.75 g (5 mmol) of **9** in 25 mL of DME was added and the mixture was stirred at room temperature overnight under N_2. The DME was evaporated, the residue was dissolved in $CHCl_3$ and H_2O, and the layers were separated. The $CHCl_3$ layer gave 2 g of an oil. The oil formed a gummy solid when triturated with H_2O. Further trituration with MeOH deposited solid which was recrystallized from MeCN to give 100 mg of methyl 3-(*p*-methoxybenzylthio)-4,6-bis(methylthio)-picolinate, mp 142–144 °C. Anal. ($C_{17}H_{19}NO_3S_3$) C, H, N, S.

Evaporation of the MeOH filtrates gave 1.4 g of a mixture which was chromatographed on silica with EtOAc–cyclohexane (1:1 by volume). The first fraction (100 mL) gave an additional 500 mg of the 4,6-dimethylthio compound. Subsequent fractions gave **13c** identical with material obtained by treating **13e** with methyl mercaptide as described below. The R_f of **13c** in thin-layer chromatographic systems using 1:1 EtOAc–cyclohexane was indistinguishable from that of **9**.

Allowing equimolar amounts of NaH, methyl mercaptan, and **13e** to react overnight under N_2 in DME gave **13c** cleanly in 60% yield.

Methyl 4-Acetamido-3-(*p*-methoxybenzylthio)picolinate (13i). A solution of 2 g (6.6 mmol) of **13f** and 10 mL of Ac_2O was stirred and heated 2 h on a steam bath. The solution was left overnight at room temperature and concentrated. The residue was azeotroped twice with C_6H_5Me to remove traces of Ac_2O and gave 1.6 g of a viscous oil homogeneous by thin-layer chromatography. A portion was converted to the hydrochloride salt for complete characterization.

3-Bromo-4-ethylpicolinonitrile. 3-Bromo-4-ethylpyridine[17] was converted to its *N*-oxide **18** with *m*-chloroperoxybenzoic acid using conditions described previously.[8] The *N*-oxide was converted to the picolinonitrile using the reaction protocol described by Matsumara et al.[10,16]

4-Ethyl-3-(*p*-methoxybenzylthio)picolinonitrile (13g). This material was prepared by subjecting 3-bromo-4-ethylpicolinonitrile to conditions previously described for similar substrates.[8,10]

4-Ethyl-3-(*p*-methoxybenzylthio)picolinamide (13h). A solution of 5 g (0.0175 mol) of **13g**, 2.8 g of KOH, 5 mL of H_2O, and 100 mL of EtOH was stirred overnight under reflux. The mixture was concentrated, diluted with H_2O, and filtered. The filtrate was extracted with $CHCl_3$; the extracts were used to dissolve the original solid and were washed with H_2O, dried, and

concentrated. The concentrate was diluted with petroleum ether and cooled. The product was collected and recrystallized.

4-Substituted 3-(*p*-Methoxybenzylthio)picolinic Acids (14a–f). A solution of 0.015 mol of the ester **13**, 20 mL of 10% NaOH, and 100 mL of EtOH was stirred at room temperature for 3 h. The solution was filtered, the pH of the filtrate was adjusted to 2–3 with dilute HCl, and the EtOH was removed. The aqueous residue was cooled and filtered. The solid was washed with H_2O, dried, and recrystallized.

4-Ethyl-3-(*p*-methoxybenzylthio)picolinic Acid (14g). The solution formed by stirring and refluxing 5.5 g (0.02 mol) of **13g**, 3 g of KOH, and 100 mL of H_2O for 60 h was cooled and filtered. The filtrate was acidified and the product was isolated as the other acids, **14a–f**.

4-Substituted 3-Mercaptopicolinic Acids (1–7). The acids **14a–g** were dissolved in trifluorocetic acid (TFA) and diluted with a solution of mercuric acetate in TFA.[8] The cherry-red solution was stirred overnight at room temperature under N_2. The TFA was removed and the residue was triturated several times with C_6H_6. The residue was then redissolved in TFA and the solution was saturated with hydrogen sulfide. The precipitated sulfides were removed and washed, and the TFA filtrates were evaporated. The residue was evaporated three times with fresh portions of C_6H_5Me and recrystallized.

2-Methyl[5,4-c]thiazolopyridine-4-carboxylic Acid (14h). A mixture of 800 mg (2.3 mmol) of **13i**, 2 g of copper sulfate pentahydrate, 20 mL of MeOH, and 35 mL of H_2O was stirred under reflux overnight. The mixture was cooled and filtered, and the filter cake was washed with H_2O and dried. The solid was dissolved in TFA and the solution was saturated with hydrogen sulfide. The copper sulfide was removed and washed with TFA. The filtrates were evaporated and the residue was azeotroped with C_6H_5Me and triturated with MeCN. The solid formed was collected and recrystallized from H_2O: yield 450 mg (86%); mp 219–221 °C dec. Anal. ($C_8H_6N_2O_2S$) C, H, N, S.

3-(*p*-Methoxybenzylthio)-4-nitropyridine *N*-Oxide (16). Using a protocol similar to that described for the preparation of **9** allowed 3-bromo-4-nitropyridine *N*-oxide (**15**)[15] to be converted to **16**. The crude product was purified by chromatography on silica.

4-Methoxy-3-(*p*-methoxybenzylthio)pyridine *N*-Oxide (17). Treating **16** with an equimolar amount of NaOMe in MeOH as was done in the preparation of **12a** yielded **17**.

Biochemistry. Hypoglycemic activity was measured in 48-h fasted male rats weighing ca. 200 g. On the morning of the test day, a zero-time tail-vein sample was obtained, followed by the oral administration of the test compound suspended in 0.5% tragacanth. A similar group of animals receiving only the vehicle served as controls. Tail-vein samples were obtained at 1, 2, and 4 h after drug administration. Glucose determinations have been described previously.[2,8] 3-MPA, after a comparable oral dose, lowered blood glucose levels 30% at 1 h, 42% at 2 h, and 45% at 4 h.[2] These values were significant at the $p \leq 0.001$ level.

References and Notes

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