mation of gentisamide sulfate (Table IV). The good agreement between the HPLC and colorimetric assay results for total gentisamide demonstrate the suitability of the HPLC assay for gentisamide and its metabolites.

In conclusion, a combination of two HPLC procedures with the same sample preparation (deproteinization and addition of internal standard) were developed for the direct determination of salicylamide and its metabolites in biological fluids. One of these metabolites, gentisamide sulfate, is reported for the first time. The excretion products of salicylamide in human urine can account for essentially the total amount of administered drug. The HPLC assays for the conjugated metabolites were developed without synthetic or isolated pure standards by a procedure that should be useful also for conjugates of other drugs.

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Synthesis, Isolation, and Characterization of Two Stereoisomeric Ring Sulfoxides of Thioridazine

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Abstract □ A selective oxidation of thioridazine to give exclusively its ring sulfoxides and a separation of the resulting products as diastereoisomeric pairs of enantiomers (DL, LD and DD, LL) are reported. These pairs were characterized by TLC, high-performance liquid chromatographic, IR, UV, ^{1}H-NMR, ^{13}C-NMR, GC–MS, and elemental analyses, and by reduction to thioridazine by lithium aluminum hydride. Structural data for the separated diastereoisomeric pairs or their nitric acid salts were obtained from NMR and IR studies. Gram quantities of each of the two diastereoisomeric pairs of enantiomers were isolated in better than 99% purity.

Keyphrases □ Thioridazine—oxidation to ring sulfoxides, separation of the diastereoisomeric pairs of the ring sulfoxides by crystallization of the nitric acid salts, ^{13}C-NMR analysis of thioridazine ring sulfoxides

A single ring sulfoxide of thioridazine has been detected by TLC (1–5), high-performance liquid chromatography (HPLC) (6, 7), and GC (8–10). Also, EKG abnormalities have been attributed "to a single ring sulfoxide" in plasma (8). Recently "two very similar ring sulfoxides" of thioridazine were identified (11) as urinary metabolites and prepared (with other products) by chemical oxidation.

Evidence is presented here that these ring sulfoxides are

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Table I—Chromatographic Detection of Oxidation Products of Thioridazine (I) and Mesoridazine (III) as TLC Spots or HPLC Peaks

Sample[a]	Compound I	IIF[b]	IIS[b]	III	IV[c]	System[d]
A	x	x	x	x		I, II, V[e], VI
B	x	x	x	x	x	I, II, V[f], VI
C				x	x	I, V[g]
D	x	x	x	x	x	I, II, VI
E	x	x	x	x	x	I, V[e]
F	x	x	x	x	x	I, V
G		x	x			I, III[h], IV[h,i], V[e]

[a] Sample A is a synthetic mixture. B–F are oxidation mixtures from hydrogen peroxide treatment and G from nitrous acid treatment. C involves the oxidation of mesoridazine. [b] IIS and IIF were found in about equal amounts in all samples of oxidized I. [c] Further identified by its intense blue fluorescence under long-wavelength UV irradiation. [d] Various solvent systems, I–IV and VI, on silica gel TLC, and an HPLC method, V, were used. [e] Chromatograms are presented in Fig. 1. [f] Approximate compound ratios 2.8:12.5:14.4:37.7:29.9, respectively. [g] Percent of IV (peak area) = 90.1. [h] Failed to separate IIF and IIS. [i] Gave R_f 0.45, close to the literature report of R_f 0.46 (13–15).

actually diastereoisomeric pairs of enantiomers (DL, LD and DD, LL). TLC and HPLC methods used to detect and distinguish these diastereoisomeric pairs and a crystallization method that yields gram amounts of each pair with better than 99% purity are described. These pure materials have been used to develop an HPLC assay of thioridazine and its metabolites in plasma (12).

RESULTS AND DISCUSSION

TLC—Literature methods (1, 2, 13–15) were used. Thioridazine (I), the pairs of enantiomeric ring sulfoxides (IIF and IIS, fast and slow migrators on silica gel, respectively), the side-chain sulfoxide of thioridazine [mesoridazine (III)], and thioridazine disulfoxide (IV) were detected (Table I) in the reaction products from hydrogen peroxide oxidation of thioridazine. Many minor products were also produced. On silica gel, the ring sulfoxides generally tend to elute between thioridazine and mesoridazine. The more polar disulfoxide elutes after mesoridazine, and the less polar sulfone, sulforidazine, elutes just after thioridazine. Both the disulfoxide and sulfone are readily distinguished by their pronounced fluorescence under long-wavelength UV irradiation. Although sulforidazine may occur in relatively high concentrations in thioridazine metabolism (16), it was not found as a product in the chemical oxidations performed in this study. Sulfoxidation occurs before any appreciable *N*-oxidation is observed.

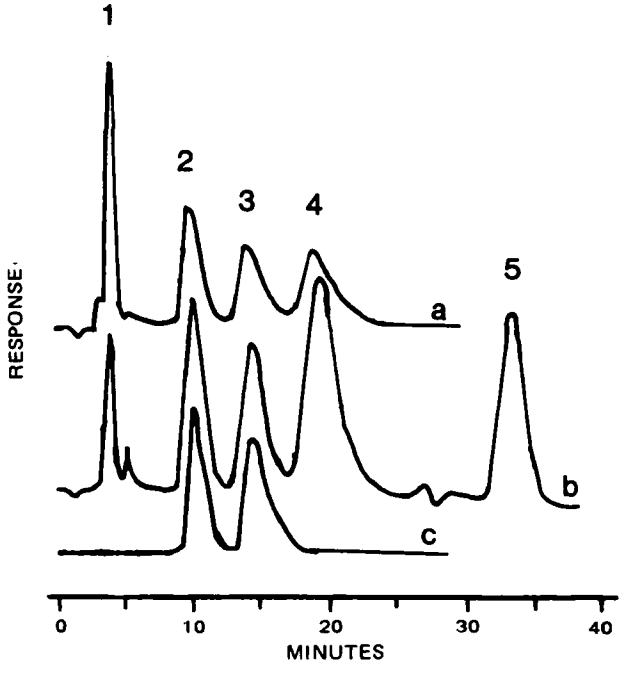

Figure 1—*High-performance liquid chromatograms. Key: a, reference mixture; b, product of reaction of thioridazine and hydrogen peroxide in refluxing alcohol, as previously described (1); and c, product of reaction of thioridazine hydrochloride and nitrous acid. (1) Thioridazine; (2 and 3) thioridazine ring sulfoxides; (4) mesoridazine; and (5) thioridazine disulfoxide. In curve b, the polarity of the mobile phase was increased after peak 4 to achieve elution of the disulfoxide.*

Table II—R_f Values and Color Detection for the Oxidation Products of Thioridazine Formed by Hydrogen Peroxide (in Acetic Acid and Aqueous Solvent) and by Nitrous Acid[a]

Compound	R_f, System I[d]	System VI, Reagents[b] Folin	H_2SO_4	100°	R_f, System II[c] Found	Reported[e]
I	0.71	B–G	—	—	0.75	0.83
IIF	0.62	—	B–V[f]	—	0.55	0.54
IIS	0.54	—	B–V[f]	—	0.50	0.54
III	0.39	P	—	—	0.43	0.45
IV	0.23	—	—	P	0.28	0.29

[a] Nitrous acid oxidation products gave only two spots for the ring sulfoxides (IIF and IIS). [b] Sprays or operations (2) done in sequence to produce the colors shown; B = blue, G = green, P = pink, V = violet. [c] Acetone–12 *N* ammonium hydroxide (100:7) (2). [d] Chloroform–95% ethanol–12 *N* ammonium hydroxide (80:20:1). [e] R_f values from the literature (2), including value for a single ring sulfoxide. [f] Initial B–G spots turned B–V after several hours.

HPLC—A highly sensitive assay method (12), which employs a silica gel column, was used to provide qualitative and semiquantitative data on the distribution of products in some of the oxidation mixtures. Figure 1 (curve a) illustrates a typical chromatogram, obtained from a mixture of ring sulfoxides (IIF and IIS) and reference standards of I and III. Products from the hydrogen peroxide oxidations (*Experimental, Methods* I–III) included both ring sulfoxides, which eluted with the same retention times as shown in Fig. 1 (curve a).

Literature Reports of a Single Ring Sulfoxide—The approach taken in this study was to show that ordinary oxidations of thioridazine, including some published methods (1, 17), gave two enantiomeric pairs (IIF and IIS) of the ring sulfoxides (two TLC spots and not one) and to examine TLC systems, including some reported in the literature (1, 2, 13–15), for their effectiveness in separating IIF and IIS. Next, the isolation of larger amounts of each ring sulfoxide (IIF and IIS) was undertaken to provide samples for identification, particularly by instrumental techniques.

Both ring sulfoxides (IIF and IIS) were found in about equal amounts in all the samples of oxidized thioridazine (Table I; samples and detection systems are described in detail in the *Experimental* section). TLC systems I and II, as well as HPLC system V, separated IIS and IIF, but TLC systems III and IV failed to give a separation. Samples A, B, and D were used to evaluate a reported TLC system (2); solvent system II and spray system VI produced the R_f values and colors presented in Table II. Although this report (2) described a single spot for thioridazine ring sulfoxide, a small but clear separation was obtained in the present work. All materials gave colors that matched those described (2).

Under conditions of more extensive oxidation, more thioridazine disulfoxide (IV) formed at the expense of the ring sulfoxides (II) and mesoridazine (III). After a reaction time of 24 hr, concentrated aqueous hydrogen peroxide gave primarily the disulfoxide (IV) and a small amount of mesoridazine (III), whereas dilute aqueous hydrogen peroxide generated the usual major products, I, IIF, IIS, III, and IV, with some minor products appearing as very faint spots on the TLC plates.

Analysis of Commercial Samples of Thioridazine Ring Sulfoxide—Two samples were assayed by HPLC system V (12). The first contained ~ 98% of IIS and 2% of IIF, and the second ~ 85% of IIS and 15% of IIF. TLC (system I) gave a rough confirmation of these results. The IR spectrum of the first commercial sample matched that of analytically pure IIS.

Study of the Reaction of Zehnder *et al.* (1)—To determine whether ring sulfoxides IIF and IIS were produced under these reaction conditions, the solution of the crude reaction product (sample E) was examined by TLC (Table I) and by HPLC (Fig. 1, curve b). Thioridazine (I), the two ring sulfoxides (IIF and IIS), mesoridazine (III), and the disulfoxide (IV) appeared at essentially the same R_f values and retention times observed for products of the hydrogen peroxide oxidations. The two ring sulfoxides were found in about equal amounts. Retention times in Fig. 1, curve b, matched those of the reference mixture (sample A) in Fig. 1, curve a. The area percentages for substances in curve b are: I, 4.4; IIF, 15.6; IIS, 17.3; III, 34.4; and IV, 23.3.

Effect of pH—Zehnder *et al.* (1) refluxed thioridazine hydrochloride with hydrogen peroxide in ethanol, mildly acidic conditions. Sample E,

Table III—Physical and Analytical Data for Thioridazine Ring Sulfoxides

Compound	Melting Point		Analyses Calc.	Found	Purity, %[a]
		Nitric Acid Salts			
VF	254.5–255.0°	C	56.10	55.87	99.9
		H	6.05	6.03	
		N	9.35	9.21	
VS	226.2–227.0°	C	56.10	56.09	99.3
		H	6.05	6.05	
		N	9.35	9.16	
V[b]	204.0–224.0°		—	—	—
		Free Bases[c]			
IIF	119.0–120.0°	C	65.25	65.00	—
		H	6.78	6.81	
IIS	141.0–142.0°	C	65.25	65.32	—
		H	6.78	6.90	
II	113.5–128.5°	C	65.25	65.06	—
		H	6.78	6.77	

[a] Based on HPLC analyses of nitric acid salts neutralized with 12 *N* ammonium hydroxide. [b] Mixture of both salts (VF and VS) obtained as the reaction product. [c] The free bases, IIF, IIS, and II, were obtained from the corresponding salts, VF, VS, and V.

obtained in this way, gave an amount of mesoridazine roughly equal to the total amount of the ring sulfoxides. By substitution of the free base of thioridazine in the refluxing medium, sample F was obtained under mildly basic conditions and was found to contain about three times as much mesoridazine as the amount of total ring sulfoxides. Thus, oxidation under mildly basic conditions favors side-chain sulfoxidation. A previous report (16) found that the rates of formation of mesoridazine and sulforidazine (side-chain sulfoxidation) in plasma are greater than that of ring sulfoxidation. One might speculate that oxidation of thioridazine under acidic conditions in the stomach could favor formation of ring sulfoxides and contribute to toxic side reactions (8).

Disadvantages of Hydrogen Peroxide Oxidations—Complex product mixtures are obtained, and intricate, inefficient separations must be used to isolate the pure ring sulfoxides from the hydrogen peroxide oxidations. Thus, a new method for the oxidation of thioridazine exclusively to its ring sulfoxides and a technique for their separation as two pairs of enantiomers should be useful for analytical studies of thioridazine metabolites and perhaps for toxicological studies.

Nitrous Acid Oxidation of Thioridazine Hydrochloride—The nitric acid salts of the ring sulfoxides (VF and VS) are generated exclusively when thioridazine hydrochloride is oxidized with nitrous oxide (sample G, Table I). The freshly prepared reaction mixture was immediately examined by HPLC system V; the product was > 99% thioridazine ring sulfoxides (Fig. 1, curve c), which eluted at the same retention times as the analytically pure sulfoxides (Fig. 1, curve a, peaks 2 and 3). TLC (Table I, system I) also showed essentially only the ring sulfoxides.

Formation of the nitric acid salts can be described by these equations (18) (T = thioridazine).

$$2HNO_2 + T(I) \rightarrow TO(II) + 2NO + H_2O \quad \text{(Eq. 1)}$$

$$4HNO_2 \rightarrow 4NO + O_2 + 2H_2O \quad \text{(Eq. 2)}$$

$$3HNO_2 \rightleftarrows HNO_3 + 2NO + H_2O \quad \text{(Eq. 3)}$$

$$2NO + O_2 \rightarrow 2NO_2 \quad \text{(Eq. 4)}$$

$$2NO_2 + H_2O \rightleftarrows HNO_3 + HNO_2 \quad \text{(Eq. 5)}$$

$$TO(II) + HNO_3 \rightarrow TO \cdot HNO_3(V) \quad \text{(Eq. 6)}$$

Nitrous acid can be an effective oxidizing agent (Eqs. 1 and 2). The nitric acid salt V (Eq. 6) is readily generated from nitrous acid, which is unstable and decomposes to nitric acid (Eq. 3), and also through other reactions (Eqs. 4 and 5). The observed oxidation with nitrous acid is not due to nitric acid. Substitution of sodium nitrate for sodium nitrite in the reaction procedure failed to give any oxidation of thioridazine.

Separation of Ring Sulfoxides as Free Bases—Attempts to isolate the ring sulfoxides (IIF and IIS) by HPLC followed by crystallization yielded only small amounts of material of ~ 95% purity, as determined by HPLC analyses. The UV spectra (λ_{max} = 279 nm, weak peak at 342 nm) of the collected fractions were identical. The free bases crystallized well from hexane or heptane, but such recrystallization failed to purify them. Vacuum sublimation (175°, 1 mm) of the mixed free bases yielded no isomeric enrichment.

Table IV—^{13}C-NMR Data (ppm) for Thioridazine Ring Sulfoxides in Deuterochloroform[a]

Peak Number	Compound IIF	IIS
1	145.50	145.50
2	138.51[b]	138.61[b]
3	138.23[b]	138.07[b]
4	132.85	132.79
5	131.98[b]	131.89[b]
6	131.65[b]	131.72[b]
7	124.60	124.69
8	121.93	121.85
9	121.06	120.96
10	118.98	119.00
11	115.73	115.61
12	112.20	112.23
13	78.73[c]	78.74[c]
14	77.33[c]	77.34[c]
15	77.14[c]	77.14[c]
16	75.53	75.54
17	62.01	61.92
18	56.78	56.80
19	44.92	44.86
20	43.25	43.18
21	30.66	30.68
22	29.23	29.28
23	25.42	25.45
24	24.08	24.13
25	15.40	15.35

[a] TMS added as an internal standard. [b] Four aromatic carbons nearest the ring sulfur. [c] Deuterochloroform lines in spectrum of IIF and IIS demonstrate instrument reproducibility to 0.01 ppm.

Separation of Ring Sulfoxides as Nitric Acid Salts—A mixture of the nitric acid salts of the ring sulfoxides (V) was separated by fractional crystallization (19) from 95% ethanol. After six recrystallizations, all cuts contained essentially one isomer and some were fairly pure, *e.g.*, 99.9% VF and 96.8% VS, as determined by peak ratios from HPLC analyses. After two more recrystallizations from fresh solvent, VS was 99.3% pure (Table III).

These high-purity samples gave TLC R_f values that matched those of the original reaction mixture (Table II). The elemental analyses of the materials were consistent for the nitric acid salts of the ring sulfoxide isomers of thioridazine, and their melting points were sharp (Table III).

Characterization of the Ring Sulfoxides as Free Bases (IIF and IIS)—The free bases were obtained from the purified nitric acid salts and gave material with sharp melting points. Elemental analyses were consistent for the isomeric ring sulfoxides of thioridazine (Table III).

NMR Studies of Thioridazine Ring Sulfoxides as Free Bases—The ^{13}C-NMR spectra of IIF and IIS are tabulated (Table IV). The fact that most of the signals in the spectra of the two compounds agreed to within 0.1 ppm shows that the two types of ring sulfoxides (IIF and IIS) are very similar. Nevertheless, a difference in stereochemistry of the sulfoxide groups of IIS and IIF is suggested by the differences in the signals from the four aromatic carbons nearest the ring sulfur.

NMR Studies of Thioridazine Ring Sulfoxides as Nitric Acid Salts—The ^{13}C-NMR spectra of VF and VS (Table V), taken at 30° in deuterium oxide, are also clearly different for the nonprotonated aromatic carbons located next to the sulfoxide group. The broadening of peaks for aliphatic carbons can be explained if the protonated piperidine coordinates with the sulfoxide group. If the change in the precession frequencies of the aliphatic carbons resulting from the formation of a complex is comparable to the rate of forming and breaking the complex, line broadening will occur.

At 90° (Table V) either the complex is forming and breaking rapidly enough to average the chemical shifts, or it is broken up and the chemical shifts are characteristic of the freely rotating side chain resulting in sharp aliphatic peaks. Merging of aromatic signals at 90° is attributed to conformational mobility of the molecules which leads to some averaging of the aromatic carbon-13 chemical shifts.

Chemical Shifts of *S*- and *N*-Methyl Proton Signals of Thioridazine and Related Compounds—As side-chain sulfoxidation increases, only the *S*-methyl signal is shifted to lower field, but ring sulfoxidation causes a shift of both the *S*- and *N*-methyl proton signals to lower field relative to thioridazine (Table VI).

The *N*-methyl proton singlets from the ring sulfoxide free bases (IIF and IIS) did not show different chemical shifts. The differences in

Table V—^{13}C-NMR Data (ppm) for the Nitric Acid Salts of the Thioridazine Ring Sulfoxides in Deuterium Oxide

Peak Number	Compound VF	VS
	At 30°[a]	
1	146.59	146.75
2	138.49[b]	138.85[b]
3	137.53[b]	137.19[b]
4	134.16	134.04
5	131.24	131.16
6	131.00	130.96
7	123.08	123.14
8	119.14	119.05
9	118.66	118.24
10	117.04	117.26
11	112.39	112.09
12	41.99	42.13
13	14.02	26.82
14	—	20.68
15	—	14.00
	At 90°[c]	
1	134.07	134.01
2	131.35	131.42
3	130.95	131.01
4	123.14	123.26
5	119.99	120.10
6	117.02	117.22
7	113.19	113.19
8	61.94	61.98
9	55.41	55.44
10	42.57	42.77
11	27.11	38.97
12	26.80	27.25
13	21.49	26.86
14	20.33	21.50
15	14.53	20.39
16	—	14.63

[a] Twelve aromatic peaks were tabulated in both VF and VS. Many of the peaks from aliphatic carbons appeared as broad areas at the baseline and were not tabulated by the spectrometer. [b] Nonprotonated aromatic carbons located next to the sulfoxide group, 0.96 ppm apart for VF and 1.66 ppm apart for VS. [c] Nine sharp aliphatic peaks were observed and tabulated. Some of the aromatic signals are merged at the higher temperature.

chemical shifts of the *N*-methyl protons in VF and VS may be due to differences in the ring sulfoxide stereochemistry, which influences their coordination with the protonated *N*-methylpiperidinyl moiety. An analogous coordination occurs between the sulfoxide group in dimethyl sulfoxide and the hydroxyl groups in alcohols (20). The lack of dissociation of the protonated amine in the rather nonpolar deuterochloroform is suggested as the cause of *N*-methyl proton splitting in these compounds. Ratios of the salts (VF and VS) in mixtures can be determined by deuterium exchange and by integration of the piperidyl *N*-methyl peaks. Such mixtures are usually soluble enough to permit accurate estimation of the ratios of VF to VS to within 2–5% with a conventional NMR spectrometer.

Reductions with Lithium Aluminum Hydride—In anhydrous ether, lithium aluminum hydride reduced a mixture of the ring sulfoxides (IIF and IIS) to a single product, thioridazine. The reduction product matched reference standard thioridazine in TLC (system I), IR spectra, and GC–MS data. Mesoridazine was also reduced under similar conditions and gave the same TLC, IR, and GLC–MS characteristics for the reaction product, thioridazine.

Mass Spectra of Ring Sulfoxides—Essentially identical spectra were obtained when IIF and IIS were passed through the GC–MS. Programmed-temperature GC of IIF and IIS gave identical retention times; the peaks of these polar compounds were broad and appeared after the thioridazine peak. Upon direct injection, postcolumn with a heated vaporizer, both materials produced spectra showing the molecular ion. The spectra of both IIF and IIS had many peaks in common with thioridazine, including the molecular-ion peak for thioridazine and the base peak for the *N*-methylpiperidyl fragment.

The ring sulfoxides theoretically exist as two diastereoisomeric pairs, and these pairs are separable by physical means such as chromatography or fractional recrystallization. These compounds are of interest because of the known metabolic conversion of thioridazine to such oxidation products. Methods for syntheses of the materials in pure form are described. Elemental analyses and IR data are consistent with the *S*-oxide structure. ^{1}H-NMR clearly rules out the *N*-oxide in favor of the *S*-oxide structure because of the chemical shift of the *N*-methyl group. ^{13}C-NMR gives the unambiguous determination of the site of this oxidation as the ring sulfur atom.

Table VI—Chemical Shifts (ppm) [a] of *S*- and *N*-Methyl Proton Signals of Thioridazine and Related Compounds

Compound (Side-Chain Group)	*S*-Methyl	*N*-Methyl
Shifts Observed After Progressive Side-Chain Sulfoxidation		
I (CH_3S)	2.40	2.20
III (CH_3SO)	2.70	2.20
VI (CH_3SO_2)	3.00	2.20
VII (CH_3SO_2)[b]	3.00	—
Shifts Caused by Ring Sulfoxidation and Splitting Generated by Subsequent Protonation[c]		
IIF (CH_3S)	2.59	2.40
IIS (CH_3S)	2.59	2.40
VF (CH_3S)[d]	2.59	2.38, 2.44[e]
VS (CH_3S)	2.59	2.30, 2.36[e]

[a] In deuterochloroform; compared to thioridazine (I). [b] Methylsulfonylphenothiazine (VII) for comparison as parent structure of sulforidazine (VI). [c] Protons were retained on the more basic piperidine nitrogens. [d] A sensitive Fourier-transform NMR instrument was needed since VF was rather insoluble in deuterochloroform. [e] J_{NH-CH_3} = 5 Hz. Centers of these doublets are chemically shifted by 0.08 ppm. Both doublets collapsed to singlets on treatment with deuterium oxide. Deuterochloroform (100%) must be used to observe sharp peaks.

EXPERIMENTAL

Materials—All chemicals were official or reagent grade. Thioridazine hydrochloride, thioridazine free base, and two samples of ring sulfoxide were obtained commercially[1]. 3-Methylsulfonylphenothiazine (21) and sulforidazine (22) were synthesized.

Instruments—A grating IR spectrophotometer[2], a UV-visible spectrophotometer with recorder[3], a 60-MHz NMR spectrometer[4], and a Fourier-transform 80-MHz NMR spectrometer[4] were used. The 60-MHz instrument was used only for ^{1}H-NMR studies of free bases. The 80-MHz instrument was operated at 79.542 MHz for ^{1}H-NMR and at 20 MHz for ^{13}C-NMR. An electron-impact (70 eV) GLC–MS[5], operated with an 88-cm × 2-mm i.d. glass column[6], was used for the lithium aluminum hydride reduction studies. A GLC–MS[7] equipped with a 61-cm × 2-mm i.d. glass column[8] was used for analysis of the ring sulfoxides; alternatively, these compounds were directly inserted into a postcolumn vaporizer operated at 270°. All UV spectra were obtained with methanol solutions. All melting points were taken on a melting-point stage[9]. All IR frequencies are reported as corrected values. A molecular model set[10] was used for structural analyses. The HPLC system is reported elsewhere (12).

TLC—All chromatograms were performed with fluorescent, precoated silica gel sheets[11], cut to 50 × 75 mm, by ascending development under equilibrated conditions in a tank lined with filter paper[12]. Spots were located by irradiation with short- or long-wavelength UV or by this sequential treatment: spray with Folin reagent[13], spray with 50% H_2SO_4, and heat at 100° for a few minutes (2).

Reactions of Thioridazine and Mesoridazine with Hydrogen Peroxide—*Method I, Oxidation in Acetic Acid*—Thioridazine and mesoridazine bases were oxidized with hydrogen peroxide in glacial acetic acid at 45° as described previously (17). The reaction product was examined by TLC with a solvent system composed of chloroform–95% ethanol–12 *N* ammonium hydroxide (80:20:1). The sample was spotted

[1] Sandoz Pharmaceuticals, Hanover, NJ 07936.
[2] Model 3-200, Pye Unicam Ltd., Cambridge, England.
[3] Spektralphotometer DM 4 and linear recorder Model 300, Carl Zeiss, Oberkochen, West Germany.
[4] Models T60-A and FT-80A, Varian Associates, Palo Alto, CA 94303.
[5] Model 5992A, Hewlett-Packard, Palo Alto, CA 94304.
[6] OV-101 (2%) and 0.2% carbowax 20M on 100/200 mesh Chromosorb W-HP, Hewlett-Packard.
[7] Olfax IIA, Vitek Systems, Inc., McDonnell Douglas Corp., Hazelwood, MO 63042.
[8] OV-225 (1.5%) on Chromosorb G, Applied Science Laboratories, State College, PA 16801.
[9] Fisher Scientific Co., Pittsburgh, PA 15219.
[10] Dreiding stereomodels, Fisher Scientific Co.
[11] Silica gel 60 F-254, 0.20-mm thick, on aluminum, E. Merck, Darmstadt, Germany.
[12] Whatman No. 1, Whatman, Inc., Clifton, NJ 07014.
[13] Phenol Reagent Solution, 2 *N*, Folin-Ciocalteau, Fisher Scientific Co.

beside reference standards of thioridazine (I) and mesoridazine (III) and synthetic samples of the thioridazine ring sulfoxides (IIF and IIS). The chromatograms were inspected by short- and long-wavelength UV irradiation and by a detection system used for locating and identifying the spots by color (1). An HPLC system (12) was also used to identify the reaction products and to obtain approximate product ratios.

Method II—Thioridazine hydrochloride (40.7 mg) was treated in four different reactions with increasing concentrations of aqueous hydrogen peroxide. The drug was dissolved in the following amounts of water, and the following amounts of hydrogen peroxide were added to the solutions over about a 2-min period: 5 ml of water, 0.1 ml of 15% H_2O_2; 15 ml of water, 0.1 ml of 15% H_2O_2; 5 ml of water, 25 μl of 15% H_2O_2; and 15 ml of water, 25 μl of H_2O_2. The studies examined whether more strenuous oxidation conditions would favor formation of the dioxide over that of the ring sulfoxides (IIF and IIS). Products from these reactions were examined by TLC as in Method I.

Method III—Thioridazine hydrochloride and 40% hydrogen peroxide were refluxed 4 hr in ethanol, as described previously (1). The crude reaction product was examined by TLC and HPLC. This oxidation process was also conducted on thioridazine free base to determine whether there was a difference in the distribution of reaction products under mildly basic conditions.

Samples Prepared for Oxidation Studies—Sample A consisted of a mixture of reference standards of I and III with synthetic IIF and IIS, prepared as described below. The rest of the samples were reaction products formed by the following oxidations: B, I with hydrogen peroxide in glacial acetic acid (17); C, III with hydrogen peroxide in glacial acetic acid; D, the hydrochloride of I with hydrogen peroxide in aqueous solution; E, the hydrochloride of I with hydrogen peroxide in refluxing ethanol (1); F, I with hydrogen peroxide in refluxing ethanol; G, I with nitrous acid.

Chromatography Systems Used to Study Oxidation Products—Systems I–IV and VI were used with silica gel TLC: I, chloroform–95% ethanol–12 *N* ammonium hydroxide (80:20:1); II, acetone–12 *N* ammonium hydroxide (100:7) (2); III, ethyl acetate–glacial acetic acid–water (5:2:2) (1); IV, a solution of 1.5 g of ammonium acetate in 10 ml of water mixed with 50 ml of methanol (13–15). System V was an HPLC method (12); identification was made by observation of an increase of peak response without appearance of new peaks after the samples were spiked with appropriate individual components of sample A. System VI: TLC plates were sprayed with Folin reagent, then with 50% sulfuric acid, and were heated at 100° for a few minutes. TLC identifications were made by observing R_f values which were identical to those of the components of sample A.

Specific Oxidation of Thioridazine to Generate Only the Ring Sulfoxides of Thioridazine and Isolation as Nitric Acid Salts (VF and VS)—To a solution of 1.521 g of thioridazine hydrochloride in 100 ml of deionized water was added 10 drops of concentrated hydrochloric acid. To this mixture a sodium nitrite solution (1 g/10 ml) was added dropwise with stirring until the solution turned from blue to yellow-brown or started to turn orange. A small portion of this reaction mixture was analyzed immediately by HPLC; the ring sulfoxides had been formed exclusively. The mixture was extracted with two 30-ml portions of chloroform. The chloroform was removed by evaporation (steam bath) and then heptane (50 ml) was added to the glassy residue, and the solvent was again removed (steam bath) to give a yellow-orange powder. Residual heptane was removed by decantation. A small amount of this decanted heptane was boiled to dryness and contained no dissolved substances. The hard granular solid (VF and VS) after air drying weighed 1.07 g. The reaction was repeated with 3- and 6-g portions of thioridazine hydrochloride to yield 2.18 and 5.02 g, respectively. Melting points are given in Table III.

The nitric acid salts of the thioridazine ring sulfoxides (5.7 g) were fractionally crystallized (19) from 95% ethanol to give 290 mg of 99.9% VF and 360 mg of 99.3% VS. Purity was determined by HPLC (12). Melting points are given in Table III.

Conversion of the Nitric Acid Salts to Free Bases—The salt (VF or VS), 140 mg, was dissolved in 10 ml of warm deionized water. Concentrated ammonium hydroxide (60 μl) was added and an oil separated. The mixture was extracted with two 5-ml portions of chloroform, and the combined extracts were passed through filter paper[9], to remove water. The chloroform was removed (steam bath, nitrogen flush), and then methanol (5 ml) was added and removed in the same manner. The product was recrystallized from hexane or heptane to give a mixture of IIF and IIS (from VF and VS). When VF and VS were treated individually, analytically pure IIF and IIS were obtained. Melting points and analyses are given in Table III. IR (Nujol mull or KBr disk): IIF, 1019 (s) and 1050 (s) cm^{-1}; IIS, 1028 (s) and 1050 (s) cm^{-1}; (cast films): both IIF and IIS, 1028 (s) and 1050 (s) cm^{-1} and superimposable from 4000 to 6000 cm^{-1}. IR sulfoxide bands: II, 1020 and 1045 cm^{-1} (S=O) (1). IR (KBr) commercial sample of ring sulfoxide (IIS–IIF = 85:15): 1028 (s) and 1050 (s) cm^{-1} and superimposable from 4000 to 6000 cm^{-1} with a spectrum of IIS; mass spectrum: *m/z* (relative abundance, percent), IIF, 386 (0.5) (M^+); 370 (M − 16), 98; IIS, 386 (2.0) (M^+); 370 (M − 16), 98.

Reduction of Thioridazine Ring Sulfoxides to Thioridazine with Lithium Aluminum Hydride—The mixed nitric acid salts VF and VS (0.1 g) were converted to the thioridazine sulfoxide free bases IIS and IIF as described directly above. A solution of the free bases in 1.3 ml of anhydrous ether was stirred under a nitrogen atmosphere, and then a slurry of 0.1 g of lithium aluminum hydride in 1.3 ml of anhydrous ether was slowly added. A vigorous reaction occurred, and the solution turned orange and then yellow. After the reaction subsided, the mixture was refluxed for 24 hr. Water was added and the mixture was extracted three times with heptane–toluene (4:1). The solvent was removed to give 35 mg of thioridazine. Mesoridazine was reduced to thioridazine in the same manner. The products were compared with thioridazine by TLC, IR spectrophotometer, and GC–MS (identical retention times with thioridazine): *m/z* 370 (M^+), 98 (base peak, *N*-methyl piperidinyl fragment); instrument correlation index for thioridazine, 0.911.

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