Convenient Oxidation of Phenothiazine Salts to Their Sulfoxides with Aqueous Nitrous Acid

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Abstract □ A simple method is reported for the preparation of gram quantities of phenothiazine sulfoxides by aqueous nitrous acid oxidation of phenothiazines at room temperature. The chiral levomepromazine gave rise to diastereoisomeric products analogous to those reported for thioridazine sulfoxidation.

Phenothiazines are among the most commonly used antipsychotic agents for the treatment of schizophrenia and manic phases of manic–depressive illness.[1] Sulfoxidation resulting in 5-sulfoxide (ring sulfoxide) metabolites is a common biotransformation pathway for all phenothiazines. Ring sulfoxides may attain steady-state plasma concentrations several times greater than that of the parent phenothiazine during maintenance therapy.[2] It is unlikely that phenothiazine ring sulfoxides contribute to the antipsychotic effects of phenothiazines, as those sulfoxides tested have demonstrated little or no dopamine receptor binding.[2] However, levomepromazine and thioridazine sulfoxides have some α-adrenergic binding activity and may contribute to peripheral autonomic side effects of the parent drugs.[3,4] While lacking antipsychotic activity, levomepromazine and thioridazine sulfoxides do possess cardiotoxic effects. Thioridazine sulfoxide has been positively correlated with ECG abnormalities and hemodynamic changes in patients receiving thioridazine.[5,6] Levomepromazine sulfoxide and thioridazine sulfoxide have produced electroconductivity and contractile-force alterations in in vitro heart preparations.[7,8] Phenothiazine sulfoxides that contain one or more chiral centers exist as stereoisomeric mixtures. Diastereoisomeric pairs of thioridazine have been identified in fatal thioridazine intoxications[9] and in in vivo metabolic studies in rats.[10]

This work provides a convenient method for the preparation of phenothiazine sulfoxides from their parent drugs by oxidation in aqueous nitrous acid. This method was previously found selective in the oxidation of thioridazine (**1a**), yielding gram quantities of thioridazine sulfoxide;[11] a chromatographically separable pair of diastereoisomers [DL, LD (**1b**) and DD, LL (**1b'**)] were isolated. In this study, the generality of sulfoxide preparation via nitrous acid was tested with five phenothiazines (Table I): chlorpromazine (**2a**), perphenazine (**3a**), prochlorperazine (**4a**), promethazine (**5a**), and levomepromazine (**6a**). The resulting sulfoxides were converted to their soluble maleate salts, which may be used as analytical standards for phenothiazine determinations in biological or pharmaceutical preparations or in pharmacologic studies.

Results and Discussion

The reaction procedure was similar to that used for thioridazine sulfoxide preparation with aqueous nitrous acid.[11] The phenothiazine free base or hydrochloride served equally well as the starting material. Maleate salts may complicate isolation of pure products, but no problem was experienced during the isolation of the two stereoisomeric products from the oxidation of chiral levomepromazine maleate.

Oxidations run by stirring the free base or salt in aqueous nitrous acid in an open Erlenmeyer flask at room temperature rapidly gave good yields of products in gram quantities (Table II). Juenge et al.[11] reported that the reaction of thioridazine with hydrogen peroxide gave a complex mixture of oxidation products and that intricate procedures were necessary to purify them. The products obtained from **2a** and **3a** by nitrous acid oxidation (**2b** and **3b**) and by hydrogen peroxide oxidation (**2b*** and **3b***) had identical melting points (Table II) and IR spectra.

In our laboratory, oxidation of **3a**, which contains the sensitive *N*-piperadinylethanol group, with hydrogen peroxide in glacial acetic acid, a method used by Turner[12] to prepare other phenothiazine sulfoxides, gave a sticky, dark product containing several substances isolated by column chromatography. Pure **3b*** was isolated from this intractable material by column chromatography; purity of this product was established by elemental analysis. However, the procedure for **2b**, simplified to use aqueous hydrogen peroxide only, gave an excellent yield of the sulfoxide, **2b***. Purity of **4b** and **5b** was shown by agreement of melting points and IR absorptions for the sulfoxide group with those given in the literature[13,14] (Table II).

Gentle room-temperature reaction with nitrous acid was particularly suitable for the oxidation of **5a**, which is subject to beta-cleavage to form 10-methylphenothiazine, probably by a free radical mechanism;[15] this substance has been found as a contaminant in promethazine drug products in our laboratory.

The stereoisomerism of **6b** and **1b** are similar in that they contain two chiral centers, but differ in that one chiral center of **6b** is configurationally pure. Consequently, thioridazine gave a chromatographically separable diastereoisomeric pair[11] of sulfoxides (four compounds) and levomepromazine gave a chromatographically separable pair of diastereoisomeric sulfoxides (two compounds, **6b** and **6b'**). Levomepromazine sulfoxide was obtained from the crude product by repeated heptane extractions. The residue left after extraction contained additional sulfoxide, as well as a second more polar material, probably the dioxide (*N*-oxide, *S*-oxide). The material obtained from the oxidation was extracted three times with hot heptane from which the product crystallized without the separation of the diastereoisomeric pair, leaving a small amount of heptane-insoluble residue. Elemental analysis of all three crops from the crystallizations agreed with the structure of the diastereoisomeric sulfoxide product. After TLC development, the crystallized sulfoxide material

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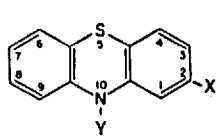
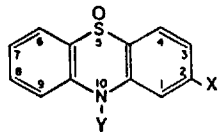
Table I—Substituted Phenothiazines and Their Sulfoxides





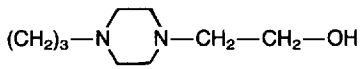
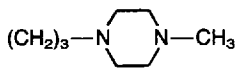
Compound	X	Y
Chlorpromazine (**2a, 2b**)	Cl	$(CH_2)_3N(CH_3)_2$
Perphenazine (**3a, 3b**)	Cl	
Prochlorperazine (**4a, 4b**)	Cl	
Promethazine (**5a, 5b**)	H	$CH_2CHN(CH_3)_2$ CH_3
Levomepromazine (**6a, 6b**)	OCH_3	$CH_2CHCH_2N(CH_3)_2$ CH_3

Table II—Physical Constants of Phenothiazine Sulfoxides from Nitrous Acid and Hydrogen Peroxide Oxidations

Reactant (Sulfoxide)[a]	Yield, %[b]	Product Melting Point, °C NO[c]	HP[d]	Lit[e]
Chlorpromazine (**2b, 2b***)	74	109–111	110–112[f]	—
Perphenazine (**3b, 3b***)	95	144–146	141–143[f]	—
Prochlorperazine (**4b**)	72	161.5–162.5	—	160–162
Promethazine (**5b**)	95	118–119	—	120
Levomepromazine (**6b**)	78[g]	182–183	—	182
Levomepromazine (**6b'**)	—	131–132	—	130–132

[a] Compounds indicated (*) were prepared by hydrogen peroxide oxidation; compounds indicated (') are stereoisomers. [b] Yield of nitrous acid oxidation product. [c] Nitrous acid oxidation product. [d] Hydrogen peroxide oxidation product. [e] Literature melting points of products **4b, 5b, 6b**, and **6b'** refer to refs 13, 14, and 16, respectively. [f] Elemental analysis is reported in the *Experimental Section.* [g] Sum of yields of **6b** and **6b'**.

showed one spot and the residue showed two spots. The diastereoisomers of levomepromazine sulfoxide were isolated.[16] Each isomer showed a single spot at R_f 0.05 after TLC development and the same chemical shift for the methyl proton absorption of their methoxide (or dimethylamino) groups; mixtures obtained from the heptane recrystallizations described earlier showed the same properties. After extraction with heptane, a small residue remained which by TLC development showed two spots for the sulfoxide and possibly the dioxide (R_f 0.05 and 0.02) under short-wavelength UV irradiation, and the latter spot fluoresced blue under long-wavelength UV irradiation.

The proton magnetic resonance spectrum (60 MHz) of this residue gave two very close, unresolved peaks for methoxide or dimethylamino functional groups because of the presence of two similar compounds, the sulfoxide and probably the dioxide. Heptane extraction probably concentrated in the residue the less polar components such as the dioxide (*N*-oxide, *S*-oxide), which migrates more slowly on silica gel. Juenge et al.[11] reported that in the case of thioridazine oxidation products, the somewhat more polar sulfone migrates just behind thioridazine, followed by the two ring sulfoxides, which are much more polar. The very polar dioxide is the slowest migrator; the *N*-oxide functional group is much more polar than the sulfoxide group. Also, the fluorescence under long-wavelength UV light of thioridazine disulfoxide and sulfone readily distinguishes them from thioridazine sulfoxide.

Water-soluble maleate salts **3c** and **4c** of **3b** and **4b**, respectively, were prepared for use as standard materials. The phenothiazine that showed greatest generation of impurities during oxidation was **3a**. The nitrous acid oxidation product, **3b**, and its maleate salt, **3c**, were checked for purity by HPLC, which showed <1% impurities. The nitrous acid oxidation method was found to be convenient and selective for the preparation of gram quantities of phenothiazine sulfoxides from the corresponding phenothiazines.

Experimental Section

Instruments—A Pye Unicam SP3 grating IR spectrophotometer (nujol mulls) and a Varian T60 60-MHz NMR spectrometer were used. All melting point measurements were made on a Fisher-Johns melting point apparatus.

Chromatography—Precoated, chromatographic sheets (silica gel $60F_{254}$ on aluminum support, E. M. Laboratories, Elmsford, NY) were developed with acetonitrile:ammonium hydroxide (9:1) for perphenazine sulfoxide and with chloroform:ethanol:ammonium hydroxide (16:3:1) for levomepromazine sulfoxide. Visualization was accomplished by fluorescence quenching under UV light (254 nm) or by fluorescence under long-wavelength UV light. Silica Sep-Pak cartridges (Waters Associates, Milford, MA, part no. 51900) were used for column chromatography.

Materials—Chlorpromazine (Smith, Kline and French, Philadelphia, PA), perphenazine (Schering, Kenilworth, NJ), prochlorperazine dimaleate (Smith, Kline and French, Philadelphia, PA), promethazine hydrochloride (Napp Chemicals, Lodi, NJ), and levomepromazine maleate (May and Baker, Dagenham, Essex, U.K.), were used as received except for prochlorperazine dimaleate, which was converted to the free base before use.

Nitrous Acid Oxidation—The phenothiazine (~0.01 mol) and 225–250 mL of deionized Milli-Q water (Millipore, Bedford, MA) were placed in an Erlenmeyer flask, and ~50–55 drops of hydrochloric acid was added with magnetic stirring. For promethazine hydro-

chloride, only ~20–25 drops of hydrochloric acid was used to prepare the acid solution. Excess (120 drops or less) aqueous sodium nitrite (1 g/10 mL water) was added. Nitrogen was bubbled through the solution for ~2 h to remove nitrogen oxide gases before workup. The solution was extracted twice with 40 mL of chloroform to remove foreign materials, and the chloroform was discarded. Concentrated ammonium hydroxide (55 to 100 drops) was added (pH 10) to cause the separation of the sulfoxide free base, which was extracted with two 100-mL portions of chloroform. The combined chloroform solution was washed twice with 100-mL portions of water. The chloroform solution was allowed to stand for 10–15 min to allow any emulsified water to separate; the solution was passed once or twice through fluted filter paper to complete removal of suspended water. The chloroform was removed by evaporation; complete removal can be effected by heating the solution on a steam bath while directing dry nitrogen at the surface through a pipette or while removing added methanol by distillation. The residue was crystallized by cooling the solution overnight in a refrigerator. Alternatively, the sticky products were repeatedly triturated with 50–100 mL of heptane at 100 °C; the products either became granular or crystallized from the heptane after being cooled overnight in a refrigerator.

The products were characterized by IR, NMR, HPLC or TLC, melting point, elemental analysis, or comparison with samples prepared by hydrogen peroxide oxidation according to literature procedures.

Chlorpromazine Sulfoxide (2b)—Nitrous acid oxidation left, after trituration with hot heptane, a pale pink product; IR 1030 cm^{-1} (S=O). The IR spectrum matched that of **2b*** from 4000 to 600 cm^{-1}.

Chlorpromazine Sulfoxide (2b*)—Hydrogen peroxide oxidation was done by mixing the hydrochloride of **1a** (0.70 g, 0.002 mol), 10 mL of 15% hydrogen peroxide, and 100 mL of water, and stirring magnetically at room temperature for 3 d. The solution was made basic with ammonium hydroxide and extracted with chloroform, and the combined chloroform extracts were completely evaporated. After overnight storage in a refrigerator the oily semisolid crystallized to a pale pink product, 0.62 g (93%). An analytical sample was prepared by crystallization twice from ethyl acetate.

Anal.—Calc. for $C_{17}H_{19}ClN_2OS$: C, 60.98; H, 5.57. Found: C, 60.98; H, 5.90.

Perphenazine Sulfoxide (3b)—Nitrous acid oxidation was used. The residue from the chloroform evaporation was triturated twice with hot heptane, leaving a pale yellow product; IR 1010 cm^{-1} (S=O). The IR spectrum matched that of **3b*** from 4000–600 cm^{-1}. The product showed no impurities by HPLC.

Perphenazine Sulfoxide (3b*)—Hydrogen peroxide oxidation was done by the method of Turner[12] starting with 88 mg of perphenazine. This product was purified by column chromatography, and the progress monitored by spotting small aliquots in methanol on TLC plates and developing them. Two tandem Sep-Paks joined by a short glass tube were conditioned with 50 mL of ether:methanol (20:1). Half of the product (40 mg) from the oxidation reaction was dissolved in methanol and applied as a thin layer to the top of the column. Elution (5-mL cuts) with 135 mL of ether:methanol (20:1) gave several separated materials. The pure sulfoxide (**3b***) was subsequently eluted with 75 mL of ether:methanol (10:1) collected in ~15 cuts, each of which showed only one TLC spot (R_f 0.24). The residue from evaporation of the combined cuts under nitrogen flush crystallized to give 15 mg of pale yellow solid.

Anal.—Calc. for $C_{21}H_{26}ClN_3O_2S$: C, 60.06; H, 6.24. Found: C, 60.21; H, 6.47.

Perphenazine Sulfoxide Dimaleate (3c)—Two solutions were formed by warming 105.0 mg (0.25 mmol) of **3b** in 0.5 mL of alcohol and 58.1 mg (0.50 mmol) of maleic acid in 0.5 mL of alcohol. The warm solutions were combined with immediate precipitation of 80 mg (49.1%) of pale orange product, mp 173–174 °C. The product showed no impurities by HPLC.

Prochlorperazine Sulfoxide (4b)—Nitrous acid oxidation was used. The residue from the chloroform evaporation was triturated twice with hot heptane until the extract was colorless, leaving a pale pink product; IR 1030 cm^{-1} (S=O).

Prochlorperazine Sulfoxide Maleate (4c)—The product, prepared from 97.5 mg (0.25 mmol) of prochlorperazine sulfoxide and 58.1 mg (0.50 mmol) of maleic acid by the same procedure described earlier for **3c**, was dried under a flush of nitrogen and gave 104 mg (67%) of white powder, mp 178.2–180.0 °C; IR 1025 cm^{-1} (S=O).

Promethazine Sulfoxide (5b)—Nitrous acid oxidation was used. The viscous semisolid from the chloroform evaporation was triturated with hot heptane, overlaid with a fresh layer of heptane, and placed in a refrigerator overnight, leaving a pink solid; IR 1028 cm^{-1}; ^1H NMR ($CDCl_3$, Me_4Si): 0.90 (d, 3, J = 6.4 Hz, $CHCH_3$) and 2.54 ppm (s, 6, $N(CH_3)_2$). The IR spectrum matched a literature spectrum.[12]

Levomepromazine Sulfoxide (6b and 6b')—Nitrous acid oxidation gave 1.99 g (78%) of a yellow, oily, crude product. Three triturations and extractions with boiling heptane gave, after cooling, three crops of crystals (**6b** and **6b'**). Each showed one dark spot by TLC, and had the following yields and mp values: 0.94 g, mp 128–138 °C; 0.47 g, mp 129–138 °C; 0.07 g, mp 145–153 °C.

The three crops of diastereoisomeric product (**6b** and **6b'**) were subjected to elemental analysis and TLC. For the first crop, the following results were obtained: TLC: R_f 0.50; IR: 1036 cm^{-1} (S=O); ^1H NMR ($CDCl_3$, Me_4Si): 2.56 (s, 6, $N(CH_3)_2$), and 3.72 ppm (s, 3, OCH_3).

Anal.—Calc. for $C_{19}H_{24}N_2O_2S$: C, 66.25; H, 7.02. Found: C, 66.38; H, 7.14; C, 66.11; H, 7.25; C, 66.25; H, 7.29.

A small amount of heptane-insoluble residue remained. It gave two TLC spots (R_f 0.50 and 0.20, latter fluoresced blue under long-wavelength UV light) and two NMR doublets (2-Hz separation each) for the methoxy and dimethylamino groups.

The diastereoisomeric pair was separated according to the method of Fouche and Horclois[16] and gave the expected melting points (Table II). Each of the isolated pairs gave the same R_f values and NMR absorptions reported earlier.

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