Preparation of Metabolites by Chemical Reaction: Conversion of Antipsychotic Phenothiazines to their Sulfoxides and Tertiary Amino Cyclic Antidepressants to their *N*-Oxide with Hydrogen Peroxide Using Titanosilicate Catalyst

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The aim of this study was to establish a preparation method needed to analyze the metabolites of an analytical procedure for antipsychotic phenothiazines and tertiary amino cyclic antidepressants by chemical reaction. These drugs were oxidized to their sulfoxide and *N*-oxide, respectively, with hydrogen peroxide using titanosilicate as the catalyst. An acidic medium (pH 3.0) containing 20% methanol was found to be optimal for the preparation of phenothiazine sulfoxide, and an alkaline medium (pH 10.5) containing 50% methanol was optimal for the preparation of the *N*-oxide of tertiary amino cyclic antidepressants. Each preparative scale reaction (2 mmol) was carried out and the crystallized product of each oxide was obtained. The amount of hydrogen peroxide needed to obtain the best yields of sulfoxide was 1.3–2.0 times the molar equivalent of the phenothiazines. The purities of the prepared crystallized product of the phenothiazine sulfoxides were good except for periciazine sulfoxide. The amounts of hydrogen peroxide needed to obtain the best yields of *N*-oxide were 4–5 times the molar equivalent of tertiary amino cyclic antidepressants. The purities of the crystals of each *N*-oxide were 99.5–100%. The characteristic mass fragment ions of each prepared oxide that were distinguishable as the *N*-oxides from parent drug could be confirmed when the collisional energy was decreased to 10 eV. ^{1}H- and ^{13}C-NMR spectral data confirmed the structure of the prepared mianserin *N*-oxide to be 2-oxide. In conclusion, a simple and rapid preparation method for oxide metabolites of phenothiazines and tertiary amino cyclic antidepressants available as analytical standards was established.

Key words —— metabolite, chemical preparation, oxide, phenothiazine, cyclic antidepressants, mass spectroscopy

INTRODUCTION

The identification and quantification of drugs ingested into human bodies require comprehensive information covering the target drugs themselves and their metabolites. To carry out a quantitative analysis, the standard substances of metabolites are essential. Some important metabolites can be obtained as commercial products, but many metabolites, especially those of new drugs, are difficult to obtain as standard samples. These problems have been solved by using experimental animals or isolated enzyme systems originating from biological materials. But defects such as low yield, high cost, and lengthy preparation time have necessitated the search for another effective method. Preparing metabolites by applying a chemical reaction to target drugs may be effective, if the reaction is specific, rapid, simple, and applicable to a broad range of organic compounds.

Drugs acting on the central nervous system are the main targets of analysis in emergency medicine and forensic analytical toxicology. The major metabolites of phenothiazine drugs in blood and urine are their corresponding sulfoxides together with the hydroxylated form.[1)] Although the sulfoxides generally lack antipsychotic activity, they may be responsible for the adverse effects (*e.g.* cardiotoxic activity[2,3)]) associated with drug intake. Conventional preparation methods for phenothiazine sulfoxide have been reported.[4–9)] The yields of these methods are not sufficient, and the phenothiazine derivatives reported have been limited to simple structural phenothiazine derivatives and drugs produced at an early stage of development.

Most of the cyclic antidepressants used clinically include a ternary amino group in their structures. Major metabolites of these cyclic antidepressants are

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the *N*-demethylated and hydroxylated forms.[1)] Although *N*-oxide is a minor metabolite,[1,10–12)] it retains its original structure and is useful for identification. Conventional preparation methods for the *N*-oxide of general ternary amino compounds are oxidation by hydrogen peroxide or organic peroxide.[13–15)] The reaction rate with hydrogen peroxide is slow and the yield is low, while the latter method represented by *m*-chloroperbenzoic acid requires the maintenance of a low reaction temperature and purification of the column chromatography system.

Hydrogen peroxide is a very attractive oxidant because it is relatively cheap and can oxidize organic compounds with the generation of water as the only theoretical by-product. Recently, many catalysts have been developed to enhance the efficiency of the oxidation ability of hydrogen peroxide. We selected titanosilicate as the catalyst for oxidation by hydrogen peroxide since the effective catalytic ability of titanosilicate to form sulfoxide from a simple structural sulfide (thioether) in the presence of hydrogen peroxide has been reported.[16–18)] Titanosilicate has been used in the field of chemical industry as a catalyst for hydrogen peroxide.[19)]

This article deals with the search for the optimal reaction conditions of reaction to form the oxide of each drug using hydrogen peroxide and titanosilicate in a small scale reaction (0.1 mmol). Larger amounts of drugs (2 mmol) were then oxidized on the basis of the optimal conditions obtained, and purification of the oxides formed was carried out using crystallization.

MATERIALS AND METHODS

Materials —— Chlorpromazine hydrochloride, promethazine hydrochloride, periciazine (synonym: pericyazine, propericiazine), imipramine hydrochloride, and 30% hydrogen peroxide (9.88 mmol/ml) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Promazine hydrochloride, fluphenazine dihydrochloride, trifluoperazine dihydrochloride, perphenazine, prochlorperazine dimaleate, clomipramine hydrochloride, amitriptyline hydrochloride, and mianserin hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Titanosilicate catalyst, TS-1, was kindly supplied by Süd-Chemie Catalysts Japan Inc. (Tokyo, Japan). The Si/Ti molar ratio in the TS-1 used was 70, and average particle size as determined by scanning electron micrographs was 100–200 nm. The absence of any band or signal in the range of 310–320 nm indicates the absence of an extra-framework octahedral TiO_2 phase in the TS-1 sample.

Small-Scale Reaction of Antipsychotic Phenothiazines with Hydrogen Peroxide Using Titanosilicate as Catalyst —— Each phenothiazine solution (0.1 mmol) was mixed with 0.2 M acetate buffer (pH 3.0) and methanol (final concentration: 20%) in a test tube with a screw plug, and stirred with the indicated amounts of 30% hydrogen peroxide and titanosilicate in a water bath at 60°C for 1 hr. The total volume of the reaction mixtures was 0.5 ml, although that of periciazine was increased to 1 ml by increasing the amounts of methanol and buffer in order to improve its solubility. As control tubes, the drug solutions were incubated in the same acetate buffer and methanol without titanosilicate and hydrogen peroxide under the same conditions. Prochlorperazine maleate was converted into the free form to improve the solubility. An equal molar amount of 35% hydrogen chloride (0.1 mmol) was added to the reaction mixtures containing free base type phenothiazine (pericyazine, perphenazine and prochlorperazine).

After the reactions, the reaction mixture was diluted with cold milli-Q water (1.5 ml) and the pH of the solution was adjusted to 10.0 with 1 M sodium carbonate. The reaction mixture was vigorously stirred with chloroform twice (1.5 ml each) for 3 min. After centrifugation, the chloroform phase was removed using a pasteur pipette, and the combined extract was dried under a stream of N_2 gas at 40°C. The residue was dissolved in methanol (HPLC grade, 1 ml), and 0.1 ml of this methanol solution was diluted to 100-fold with HPLC eluting solution, and then analyzed by HPLC.

Small-Scale Reaction of Cyclic Antidepressants with Hydrogen Peroxide Using Titanosilicate as Catalyst —— Each methanol solution of the HCl salts of cyclic antidepressants (0.1 mmol) was mixed with 1 M carbonate buffer (pH 10.5) and methanol (final volume: 50%) and stirred with the indicated amounts of 30% hydrogen peroxide and titanosilicate in a water bath at 60°C for 1 hr (total volume: 0.5 ml). As control tubes, the drug solutions were incubated in the indicated buffer and methanol without titanosilicate and hydrogen peroxide under the same conditions. After the reaction, the reaction mixture was diluted with cold milli-Q water (1.5 ml) and extracted with chloroform twice (1.5 ml each). The rest of the procedure was the same as that for phenothiazines described above.

Preparation of Phenothiazine Sulfoxide —— Each solid phenothiazine (2 mmol) was transferred to an Erlenmeyer flask, and dissolved in 2 ml of methanol and a calculated amount (8 ml – volume of hydrogen peroxide) of 0.2 M sodium acetate buffer (pH 3.0). Titanosilicate (100 mg) and the indicated amount of 30% hydrogen peroxide were added. The reaction mixture (10 ml) was stirred in a water bath at 60°C for 1 hr. Prochlorperazine maleate was converted to its free base. An equal molar amount of 35% hydrogen chloride (2 mmol) was added to the reaction mixtures of the free base of phenothiazine (pericyazine, perphenazine and prochlorperazine).

After the reactions, the reaction mixture was adjusted to pH 10.0 with 0.1 M sodium carbonate, transferred to a separatory funnel, and extracted with chloroform twice (15 ml each). The combined extract was dehydrated with solid sodium sulfate and then a 10% hydrogen chloride solution in methanol (1.5 times molar equivalent) was added to make the HCl salt. After evaporating the chloroform in a vacuum evaporator, each phenothiazine sulfoxide in the residue was crystallized from ethanol, methanol-acetone, or acetone.

Preparation of *N*-Oxide of Antidepressants —— Each solid tricyclic antidepressant (2 mmol) was transferred to an Erlenmeyer flask, and dissolved in 5 ml of methanol and a calculated amount (5 ml – volume of hydrogen peroxide) of 1 M sodium carbonate buffer (pH 10.5). Titanosilicate (200 mg) and the indicated amount of 30% hydrogen peroxide were added. In the case of mianserin, the scale of the reaction was decreased to one-fourth because of the high cost of mianserin; therefore, 0.4 mmol of mianserin was reacted with hydrogen peroxide and titanosilicate in 2 ml of reaction mixture. The reaction mixture was stirred in a water bath at 60°C for 1 hr. Sodium carbonate (1 M) was added to make the reaction mixture alkaline (pH 10.0), and then it was extracted with chloroform twice (15 ml each). The combined extract was dehydrated with sodium sulfate and then 10% hydrogen chloride solution in methanol (1.5 times molar equivalents) was added. After evaporation of the chloroform, the *N*-oxides of the antidepressants in the residue were crystallized from acetone or methanol-acetone. As an exceptional case, amitriptyline was crystallized as the free base.

HPLC Analysis —— Quantitative analysis of the chloroform extract of the reaction mixture and obtained crystals was performed using an octadecylsilane column (Mightysil RP-18, 4.6 × 250 mm, 5 μm, Kanto Chemicals Co., Inc., Tokyo, Japan) with the eluting solution of 67 mM phosphate buffer (pH 3.0) - CH_3CN (91 : 49, v/v)[20] at a flow rate of 1.2 ml/min. The wavelengths of the UV detector for phenothiazine were set at 247–260 nm depending on each drug as described in the Results. The wavelength setting for the cyclic antidepressants was 215 nm. Each injection volume of sample solution was 10 μl. The HPLC system consisted of a L-7100 liquid chromatograph equipped with a L-7420 spectrophotometric detector and a L-7500 integrator (Hitachi, Ltd., Tokyo, Japan). The analytical data are expressed as the composition that indicates the mean ratio of the integration value of each peak to that of starting drug in the control tube.

TLC Analysis —— TCL analysis was carried out to confirm the purities of the prepared oxides using Silica gel 60 F_{254} (Merck, Darmstadt, Germany) plates. The solvent systems were chloroform : ethanol : 28% ammonium hydroxide (45 : 10 : 1, v/v) for phenothiazines, and methanol-28% ammonium hydroxide (200 : 3, v/v) for cyclic antidepressants.

Spectroscopy —— Mass spectrometry was carried out with a JEOL JMS-700 MStation and JMS-AX505HA mass spectrometers (JEOL Ltd., Akishima, Japan). ^1H and ^{13}C NMR spectra were obtained on a Varian XL-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). Samples were dissolved in methanol-d_4 (CD_3OD). ^1H and ^{13}C NMR chemical shifts referred to the signals of deuterated solvent (CD_3OD) at 3.31 ppm and 49.0 ppm, respectively.

RESULTS

Small-Scale Survey for Optimal Conditions: Sulfoxidation of Phenothiazines

To measure the ratio of sulfoxide formed to starting phenothiazine using an HPLC system with a spectrophotometric detector, the wavelength showing the same absorbance for phenothiazines and their sulfoxides at the same concentration should be selected. The UV spectra of 20 μM chlorpromazine (CPZ), 20 μM CPZ sulfoxide, and four sets of solutions consisting of different concentration ratios of CPZ to CPZ sulfoxide (4 and 16 μM, 8 and 12 μM, 12 and 8 μM, and 16 and 4 μM) dissolved in HPLC eluting solution intersected each other at 251 nm (absorbance: 0.490). Therefore, the UV detector was set at 251 nm to measure the ratio of formed CPZ

Table 1. Effects of pH, Methanol Concentration, and Amount of Titanosilicate Added on the Formed Ratio of CPZ Sulfoxide[a)]

Exp. No.	pH[b)]	Methanol (%)	Titanosilicate (mg)	Composition (%)			
				Sulfoxide	Other product	Unreacted CPZ	Total
1	3.0	20	5	91.5	1.5	0.5	93.5
	5.0	20	5	87.6	0.2	1.9	89.7
	7.0	20	5	70.1	2.9	14.5	87.5
	10.5	20	5	0.4	2.7	77.2	80.3
2	3.0	0	5	66.7	1.4	0.4	68.5
	3.0	10	5	75.7	1.0	0.4	77.1
	3.0	20	5	90.9	0.2	0.6	91.7
	3.0	30	5	87.5	0.2	1.1	88.8
3	3.0	20	3	86.3	0.1	2.3	88.7
	3.0	20	5	94.3	0.8	0.5	98.1
	3.0	20	10	94.4	0.6	0.1	95.1
	3.0	20	15	93.0	0.8	0.3	94.1

a) Chlorpromazine hydrochloride (0.1 mmol) was reacted with 20 μl (0.2 mmol) of 30% H_2O_2 and each amount of titanosilicate in the presence of the indicated buffer and indicated amount of methanol at 60°C for 1 hr with stirring (total volume: 0.5 ml). *b*) The following buffers were used: 0.2 M acetate buffer (pH 3.0 and pH 5.0), 0.25 M phosphate buffer (pH 7.0), and 1.0 M carbonate buffer (pH 10.5).

sulfoxide to starting CPZ.

The formation of sulfoxide from phenothiazine by hydrogen peroxide and titanosilicate was found to be affected by the pH and methanol concentration of the reaction medium, and by the amount of titanosilicate added. Table 1 shows these effects on the ratio of formed CPZ sulfoxide to starting CPZ. Exp. 1 in Table 1 indicates that an acidic medium, especially pH 3.0, was essential. Exp. 2 and 3 suggest that the medium containing 20% methanol was optimal and that an amount of titanosilicate higher than 5 mg per 0.1 mmol of CPZ was necessary to obtain a satisfactory formed ratio of CPZ sulfoxide.

Eight antipsychotic phenothiazines used clinically, including CPZ (Fig. 1), were oxidized with various amounts of hydrogen peroxide with titanosilicate (5 mg) under the optimum conditions of CPZ stated above. These results suggest that optimal amounts of hydrogen peroxide were found to be different, mainly depending on the base type of the *N*-side chain of the phenothiazines. Therefore, large-scale reactions were carried out to determine the amounts of hydrogen peroxide that produced the highest formed ratios of phenothiazine sulfoxides.

Small-Scale Survey for Optimal Conditions: *N*-Oxidation of Cyclic Antidepressants

The UV spectra of the *N*-oxides of antidepressants were compatible with those of each parent antidepressant. Therefore, the UV detector of the HPLC system was set to a sensitive short wavelength (215 nm) to measure the ratio of formed *N*-oxide to the starting antidepressant.

The *N*-oxidation of antidepressants by the combination of hydrogen peroxide and titanosilicate was affected by the pH and methanol concentration of the reaction medium, and by the amount of titanosilicate added. Table 2 indicates the experimental data showing these effects on the ratio of formed imipramine *N*-oxide to starting imipramine. Exp. 1 in Table 2 reveals that an alkaline medium (pH 10.5) was essential for the *N*-oxide formation. Exp. 2 and 3 show that the medium containing 50% methanol was optimal and that more than 10 mg of titanosilicate was necessary to obtain a good formed ratio of antidepressant *N*-oxides. The role of methanol in the reaction medium was assumed to improve the solubility of antidepressants in alkaline reaction medium.

The optimal amounts of hydrogen peroxide to form the *N*-oxide of three tricyclic and one tetracyclic antidepressants used clinically (Fig. 2) were determined under the optimal conditions of imipramine described above. These results suggest that the optimal amount of hydrogen peroxide for tricyclic antidepressants differed from that for tetracyclic antidepressants. Therefore, large-scale reactions were carried out to determine the optimal amounts of hydrogen peroxide.

Table 2. Effects of pH, Methanol Concentration, and Amount of Titanosilicate Added on the Formed Ratio of Imipramine (IMP) *N*-Oxide[a)]

Exp. No.	pH[b)]	Methanol (%)	Titano-silicate (mg)	Composition (%)			
				N-oxide	Other product	Unreacted IMP	Total
1	5.0	50	10	3.8	0	89.6	93.4
	7.0	50	10	28.2	0	65.1	93.3
	10.5	50	10	92.2	0	1.7	94.0
2	10.5	20	10	6.8	0.4	84.2	91.4
	10.5	35	10	71.3	1.2	16.6	89.1
	10.5	50	10	92.2	0	1.7	94.0
	10.5	60	10	92.9	0	0.1	93.0
3	10.5	50	5	90.2	0	0.8	91.0
	10.5	50	10	92.2	0	1.7	94.0
	10.5	50	15	93.1	0	0.9	94.0
	10.5	50	20	92.3	0	0.4	92.7

a) Imipramine hydrochloride (0.1 mmol) was reacted with 40 μl (0.4 mmol) of 30% H_2O_2 and each amount of titanosilicate in the presence of the indicated buffer and indicated amount of methanol at 60°C for 1 hr with stirring (total volume: 0.5 ml). *b*) Buffers were the same as those listed in Table 1.

Table 3. Effect of H_2O_2 Concentration on Formed Ratio of Chlorpromazine Sulfoxide in Large-Scale (2 mmol) Reactions[a)]

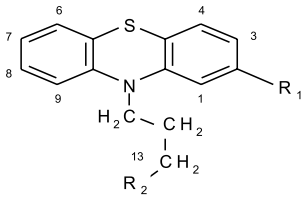
30% H_2O_2, ml (molar ratio to drug)	Composition (%)			
	Sulfoxide	Other product	Unreacted drug	Total
0.40 (2.0)	69.0	24.9	0	93.9
0.32 (1.6)	70.1	26.3	0.1	96.5
0.26 (1.3)	95.7	1.3	2.3	99.3
0.20 (1.0)	87.2	0.4	8.4	96.0

a) Chlorpromazine HCl (2 mmol) was reacted with the indicated amount of 30% H_2O_2 and titanosilicate (100 mg) in the presence of 0.2 M acetate buffer (pH 3.0) and methanol (final 20%) at 60°C for 1 hr with stirring (total volume: 10 ml).

Preparation of Phenothiazine Sulfoxides

In order to obtain practically applicable conditions and to obtain each phenothiazine sulfoxide as a pure sample, the amounts of starting drugs were scaled up from 0.1 mmol to 2 mmol (total reaction volume: 10 ml). Table 3 shows the changes in the formed percentage of CPZ sulfoxide according to the amount of hydrogen peroxide. The large-scale reactions proceeded effectively, and even an equal molar amount of hydrogen peroxide to CPZ could convert CPZ to its sulfoxide with a better formed ratio (over 85%), despite the fact that 8.4% of the CPZ remained unreacted. In the CPZ data, an amount of hydrogen peroxide larger than the optimal amount (2.6 mmol) to 2 mmol of CPZ resulted in the marked increase of another oxidized product believed to be a sulfone. In the end, 1.3 times the molar equivalent of hydrogen peroxide was shown to be adequate to obtain the best yield of CPZ sulfoxide. Table 4 summarizes the formed ratio of the eight phenothiazine sulfoxides in chloroform extract under the optimal amounts of hydrogen peroxide, together with each wavelength used for the HPLC measurements (isosbestic point). The optimal amounts of hydrogen peroxide changed depending on the amine type of the *N*-side chain of the phenothiazines. The appropriate amounts of hydrogen peroxide were found to be 1.3–1.4 times the molar equivalent of both aliphatic amine and piperizine base type, and 1.5–2.0 times the molar equivalent of piperazine base type.

After the large-scale sulfoxidation of each phenothiazine was carried out, sulfoxides in the residue of the chloroform extract from the reaction mixture were converted to the HCl salts, and crystallized from ethanol, acetone + methanol, or methanol. Table 4 also summarizes the yields and purities of the obtained crystallized products calculated from HPLC analysis. Although the data are not shown in Table 4, TLC analysis of all crystallized products



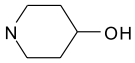
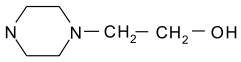
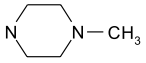
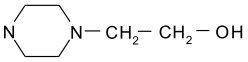
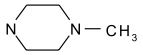
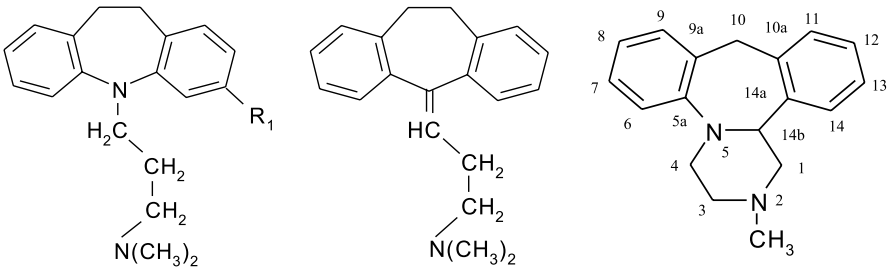
Phenothiazines	R_1	R_2
Chlorpromazine HCl	Cl	$N(CH_3)_2$
Promazine HCl	H	$N(CH_3)_2$
Promethazine HCl	H	CH_2-CH (CH_3) - $N(CH_3)_2$ [a]
Pericyazine	CN	
Fluphenazine HCl	CF_3	
TrifluoperazineHCl	CF_3	
Perphenazine	Cl	
Prochlorperazine	Cl	

Fig. 1. Structures of Antipsychotic Phenothiazines Used in the Present Study
a) Indicate whole side chain.

Table 4. Conditions of Large-Scale (2 mmol) Reactions and Properties of Phenothiazine Sulfoxides Obtained from Chloroform Extracts[a)]

Sulfoxide (as HCl salt)	30% H_2O_2, ml (molar ratio to drug)	Measured wavelength (nm)	Formed sulfoxide in chloroform extract (%)	Yield of crystallized product (%)	Purity of crystallized product (%)
Aliphatic amine type					
Chlorpromazine sulfoxide	0.26 (1.3)	251	95.7	71.2	98.4
Promazine sulfoxide	0.28 (1.4)	244	92.0	55.6	98.0
Promethazine sulfoxide	0.26 (1.3)	244	93.2	53.9	98.2
Piperidine base type					
Periciazine sulfoxide	0.30 (1.4)	260	85.2	46.5	95.2
Piperazine base type					
Fluphenazine sulfoxide	0.40 (2.0)	247	88.7	75.3	99.2
Trifluoperazine sulfoxide	0.30 (1.5)	247	82.0	62.6	99.0
Perphenazine sulfoxide	0.40 (2.0)	249	86.3	72.5	99.8
Prochlorperazine sulfoxide	0.40 (2.0)	249	78.7	73.8	99.8

a) Each phenothiazine (2 mmol) was reacted with the indicated amount of 30% H_2O_2 and titanosilicate (100 mg) in the presence of 0.2 M acetate buffer (pH 3.0) and methanol (final 20%) at 60°C for 1 hr with stirring (total volume: 10 ml).



Imipramine $R_1 = H$ Amitriptyline Mianserin

Clomipramine $R_1 = Cl$

Fig. 2. Structures of Tertiary Amino Cyclic Antidepressants Used in the Present Study

Table 5. Conditions of Large-Scale Reactions and Properties of Tertiary Amino Cyclic Antidepressant *N*-Oxides Obtained from Chloroform Extracts[a)]

N-Oxides	Starting amount (mmol)	30% H_2O_2, ml (molar ratio to drugs)	Formed *N*-oxide in chloroform extract (%)	Yield of crystal (%)	Purity of crystal (%)
Tricyclic type					
Imipramine *N*-Oxide (HCl salt)	2	0.8 (4.0)	85.7	73.5	100
Clomipramine *N*-Oxide (HCl salt)	2	0.8 (4.0)	85.4	69.8	99.9
Amitriptyline *N*-Oxide (Free type)	2	0.8 (4.0)	70.8	44.4	100
Tetracyclic type					
Mianserin *N*-Oxide (HCl salt)	0.4	0.2 (5.0)	88.6	66.8	99.5

a) Each cyclic antidepressant (2 mmol) was reacted with the indicated amount of 30% H_2O_2 and titanosilicate (200 mg) in the presence of 1.0 M carbonate buffer (pH 10.5) and methanol (final 50%) at 60°C for 1 hr with stirring (total volume: 10 ml).

confirmed that any spot which could not be detected by HPLC, such as an inorganic salt, was not included in the crystals at all.

Preparation of *N*-Oxides of Ternary Amino Cyclic Antidepressants

Table 5 summarizes the results of the large-scale reaction of ternary amino cyclic antidepressants and the yields and purities of the obtained crystals. Large-scale *N*-oxidations of three tricyclic antidepressants by 4 times the molar equivalent of hydrogen peroxide proceeded without production of any by-product. The purities of prepared crystals of three kinds of *N*-oxide came close to 100%.

However, *N*-oxidation of mianserin was distinct from the tricyclic drugs. Five times the molar equivalent of hydrogen peroxide converted mianserin to 88.6% of its *N*-oxide together with 7.9% of by-product. This by-product, however, could be effectively removed by the process of crystallization from acetone and methanol, resulting in pure crystals (99.5%). This *N*-oxide was confirmed by NMR spectrometry to be a 2-oxide, as described in a latter section.

Mass Spectrometry of Phenothiazine Sulfoxides and Ternary Amino Cyclic Antidepressant *N*-Oxide

FAB mass spectra of all oxides together with starting drugs were measured to obtain the *m*/*z* values of positive molecular ion $[M+1]^+$. The observed *m*/*z* values of each positive ion agreed with the sum of that of starting drugs and 16 (atomic weight of oxygen) crystallized products.

The electronic ionization (EI) mass spectra of all pairs of starting drugs and their oxides were measured to determin which characteristic fragment ions are useful for confirmation of oxide structure. Under usual collisional energies (70 eV), the EI mass spectra of starting drug and its oxide showed almost

Table 6. EI-Mass Spectra of Drugs and their Oxides Measured at Low Collisional Energies (10 eV)

Drug and its Oxide	MW	Molecular Ion and Main Fragment Ions, *m/z* (Rerative Intensity, %)[a)]
Chlorpromazine	318.87	318 (100), 86 (5.4)
sulfoxide	334.87	**334 (13.5)**, 318 (100), **289 (7.3)**, **233 (18.1)**, 86 (6.7)
Pericyazine	365.50	365 (100), 224 (6.7), 142 (9.5), 114 (17.5)
sulfoxide	381.50	**381 (12.4)**, 365 (100), **237 (10.0)**, 224 (27.2), 142 (11.1), 114 (25.6)
Fluphenazine	437.53	437 (100), 419 (15.4), 406 (21.3), 393 (7.2), 267 (24.0)
sulfoxide	453.53	**451 (7.8)**, 437 (100), 419 (17.0), 406 (22.6), 393 (20.1), **353 (21.6)**, 267 (52.6)
Trifluoperazine	407.50	407 (100), 267 (20.6)
sulfoxide	424.50	**422 (2.1)**, 407 (100), **353 (6.1)**, 267 (28.7)
Perphenazine	403.98	403 (100), 233 (12.2)
sulfoxide	419.98	403 (100), **319 (14.7)**, 233 (28.2)
Prochlorperazine	373.95	373 (100), 233 (8.1)
sulfoxide	389.95	373 (100), **319 (9.5)**, 233 (13.2)
Imipramine	280.41	280 (100), 235 (28.9), 195 (16.8), 85 (13.7)
N-oxide	296.41	**294 (74.6)**, 280 (100), **266 (M-30, 23.5)**, 235 (56.4), 195 (62.0), 85 (13.6)
Clomipramine	314.86	314 (100), 269 (73.7), 229 (6.7), 85 (36.1)
N-oxide	330.86	**328 (77.0)**, 314 (88.3), **300 (M-30, 23.2)**, 269 (100), 255 (13.5), 242 (20.0), 229 (48.5), 85 (30.8)
Amitriptyline	277.41	277 (4.3), 58 (100)
N-oxide	293.41	**232 (100)**, **217 (4.5)**, **61 (4.9)**
Mianserin	264.37	264 (100)
N-oxide	280.37	**280 (6.6)**, 264 (100), **250 (M-30, 4.7)**, 220 (4.5), 72 (2.0)

a) Bold figures represent the characteristic fragments useful for confirmation of oxide structure.

the same pattern, except for the weak molecular ion of drug oxide. However, the differences in spectra became apparent when each pair of samples underwent fragmentation by low collisional energies (10 eV), as shown in Table 6.

Four sulfoxides of phenothiazines comprised of *N*-side chains containing a piperazine ring produced a common sulfoxide-specific fragment, *i.e.*, fluphenazine sulfoxide and trifluoperazine sulfoxide produced fragment ions at *m/z* 353, and perphenazine sulfoxide and prochlorperazine sulfoxide produced fragment ions at *m/z* 319. By high-resolution mass analysis, the molecular formulas of these two kinds of fragments were found to be $C_{17}H_{16}ON_2SF_3$ and $C_{16}H_{16}ON_2SCl$, respectively. These results suggest that cleavage of the piperazine ring occurred, resulting in the formation of fragments containing $-N^+H = CH_2$ at a side chain terminal, as shown in Fig. 3. In contrast, periciazine sulfoxide containing a piperizine ring produced fragment ions at *m/z* 237, and the molecular formula ($C_{14}H_9N_2S$) of this fragment suggested the structure of a deoxygenated phenothiazine ring containing an *N*-methylene group (Fig. 3).

The spectrum of imipramine *N*-oxide showed a characteristic fragment ion at *m/z* 266 (23.5) together with a fragment ion (M-2) at *m/z* 294 (74.6). The

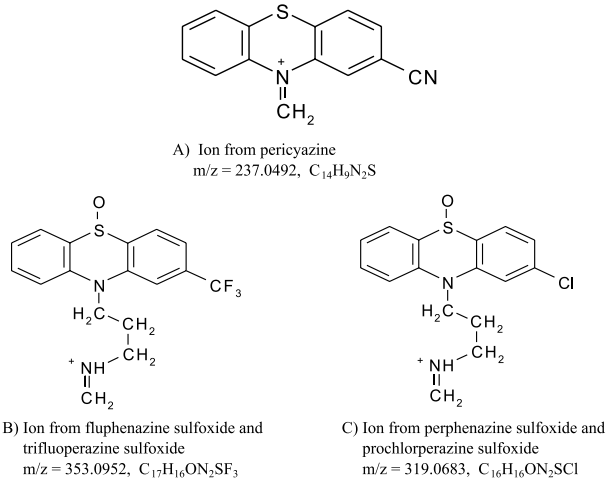


Fig. 3. Fragment Ions Formed from Phenothiazine Sulfoxides at Low Collisional Energies (10 eV)

former fragment corresponded to M-30, and its molecular formula $C_{18}H_{22}N_2$ determined by high-resolution mass analysis suggests that this fragment ion was generated by the release of formaldehyde from the *N*-oxide and -methyl groups bonded to the terminal nitrogen of the side chain. The spectrum of clomipramine *N*-oxide showed a similar characteristic fragment ion (M-30) at *m/z* 300 (23.2), $C_{18}H_{21}N_2Cl$, generated by the same process together

Table 7. Marked Changes of ^{1}H- and ^{13}C-NMR Chemical Shifts of Phenothiazines Accompanied by Sulfoxide Formation

Position	$\Delta\delta^{a)}$			
	Chlorpromazine	Pericyazine	Fluphenazine	Prochlorperazine
^{1}H-NMR				
1	0.72	0.92	0.78	0.79
4	0.84	0.92	0.85	0.89
6	0.81	0.94	0.82	0.87
9	0.62	0.80	0.73	0.78
^{13}C-NMR				
4	4.9	4.1	3.9	5.0
6	5.6	3.4	2.9	4.1
8	4.3	5.5	7.0	6.0
9a	−6.6	−6.5	−5.5	−6.5
10a	−7.1	−7.8	−6.7	−7.1

a) Positive numbers indicate low field shift.

with two molecular related ions (M, M-2) at *m/z* 330 (27.9) and 328 (77.0). The spectrum of mianserin was very simple and its molecular ion was observed as the sole fragment, whereas that of mianserin *N*-oxide showed a characteristic fragment ion (M-30) at *m/z* 250 (4.7), $C_{17}H_{18}N_2$, together with molecular ion. Consequently, the fragment ion corresponding to M-30 could be found in the spectra of imipramine *N*-oxide, clomipramine *N*-oxide, and mianserin *N*-oxide.

NMR Spectrometry of Phenothiazine Sulfoxides and Ternary Amino Cyclic Antidepressant *N*-Oxide

The ^{1}H- and ^{13}C-NMR spectra of all the prepared drug oxides were measured and compared with those of the starting drugs. Table 7 shows the marked changes in the chemical shifts of representative phenothiazines accompanied by sulfoxide formation, and expresses these changes as the difference in chemical shifts between the sulfoxides and starting drugs. Chemical shifts at H-1, -4, -6, -9, and at C-4, -6, -8 moved to a low magnetic field, and those of C-9a, -10a to a high magnetic field. In the case of CPZ, the signal changes were consistent with results reported previously.[21,22)]

The ^{1}H- and ^{13}C-NMR spectra of imipramine *N*-oxide, clomipramine *N*-oxide, and amitriptyline *N*-oxide revealed a common low field shift of a neighboring carbon and proton of the terminal nitrogen (data not shown). A comparison of the spectrum of mianserin with that of its *N*-oxide, containing two ternary amino nitrogens (2- and 5-) in a condensed ring, suggested the position of the incorporated oxygen. Namely, the low field shift of ^{13}C-NMR spectra at C-1, -3 and *N*-methyl-carbon (α position of N-2) and the high field shift at C-4, -14b (β position of 2-N) indicated the structure of the prepared mianserin *N*-oxide to be 2-oxide, as shown in Table 8 and Fig. 2. The low field shifts of the ^{1}H-NMR spectra at H-1, -3 and *N*-methyl-protons were also confirmed as the 2-position.

DISCUSSION

The oxidation of phenothiazine and antidepressant with hydrogen peroxide and titanosilicate proceeds quantitatively to completion after 1 hr with the production of a small amount of by-product and unreacted starting drug. Therefore, crystallization was effective as a means of purification. This makes it possible to obtain a pure sample within a short time period. The results of this study show that titanosilicate is an effective catalyst for hydrogen peroxide. It exerts a catalytic effect on a small molar equivalent of hydrogen peroxide in the presence of concentrated drugs such as 0.2 M.

Although the oxidation of a phenthiazine ring proceeds *via* a successive reaction from sulfide to sulfone, intermediate sulfoxide can be obtained quantitatively by using an optimal amount of hydrogen peroxide. Another possible by-product is the *N*-oxide of the side chain of the phenothiazine ring, but maintaining acidic conditions in the reaction mixture can prevent *N*-oxidation. In contrast, in spite of the necessity of a larger amount of hydrogen peroxide and titanosilicate, the oxidation of tricyclic

Table 8. ^{1}H- and ^{13}C-NMR Chemical Shifts of Mianserin and its *N*-Oxide

Position	^{1}H-NMR			^{13}C-NMR		
	mianserin	*N*-oxide	$\Delta\delta^{a)}$	mianserin	*N*-oxide	$\Delta\delta^{a)}$
1	3.39 / 3.53$^{b)}$	4.08 / 3.80$^{b)}$	0.69 / 0.27$^{b)}$	61.38	71.19	9.81
3	3.35 / 3.68$^{b)}$	3.98 / 3.92$^{b)}$	0.63 / 0.24$^{b)}$	54.91	65.60	10.69
4	3.62 / 3.53$^{b)}$	3.45 / 3.93$^{b)}$	−0.17 / 0.40$^{b)}$	49.73	46.47	−3.26
5a				147.89	147.77	−0.12
6	7.07	7.13	0.06	120.18	120.30	0.12
7	7.18	7.20	0.02	128.61	128.64	0.03
8	6.92	6.94	0.02	124.60	124.67	0.06
9	7.14	7.15	0.01	127.72	127.76	0.04
9a				141.28	141.28	0
10	3.42 / 4.80$^{b)}$	3.42 / 4.84$^{b)}$	0 / 0.04$^{b)}$	39.04	39.07	0.03
10a				141.31	141.77	−0.49
11	7.23	7.24	0.01	129.82	129.99	0.17
12	7.15	7.16	0.01	129.34	129.45	0.11
13	7.15	7.16	0.01	128.11	128.19	0.08
14	7.09	7.07	−0.02	130.60	130.66	0.06
14a				134.88	134.04	−0.84
14b	4.34	4.65	0.31	63.34	61.91	−1.43
N-CH_3	2.96	3.64	0.68	43.62	58.24	14.62

a) Positive numbers indicate a low field shift. *b*) Assigned in the order of axial/equatorial.

antidepressants proceeds smoothly without formation of any by-product and with only a small amount of remaining starting drugs, yielding a very pure crystals of antidepressant *N*-oxide. Highly pure crystals of mianserin can be obtained from the residue of the chloroform extract including a comparatively high ratio of by-product (7.4%).

Mass spectrometry has been used for the identification because it can measure a very small amount of sample. The characteristic fragment pattern of each prepared oxide useful for confirmation of oxide structure were ascertained by decreasing the collisional energies. The result of NMR spectrometry of the formed oxides suggested that this information is useful for identifying the oxide formation of members in condensed aromatic rings, such as sulfoxidation of the phenothiazine ring and oxidation of nitrogen in the tetracyclic ring of mianserin. The chemical shift assignments of the ^{1}H- and ^{13}C-NMR spectra of mianserin in $CDCl_3$ have also been reported.[23,24] The prepared mianserin 2-oxide was one of the three major metabolites formed by incubation with human liver microsomes,[25] and was identified in urine from humans who ingested mianserin.[26]

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