Oxidative Degradation of Pharmaceutically Important Phenothiazines I: Isolation and Identification of Oxidation Products of Promethazine

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Abstract □ The thermal degradation of promethazine in water in the presence of oxygen was studied. After degradation, the products were isolated by TLC. Identification was carried out by comparison of the isolated compounds with reference compounds. Melting points, spectral data, and polarographic and chromatographic behavior were compared. The following products were identified: 10-methylphenothiazine, phenothiazine, 3*H*-phenothiazine-3-one, phenothiazine 5-oxide, promethazine 5-oxide, 7-hydroxy-3*H*-phenothiazine-3-one, acetaldehyde, formaldehyde, and dimethylamine.

Keyphrases □ Promethazine—oxidative thermal degradation products isolated and identified □ Oxidation—promethazine, thermal degradation products isolated and identified □ Degradation, oxidative thermal—promethazine, products isolated and identified □ Phenothiazines—promethazine, oxidative thermal degradation products isolated and identified □ Antiemetics—promethazine, oxidative thermal degradation products isolated and identified

The separation of phenothiazines and their oxidation products, mostly by TLC or paper chromatography, was described previously (1–10). Details were given about the identity of the products obtained, but the purity of the isolated fractions was unknown. Moreover, many reports (11–23) described the separation of different phenothiazines, also by TLC, paper chromatography, and GLC. These data were used for the development of a method to separate promethazine and its degradation products.

EXPERIMENTAL

Materials—Since promethazine hydrochloride[1] gave a single spot with several TLC solvent systems, it was used as supplied, as were 10-methylphenothiazine[2], phenothiazine[3], 3*H*-phenothiazine-3-one[2], 10-methylphenothiazine 5-oxide[2], and promethazine 5-oxide[1]. Phenothiazine 5-oxide was synthesized according to Pummerer and Gassner (24); 7-hydroxy-3*H*-phenothiazine-3-one was synthesized according to Vidal as modified by Inukai and Ueda (25). All other materials were reagent grade, and deionized water was used throughout.

Methods—Promethazine hydrochloride, 500 mg, was dissolved in 100 ml of water, and oxygen was bubbled through the solution for 30 min. The solution was placed into a screw-capped bottle and kept at 65° in the dark for 48 hr. After cooling to room temperature, the sample was transferred into a separator, made alkaline with 4 *N* NaOH, and extracted with dichloromethane. The remaining water layer was made acid with 4 *N* H_2SO_4 and extracted with dichloromethane.

Both organic phases were applied to a silica gel[4] TLC plate, 0.25 mm thick (26), as zones 15 cm long × 1 cm wide. Dichloromethane was evaporated by heating the plate at the lower side. After equilibration of the plate with the vapor of the solvent for 10 min, the chromatogram was developed for 15 cm, using acetone–6 *N* ammonia (100:2) as the solvent.

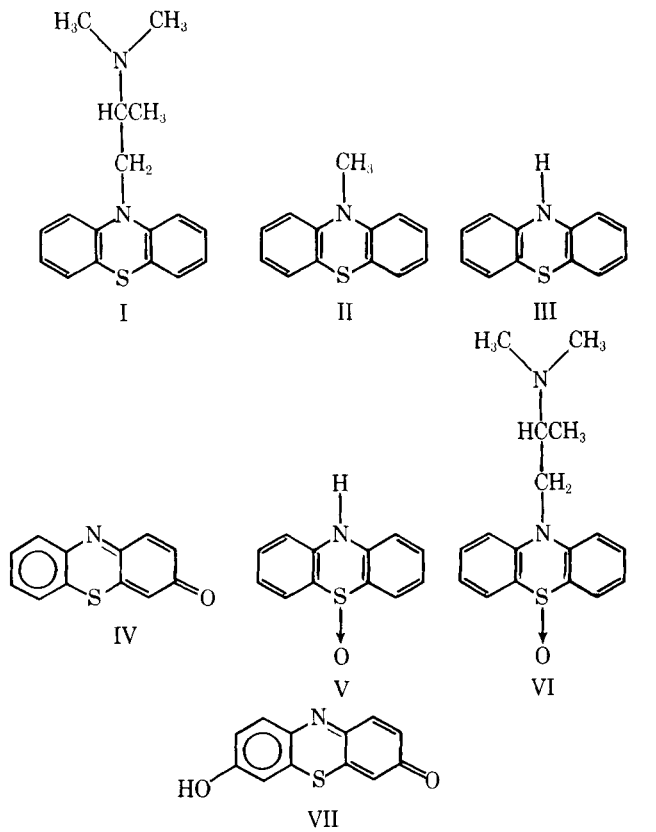
During the procedure, the system was protected from light to avoid uncontrolled degradation. After development, the zones on the chromatogram were detected in UV light (254 nm). All compounds except H were derived from the alkaline dichloromethane fraction. Compound H was derived from the acidic dichloromethane fraction.

For the identification of the degradation products, the zones were extracted with either dichloromethane or 96% ethanol. Identification was carried out using data obtained from TLC, GLC, spectroscopy (UV, IR, and mass[5]), polarography, and melting points. For the isolation of Compounds A_1 and A_2, zone A was extracted with dichloromethane. The extract was applied onto a silica gel plate, and the chromatogram was developed for 15 cm with *n*-hexane–chloroform (75:25). Both zones obtained were extracted with dichloromethane, recrystallized from acetone–water (1:1), and dried over 96% H_2SO_4 under reduced pressure in the dark.

Some volatile degradation products were isolated by precipitation as the 2,4-dinitrophenylhydrazones (aldehydes) or 3,5-dinitrobenzamides (amines) (27). Purification was carried out by recrystallization from ether and water. Identification of the volatile compounds was completed using TLC, GLC, spectroscopy, and melting-point data. Moreover, the sample was distilled, and the distillate was subjected to color reactions.

RESULTS AND DISCUSSION

Nine zones could be observed on the chromatogram obtained (Table I). Zone D has the same R_f value as promethazine, zone B was red, and zone H was violet.



[1] Rhône Poulenc, Paris, France.
[2] Provided by Dr. C. D. M. Ten Berge, Groningen, The Netherlands.
[3] Merck-Schuchardt, Munich, West Germany.
[4] GF 254, E. Merck, Darmstadt, West Germany.
[5] Mass spectra were recorded at the Analytisch-Chemisch Laboratorium, Rijksuniversiteit Utrecht, The Netherlands.

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Table I—TLC [a] Separation of Promethazine and Degradation Products

Zone[b]	R_f	Color in Daylight
A	0.80	Colorless
B	0.72	Red
C	0.61	Colorless
D	0.48	Colorless
E	0.38	Colorless
F	0.28	Colorless
G	0.19	Colorless
H	0.10	Violet
I	0.00	Brownish

[a] The sorbent was silica gel, the solvent was acetone–6 *N* ammonia (100:2), and detection was by UV light (254 nm). [b] All zones except H were extracted from an alkaline medium; H was extracted from an acidic medium.

Table II—R_f Values [a] of Phenothiazone, 10-Methylphenothiazine, and Fraction A

Solvent	Phenothiazine[b]	10-Methylphenothiazine	Fraction A A_1	Fraction A A_2[b]
Acetone–6 *N* ammonia (100:2)	0.76	0.76	0.76	0.76
n-Hexane	0.03	0.17	0.17	0.03
Chloroform	0.65	0.79	0.79	0.65
Toluene	0.54	0.71	0.71	0.54
Dichloromethane	0.81	0.85	0.85	0.81
n-Hexane–acetone (9:1)	0.20	0.42	0.42	0.20
n-Hexane–chloroform (75:25)	0.19	0.44	0.44	0.19

[a] The sorbent was silica gel. [b] Turned green under light.

Table III—R_f Values [a] of 3*H*-Phenothiazine-3-one and Fraction B

Solvent	3*H*-Phenothiazine-3-one	Fraction B
Acetone	0.64	0.64
Acetone–6 *N* ammonia (100:2)	0.69	0.69
Methanol	0.67	0.67
Methanol–6 *N* ammonia (100:2)	0.67	0.67
96% Ethanol	0.61	0.61
96% Ethanol–6 *N* ammonia (100:2)	0.62	0.62
Chloroform	0.17	0.17
n-Hexane	0.00	0.00

[a] The sorbent was silica gel.

Compound D—The R_f values of Compound D in several TLC systems were identical with those of promethazine (I) in the same systems. In all cases, only one spot was observed. The UV spectrum of D in 1 *N* HCl and IR spectrum of D in sodium chloride were identical with those of I in the same media.

Compound A_1—Table II shows that R_f values of Compound A_1 and 10-methylphenothiazine (II) were the same. The melting point of A_1 was 107°, and a mixture of equal parts of A_1 and II melted at 107°.

The UV spectra of A_1 and II in dichloromethane were identical, with a maximum at 255 nm. The IR spectra of both compounds in sodium chloride were also identical. The parent peak of A_1 in the mass spectrum was *m/e* 213, corresponding to the formula $C_{13}H_{11}NS$. The data showed A_1 to be II.

Compound A_2—The R_f values of Compound A_2 and phenothiazine (III) in several chromatographic systems were the same (Table II). The melting point of A_2 was 178–182°, while a mixture of A_2 and III melted at 178–181°.

The UV spectra of A_2 and III in dichloromethane were identical, with a maximum at 255 nm. The IR spectra of both compounds were also identical. The mass spectrum of A_2 showed a parent peak at *m/e* 199, corresponding to the formula $C_{12}H_9NS$. These data led to the conclusion that A_2 was III.

Compound B—Compound B was red, as is 3*H*-phenothiazine-3-one (IV), and was identified according to Roseboom and Fresen (28). The R_f values of B and IV in several systems were identical (Table III). The melting point of B was 146–150°, and the melting point of a mixture of equal parts of B and IV was 149–155°. (The melting point of B was a few degrees below the melting point of IV. The fraction could not be purified by recrystallization.)

Table IV—R_f Values of Phenothiazine 5-Oxide, 10-Methylphenothiazine 5-Oxide, and Fraction C

Sorbent	Solvent	Phenothiazine 5-Oxide	10-Methylphenothiazine 5-Oxide	Fraction C
Silica gel	Acetone–6 *N* ammonia (100:2)	0.59	0.59	0.59
Silica gel	Acetone–water (95:5)	0.75	0.75	0.75
Silica gel	Acetone	0.57	0.57	0.57
Silica gel	Acetone–dichloromethane (6:4)	0.40[a]	0.40[a]	0.40[a]
Alumina, basic	Chloroform	0.23	0.67	0.23

[a] Tailing.

Table V—R_f Values [a] of Promethazine 5-Oxide and Fraction F

Solvent	Promethazine 5-Oxide	Fraction F
Acetone–6 *N* ammonia (100:2)	0.21	0.21
1-Butanol–acetic acid–water (88:5:7)	0.14	0.14
Acetone–water–acetic acid (5:4:1)	0.33	0.33
3 g of ammonium acetate in 20 ml of water and methanol to 100 ml	0.35	0.35

[a] The sorbent was silica gel.

The UV and visible light spectra of B and IV in water were identical, with maxima at 530, 385, and 289 nm. Shoulders were observed at 269 and 238 nm. The IR spectra of both compounds were also identical, with a strong maximum at 1620 cm^{-1} due to the C=O group. The mass spectrum showed a parent peak at *m/e* 213, corresponding to the formula $C_{12}H_7NOS$. These data showed B to be IV.

Compound C—The melting point of Compound C was 257–258.5°. Its UV spectra in acidic and alkaline ethanolic media were identical. The IR spectrum in sodium chloride showed a strong maximum at 973 cm^{-1}. The mass spectrum showed a parent peak at *m/e* 215. Calculation of an exact mass yielded a value of *m/e* 215.0401, corresponding to the formula $C_{12}H_9NOS$. Compound C could be reduced at the dropping mercury electrode with an $E_{1/2}$ of −590 mv *versus* the saturated calomel electrode in 96% ethanol–4 *N* H_2SO_4 (25:75).

The data obtained from the mass spectrum showed C to be an oxidation product of phenothiazine containing one oxygen atom. The data from the UV spectra showed that no hydroxyl group was present, while the IR spectral data indicated the possible presence of an SO group with a maximum at about 1000 cm^{-1} (29). The polarographic behavior of C also indicated the presence of a reducible SO group. The melting point of phenothiazine 5-oxide (V) is 257–258° (30), which was in good agreement with the melting point of C. The mixed melting point of C and V was also 258°. The R_f values of C and V (Table IV) were identical, while 10-methylphenothiazine 5-oxide had a different R_f value in one system. All these data showed C to be V.

Compound F—Zone F showed the strongest absorption under UV light (254 nm) and contained probably the main degradation product. Compound F and promethazine 5-oxide (VI) had the same R_f values in several chromatographic systems (Table V). Compound F melted at 120°, and a mixture of F and VI (1:1) melted at 119–120°.

The UV absorption spectra of F and VI in water were identical, with maxima at 334, 293, 268, and 234 nm, in agreement with the values of Kofoed *et al.* (31). The IR spectra of both compounds were identical as well, with a maximum at 1020 cm^{-1} due to the SO group (29). The mass spectrum of F showed a parent peak at *m/e* 300 in agreement with the formula $C_{17}H_{20}N_2OS$. All these data led to the conclusion that F and VI were identical.

Compound H—After extraction of the original sample in an alkaline medium, the remaining water phase had a violet color, which turned red when acidified. The dye was extracted from the acidic medium with dichloromethane. Chromatographic analysis showed that only one compound was obtained. The melting point of H was above 360°.

Absorption spectra of H in different solvents showed that, when the solutions were made alkaline, the absorption maximum shifted from about 510 to about 600 nm (Table VI) while the extinction of the ethanolic

Table VI—Absorption Maxima of Fraction H in UV and Visible Light

Solvent	λ_{max}, nm
Water, acidic[a]	244, 286, 502
Water, alkaline[b]	278, (510), 590
96% Ethanol, acidic[a]	246, (275), 429, 515
96% Ethanol, alkaline[b]	231, 277, 309, (565), 600
Acetone, acidic[a]	510[c]
Acetone, alkaline[b]	565, 609[c]

[a] Three milliliters of solution plus 1 drop of 6 *N* HCl. [b] Three milliliters of solution plus 1 drop of 6 *N* NaOH. [c] Spectra were recorded in the 330–700-nm region. Numbers in parentheses are shoulders.

Table VII—R_f Values of 7-Hydroxy-3*H*-phenothiazine-3-one and Fraction H

Sorbent	Solvent	7-Hydroxy-3*H*-phenothiazine-3-one	Fraction H
Silica gel	Acetone–water (95:5)	0.42	0.42
Silica gel	Acetone–6 *N* ammonia (100:2)	0.06	0.06
Silica gel	Acetone–6 *N* ammonia (90:10)	0.56	0.56
Silica gel	*n*-Hexane–chloroform (75:25)	0.00	0.00
Alumina, basic	Acetone–6 *N* ammonia (90:10)	0.50	0.50
Alumina, basic	Acetone–water (90:10)	0.37	0.37
Alumina, basic	1. Benzene–acetone (95:5)[a] 2. Benzene–acetone–acetic acid (95:5:5)[a]	0.24	0.24

[a] Subsequent development.

Table VIII—R_f Values[a] of the 2,4-Dinitrophenylhydrazones of Formaldehyde (A), Acetaldehyde (B), and Some Degradation Products of Promethazine (C)

Solvent	A	B	C
Nitrobenzene–cyclohexane (1:2)	0.34	0.43	0.34 0.43
Nitrobenzene–chloroform–*n*-hexane (1:2:8)	0.37	0.49	0.37 0.49

[a] The sorbent was alumina GF 254 (E. Merck, Darmstadt, West Germany).

solution increased by a factor of 4.4. Moreover, the acetone solution showed an intense fluorescence. The mass spectrum showed a parent peak at *m/e* 229, corresponding to the formula $C_{12}H_7NO_2S$.

The data obtained led to the conclusion that the compound was an acid, probably a phenol. Moreover, the spectra indicated a similarity in structure between Compound H and 3*H*-phenothiazine-3-one (IV). Some investigators (25, 32, 33) discussed a compound, 7-hydroxy-3*H*-phenothiazine-3-one (VII), with the same spectral properties and melting point. The R_f values of H and VII in several chromatographic systems were identical (Table VII). The spectra in the UV and visible light region, as well as the IR spectra of both compounds, were identical. The data obtained showed H to be VII.

Compounds E, G, and I were not identified. They only occurred in traces during the degradation of promethazine.

Identification of Volatile Degradation Products—On oxidation of promethazine (with persulfate or on boiling), some volatile degradation products are formed, such as formaldehyde, acetaldehyde, and dimethylamine (34) or acetaldehyde and dimethylamine (35).

Aldehydes—The precipitate of the 2,4-dinitrophenylhydrazones was analyzed chromatographically (Table VIII). GLC analysis on Chromosorb W with 5% SE-30 showed the retention times of the 2,4-dinitrophenylhydrazones to be identical to those of the 2,4-dinitrophenylhydrazones of acetaldehyde and formaldehyde. After distillation of a few milliliters of the original sample, the reaction with chromotropic acid in 96% H_2SO_4 (36) and Schiff's reaction (37), both very selective for formaldehyde, were positive in the distillate.

Finally, GLC analysis of the headspace of the decomposed promethazine solution on Chromosorb WAW DMC-8 with 50% Carbowax 1500 showed a peak with a retention time identical to that of acetaldehyde (formaldehyde is not detectable with a flame-ionization detector). These data showed the volatile aldehydes to be acetaldehyde and formaldehyde.

Table IX—R_f Values[a] of the 3,5-Dinitrobenzamides of Dimethylamine (A) and a Degradation Product of Promethazine (B)

Solvent	A	B
Chloroform–ethanol (99:1)	0.57	0.57
Ethanol–6 *N* ammonia (75:25)	0.53	0.53
1-Butanol–acetic acid–water (4:1:5)	0.76	0.76

[a] The sorbent was silica gel.

Dimethylamine—The R_f values of the 3,5-dinitrobenzamide obtained were identical with those of the 3,5-dinitrobenzamide of dimethylamine (Table IX). The melting point of the isolated compound was 108–112°, and the melting point of a mixture of the isolated and the synthesized compound was 110–112°. The IR spectra of both compounds were identical.

GLC analysis of the headspace of the original sample on Chromosorb WAW DMC-8 with 50% carbowax 1500 showed a peak with the same retention time as dimethylamine. The data obtained led to the conclusion that the isolated amine was dimethylamine.

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Oxidative Degradation of Pharmaceutically Important Phenothiazines II: Quantitative Determination of Promethazine and Some Degradation Products

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Abstract □ Methods for the determination of promethazine and several degradation products, which can be used in kinetic studies, were developed. All determinations were carried out after isolation of the compounds by TLC. Promethazine was determined by oxidation with vanadyl sulfate in an acidic medium. The method was suitable for approximately 3 mg of the compound. 3*H*-Phenothiazine-3-one was determined spectrophotometrically at 492 nm in acetone. The compound could be determined in amounts of 30–40 μg. Promethazine 5-oxide was determined spectrophotometrically using the acid dye method; 100–200 μg could be determined. 7-Hydroxy-3*H*-phenothiazine-3-one could be determined spectrophotometrically at 600 nm after extraction of the other compounds from the solution. The medium had to be alkaline and contain water in a fixed ratio. The method was suitable for 15–30 μg of the compound.

Keyphrases □ Promethazine—polarographic analysis, prepared samples □ Degradation products, various—of promethazine, spectrophotometric analyses, prepared samples □ Polarography—analysis, promethazine, prepared samples □ Spectrophotometry—analyses, various degradation products of promethazine, prepared samples □ Antiemetics—promethazine, polarographic analysis, prepared samples □ Phenothiazines—promethazine, polarographic analysis, various degradation products, spectrophotometric analyses, prepared samples

Previously (1), the isolation and identification of some products formed on storage of promethazine dissolved in water in the presence of oxygen were described. Literature methods for the determination of promethazine are not useful for the study of its degradation kinetics because of interference by the degradation products. Moreover, no suitable methods are available for the determination of the degradation products.

This paper describes suitable methods for the quantitative determination of promethazine, promethazine 5-oxide, 3*H*-phenothiazine-3-one, and 7-hydroxy-3*H*-phenothiazine-3-one. The method for the determination of promethazine is also suitable for other phenothiazine derivatives. All quantitative determinations were carried out after separation by TLC.

EXPERIMENTAL

Materials—All materials were described previously (1).

Preparation of Reagents—The acid mixture contained 16 parts of 4 *N* sulfuric acid mixed with nine parts of 85% phosphoric acid.

To prepare 0.002 *M* ferrous ammonium sulfate, about 0.20 g of ferrous ammonium sulfate was dissolved in 50 ml of water. To the solution were added 62 ml of 4 *N* sulfuric acid and water to a volume of 250.0 ml. The strength of the solution was determined by titration with potassium dichromate.

To prepare 0.005 *M* vanadyl sulfate ($VOSO_4$), about 0.58 g of ammonium vanadate(V) was dissolved in 10 ml of 6 *N* ammonia and 200 ml of water. To the solution were added 250 ml of 4 *N* sulfuric acid and water to a volume of 1 liter. The strength of the solution was determined with the 0.002 *M* ferrous ammonium sulfate.

Acetate buffer, pH 4.6, was prepared by dissolving 26.6 g of sodium acetate in water and adding 15.9 g of 97% acetic acid and water to a volume of 100.0 ml. The pH was adjusted to 4.6.

A saturated solution of tropaeolin 00 in water also was prepared. A sulfuric acid–methanol solution was prepared by mixing one part of 96% sulfuric acid with 99 parts of methanol.

Procedures—For the determination of promethazine, an amount of the sample corresponding to about 3 mg of promethazine was transferred into a separator. Extraction and separation by TLC were carried out as described (1). The zone containing promethazine was transferred into a 50-ml beaker, and 5.00 ml of 0.005 *M* vanadyl sulfate and 25 ml of acid mixture were added. The mixture was stirred until the red color disappeared completely. Excess vanadyl sulfate was determined with 0.002 *M* ferrous ammonium sulfate, with biamperometric determination of the end-point according to Van Pinxteren and Verloop (2) with a potential of 150 mv between the platinum electrodes.

The equivalent weight is the molecular weight divided by 2.

The oxidation mechanism of promethazine was studied using polarography[1] with a platinum electrode as the indicator electrode and a saturated calomel electrode (SCE) as the reference electrode.

After separation by TLC as described previously (1), 3*H*-phenothiazine-3-one was determined. The dye was extracted from the silica gel with acetone. The solution was filtered through a small plug of cotton, and acetone was added to a volume of 10.0 ml. The absorbance of the solution was measured[2] at 492 nm in a 1- or 2-cm cell with acetone as a blank.

The determination of promethazine 5-oxide was carried out using the acid dye method described by Häussler and Hajdú (3). After extraction of the sample and separation by TLC as described previously (1), the zone containing promethazine 5-oxide was transferred into a separator and mixed with 10 ml of pH 4.6 acetate buffer and 5 ml of tropaeolin 00 (Mixture A). A blank was prepared using a zone of the same size from the same chromatogram containing no compounds (Mixture B). Both mixtures were extracted with portions of 5 ml of chloroform until the color of the chloroform phases of both systems was the same. The chloroform phases were filtered through a plug of cotton into 50-ml volumetric flasks, and 3.0 ml of sulfuric acid–methanol and chloroform to a volume of 50.0 ml were added. The absorbance of Mixture A was measured at 545 nm in a 0.5- or 1-cm cell with Mixture B as a blank.

To obtain reproducible results and to lower the blank values, all glassware was cleaned carefully, freshly prepared solvent was used always,

[1] Radiometer polarograph PO3m.
[2] Unicam SP 500 spectrophotometer.

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