(33) D. E. Houston, E. B. Kester, and F. De Eds, *J. Am. Chem. Soc.*, 71, 3819 (1949).
(34) G. M. Nano, P. Sancin, and G. Tappi, *Pharm. Acta Helv.*, 38, 623 (1963).
(35) T. Waaler, *ibid.*, 35, 168 (1960).
(36) F. Feigl, "Spot Tests in Organic Analysis," 6th ed., Elsevier, Amsterdam, The Netherlands, 1960, p. 349.
(37) *Ibid.*, p. 223.

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

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Received August 8, 1977, from the *Farmaceutisch Laboratorium, Rijksuniversiteit Utrecht, Catharijnesingel 60, Utrecht, The Netherlands.* Accepted for publication December 14, 1977.

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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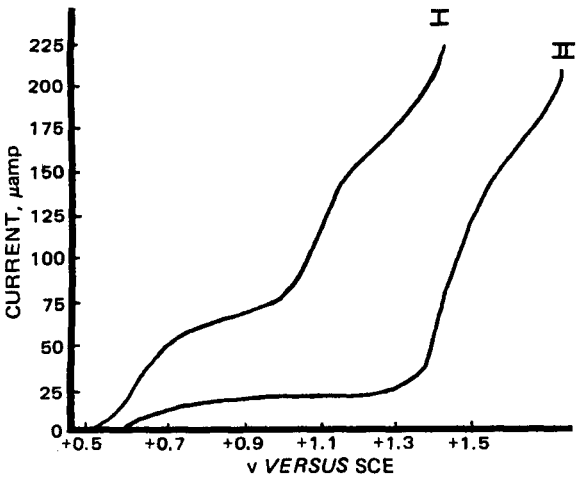

Figure 1—*Voltammogram of promethazine in 4 N sulfuric acid. Key: curve I, 2 × 10⁻⁴ M promethazine hydrochloride in 4 N sulfuric acid; and curve II, 4 N sulfuric acid.*

and the chromatograms were dried with a warm air stream immediately after development.

For the determination of 7-hydroxy-3*H*-phenothiazine-3-one, 3.00 ml of the sample was transferred into a separator, 1.00 ml of 4 *N* sodium hydroxide was added, and the mixture was extracted with dichloromethane until the organic phase remained colorless. The water layer was transferred quantitatively into a volumetric flask, and 96% ethanol was added to a volume of 10.0 ml. The absorbance of the solution was measured at 600 nm in a 1- or 2-cm cell with water as a blank.

RESULTS AND DISCUSSION

The polarogram of the oxidation of promethazine in 4 *N* sulfuric acid showed two steps (Fig. 1). The first was the loss of one electron and the formation of a red-colored radical ($E_{1/2}$ = +0.65 v *versus* the saturated calomel electrode). The second step was the loss of another electron with the formation of a colorless compound (3) ($E_{1/2}$ = +1.02 v *versus* the saturated calomel electrode). Since the radical was unstable, an oxidimetric determination method was used to measure the formation of the colorless product.

The oxidation potential of a mixture of 5.00 ml of 0.005 *M* vanadyl sulfate and 25 ml of acid mixture was +1.03 v *versus* the saturated calomel electrode, which was in good agreement with the $E_{1/2}$ of the second step of the oxidation of promethazine. This result indicated that vanadyl sulfate was adequate for performing the oxidation. Subsequent determinations of promethazine after each step of the isolation procedure showed neither degradation of promethazine during the isolation procedure nor interference from the silica gel in the determination.

Table I gives the results obtained with this method. Moreover, the procedure is suitable for the determination of N-10 substituted phenothiazines in general (Table II). Finally, the oxidation product formed was identified as promethazine 5-oxide according to the procedure described previously (1).

The absorption maximum of 3*H*-phenothiazine-3-one in acetone is 492 nm. With this solvent, the compound could be eluted easily from the silica gel. In the 20–100-μg range, Beer's law was obeyed; the molar absorptivity of 3*H*-phenothiazine-3-one is 7130. Table III shows the results of the determination of 3*H*-phenothiazine-3-one with the procedure described.

Table I—Determination of Promethazine Hydrochloride

Amount, mg	Recovery[a], %
3.00	100
3.04	101
3.01	102
2.99	99
3.02	99
3.08	101
Average ± *SD* = 100.3 ± 1.0%	

[a] Average of three determinations.

Table II—Determination of Several Phenothiazines

Compound	Amount, mg	Recovery, %
Chlorpromazine hydrochloride	3.02	100
Cyanopromazine hydrochloride	3.01	102
Methoxypromazine hydrochloride	2.89	99
Methylpromazine hydrochloride	3.06	101
Promazine hydrochloride	3.12	100
Promethazine hydrochloride	2.99	102
Triflupromazine hydrochloride	3.08	101

Table III—Determination of 3*H*-Phenothiazine-3-one

Amount, μg	Recovery, %
31	90
31	91
50	94
50	91
51	94
70	93
70	93
72	93
Average ± *SD* = 92.4 ± 1.5%	

Table IV—Determination of Promethazine 5-Oxide

Amount, μg	Recovery, %
99	97
99	106
115	101
115	104
149	104
149	103
173	103
173	97
Average ± *SD* = 101.9 ± 3.5%	

Table V—Determination of Promethazine 5-Oxide (150 μg) after Isolation by TLC with Different Solvents

Solvent	Recovery, %
Acetone–6 *N* ammonia (100:2)	102
Methanol–6 *N* ammonia (100:2)	90
Ether–6 *N* ammonia (100:2)	80
Water–6 *N* ammonia (100:2)	79
Methanol–6 *N* ammonia–isopropylamine (100:1:1)	79
No development	81

Promethazine 5-oxide forms an ion-pair with tropaeolin 00 at pH 4.6, extractable with chloroform. Tropaeolin 00 in acidified chloroform–methanol solution has an absorption maximum at 545 nm. Since the ion-pair was destroyed after extraction by adding sulfuric acid–methanol, all determinations were carried out at this wavelength. In the 40–200-μg range of promethazine 5-oxide, Beer's law was obeyed. The molar absorptivity of the ion-pair is 48,590. The molar absorptivity of tropaeolin 00 under the same conditions is 40,400 (3), which shows promethazine and tropaeolin 00 to be present in the complex in a 1:1 ratio. Table IV shows the results of some determinations of promethazine 5-oxide with the described method.

The blank showed an absorbance that could not be neglected. This response sometimes was due to the solvent used in the TLC separation of the compounds, so other solvents were tried. Table V shows that the absorbance of the blank could be decreased to almost zero, but the recovery also decreased. This phenomenon also occurred when promethazine 5-oxide was applied onto a silica gel layer without development of the chromatogram (Table V). Apparently, the extraction of the ion-pair from the sorbent was not complete. Only after developing the chromatogram with acetone–6 *N* ammonia (100:2) were good results obtained (Table VI).

The fact that the acetone–6 *N* ammonia mixture is highly reactive (4) might be an explanation. Possibly, an intermediate formed in the reaction decreases the activity of the sorbent so that a complete extraction can

Table VI—Determination of 7-Hydroxy-3*H*-phenothiazine-3-one

Amount, μg	Recovery, %
16.4	89
18.2	93
18.4	94
19.6	96
24.6	93
27.3	95
27.8	94
29.4	99
Average ± *SD* = 94.1 ± 3.2%	

be carried out. This reactivity is also an explanation for the higher blank values obtained with the solvent acetone–6 *N* ammonia (100:2). One product formed, isopropylamine, also forms a complex with tropaeolin 00 that is extractable with chloroform. The use of freshly prepared solvent, careful cleaning of the glassware, and fast drying of the developed chromatograms were necessary to reduce the absorbance of the blank.

Although 7-hydroxy-3*H*-phenothiazine-3-one was the only compound formed during degradation of promethazine with an absorption maximum at 600 nm in an alkaline medium, the other products and promethazine itself interfered by reducing the compound to the colorless 3,7-dihydroxyphenothiazine. Therefore, the other compounds had to be removed by extraction with dichloromethane from the alkaline medium. The resulting water phase was diluted with 96% ethanol to get a clear solution. In the 5–40-μg range, Beer's law was obeyed. The molar absorptivity of the compound under this condition is 58,900. The results obtained with this method (Table VI) were quite satisfactory, provided that the ratio between water and ethanol in the solvent remained constant.

REFERENCES

(1) W. J. M. Underberg, *J. Pharm. Sci.*, **67**, 1128 (1978).

(2) J. A. C. Van Pinxteren and M. E. Verloop, *Pharm. Weekbl.*, **96**, 545 (1961).

(3) A. Häussler and P. Hajdú, *Arzneim.-Forsch.*, **12**, 411 (1962).

(4) D. C. Borg and G. C. Cotzias, *Proc. Natl. Acad. Sci. USA*, **48**, 617 (1962); through A. H. Beckett and S. H. Curry, *J. Pharm. Pharmacol.*, **15**, 246T (1963).

Oxidative Degradation of Pharmaceutically Important Phenothiazines III: Kinetics and Mechanism of Promethazine Oxidation

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Received August 8, 1977, from the *Farmaceutisch Laboratorium, Rijksuniversiteit Utrecht, Catharijnesingel 60, Utrecht, The Netherlands.* Accepted for publication December 14, 1977.

Abstract □ The kinetics of the thermal degradation of promethazine in an acidic medium under various conditions were investigated. The degradation of promethazine and the formation of some degradation products were studied under aerobic and anaerobic conditions. The influence of pH, metal ions such as copper(II) and iron(III), and antioxidants was investigated. In an oxygen-saturated medium, promethazine generally followed first-order kinetics. Increasing the pH increased the degradation rate to a limiting value at pH 5. Addition of copper(II) increased the degradation rate over the whole process, while iron(III) caused an increase for only a short time. Ascorbic acid sometimes increased the degradation rate, while higher concentrations of hydroquinone also accelerated the degradation. Pyrosulfite did not have any influence. Under anaerobic conditions, promethazine degraded only in the presence of copper(II) and iron(III) ions. As a result of the studies on the qualitative and quantitative aspects of the oxidation process, a mechanism for the oxidative degradation of promethazine is suggested. Promethazine 5-oxide and a number of degradation products without intact side chains are formed *via* a semiquinone free radical. The influence of several factors on the degradation process is discussed.

Keyphrases □ Promethazine—thermal degradation kinetics in acidic medium, effect of anaerobic and aerobic conditions, pH, metal ions, and antioxidants □ Degradation, thermal—promethazine, kinetics in acidic medium, effect of anaerobic and aerobic conditions, pH, metal ions, and antioxidants □ Oxidation—promethazine, thermal degradation kinetics in acidic medium, effect of anaerobic and aerobic conditions, pH, metal ions, and antioxidants □ Antiemetics—promethazine, thermal degradation kinetics in acidic medium, effect of anaerobic and aerobic conditions, pH, metal ions, and antioxidants □ Phenothiazines—promethazine, thermal degradation kinetics in acidic medium, effect of anaerobic and aerobic conditions, pH, metal ions, and antioxidants

Little information is available on the quantitative aspects of promethazine degradation. Chalabala *et al.* (1) mentioned the stability of a solution containing aminopyrine, trimecaine, and promethazine at pH 5.5–6.0 in the presence of sodium pyrosulfite, thiourea, or sodium formaldehyde sulfoxylate. One study (2) found that promethazine degradation followed first-order kinetics. The degradation rate decreased on changing the pH from 3.0 to 5.0. In another study, the degradation rate of the reaction was reported to be pH independent (3). Other studies (4, 5) also described first-order kinetics for the degradation reaction. However, at pH values over 5.5, degradation no longer followed first-order kinetics (5). Moreover, promethazine was most unstable at pH 4.3.

In this paper, the kinetics of promethazine degradation under varying conditions are described and the degradation mechanism is discussed.

EXPERIMENTAL

Materials—All materials used were described previously (6).

Methods—For the study of promethazine degradation under aerobic conditions, about 50 mg of promethazine hydrochloride, accurately weighed, was dissolved in a few milliliters of water. To the solution were added 12.5 ml of acetate buffer according to Walpole (7) to get the right pH (at 65°), any other necessary additive previously adjusted to the right pH, and water to a volume of 50.0 ml. The solution was saturated with oxygen, and 4 ml was transferred into 10-ml ampuls in an oxygen atmosphere according to a previous method (8). The ampuls were kept at 65° in the dark.

The isolation and determination of promethazine, promethazine 5-oxide, 3*H*-phenothiazine-3-one, and 7-hydroxy-3*H*-phenothiazine-3-one were carried out as described previously (6, 9).

For the study of promethazine degradation under anaerobic conditions, the oxygen was removed by bubbling carbon dioxide through the solution for 5 hr. The degradation was carried out at 100°.

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