cycloheximide, m.p. 151–153°; mixed m.p. 150 to 151.5° with an authentic sample of *epi*-deoxycycloheximide. $\tilde{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1730, 1700, and 1680 (C = 0). The *epi*-deoxycycloheximide was further identified by thin-layer chromatography on silica gel when compared with the authentic sample of *epi*-deoxycycloheximide ($R_f$ = 0.15, in chloroform containing 1% methanol).

**Attempted Isomerization of *epi*-Deoxycycloheximide.**—A solution of 0.10 Gm. (0.38 mmole) of *epi*-deoxycycloheximide and *p*-toluenesulfonic acid (0.08 Gm., 0.41 mmole) in 2.0 ml. of dimethylformamide was refluxed for 40 minutes. The reaction mixture was allowed to cool to room temperature and then poured onto ice. After the ice had melted, the crystals were collected by filtration and dried *in vacuo;* yield, 0.07 Gm. of *epi*-deoxycycloheximide, m.p. 140°. On recrystallization from ethanol and water, the crude material gave the pure sample, m.p. 149.5 to 152°; mixed m.p. 150–152° with the authentic sample of *epi*-deoxycycloheximide. $\tilde{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1730, 1700, and 1680 (C = 0). The *epi*-deoxycycloheximide was further identified by thin-layer chromatography on silica gel when compared with the authentic sample of *epi*-deoxycycloheximide ($R_f$ = 0.15 chloroform containing 1% methanol).

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# Photometric Determination of Pentylenetetrazol in Pharmaceutical Preparations

By ROBERT A. DAOUST

**Pentylenetetrazol is quantitatively precipitated from aqueous solution by cuprous chloride reagent. The pentylenetetrazol–cuprous chloride complex is dissolved in concentrated nitric acid; the solution is adjusted to pH 4 with acetate buffer and sodium hydroxide solution, and the deep blue color of the copper complex developed with tetraethylenepentamine is determined photometrically at 580 mμ. The method can be applied to samples containing from 2–10 mg. pentylenetetrazol and furnishes a rapid means of determining small amounts of the drug in the presence of other ingredients in pharmaceutical preparations.**

PENTYLENETETRAZOL, a white, odorless, crystalline drug with a slightly pungent bitter taste is applied extensively as a central nervous system stimulant in the treatment of schizophrenia in shock therapy of psychoses and as an analeptic in narcotic poisoning.

This paper describes a method for the photometric determination of pentylenetetrazol in pharmaceutical liquids and tablets; the method can be performed rapidly and simply on small amounts of samples suitable for pharmaceutical control work. A gravimetric method which is determined by salting the drug from its aqueous solution with ammonium sulfate, followed by extraction with carbon tetrachloride has been officially adopted by "The National Formulary" (1, 2). Another gravimetric method involving precipitation with phosphotungstic acid is favored by some workers (3, 4). Numerous procedures have been reported for the assay of pentylenetetrazol: refractometric (5, 6), indirect polarographic (7), complexometric (8–10), colorimetric (11, 12), and volumetric and potentiometric methods (12).

The principle of the method proposed involves the precipitation of pentylenetetrazol from its aqueous solution with an excess of cuprous chloride reagent to give an insoluble, silver-white, crystalline, double salt complex which is then dissolved in concentrated nitric acid. The acid decomposes the pentylenetetrazol–cuprous chloride complex into its components and simultaneously oxidizes the cuprous salt to the cupric form. The acid solution is adjusted to pH 4 with acetate buffer and sodium hydroxide solution, and the absorbance of the deep blue color of the copper complex developed with tetraethylenepentamine

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(13) is measured at 580 mμ with a suitable spectrophotometer.

## EXPERIMENTAL

**Reagents.**—*Dister's Reagent* (14). A 1.250 Gm. quantity of cuprous chloride (reagent grade) is dissolved in distilled water to make 100 ml. of an aqueous solution containing 10 Gm. of ammonium chloride U.S.P. and 1 Gm. of sodium *meta*-bisulfite (reagent grade). This solution, if kept in a tightly stoppered amber bottle in a refrigerator immediately after use, is stable for several weeks.

*Pentylenetetrazol Standard Solution.* One-hundred milligrams of dried crystalline pentylenetetrazol N.F. is dissolved in about 50 ml. distilled water and the solution is diluted to the mark of a 100-ml. glass-stoppered volumetric flask. Each milliliter of this solution contains 1.0 mg. pentylenetetrazol.

*Acetate Buffer Solution* (15). A 16.6 Gm. quantity of anhydrous sodium acetate (reagent grade) is dissolved in about 100 ml. distilled water; 24 ml. glacial acetic acid is added and the mixture diluted to 200 ml.

*Sodium Hydroxide Solution,* 6% (w/v). Twelve grams of sodium hydroxide pellets (reagent grade) is dissolved in distilled water to make 200 ml. of solution.

*Tetraethylenepentamine Solution,* 2% (w/v). Four milliliters of tetraethylenepentamine (technical grade) is mixed with distilled water to make 200 ml. of solution.

TABLE I.—RELATION BETWEEN CONCENTRATION AND ABSORBANCE OF PENTYLENETETRAZOL

| Concn., mg./50 ml. | Absorbance, S.D. |
|---|---|
| 2 | 0.095 ± 0.008 |
| 4 | 0.177 ± 0.006 |
| 5 | 0.228 ± 0.004 |
| 6 | 0.267 ± 0.007 |
| 8 | 0.356 ± 0.011 |
| 10 | 0.444 ± 0.005 |

*Dilute Acetic Acid Solution,* 1% (v/v). One milliliter of glacial acetic acid is mixed with distilled water to make 100 ml. of solution.

*Concentrated Nitric Acid* (reagent grade).

**Preparation of the Calibration Graph.**—Aliquots of 2, 4, 6, 8, and 10 ml. of the standard solution of pentylenetetrazol are pipeted in order into each of five 50-ml. Erlenmeyer flasks. Each of the first four solutions is diluted to 10 ml. with distilled water, and 2 ml. of Dister's reagent is added to each of the five solutions. The flasks are stoppered and placed in an ice bath for 20 minutes with swirling a few times at 5-minute intervals to insure complete precipitation.

The precipitate is then filtered with suction through a 30-ml. sintered Pyrex glass filter funnel of medium porosity attached to a 500-ml. Pyrex glass suction flask; the filtrate, which is collected in a 18 x 150-mm. test tube placed inside of the flask, is rejected. The precipitate is washed twice with 5-ml. portions of cold dilute 1% acetic acid solution and the washings rejected.

The residue is dissolved by adding 1 ml. of concentrated nitric acid directly onto the sintered-glass funnel using a safety pipet or 50-ml. glass syringe. The filtrate is collected in the same manner in the test tube. The walls and plate of the glass funnel are washed with about 5 ml. of cold distilled water and aspirated through into the filtrate. The test tube (containing the filtrate) is carefully lifted out of the flask by a wire bent into a hook placed around the test tube. The filtrate is then poured into a 50-ml. glass-stoppered volumetric flask by means of a glass buret funnel. The washing is repeated with another 5 ml. portion and this is poured into the flask also. Ten milliliters each of the acetate buffer, 6% aqueous sodium hydroxide solution, and 2% aqueous tetraethylenepentamine solution is pipeted in succession into the acid solution; diluted to the mark with distilled water, stoppered, and mixed well. A blank solution is prepared by pipeting in succession into another 50-ml. glass-stoppered volumetric flask (containing a little distilled water) 1 ml. of concentrated nitric acid, 10

TABLE II.—RESULTS OF ASSAYS FOR PENTYLENETETRAZOL ON VARIOUS PHARMACEUTICAL LIQUIDS (100 MG./5 ML.) AND TABLETS (100 MG./TABLET)

| Vita-Metrazol[a] | | Metrazol-Liquidum[b] | |
|---|---|---|---|
| Absorbance of Sample[e] | Recovery, % | Absorbance of Sample[f] | Recovery, % |
| 0.240 | 100.0 | 0.235 | 100.0 |
| 0.230 | 95.8 | 0.235 | 100.0 |
| 0.235 | 97.9 | 0.230 | 97.8 |
| 0.235 | 97.9 | 0.230 | 97.8 |
| 0.238 | 99.1 | 0.230 | 97.8 |
| Mean | 98.2 | | 98.7 |
| S.D. | ±1.6 | | ±1.2 |

| Nico-Metrazol[c] | | Metrazol Tablets[d] | | |
|---|---|---|---|---|
| Absorbance of Sample[g] | Recovery, % | Absorbance of Sample[h] | Wt. Sample, Gm. | Recovery, % |
| 0.230 | 102.2 | 0.225 | 0.3368 | 97.4 |
| 0.230 | 102.2 | 0.235 | 0.3359 | 101.9 |
| 0.230 | 102.2 | 0.235 | 0.3362 | 101.9 |
| 0.225 | 100.0 | 0.240 | 0.3374 | 103.7 |
| 0.235 | 104.4 | 0.225 | 0.3368 | 97.4 |
| Mean | 102.2 | | | 100.5 |
| S.D. | ±1.6 | | | ±2.9 |

[a] Each 5 ml. contains 100 mg. Metrazol, 10 mg. niacinamide, 1 mg. pyridoxine hydrochloride, 1.4 mg. riboflavin sodium phosphate, and 2 mg. *d*-panthenol dissolved in 100 ml. of 15% ethanol. [b] Each 5 ml. contains 100 mg. Metrazol and 1 mg. thiamine hydrochloride in 15% ethanol. [c] Each 5 ml. contains 100 mg. Metrazol and 50 mg. nicotinic acid in 15% ethanol. [d] Based on an average tablet weight of 0.3353 Gm. [e] Absorbance of standard of 5 mg. concentration = 0.240. [f] Absorbance of standard of 5 mg. concentration = 0.235. [g] Absorbance of standard of 5 mg. concentration = 0.225. [h] Absorbance of standard of 5 mg. concentration = 0.230.

ml. each of acetate buffer, 6% sodium hydroxide solution, and 2% tetraethylenepentamine solution; the solution is diluted to the mark with distilled water, stoppered, and mixed well. The zero absorbance of the Beckman spectrophotometer, model DB, is set with this solution.

After allowing the solutions to stand for 10 minutes, the absorbance of the deep blue copper tetraethylenepentamine complex is measured at its maximum absorption, 580 mμ. The calibration graph of pentylenetetrazol, plotted on a linear scale at 2-mg. increments in the range from 2–10 mg., indicated that the method obeys Beer's law with good agreement. It is a straight line which passes through the origin.

Table I shows the relation between the absorbance and concentration of the standard solutions of pentylenetetrazol (which is directly proportional to the copper tetraethylenepentamine complex). Each value of the absorbance is the mean of ten determinations.

**Procedure for Tablets.**—The average tablet weight is ascertained from the weight of 20 tablets weighed accurately. Transfer a sample of the pulverized tablets, containing approximately 100 mg. pentylenetetrazol, into a 100-ml. glass-stoppered volumetric flask, dissolve in distilled water, fill up to the mark, stopper, mix well, and filter through paper, rejecting the first portion of the filtrate. Pipet 5 ml. of the filtrate, containing approx. 5 mg. pentylenetetrazol, into a 50-ml. Erlenmeyer flask and add 5 ml. of distilled water and 2 ml. of Dister's reagent. Stopper the flask, place in an ice bath, and follow the procedure described for the *Preparation of the Calibration Graph*. The amount of pentylenetetrazol present can be determined from the graph, or comparison with the absorbance of a solution obtained by similar treatment of 5 ml. of the *Standard Solution*.

**Procedure for Liquids.**—Pipet an aliquot of the liquid sample, containing approx. 100 mg. pentylenetetrazol, into a 100-ml. glass-stoppered volumetric flask, dilute to the mark with distilled water, stopper, and mix well. Proceed by the directions given for the *Procedure for Tablets* beginning with "Pipet 5 ml. . . ." then calculate

$$\frac{\text{A sample}}{\text{A standard}} \times \frac{5\text{ mg.}}{50\text{ ml.}} \times \frac{100\text{ ml.}}{\text{wt. sample (Gm.)}} \times \frac{\text{average tablet wt.}}{1\text{ tablet}} \times \frac{50\text{ ml.}}{5\text{ ml.}} = \text{mg./tablet}$$

$$\frac{\text{A sample}}{\text{A standard}} \times \frac{5\text{ mg.}}{50\text{ ml.}} \times \frac{100\text{ ml.}}{\text{vol. sample (5 ml.)}} \times \frac{50\text{ ml.}}{5\text{ ml.}} = \text{mg./5 ml.}$$

Table II shows the results of assays carried out on pentylenetetrazol pharmaceutical preparations manufactured by the Knoll Pharmaceutical Co., Orange, N. J. The absorbance of a solution obtained by similar treatment of 5 ml. of the *Standard Solution* was measured at the same time as that of the sample.

## DISCUSSION

It was observed that small concentrations up to 4 mg. pentylenetetrazol were not precipitated immediately with Dister's reagent at room temperature, except on longer standing with frequent swirling. For this reason, the solutions were chilled in an ice bath for 20 minutes with occasional swirling of the stoppered flasks.

It is evident that other active ingredients and materials usually present with pentylenetetrazol do not interfere with the photometric method.

Ephedrine sulfate is not precipitated by Dister's reagent, so it should not interfere if present with pentylenetetrazol. On the other hand, quinine sulfate and quinidine sulfate form yellow flocculent precipitates with Dister's reagent, so the procedure cannot be applied directly if these alkaloids are present, unless they are separated by chromatographic adsorption or some other technique. However, the alkaloids can be determined satisfactorily by this method, although the sensitivity is about half that of pentylenetetrazol.

Eight solutions of pentylenetetrazol can be assayed in about 2 hours by the proposed method.

## SUMMARY

Pentylenetetrazol can be determined quantitatively in the presence of active ingredients and materials usually present in pharmaceutical liquids and tablets by precipitation from aqueous solution with cuprous chloride reagent. The double salt complex formed is decomposed into the copper ion which is determined photometrically. The method proposed is sensitive and gives reproducible results on small samples. Since the method is not specific for pentylenetetrazol, it may be possible to apply it satisfactorily to other substances which react quantitatively with the cuprous chloride reagent.

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