could result in apparent theophylline levels well above the therapeutic range.

A relatively large number of materials are used in the manufacture of butyl rubber stoppers, and the identification of the interfering material would be a major project. Telephone conversations with the manufacturer of the tubes led to no clues as to what the material might be. Therefore, the finding of this study indicates that any laboratories analyzing blood samples by GLC should determine whether the collection tubes will interfere with the analysis. Such an analysis can be performed quite easily by the methods used in this study.

REFERENCES

(1) F. A. Chrzanowski, P. J. Niebergall, J. G. Nikelly, E. T. Sugita, and R. L. Schnaare, *Biochem. Med.*, 11, 26(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 2, 1975, from the *Department of Pharmacy, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.*, and the ‡*Pulmonary Disease Section of the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.*

Accepted for publication July 3, 1975.

Supported in part by National Institutes of Health Clinical Research Center Grant RR 00040.

The authors acknowledge the comments and assistance of Dr. Karl Engelman, Director of the Clinical Research Center of the Hospital at the University of Pennsylvania.

ˣ To whom inquiries should be directed. Present address: Marion Laboratories, Inc., Kansas City, MO 64137

Determination of Tinidazole in Tablets by dc Polarography

MARJAN SLAMNIK

Abstract ☐ A polarographic method was developed for determining tinidazole in tablets. The substance is extracted from the sample with water, pH 6.8 Britton–Robinson buffer is added to an aliquot, and the solution is polarographed at the dropping mercury electrode *versus* the saturated calomel electrode. The polarographic wave is well developed and enables a fairly precise quantitative determination. The method of standard addition is used.

Keyphrases ☐ Tinidazole—polarographic analysis, commercial tablets ☐ Polarography—analysis, tinidazole, commercial tablets ☐ Antitrichomonal agents—tinidazole, polarographic analysis, commercial tablets

Tinidazole[1], 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole, is an antitrichomonal agent and has a chemical structure similar to that of metronidazole, 2-methyl-5-nitro-1*H*-imidazole-1-ethanol, *i.e.*, a 2-methyl-5-nitroimidazole ring. The mechanism of the polarographic reduction of metronidazole is known (1), and from the chemical similarity it can be concluded that tinidazole undergoes the same polarographic reduction mechanism. Tinidazole and metronidazole also have similar dc polarographic behavior including a four-electron reduction wave corresponding to the reduction of the nitro group, the same slope of the $E_{1/2} = f/\text{pH}$ plot, and close values of $E_{1/2}$.

For the quantitative determination of tinidazole, the more positive wave was used. This wave is evident in a Britton–Robinson buffer of pH 6.8 and has the $E_{1/2}$ at −0.47 v *versus* the saturated calomel electrode. The more negative wave, which is not well developed, does not interfere. Tinidazole is stable at pH 6.8.

[1] Pfizer Inc.

EXPERIMENTAL

Apparatus—A recording polarograph[2] was equipped for dc polarography and had a drop timer[3]. A dropping mercury electrode with an outflow velocity of 2 mg/sec at a height of 60 cm of the mercury head was used. It was placed in a standard beaker-type cell with a saturated calomel electrode.

Reagents and Chemicals—Britton–Robinson buffer, pH 6.8, was prepared by mixing 100 ml of Solution A and 50 ml of Solution B. Solution A consisted of 0.04 *M* acetic acid, 0.04 *M* orthophosphoric acid, and 0.04 *M* boric acid. Solution B was 0.2 *M* sodium hydroxide.

For the standard solution, 60 mg of tinidazole standard was dissolved in water in a 100-ml volumetric flask (amber glass).

Procedures—Weigh a portion of finely ground sample, 100–150 mg of tinidazole, into a 250-ml volumetric flask (amber glass). Add 200 ml of water, shake well for at least 30 min, and dilute to volume with water. Filter through filter paper with fine pores, discarding the first 20 ml of filtrate. Then pipet 5 ml of the clear filtrate into a 50-ml volumetric flask, add 0.3 ml of a 0.2% aqueous solution of polyethylene glycol ether of monoisooctylphenol[4] or the same amount of a 0.2% aqueous solution of gelatin, and dilute to volume with the Britton–Robinson buffer. Pipet 25 ml of the prepared solution into a polarographic cell and purge with oxygen-free nitrogen for 10–15 min. Record the polarogram *versus* the saturated calomel electrode from 0.0 to 0.9 v applied voltage on the dropping mercury electrode with the sensitivity of 7 μamp for the full scale of the recorder. Apply a suitable damping and use a drop timer, the drop time being 2–3 sec.

After obtaining the polarogram, add 1 ml of the standard solution to the solution in the cell, deaerate with oxygen-free nitrogen for 1 min, and again record the polarogram under the same conditions. Measure the wave heights *H* and *h* (Fig. 1) and calculate:

$$\text{mg of tinidazole per tablet} = \frac{ahb \times 1000}{(1.04H - h)(w)} \qquad \text{(Eq. 1)}$$

where a = milligrams of tinidazole reference standard in 100 ml of standard solution, w = weight of sample (milligrams) taken for the

[2] Polarimeter type PO 4, Radiometer, Copenhagen, Denmark.
[3] Type DLT 1, Radiometer, Copenhagen, Denmark.
[4] Triton X-100, Rohm & Haas Co.

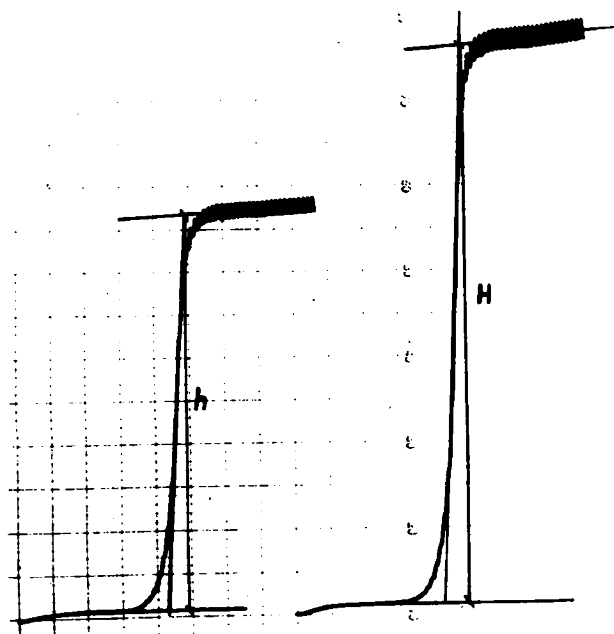

Figure 1—*Polarographic waves of tinidazole before and after standard addition.*

determination, h = wave height of tinidazole before standard addition, H = wave height of tinidazole after standard addition, and b = average weight of a tablet (grams).

RESULTS AND DISCUSSION

The polarographic wave of tinidazole obtained under the described conditions is well developed, and the precision of the proposed method is evident from Table I. The polarographic current was diffusion controlled and increased linearly with concentration in the range used for the determination.

The method of standard addition was preferred because it is more rapid than the concentration–diffusion current plot method.

Table I—Results of 10 Polarographic Analyses of Tinidazole in Tablets[a]

Analysis	Tinidazole per Tablet, mg
1	150
2	148
3	156
4	153
5	147
6	151
7	151
8	146
9	149
10	154
Average	150.5
SD	±2%

[a] Fasigyn tablets, Pfizer; analyzed by Pfizer Specification No. 3-FPS-255. This sample contains 152 mg of tinidazole/tablet.

Since the currents are diffusion controlled and possess the normal temperature coefficient of less than 2%/degree, no thermostating of the cell is necessary and the curves for the solution to be analyzed and for the solution after standard addition are recorded at room temperature.

REFERENCES

(1) D. Dumanović, J. Volke, and V. Vajgand, *J. Pharm. Pharmacol.*, 18, 507(1966).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 4, 1975, from the *Research-Development Institute, KRKA Pharmaceutical and Chemical Works, Novo mesto, Yugoslavia.*

Accepted for publication July 2, 1975.

The author thanks Pfizer Inc. for samples and the Pfizer Inc. Publication Committee for help in the preparation of this paper. The author also gratefully acknowledges Dr. J. Volke, Polarographic Institute of J. Heyrovský, Prague, Czechoslovakia, for help in correcting this article.

Isolation of Pinastric Acid and Ergosterol from *Parmelia caperata* (L.) Arch.

T. SERRA and J. POLÓNIA ˣ

Abstract □ Among other common compounds, pinastric acid and ergosterol were isolated for the first time from *Parmelia caperata.* The isolation of these compounds is described; identification was made from the melting point and UV, IR, and mass spectral data.

Keyphrases □ Pinastric acid—isolated from *Parmelia caperata,* UV, IR, and mass spectral data □ Ergosterol—isolated from *Parmelia caperata,* UV, IR, and mass spectral data □ *Parmelia caperata*—isolation of pinastric acid and ergosterol, UV, IR, and mass spectral data □ Lichens—*Parmelia caperata,* isolation of pinastric acid and ergosterol, UV, IR, and mass spectral data

Parmelia caperata (L.) Arch. is a widely studied lichen (1), but there are no references to the identification of pulvinic acid derivatives and sterols among the several compounds isolated from this species. The present paper reports the extraction and identification of pinastric acid and ergosterol from a Portuguese specimen of *P. caperata,* in which several common compounds, such as (+)-usnic, caperatic, and protocetraric acids, also were found.

Pinastric acid is a methoxy derivative of vulpinic acid, the latter having shown antibiotic activity (2). Because of its chemical constitution, pinastric acid cannot be considered an artifact formed during extraction but may be a term in a probable biogenetic chain: pulvinic acid → vulpinic acid → pinastric acid → leparinic acid → leparinic acid methyl ether.