# Analysis of Phenobarbital in Relation to its Degradation Products

By FREDERICK TISHLER†, LEE F. WORRELL‡, and JOSEPH E. SINSHEIMER

**The effect of the degradation products of phenobarbital upon six of its quantitative literature assays has been studied. Two new colorimetric procedures and an infrared method for the determination of phenobarbital are described and the effects of degradation products upon these assays is also discussed. Differential ultraviolet spectrophotometric analysis was found to be the assay procedure most useful for the determination of phenobarbital in the presence of its degradation products. This analysis has also been successfully applied to phenobarbital in various dosage forms.**

THE LITERATURE contains little information of the effects of the degradation products of barbiturates upon the quantitative assays of the respective barbiturates. Jackson, *et al.* (1), have shown that the decomposition products of barbital did not interfere with its ultraviolet spectrophotometric assay as described by Goldbaum (2).

Since the instability of barbiturates (3-5) and the isolation of their decomposition products (6, 7) are well described in the literature, it was the purpose of this investigation to study the effects of the hydrolysis products of phenobarbital upon its most widely used quantitative assays. The decomposition products of phenobarbital which were used in this study included the following: phenylethylacetylurea, phenylethylmalonic acid, phenylethylmalonamide, α-phenyl-*n*-butyric acid, α-phenyl-*n*-butyramide, and urea.

The quantitative methods chosen for the study included: (*a*) the U.S.P. gravimetric assay (8), (*b*) a volumetric precipitation titration (9), (*c*) a nonaqueous titration (10), (*d*) the cobalt colorimetric procedure (11), (*e*) a direct ultraviolet spectrophotometric method (12), and (*f*) a differential ultraviolet spectrophotometric method (2, 13, 14). In addition to the above literature procedures, two new colorimetric assays were developed and their applicability for the analysis of phenobarbital in the presence of its degradation products was studied.

## EXPERIMENTAL

### *Apparatus and Materials*

A Beckman model DU spectrophotometer and a Zeromatic pH meter, and a Perkin-Elmer model 21 infrared spectrophotometer were the instruments used in this investigation. The melting points reported are corrected.

Phenobarbital, U.S.P. grade, was recrystallized from dilute ethanol, m.p. 176-178°.

Phenylethylacetylurea was prepared by thermal degradation of a 5 per cent aqueous solution of sodium phenobarbital and isolation of the precipitate. Recrystallization from dilute ethanol gave a melting point of 147-149° (3, 6, 7).

Phenylethylmalonic acid was prepared by the hydrolysis of the diethylester of the acid with potassium hydroxide according to the procedure of Tasselly, *et al.* (15). Recrystallization from benzene gave a melting point of 155-158° with liberation of carbon dioxide (6, 7).

Phenylethylmalonamide was prepared by heating the corresponding acid with phosphorus pentachloride on a steam bath for 6 hours. The phosphorus oxychloride formed was distilled off with the aid of a steam bath and water aspirator. The crude phenylethylmalonyl chloride was then added to a well cooled ether solution saturated with ammonia. The precipitated amide was dissolved in acetone and, after evaporation of the solvent, was recrystallized from dilute ethanol, m.p. 119-121° (6, 7).

α-Phenyl-*n*-butyric acid, m.p. 42-44°, and α-phenyl-*n*-butyramide, m.p. 83-84° were purchased from Eastman Organic Chemicals and recrystallized from dilute ethanol.

Urea, A.R., m.p. 132-133°, was used as purchased from Mallinckrodt.

Sörenson phosphate buffer, pH 7.38. The buffer is prepared by mixing 20 ml. of sodium acid phosphate solution (9.21 Gm. of $NaH_2PO_4 \cdot H_2O$/1000 ml.) and 80 ml. of dibasic sodium phosphate solution (9.47 Gm. of $Na_2HPO_4$/1000 ml.).

### *Elimination of Acidic Impurities*

The following procedure was applied to mixtures of phenobarbital and its acidic degradation products, each of which was present in an amount equivalent to a 50% decomposition of 0.1 Gm. of phenobarbital: The mixture, after evaporation of any solvent, was mixed with three 10-ml. portions of petroleum ether (40-60°), in which phenobarbital is insoluble and α-phenyl-*n*-butyric acid is soluble. Each portion was decanted, filtered, and the petroleum ether solution discarded. The filter paper was washed with several portions of acetone and added to the petroleum ether-washed mixture. After evaporation of the solvent, 30 ml. of Sörenson phosphate

Received October 25, 1961, from the University of Michigan, College of Pharmacy, Ann Arbor.
Accepted for publication November 2, 1961.
Abstracted in part from a thesis presented to the University of Michigan Graduate School by Frederick Tishler in partial fulfillment of the requirements for the Ph.D. degree.
† Fellow of the American Foundation for Pharmaceutical Education 1959-1961. Present address: Ciba Pharmaceutical Products, Summit, N. J.
‡ Present address: University of Texas, College of Pharmacy, Austin.

buffer (pH 7.38) was added and the mixture stirred with a magnetic stirrer for one-half hour. Twenty milliliters of chloroform was added and the mixture stirred for an additional 5 minutes. The chloroform layer was drawn off by use of a separator and the aqueous phase was extracted with two additional 20-ml. portions of chloroform. The combined chloroform extract, after evaporation of the solvent, was then employed in the various analyses.

*Assays*

**Differential Ultraviolet Analysis.**—A sample of about 0.100 Gm. of phenobarbital is dissolved in an ammonia-ammonium chloride buffer (pH 10.0 ± 0.2) in a 1000-ml. volumetric flask. One 10-ml. portion of this solution is diluted with the buffer, pH 10.0, in a 100-ml. volumetric flask. A second 10-ml. sample is transferred to a 100-ml. volumetric flask, diluted to volume with buffer, pH 10.0, and 10 ml. of 1.0 *N* hydrochloric acid (final pH 1.5 ± 0.2). The absorbance of the alkaline solution is determined relative to the acid solution in the reference cell at 241 mμ. A blank solution was prepared and was found to have no contribution due to reagents and solvents at 241 mμ.

**Dimethylglyoxime-Thiosemicarbazide Colorimetric Procedure.**—A sample of phenobarbital, 0.005 Gm./10 ml. of 0.1 *N* sodium hydroxide, is decomposed at 100° for 90 minutes. Five milliliters of 1.0 *N* hydrochloric acid is added immediately to stop the degradation. A 1-ml. quantity of dimethylglyoxime solution (3% w/v in concentrated hydrochloric acid) and 1.0 ml. of thiosemicarbazide solution (1% w/v in 70% ethanol) are added. The mixture is heated for 105 min. at 100°. After cooling, the solution is diluted to volume in a 25-ml. volumetric flask with distilled water and the absorbance determined at 532 mμ relative to distilled water.

**Copper-Pyridine Colorimetric Procedure.**—The sample of phenobarbital, approximately 0.050 Gm., is dissolved in 4 ml. of a 4% ammonium hydroxide solution. To this solution is added 2 ml. of a copper-pyridine reagent (2 ml. of pyridine and 25 ml. of a 3% copper sulfate solution). After 3 hours, the solution is filtered with aid of a filtering stick and washed with several small portions of distilled water. To the suspension of the colored precipitate in distilled water, 5 ml. of concentrated ammonium hydroxide solution is added, and the precipitate decomposed in a steam bath. The solution is quantitatively transferred to a 500-ml. volumetric flask and diluted to volume with distilled water. The amount of copper present is determined colorimetrically with sodium diethyldithiocarbamate by the procedure of Charlot and Bezier (16) on a 2-ml. aliquot sample. The amount of copper is related in turn to the concentration of phenobarbital present.

**Quantitative Infrared Analysis of Phenobarbital in Combination with Phenylethylacetylurea.**—*Method I.*—The sample to be determined is dissoved in an amount of reagent chloroform so that the concentration of phenobarbital is 1% or less. The absorbances at 5.75 and 6.39 μ are determined from the spectrum produced in an 0.1-mm. cell with chloroform as the blank and the results compared to standards. The 5.75 μ band represents the concentration of phenobarbital and the 6.39 μ band represents the concentration of the ureide.

*Method II.*—The spectrum is obtained as directed under *Method I.* The amount of phenobarbital present is calculated from its 5.75 μ band. A blank sample is made containing this amount of phenobarbital in chloroform and the unknown sample is run against this blank. The concentration of ureide is determined from its 5.83 μ band.

## DISCUSSION

The quantitative literature assays of phenobarbital were tested for interference using an amount of each degradation product corresponding to a complete hydrolysis of phenobarbital. Several of the decomposition products were found to interfere with the assays. The acidic decomposition products interfered with the gravimetric, nonaqueous titration, and cobalt colorimetric procedures. The use of a Sörenson phosphate buffer, as described above, was found to be the method of choice for the removal of acidic decomposition products. Rotondaro (17) has employed a bicarbonate purification procedure but in our hands, inconsistent recovery of the barbiturate was obtained due to the variation in pH of the bicarbonate solution.

The ultraviolet spectra for the degradation products of phenobarbital under the same conditions used by Mattson (12) for his determination of barbiturates are given in Fig. 1. At the wavelength of 240 mμ used in this analysis, the ureide would cause the greatest interference. Since differential ultraviolet spectrophotometric methods have been shown to be more selective for barbiturates, the

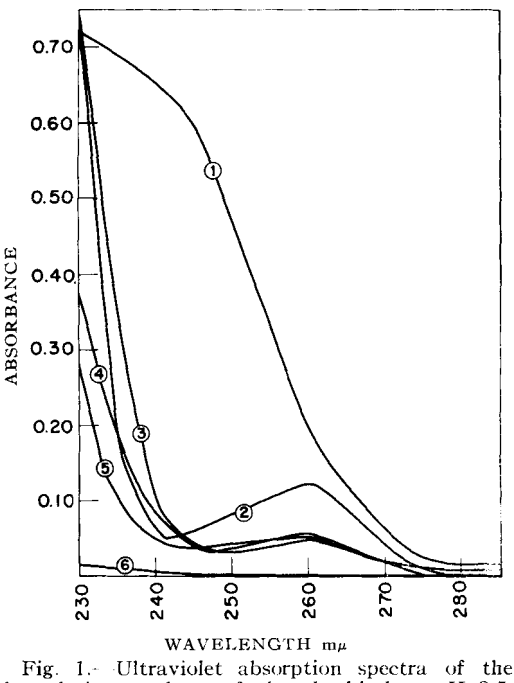


Fig. 1.—Ultraviolet absorption spectra of the degradation products of phenobarbital at pH 9.5. 1, Phenylethylacetylurea (0.1 mg./ml.); 2, α-phenyl-*n*-butyric acid (0.1 mg./ml.); 3, phenylethylmalonic acid (0.05 mg./ml.); 4, phenylethylmalonamide (0.05 mg./ml.); 5, α-phenyl-*n*-butyramide (0.05 mg./ml.); 6, urea (0.1 mg./ml.).

TABLE I.—COMPARISON OF ASSAYS

| Technique | Sensitivity, (Sample Size) | Accuracy and Precision, Av. % Recovery ± S. D. | |
|---|---|---|---|
| Gravimetric | 0.200–0.250 Gm. | 98.1 ± 0.7[a] | ... |
| Volumetric precipitation | 0.100 Gm. | 101.9 ± 1.0 | 102.1 ± 1.1[b] |
| Nonaqueous titration | 0.100 Gm. | 99.9 ± 0.1 | 99.9 ± 0.2[c] |
| Cobalt colorimetric | 0.005 Gm./25 ml. | 100.2 ± 1.5 | 98.3 ± 1.7[c] |
| Differential ultraviolet method | 0.001 Gm./100 ml. | 99.9 ± 0.1 | 99.9 ± 0.1[b] |
| Dimethylglyoxime-thiosemicarbazide method | 0.005 Gm./25 ml. | 99.2 ± 0.9 | ... |
| Copper-pyridine method | 0.005 Gm./50 ml. | 99.8 ± 1.0 | 99.7 ± 1.0[b] |
| Infrared analysis | 0.125 Gm./25 ml. | ... | 97.8 ± 1.0[d] |

[a] The results in this column are based on the analysis of pure samples of phenobarbital. [b] Determined in the presence of all the degradation products. [c] Determined after the elimination of interfering degradation products. [d] Determined in the presence of phenylethylacetylurea.

influence of the degradation products on this technique was studied.

Williams and Zak (13) and Goldbaum (2) have employed the difference maxima obtained from the spectra of the first and second ionic species of the barbiturate for quantitative purposes, while Walker, *et al.* (14), used the difference maxima obtained from the nonionized and first ionized species for the determination of barbiturates in urine and blood. Since the difference maxima involving the second ionic species depends on the use of extremely basic and, therefore, unstable solutions of phenobarbital, this procedure was not employed. The method of Walker, *et al.*, was further investigated and modified as described in the experimental procedure. The absorbance of each of the degradation products at 241 mμ was found to be constant between the pH range of 1 to 12. Thus, the degradation products do not interfere with this differential method of analysis.

In addition to the literature assays, two new colorimetric procedures have been developed and their usefulness for the assay of phenobarbital in the presence of its degradation products was investigated. The dimethylglyoxime-thiosemicarbazide procedure is based upon a qualitative test of urea compounds as described by Ohkuma (18). Since this method depends on the decomposition of phenobarbital with the formation of urea and phenylethylacetylurea, a sample should be tested prior to hydrolysis for a positive reaction due to these degradation products. The other decomposition products do not interfere with the analysis. At the end of 1 hour, the blank used in this determination becomes cloudy. However, since the absorbance due to the blank is negligible at 532 mμ, distilled water is employed as the blank.

Copper-pyridine derivatives of barbiturates have been investigated by Zwikker (19), Fouchet (20), and Levi and Hubley (21). The formation of this phenobarbital derivative followed by the quantitative determination of its copper content was found to be useful for the determination of phenobarbital in combination with its degradation products.

Infrared spectroscopy was found to be a useful procedure in the qualitative and quantitative determination of phenobarbital both alone and in the presence of its main degradation product phenylethylacetylurea. Phenobarbital exhibits a carbonyl doublet which serves as a criterion of the purity of phenobarbital. When the ratio of the 5.75 and 5.89 μ carbonyl doublet for phenobarbital is not 1 to 1.26, the presence of ureide is indicated. Method I or method II can be used to determine the concentration of phenobarbital and phenylethylacetylurea. Method II should be employed when the concentration of the ureide is less than 0.25% since the 6.39 μ band of the ureide does not follow Beer's law below this concentration. A comparison of the methods investigated is summarized in Table I. The nonaqueous titration and the differential ultraviolet method are highly satisfactory with regard to accuracy, precision, and efficiency. Where degradation products are present, the differential ultraviolet method offers the additional advantage of eliminating a prior separation of interfering products. The differential ultraviolet procedure has been used in these laboratories in the kinetic study of the degradation of phenobarbital (22).

TABLE II.—DIFFERENTIAL ULTRAVIOLET ANALYSIS OF PHENOBARBITAL ELIXIR U.S.P.

| | | |
|---|---|---|
| Amount Present | | 4.00 Gm./L. |
| Found | 1 | 3.98 |
| | 2 | 3.99 |
| | 3 | 3.98 |
| | 4 | 3.99 |
| | 5 | 4.00 |
| Average | | 3.99 Gm./L. |

To serve as a basis for the quantitative determination of phenobarbital preparations, the difference in molecular extinction coefficient, $\Delta\epsilon$, was determined from standard solutions. The average of 10 determinations gave a value of 9404 for a molar solution of phenobarbital at a pH of 10 ± 0.2 over that of a molar solution of a pH of 1.5 ± 0.2 at 241 mμ. To test the applicability of the differential analysis to a pharmaceutical formulation, phenobarbital elixir U.S.P. (8) was prepared and analyzed for its phenobarbital content. In Table II are presented the results of the analysis. No interference of the components of the elixir was found in the differential ultraviolet analysis, while Mattson (12) reported that the amaranth interfered with the direct ultraviolet procedure and an elixir base was necessary as a blank.

Ephedrine and amphetamine do not show changes in ultraviolet spectra under the conditions of the differential ultraviolet method, and therefore the general usefulness of this method is further illustrated in the analysis of phenobarbital combinations of the above compounds.

## REFERENCES

(1) Jackson, G. R., Jr., Weschler, J. R., and Dannley, R. L., *Anal. Chem.*, **26**, 1661(1954).
(2) Goldbaum, L. R., *ibid.*, **24**, 1604(1952).
(3) Nielsen, L., *Dansk Tidsskr. Farm.*, **7**, 137(1933).

(4) Bailey, A. E., *Pharm. J.*, **136**, 620(1936).
(5) Husa, W. J., and Jatul, B. B., THIS JOURNAL, **33**, 217 (1944).
(6) Fretwurst, F., *Arzneimittel-Forsch.*, **8**, 44(1958).
(7) Aspelund, H., *Acta Acad. Aboensis, Math. et Phys.*, **20**, No. 4(1955).
(8) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 524.
(9) Autian, J., and Allen, B. F., *Drug Standards*, **22**, 164 (1954).
(10) Vespe, V., and Fritz, J. S., THIS JOURNAL, **41**, 197 (1952).
(11) Mattson, L. N., and Holt, W. L., *ibid.*, **38**, 55(1949).
(12) Mattson, L. N., *ibid.*, **43**, 22(1954).
(13) Williams, L. A., and Zak, B., *Clin. Chim. Acta*, **4**, 170 (1959).
(14) Walker, J. T., Fischer, R. S., and McHugh, J. J., *Am. J. Clin. Pathol.*, **18**, 451(1948).
(15) Tasselly, E., Belot, A., and Descombes, M., *Compt. rend.*, **186**, 149(1928).
(16) Charlot, G., and Bezier, D., "Quantitative Inorganic Analysis," John Wiley & Sons, Inc., New York, N. Y., 1957, p. 421.
(17) Rotondaro, F. A., *J. Assoc. Offic. Agr. Chemists*, **38**, 809(1955).
(18) Ohkuma, S., *J. Pharm. Soc. Japan*, **75**, 1291(1955).
(19) Zwikker, J. J. L., *Pharm. Weekblad*, **68**, 975(1931).
(20) Fouchet, M., *J. Pharm. Chim.*, **20**, 403(1934).
(21) Levi, L., and Hubley, C. E., *Anal. Chem.*, **28**, 1591 (1956).
(22) Tishler, F., Sinsheimer, J. E., and Goyan, J. E., THIS JOURNAL, **51**, 214(1962).

# Pharmaceutical Investigation of Selected Alberta Bentonites I

## Geology and Identification

By ARTHUR J. ANDERSON† and ELMER M. PLEIN

**Bentonite samples were collected from a number of Alberta deposits. Of these, 23 were indexed in table form to show location and other information dealing with the parent beds. On a selected suite of samples, identification as bentonites was confirmed by differential thermal and X-ray diffraction techniques.**

EARLY INFORMATION concerning bentonite deposits in western Canada for the most part is found as brief reports in publications of the Canadian government (1–4). Of particular value in this respect are the comprehensive surveys of Spence (5) and Spence and Light (6).

In 1949 the discovery of major oil reserves in Alberta brought an unprecedented demand for the high-swelling type of clay. With no known reserves of suitable quality in Canada, all supplies, of necessity, were imported from the United States. In recent years, however, intensive subsurface exploration programs carried out by commercial interests resulted in the location of a number of reserves considerably better in quality than those previously described. Of these, deposits uncovered at Rosalind and Onoway were considered sufficiently important to warrant commercial development. Mines and processing plants were established at both locations with production designed to meet the needs of the petroleum and associated industries.

Our interest in the material arose when claims were made that certain beds contained bentonite comparable in some respects to that obtained from the Black Hills region of Wyoming and South Dakota. A subsequent inquiry revealed that insufficient information was available to allow for an adequate pharmaceutical evaluation of bentonites from any of the known Alberta deposits. For that reason, a study was undertaken with a view to determining the presence of reserves of pharmaceutical grade clay in the province.

### GEOLOGY

Byrne (7) states that in Alberta, thin beds of bentonite are fairly common throughout the Cretaceous and Tertiary eras. However, thick accumulations have been reported only from the upper Cretaceous, with the Edmonton formation undoubtedly being the most important for future prospecting (Fig. 1). The occurrences found in other levels of the upper Cretaceous as well as in the Paskapoo member of the Tertiary are considered to have little commercial importance.

Received August 21, 1961, from the College of Pharmacy, University of Washington, Seattle 5.
Accepted for publication October 11, 1961.
Based on a thesis presented to the Graduate School of the University of Washington by Arthur J. Anderson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
This research was supported in part by the Canadian Foundation for the Advancement of Pharmacy.
† Present address: Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada.