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STUDIES ON BARBITURATES. III. CHEMICAL ASSAY OF BARBITURATES.*

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In a previous paper Koppanyi, Murphy and Krop (1) presented a modification of their original quantitative barbiturate test (2) using cobaltous acetate and isopropylamine dissolved in absolute methyl alcohol as reagents. This test was utilized in the following work as a method of chemical assay of various preparations containing barbiturates.

EXPERIMENTAL.

The test depends upon the formation of a bluish or reddish purple color produced by cobaltous acetate in an alkaline medium when barbiturates are present. The barbiturate to be assayed is either directly dissolved in chloroform or extracted with chloroform from an aqueous solution. To 2 cc. of this chloroform solution are added 0.1 cc. of 1.00 per cent cobaltous acetate ($CO(CH_3COO)_2.4H_2O$) in absolute methyl alcohol and 0.6 cc. of a 5.00 per cent (by volume) solution of isopropylamine in absolute methyl alcohol. A reddish violet color develops which is compared in a standard colorimeter with the color produced by a standard made up of the barbiturate under consideration.

As is generally the case with colorimetric tests, the concentration of the standard must be near that of the unknown. In order to select such a standard a rough approximation of the strength of the unknown is first made. Two cc. of each of the following solutions of the barbiturate in chloroform are placed in test-tubes: 0.040%, 0.060%, 0.080%, 0.100% and 0.120%. To each tube 0.1 cc. of cobaltous acetate reagent and 0.6 cc. of isopropylamine reagent are added. The tubes are then stoppered and set aside. The unknown solution treated similarly is then compared with the color of the standards, and a standard close to the unknown is chosen. The series of color standards can be kept for several hours in stoppered test-tubes.

If the concentration is above the range of the standards, the unknown solution may be diluted to bring it within the range covered by the series of standards. If it is weaker, it may be concentrated by evaporation on a water-bath.

After a standard has been chosen, 2 cc. each of the standard solution and the unknown are placed in two test-tubes. The reagents are added and the standard and unknown are compared in the microcups of a standard colorimeter.

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The amount of the barbiturate in the original sample can be calculated by the following formula:

$$\frac{\text{Reading of standard} \times \text{Concentration of standard (mg. per cc.)} \times \text{Salt correction factor} \times \text{Cubic centimeters of chloroform extract}}{\text{Reading of unknown}} = \text{Milligrams of barbiturate in sample.}$$

The salt correction factor is applied in cases where the salt of a malonyl urea derivative is being assayed. It is found by dividing the molecular weight of the salt by the molecular weight of the acid form of the barbiturate.

If the barbiturate is soluble in chloroform the assay is made by simply dissolving an accurately weighed amount in chloroform. This solution is then compared with standards as described.

Preparations of barbiturates such as tablets or capsules which contain inert substances to provide bulk, can be best assayed by first dissolving the tablet or the contents of the capsule in a small amount of water in a separatory funnel, and then extracting this aqueous solution or suspension with chloroform. If the barbiturate is in the form of its salt the aqueous portion must always be acidulated with 5 per cent sulphuric acid. This converts the salt into a form which is soluble in chloroform. Three extractions should be made: The first with a volume of chloroform approximately five times that of the aqueous volume, the second and third with a volume of chloroform equal to twice the aqueous volume. The chloroform extracts are drawn off from the separatory funnel and filtered through a small filter into a volumetric flask of a size to hold ten times the volume of water used. Chloroform is washed through the filter to make up to the final volume.

Elixirs or solutions of barbiturates are pipetted into a separatory funnel and extracted with chloroform as above. For barbital there is a theoretical extraction of 99.48 per cent by this procedure.

Accuracy of Procedure.—With a view of determining the error of reading the colors and the amount of loss by the various extractive or other manipulations, a series of experiments with barbiturates of known concentrations was undertaken. The various barbiturates used were in most cases furnished in a pure form through the kindness of the manufacturers. Nitrogen determinations by the Kjeldahl method indicated that all of the samples of barbiturates used were close to 100 per cent purity. They were kept in a desiccator, and solutions were made up immediately before use. The error of making up the solutions and preparing them for colorimetric determinations was within the allowable error for analytical work.

Experiments were first carried out using chloroform solutions of the barbiturates. A number of solutions of different concentrations were tested against standards, the concentrations of which lay close to that of the solution being tested. Results are summarized in Table I. These show the sources of error which may be expected from the assay of barbiturates by this procedure. It can be seen that there are two sources of error in this procedure: *First,* the error that results from matching the colors in the colorimeter, and, *second,* the error that occurs when the concentration of the standard is not close to that of the unknown.

TABLE I.—RECOVERY OF BARBITURATES FROM CHLOROFORM SOLUTIONS.

Barbiturates.	Concentration of Sample. Gm. per 100 Cc.	Concentration or Standard. Gm. per 100 Cc.	Recovery. Average of 3 Determinations.	Per Cent Recovery.
Barbital	0.040	0.060	0.0429	107.1
Barbital	0.060	0.060	0.0594	99.0
Barbital	0.080	0.060	0.0763	95.5
Phenobarbital	0.060	0.080	0.0655	108.3
Phenobarbital	0.080	0.080	0.0816	102.0
Phenobarbital	0.100	0.080	0.0935	93.5
Amytal	0.100	0.120	0.1042	104.2
Amytal	0.120	0.120	0.1216	100.5
Amytal	0.140	0.120	0.1309	93.5

Other experiments in which aqueous solutions of water-soluble barbiturates were extracted with chloroform are embodied in Table II. Sodium barbital for example was dissolved in water, acidulated and extracted with chloroform. In addition to the two sources of error given above, there is now an additional possibility of loss in the extraction process.

TABLE II.—RECOVERIES OF BARBITURATES FROM AQUEOUS SOLUTIONS.

Barbiturate.	Concentration of Aqueous Solution, Per Cent.	Amount of Solution Extracted, Cc.	Amount of Barbiturate in Sample, Gm.	Final Volume of Chloroform Extract, Cc.	Recovery. Average of 3 Determinations, Gm.	Per Cent Recovery.
Sodium barbital	1.000	10	0.100	100	0.0982	98.2
Sodium phenobarbital	1.000	10	0.100	100	0.1010	101.0
Sodium phenobarbital	0.800	10	0.080	100	0.0785	98.1
Sodium ortal	0.800	10	0.080	100	0.0753	94.1

We prepared powders in which barbiturates were mixed with lactose. These powders were then placed in a separatory funnel, dissolved in water and extracted with chloroform. The results of this type of assay are embodied in Table III.

TABLE III.—RECOVERIES OF BARBITURATES FROM POWDERS MADE WITH LACTOSE.

Barbiturate.	Amount of Drug in a 1-Gm. Sample, Gm.	Amount of Water, Cc.	Volume of Chloroform Extract, Cc.	Recovery. Average of 3 Determinations, Gm.	Per Cent Recovery.
Sodium phenobarbital	0.100	10	100	0.0974	97.4
Sodium phenobarbital (gr. IV saccharine added)	0.100	10	100	0.0973	97.3
Phenobarbital	0.100	10	100	1.0041	100.4
Phenobarbital	0.080	10	100	0.0815	101.9
Amytal	0.060	10	100	0.0582	97.0
Amytal	0.080	10	100	0.0744	93.0
Neonal	0.080	10	100	0.0764	95.5
Sodium barbital	0.120	10	100	0.1130	94.2
Phanodorn	0.100	10	100	0.0990	99.0

We believe that with careful manipulation, and with the choice of a proper standard this assay can be performed with an error of not more than 6 per cent.

Specificity of the Test.—Of chloroform-soluble substances, theobromine. theophylline and thymine show a positive test. Certain urea derivatives such as biuret and oxamide give positive tests but do not enter into the chloroform frac-

tions in detectable mounts. Guanidine and creatinine give a positive test in alcoholic solutions only.

None of the common drugs which are combined with the barbiturates in the proprietary preparations investigated interfere with this test.

Pharmaceutical Applications.—Using this test we assayed a number of barbiturate preparations purchased on the open market. Tablets, capsules and elixirs were assayed by this method. These preparations are usually mixed with some inert substance to provide bulk for the preparation, or are fixed mixtures.

The results of these determinations are embodied in Table IV. It can be seen that some of these proprietary preparations tested do not come up to the strength stated on the label.

TABLE IV.—AN INVESTIGATION OF SOME PROPRIETARY BARBITURATE PREPARATIONS.

Barbiturate.	Type of Preparation.	Amount of Barbiturate in Sample from Label. Grains.	Grams.	Amount Found, Average of 5 Determinations.	Per Cent of Labeled Amount.	Variation.
Sodium veronal	Tablet	5	0.3240	0.3101	95.6	93 5 to 98.9
Veronal	Tablet	5	0.3240	0.2759	84.8	82.1 to 90.8
Amytal	Tablet	1 1/2	0.0972	0.0922	94.7	93.5 to 95.7
Amytal	Capsule (with amido pyrine)	1 1/2	0.0972	0.0915	94.5	87.1 to 98.9
Sodium luminal	Tablet	1 1/2	0.0972	0.0812	83.6	82.3 to 84.6
Luminal	Elixir	1/4 gr. in 4 cc.	0.0405	0.0388	95.8	94.1 to 99.0
Veronal	Elixir	2 gr. in 4 cc.	0.1665	0.1020	61.4	58.5 to 63.3
Novasurol	10% solution in ampul	...	0.300	0.1878	62.5	57.6 to 69.0

This method of assay is not limited to malonyl-urea hypnotics. Novasurol, for example, a commonly used mecurial diuretic composed of diethyl barbituric acid and mercurichlorphenyl oxyacetate may also be assayed by this method. In the presence of an acid this compound is broken down, and diethyl barbituric acid is liberated. This may then be extracted and estimated. From the amount of barbital present the amount of novasurol can be calculated by multiplying the amount of barbital in Gm. by the factor 3.211.

Correlation of Color with Molecular Weight.—In a previous paper (2) it was postulated that the color-giving portion of barbiturate molecule is the malonyl-urea residue in which case we would expect the intensity of color to vary with the amount of malonyl urea present. The increase in molecular weight among the commonly used barbiturates is not due to a change in the malonyl-urea residue but to the alkyl radicals attached to the central carbon atom. Thus solutions of barbiturates of equal percentage should show intensities varying inversely as the molecular weight, whereas equimolar solutions of different barbiturates should show close agreement in color depth.

A 0.5 per cent solution of barbital was used as a standard against which other barbiturates of the same concentration were compared. The barbiturates used were the pure dried substances and were dissolved in chloroform immediately before use. Pernoston and nostal being difficulty soluble in chloroform were dissolved in absolute methyl alcohol in which case the barbital standard was also made up in absolute methyl alcohol.

The comparison of barbiturate solutions of different molecular weight but equal concentrations verified the assumption that the depth of color of these solutions would vary inversely as the molecular weight. Thus a 0.1 per cent solution of pernoston when compared with 0.1 per cent solution of barbital appeared much lighter in color. Due to the great difference in the molecular weights of these two compounds (barbital equals 184.112, pernoston equals 303.052) it was necessary to use barbital in 0.05 per cent solution in order to get an accurate reading of the two colors.

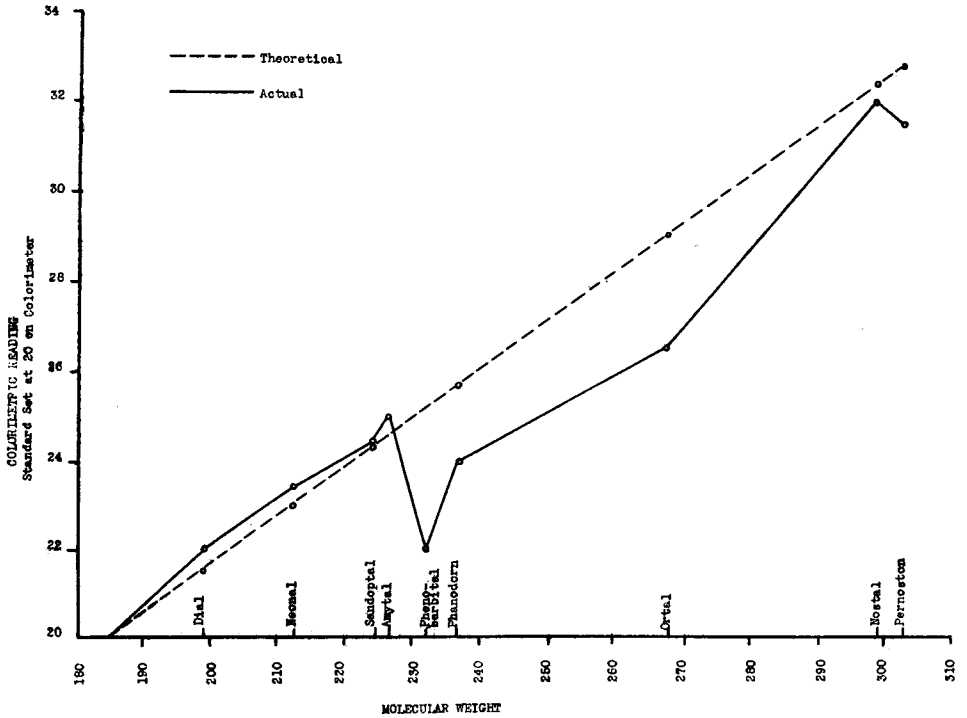

Fig. 1.—Shows the relationship of the molecular weight to the colorimetric reading. The actual colorimetric readings of the different barbituric acid derivatives, with a few exceptions, almost coincide with the line representing the theoretical reading.

The experiments as summarized in Table V, indicate that the theoretical prediction, namely, that the depth of color will vary inversely with the molecular weight is fulfilled by the different barbiturate derivatives, with the exception of phenobarbital, phanodorn and ortal.

It is possible to determine in a general way the molecular weight of an unknown barbiturate if it is in the pure dry state. Using the barbital as a standard the difference in depth of color indicates whether the molecular weight is near or far from the molecular weight of barbital.

SUMMARY.

1. The isopropylamine quantitative barbiturate test was utilized in assaying pharmaceutical preparations containing malonyl-urea derivatives.

2. This colorimetric chemical assay is accurate within 6 per cent.

TABLE V.—RELATION OF DEPTH OF COLOR TO MOLECULAR WEIGHT.

Barbiturate.	Chemical Structure.	Molecular Weight.	Theoretical Reading, Standard at 20.	Actual Reading, Standard at 20.
Barbital	Diethyl barbituric acid	184.112	20.0	20.0
Dial	Diallyl barbituric acid	198.112	21.5	22.2
Neonal	*n*-Butyl-ethyl barbituric acid	212.144	23.0	23.4
Sandoptal	Iso-butyl allyl barbituric acid	224.144	24.3	24.5
Amytal	Isoamyl ethyl barbituric acid	226.160	24.6	25.0
Phenobarbital	Phenyl ethyl barbituric acid	232.112	25.2	22.0
Phanodorn	Cyclohexenyl ethyl barbituric acid	236.141	25.7	24.0
Ortal	*n*-Hexyl ethyl barbituric acid	268.202	29.1	26.5
Nostal	Isopropyl bromallyl barbituric acid	289.036	31.4	32.0
Pernoston	Sec-butyl bromallyl barbituric acid	303.052	33.0	31.5

3. Some of the proprietary barbiturate preparations investigated did not come up to the strength stated on the label.

4. The isopropylamine test for barbiturates is sensitive enough for an approximation of the molecular weight of different barbiturates.

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A NEW METHOD OF DETERMINING ACETYLSALICYLIC ACID IN THE PRESENCE OF MEDICINAL PRODUCTS.*

BY RICHARD M. HITCHENS.

Acetylsalicylic acid is employed so extensively in pharmaceutical preparations that its estimation in such mixtures is of considerable importance.

Many methods have been proposed for its determination. The association of Official Agricultural Chemists has carried out several systematic investigations and has proposed several methods of analysis (1). One method, applicable in the absence of acidic or basic substances, consists of titrating a cold alcoholic solution of the acetylsalicylic acid with standard alkali to a phenolphthalein end-point, thus neutralizing the free carboxyl group present in the molecule, then adding excess of standard alkali to hydrolyze the sodium acetylsalicylate to sodium salicylate and sodium acetate, and back titrating to a phenolphthalein end-point. The original titration to phenolphthalein should be exactly one-half of the total titration. Another method consists of a chloroform extraction of the acetylsalicylic acid from a water suspension, or a dry chloroform extraction of alkaline excipients is present. After removal of the chloroform the acetylsalicylic acid is determined as such if no other chloroform-soluble substances are present. Otherwise it is hydrolyzed to salicylic and acetic acids. The salicylic acid thus obtained is determined either by volumetric bromination to tribromphenol or gravimetrically by alkaline iodination to the complex $(C_6H_2I_2O)x$, structure uncertain.

In none of the methods found in the literature is acetylsalicylic acid itself separated from other organic substances and determined as such. Such a method,

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