These results indicated that all four samples are well below the limit set by the U. S. P. for moisture. The only significant fact is that the sample which was really open to the air (kept in a paper sack) was lowest of the four in moisture.

The fact that these three old samples of varying age, stored under ordinary conditions are still equal in activity to the present high U. S. P. XI standard for ergot as crude drug should be strongly indicative of a remarkable stability of potency. These tests should show that the conditions of storage of ergot crude drug as made mandatory in the U. S. P. XI, namely, in water-proof and air-tight containers, are not necessary. They are undoubtedly included because of the known instability of the average extract of ergot and of drug which has been allowed to mold but they place an unnecessary burden upon the importer and the manufacturer who know how to store the drug under proper but ordinary conditions.

It appears that keeping ergot dry is a sufficient protection of activity and that the U. S. P. requirements for storage may well be changed by interim revision to "Preserve ergot under all conditions of storage and transportation in a dry place."

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THE ASSAY OF THEOPHYLLINE, THEOPHYLLINE MONOETHANOLAMINE AND THEOPHYLLINE WITH ETHYLENE DIAMINE, U. S. P.*

BY ASA N. STEVENS AND DALE T. WILSON.[1]

A few years ago it became necessary for this laboratory to examine the available assay methods for the determination of theophylline. Of the various methods studied two appeared to offer possibilities. These were tried and the following observations were noted.

Kochum (1) described a volumetric method with 0.1*N* $AgNO_3$ using potassium dichromate as an internal indicator. The results obtained by this method varied so widely, due to the inconsistency in the end-point of the titration, that the method was abandoned.

Schmitt (2) used a gravimetric method, recommending silver ammonium chloride as the reagent. The results were found to vary unless the silver chloride precipitation was carried out in a dark room and, further, that a difference of 0.5 mg. in the weight of the silver chloride obtained made a difference of 1.11 per cent in the results.

Finding these methods unsuited to our purpose an attempt was made to develop a more satisfactory procedure. As a result of this investigation the following method is proposed.

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METHOD.

To 0.2 Gm. of theophylline, accurately weighed, in a 200-cc. flask, add 50 cc. of distilled water and 7 cc. of ammonia water. Warm the mixture on a steam-bath until complete solution is effected. Add 20 cc. of 0.1*N* $AgNO_3$, mix and continue to warm on a steam-bath for 15 minutes. Filter with the aid of vacuum while still warm, washing the precipitate with three 10-cc. portions of distilled water. Cool and acidify the combined filtrate and washings, using nitric acid. Add 2 cc. of ferric ammonium sulfate T.S. and titrate the excess silver nitrate with 0.1*N* KCNS.

Each cc. of 0.1*N* $AgNO_3$ is equivalent to 0.0180 Gm. of anhydrous theophylline.

The results obtained by applying this method to the assay of theophylline are tabulated below. It will be noted that the last sample contained starch as an added diluent. This addition was made in order to determine whether or not theoretical results could be obtained in the presence of a foreign material.

TABLE I.

Sample Wt.	Starch.	Cc. *N*/10 $AgNO_3$ Required.	Anhydrous Theophylline %.	Theoretical %.
0.2 Gm.	....	10.10	90.90	90.90
0.2 Gm.	....	10.10	90.90	90.90
0.2 Gm.	....	10.10	90.90	90.90
0.2 Gm.	....	10.10	90.90	90.90
0.2 Gm.	....	10.10	90.90	90.90
0.2 Gm.	0.2 Gm.	10.10	90.90	90.90

THEOPHYLLINE MONOETHANOLAMINE.

Samples of theophylline monoethanolamine were then assayed for their theophylline content using this method. In this series of assays an excess of monoethanolamine was added to two samples while another sample was mixed with starch. The results appear in Table II.

TABLE II.

Sample Wt.	Starch.	Monoethanolamine.	Cc. *N*/10 $AgNO_3$ Required.	Anhydrous Theophylline %.	Theoretical %.
0.2 Gm.	....	....	8.2	73.80	74.68
0.2 Gm.	....	....	8.2	73.80	74.68
0.2 Gm.	....	Excess	8.2	73.80	74.68
0.2 Gm.	....	Excess	8.3	74.70	74.68
0.2 Gm.	....	....	8.3	74.70	74.68
0.2 Gm.	0.5 Gm.	....	8.4	75.60	74.68
0.2 Gm.	....	....	8.3	74.70	74.68

In order to further check the reliability of the method a number of samples containing theophylline and monoethanolamine were assayed. Each contained a different amount of theophylline. Two of the samples in this series contained talc and two of them starch, as added materials. The results obtained in these assays follow.

TABLE III.

Sample.	Diluent.	Cc. *N*/10 $AgNO_3$ Required.	Theophylline Found Grams.	Theophylline (Theoretical) Grams.	Error %.
A	....	10.30	0.1854	0.1859	−0.26
B	Talc	8.10	0.1458	0.1460	−0.30
C	Talc	10.80	0.1944	0.1925	+0.90
D	Starch	10.70	0.1674	0.1689	−0.69
E	Starch	10.50	0.1890	0.1886	−0.21

THEOPHYLLINE WITH ETHYLENE DIAMINE.

The following data show the comparative results obtained for theophylline and theophylline with ethylene diamine when assayed according to the U. S. P. XI method and by the proposed method herein described.

TABLE IV.—THEOPHYLLINE.

Assay Method.	Wt. of Sample Taken Gm.	Wt. of Anhydrous Theophylline Found Gm.	Cc. $N/10$ $AgNO_3$ Required.	Anhydrous Theophylline %.
U. S. P. XI	2.0	1.8347	...	91.74
U. S. P. XI	2.0	1.7922	...	89.61
Proposed	0.2	..	11.10	99.90
Proposed	0.2	..	11.00	99.00

TABLE V.—THEOPHYLLINE WITH ETHYLENE DIAMINE.

Assay Method.	Sample No.	Wt. of Sample Taken Gm.	Wt. of Anhydrous Theophylline Found Gm.	Cc. $N/10$ $AgNO_3$ Required.	Anhydrous Theophylline %.
U. S. P. XI	A	2.0	1.4077	..	70.39
U. S. P. XI	A	2.0	1.4012	..	70.06
Proposed	A	0.2	..	9.40	84.60
Proposed	A	0.2	..	9.30	83.70
U. S. P. XI	B	2.0	1.3006	..	65.03
U. S. P. XI	B	2.0	1.3175	..	65.88
Proposed	B	0.2	..	8.95	80.55
Proposed	B	0.2	..	8.85	79.65
U. S. P. XI	C	2.0	1.4024	..	70.12
U. S. P. XI	C	2.0	1.4156	..	70.78
Proposed	C	0.2	..	9.00	81.00

COMMENTS.

All the samples of theophylline with ethylene diamine taken for assay were previously dried over sulfuric acid for 48 hours as specified in the U. S. P. XI. The results show that theoretical values are more nearly attained by the method described in this paper than by the method of the U. S. P. XI.

Theoretically the results shown in Table IV should amount to nearly 100 per cent since the assay sample, which had previously been dried over sulfuric acid for 48 hours, was further dried for 17 hours at 100° C. without loss of weight. This test was made in order to prove that the pure theophylline taken for these assays contained no moisture or water of crystallization.

Theophylline with ethylene diamine, according to the U. S. P. XI, should assay not less than 75 per cent and not more than 85 per cent anhydrous theophylline. The assay results in Table V show that the proposed method gives results which are within the official tolerance, while the assay method of the U. S. P. XI gives results that do not meet these standards.

In addition to yielding sub-standard results the U. S. P. XI assay method requires a considerable amount of time. We believe that a shorter, less complicated and more accurate method of assay should be used for the determination of theophylline. The method described in this paper apparently meets these requirements.

CONCLUSION.

1. An accurate method has been developed for the assay of theophylline, which is considerably less involved than the method described in the U. S. P. XI.

2. Its applicability to the assay of Theophylline Monoethanolamine and Theophylline with Ethylene Diamine has been demonstrated.

3. Evidence is presented to show that the method described gives accurate results in the presence of monoethanolamine, ethylene diamine and starch.

The authors wish to express their appreciation to Dr. Edward J. Hughes for his friendly criticisms and advice.

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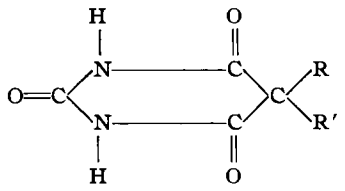
2-METHYL-ALLYL SUBSTITUTED BARBITURIC ACID DERIVATIVES.*

BY EDWARD E. SWANSON AND WILLIAM E. FRY.[1]

As previously observed (1), there is a distinct relationship between the pharmacological action and chemical structure of certain barbituric acid derivatives. In the primary or secondary alkyl substituted compounds, with an increasing number of C-atoms in the alkyl substituted group, both the minimal anesthetic dose (M. A. D.) and the minimal lethal dose (M. L. D.) become relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to produce anesthesia or death in rats again increases. Furthermore, as the alkyl chain lengthens, the ratio between the M. L. D. and M. A. D., or therapeutic index, appears to be gradually greater, and the duration of action becomes shorter. More recently, it has been reported (2) that the substitution of a methyl or an ethyl group on the nitrogen (nitrogen alkyl substituted barbituric acid derivatives) also obviously reduces the duration of action, and this shorter duration of action is independent of the quantity of drug administered.

The present investigation deals with the evaluation of a number of new methallyl (2-methyl-allyl) substituted barbituric acid derivatives synthesized by Doran and Shonle (3), with the general formula:



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* Scientific Section, A. PH. A., Dallas meeting, 1936.

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