The assays on the preparations herein reported were all made by one of us (L. W. R.), who has had considerable experience with the official Cock's Comb method as well as with the Broom and Clark method. Some prefer the Broom and Clark method, in which the inhibiting action of ergot is observed on a strip of rabbit-uterus muscle—but a considerable margin of error is acknowledged for it.

The British Pharmacopœia has adopted the colorimetric determination of alkaloids by chemical methods, on the ground that while this includes both the inactive and active alkaloids the error "is probably less" than is found in biological methods.

These facts are mentioned simply to make plain that the activity figures in the above table cannot be taken with the same confidence as in the chemical assays of other alkaloidal drugs. Some of these assays were repeated because the second assay showed a materially higher activity than the first—which is, of course, contradictory. In such cases the lower figure of the (repeated) assays is given. In all tests at least two roosters were used and in several cases three, for each test.

The best prospect seems to be to use diluted alcohol with hypophosphorous acid for this preparation. In the sample prepared two years ago an equivalent of 40 cc. of U. S. P. Hypophosphorous Acid (30%) per liter was needed to produce a p_H of 4.15. In the one made a year ago—and reported in the above table an equivalent of 26 cc. per liter was required.

With tartaric acid, the sample in the table contains 30 Gm. per liter.

Better results in extraction may be expected when the acid is used in the menstruum rather than added to the fluidextract. The amount of acid to be directed will vary with the alcoholic strength of the menstruum—less being needed for high strength alcohol than for low.

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THE STANDARDIZATION OF ERGOT.*[1]

BY ASA N. STEVENS.

A COMPARATIVE STUDY OF THE BRITISH PHARMACOPŒIA ASSAY FOR EXTRACTUM ERGOTÆ LIQUIDUM AND THE MODIFICATION OF SMITH'S QUANTITATIVE COLORIMETRIC ASSAY.

A colorimetric method of assay for Ergot and its preparations is given official recognition in the recently revised "British Pharmacopœia 1932." The close relationship that exists between the British Pharmacopœia assay for Liquid Extract of Ergot and the Modification of Smith's Quantitative Colorimetric (1) Assay, which was outlined by the writer in an earlier paper, has made it desirable to undertake a comparative study of the two methods as they apply to the assay of Fluidextract of Ergot U. S. P.

It is the purpose of this paper, therefore, to present and to compare the results that have been obtained by the use of both methods.

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* Scientific Section, A. PH. A., Madison meeting, 1933.

[1] From the Analytical Laboratories, Eli Lilly & Co.

The assay procedure now given in the British Pharmacopœia for Liquid Extract of Ergot is quoted as follows:

"Assay: Introduce 10 mls. into a separator, add 50 mls. of water, render slightly alkaline with dilute solution of ammonia, and extract with four successive portions of 40, 25, 20 and 15 mls. of anæsthetic ether. Wash the mixed ethereal solutions with three successive portions of 25 mls. of water mixed with 0.2 mls. of dilute solution of ammonia, then wash once with 25 mls. of water. Shake with four successive 10-ml. portions of a 1% w/v solution of tartaric acid in water: separate, and mix the aqueous liquids, transfer to a porcelain dish, remove the dissolved ether by gentle warming in a current of air and add sufficient water to produce 40 mls. or other suitable volume. Mix 1 ml. with 2 mls. of solution of dimethylaminobenzaldehyde and place in warm water until the temperature reaches 45 degrees. Remove from the water-bath and expose to bright light for a period varying from 10 minutes to two hours, according to the intensity of the light, until the blue-violet colour which is produced reaches its maximum. In the same manner mix 1 ml. of solution of ergotoxine ethanesulphonate (0.012% w/v in 1% tartaric acid) with 2 mls. of solution of dimethylaminobenzaldehyde, heat to 45 degrees and expose to the same source of light for the same length of time. Determine the ratio of the colour intensities by comparing them in a suitable colorimeter. The colour produced by 1 ml. of the solution of ergotoxine ethanesulphonate is equivalent to that produced by 0.0001 Gm. of total alkaloids under identical conditions. The acid solution of the alkaloids should be suitably diluted so that the colour, produced during the test, does not differ by more than 20% from that produced in the solution of ergotoxine ethanesulphonate."

The details of the Modification of Smith's Quantitative Colorimetric Assay are given in the February 1933 issue of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION on page 102 and are not repeated at this time.

Table I shows the results obtained by the use of both methods on the same samples of Fluidextract of Ergot.

TABLE I.

Sample No.	Modified Method % Activity.	B. P. Method % Activity.
1	114.0	130.0
2	100.0	100.0
3	115.0	108.0
4	90.0	93.0
5	115.0	95.0
6	103.0	103.0
7	57.0	53.0
8	80.0	73.0
9	95.0	94.0
10	115.0	115.0
Average	98.4	96.4

It will be noted that in nearly every case there is a close agreement in the results that have been obtained, notwithstanding the fact that each method differs with regard to the amount of the alkaloidal salt that is used as the basis for color comparison. In the British Pharmacopœia method 0.00012 Gm. of ergotoxine ethanesulphonate is given as the standard while in the Modification of Smith's Quantitative Colorimetric Assay 0.0001 Gm. of ergotamine tartrate is used.

With this thought in mind, a series of tests were made in order to study the behavior of the foregoing quantities of ergotoxine ethanesulphonate and of ergotamine tartrate when used, separately, as standards in only one of the two methods. For this purpose the British Pharmacopœia method was selected and the procedure changed in order to permit the use of a Watkins' (2) extractor, instead of a sepa-

rator, for making the first ether extraction, thereby avoiding the tendency to form emulsions. Also, the acid solution of dimethylaminobenzaldehyde and alkaloid was not heated. The results obtained on a series of samples of Fluidextract of Ergot U. S. P. are given in Table II.

TABLE II.

Sample No.	Ergotoxine Ethanesulphonate % Activity.	Ergotamine Tartrate % Activity.
1	92.0	106.0
2	80.0	100.0
3	49.8	52.5
4	89.5	106.0
5	100.0	102.0
6	64.0	72.0
7	95.0	104.0
8	80.0	83.0
9	52.0	64.0
Average	78.0	87.7

DISCUSSION.

It will be noted in Table I that slightly lower results are generally obtained by the British Pharmacopœia method. The British Pharmacopœia standard also gives consistently lower figures when compared with the Modification of Smith's Quantitative Colorimetric Assay's standard as is indicated by Table II. This is probably due to the fact that the British Pharmacopœia method calls for a correspondingly larger amount of an alkaloidal salt of ergot as the basis for color comparison. When the prescribed quantities of the two standards were subjected to colorimetric comparison in 1 cc. of 1% tartaric acid solution, 0.0001 Gm. of ergotamine tartrate was found to have a color value equal to about 80% of that shown by 0.00012 Gm. of ergotoxine ethanesulphonate. On the other hand, when exactly the same concentrations of the two salts were compared on the same basis the readings obtained were identical.

The use of 50% sulphuric acid, which is prescribed by the British Pharmacopœia as the solvent for dimethylaminobenzaldehyde, offers a distinct advantage over the concentrated acid that is used in the Modification of Smith's Quantitative Colorimetric Assay in that very little heat is developed when the solution is added to the solution containing the ergot alkaloid. This does away with the necessity of chilling the mixture which is always required in the Modification of Smith's Colorimetric Assay procedure.

A better blue color is obtained if the solution of dimethylaminobenzaldehyde and alkaloid is not heated.

CONCLUSIONS.

1. A comparative study has been made between the British Pharmacopœia assay method for Liquid Extract of Ergot and the Modification of Smith's Quantitative Colorimetric Assay as they are applied to the assay of Fluidextract of Ergot U. S. P.

2. A difference in the amount of alkaloidal salt used as the basis for colorimetric comparison has been pointed out.

3. The use of 50% sulphuric acid as the solvent for dimethylaminobenzaldehyde and of a Watkins' extractor in making the first ether extraction have been cited as advantages.

4. A better blue color is obtained if the solution of dimethylaminobenzaldehyde and alkaloid is not heated.

The author wishes to express his appreciation to Mr. E. J. Hughes for his friendly criticism.

BIBLIOGRAPHY.

(1) A. N. Stevens, "The Standardization of Ergot—A Modification of Smith's Quantitative Colorimetric Assay," JOUR. A. PH. A., Vol. 22, 2 (1933), 99.

(2) S. Palkin, A. G. Murray and H. K. Watkins, "Automatic Devices for Extracting Alkaloidal Solutions," *Ind. Eng. Chem.*, 17 (1925), 612.

LACTUCARIA. I. THE MYDRIATIC ACTIVITY OF LACTUCARIA BY THE MUNCH METHOD.*[1]

BY JAMES C. MUNCH, HARRY J. PRATT AND GEORGE E. BYERS.

Lactucarium is the "dried milk-juice of *lactuca virosa* Linné (Fam. *Compositæ*)" (34). It was official from the First U. S. P. in 1820 to the Ninth of 1916, but was dropped from the recognized drugs in U. S. P. X (24). It has been used in homeopathic medicine in which it is described as the concrete juice of *L. virosa*, *L. sativa*, *L. scariola* and *L. altissima* (1, 20).

If lactucarium were capable of producing the effects attributed to it, it would be very miraculous. Descriptions in Folklore and in early scientific papers affirmed that it was a sedative, resembled opium in its effect, could be used as a substitute for opium, and later still that it contained hyoscyamine or some of the other mydriatic alkaloids (2, 3, 4, 8, 9, 10, 11, 12, 18, 22, 23, 25, 28, 29, 30, 31, 32, 35, 36, 37).

Apparently, the introduction of lactucarium as the dried juice of *L. virosa* was due to some inconclusive experiments by John Redman Coxe, presented at the meeting of the American Philosophical Society in 1797, at Philadelphia. Comparing the effects with those of opium, he decided that the two were similar in action, and called the material "lettuce opium." A careful scrutiny of his report fails to show any basis for this startling deduction (8, 9). Work by Duncan, Sr., in Edinburgh at about the same time appears to be responsible for the introduction of this product into the Dublin and Edinburgh Pharmacopœias (11).

To prepare lactucarium, various species of *lactuca* appear to have been used (*virosa*, *scariola*, *sativa*, *canadensis*, *septiva* and *altissima*, being most frequently reported (1, 2, 6, 7, 8, 12, 14, 16, 19, 20, 21)).

The juice is collected by cutting off the top of the lettuce plant in June, when it is just ready to blossom. The latex is collected daily. As needed, new incisions are made in the stalk. The combined latex is dried, forming irregular brown lumps of a narcotic odor and bitter taste (6, 24, 37).

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* Scientific Section, A. PH. A., Madison meeting, 1933.

[1] Joint communication from the Department of Research, School of Pharmacy, Temple University, and Department of Pharmacology, Sharp and Dohme, Philadelphia, Pa.