from their glycosides indicates that they are present in a true glycosidic linkage rather than in an ether linkage as reported for barbaloin. From the results of animal feedings, it was found that dialysis gave the most expedient way of separating inert material from the active ingredients in cascara fluidextract. The lipid fraction from cascara bark was practically inert. Isoemodin was only slightly active in a 75-mg. dosage. The ethyl acetate and alcohol extracts from bark were less active than the standard fluidextract, on either a total solids basis, or on a basis of bark extracted.

Bubbling air through the fluidextract for three hours at boiling water-bath temperature caused a loss in activity of approximately 50 per cent.

The activity of the fluidextract was not altered appreciably by (*a*) complete hydrolysis of the glycosides present or (*b*) extraction of the free anthraquinones.

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THE STANDARDIZATION OF ERGOT—A COMPARISON OF RESULTS OBTAINED BY THE COLORIMETRIC, THE COCK'S COMB AND THE BROOM AND CLARK METHODS OF ASSAY.*

BY ASA N. STEVENS.[1]

Van Urk (1) was among the first to report a color reaction between the ergot alkaloids and para-dimethyl-amino-benzaldehyde. Smith (2) later modified van Urk's procedure by directly mixing a one per cent tartaric acid solution of the alkaloids with concentrated sulfuric acid containing para-dimethyl-amino-benzaldehyde. Allport and Cocking (3) then modified Smith's reagent by altering the acid concentration and introducing ferric chloride as a catalyst. The Allport and Cocking reagent is now used by a majority of workers and has been adopted by the British Pharmacopœia, 1932, Addendum 1935 (4), in the estimation of the ergot alkaloids.

Smith (2) was perhaps the first to propose a quantitative colorimetric assay for the alkaloids in fluidextract of ergot. Other methods of the same general nature appear in the British Pharmacopœia, 1932 (5) and the Methods of Analysis of the Association of Official Agricultural Chemists, Fourth Edition, 1935. In each of these methods only those alkaloids which are sparingly soluble in water are deter-

* Scientific Section, A. PH. A., New York meeting, 1937.

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mined. All of them fail to take into account the alkaloids which are readily soluble in water.

The method of Hampshire and Page (6) differs from the methods mentioned above in that the total alkaloids also are determined: those alkaloids which are readily soluble in water being calculated by difference.

A modification of Smith's quantitative colorimetric assay for the fluidextract of ergot has already been reported by the author in a previous communication (7) along with a comparative study of the results obtained using this modified procedure, the U. S. P. X (Cock's Comb) and the Broom and Clark (8) methods of physiological standardization.

It is the object of this paper to compare the results obtained by the colorimetric, the Cock's Comb (U. S. P. XI) and Broom and Clark (8) methods of assay.

For some time the author has been supplementing the official assay of various lots of fluidextract of ergot with the colorimetric determination of total alkaloids. This was accomplished by the following method.

METHOD FOR TOTAL ALKALOIDS.

An aliquot portion of the sample to be tested is pipetted into a Watkins (9) extractor and diluted to about 50 cc. with distilled water. This aqueous suspension is then made faintly alkaline to litmus with three per cent ammonia. A small piece of litmus is placed in the aqueous suspension as an indicator. Ether is then added and the alkaline solution extracted for four hours on a water-bath using just enough heat to cause continuous evaporation.

At the end of this time the apparatus is dismantled and the total ether extract transferred to a separatory funnel. The ether is extracted with one per cent aqueous tartaric acid solution, using 10-, 10-, 10-, 5- and 5-cc. portions, respectively. Test for complete extraction by shaking with an additional 5 cc. of 1 per cent tartaric acid solution. Add to 2 cc. of this additional acid extract 4 cc. of the para-dimethyl-amino-benzaldehyde reagent. No blue color should develop.

The combined aqueous tartaric acid solutions are then gently heated on a water-bath, in order to remove the ether, and then diluted to 50 cc.

Two tubes containing 1 and 2 cc., respectively, of this solution are prepared. To the first 1 cc. of distilled water is added. Then to each tube 4 cc. of $M/60$ para-dimethyl-amino-benzaldehyde in 65 per cent sulfuric acid containing 0.01 per cent ferric chloride is added. The tubes are then thoroughly mixed and allowed to stand for one-half hour. A blue color develops.

The tubes are then compared in a colorimeter with standards containing known quantities of ergotoxine ethanesulfonate. From this data the quantities of the alkaloid in the sample tested can be calculated.

Typical results, obtained by this procedure, as compared with those obtained by the Cock's Comb (U. S. P. XI) and the Broom and Clark methods of assay are shown in Table I. An average of the results obtained on thirty-three samples is also indicated.

TABLE I.

Sample No.	Cock's Comb (U. S. P. XI) % Activity.	Broom and Clark % Activity.	Colorimetric (Total Alkaloids) % Activity.
A	100	80	149.7
B	130	100	132.4
C	100	90	170.8
D	100	80	152.8
E	100	100	80.0
F	50	30	38.4
G	125	90	128.0
Average—33 samples	77.5	67.6	105.9

NOTE: U. S. P. XI Standard, ergotoxine ethanesulfonate, has been used in all determinations reported in this paper.

It will be noted in Table I that the colorimetric results obtained for the total alkaloids in fluidextract of ergot are, on the average, higher than those obtained by either of the physiological methods of assay.

Table II gives the colorimetric results obtained, on samples of fluidextract of ergot, for those alkaloids which are sparingly soluble in water, and for the total alkaloids. Assay results obtained by the official Cock's Comb method are also tabulated for comparison.

TABLE II.

Sample No.	Cock's Comb (U. S. P. XI) % Activity.	Colorimetric. Total Alkaloids % Activity.	Colorimetric. Alkaloids Sparingly Soluble in Water % Activity.
1	95	126.8	99.6
2	80	126.7	82.5
3	112	102.7	79.9
4	100	144.0	86.4

The results in Table II are interesting in that they indicate that the Cock's Comb method of assay has evaluated only those alkaloids in the fluidextract of ergot which are sparingly soluble in water. This apparently explains the difference recorded in Table I between the colorimetric assay figures for total alkaloids and those obtained by the physiological assays.

DISCUSSION.

Since the discovery that there are alkaloids in ergot which are readily soluble in water, Barger (10) has pointed out that the only specific biological test for these substances lies in the use of the puerperal (human) uterus. Davis, Adair, Chen, Swanson (11) have already maintained "that the Broom and Clark method is not suitable for the evaluation of the new ergot principle." The data presented in this paper quite definitely indicates that the U. S. P. XI method of assay fails to determine these new alkaloids.

The investigations reported by Smelt (12); Swoop, Cartland and Hart (13); Swanson, Powell, Stevens and Stuart (14), and the author (7) have demonstrated that Smith's quantitative colorimetric assay method, and certain modifications thereof, are as accurate a measure of those sparingly water-soluble alkaloids in fluidextract of ergot as are the Cock's Comb and the Broom and Clark methods of assay.

Obviously the modified Smith (7) assay procedure determines only those alkaloids which are sparingly soluble in water.

It is possible, however, to determine the total alkaloids in fluidextract of ergot by the method described elsewhere in this paper.

Therefore it seems reasonable to assume that the alkaloids which are readily soluble in water may be estimated as the difference between the result obtained for total alkaloids and that obtained for the alkaloids sparingly soluble in water. These alkaloids could then be calculated either as ergotoxine ethanesulfonate or as the new ergot alkaloid.

SUMMARY.

Data have been presented showing the comparative results obtained upon assaying various samples of fluidextract of ergot by the (*a*) Cock's Comb (U. S. P. XI); (*b*) the Broom and Clark; (*c*) the modified Smith quantitative colorimetric and (*d*) the colorimetric method described in this paper.

These results quite definitely indicate that the U. S. P. XI method of assay determines only those alkaloids in fluidextract of ergot which are sparingly soluble in water.

A method for the determination of total alkaloids in fluidextract of ergot has been given in detail.

The author is indebted to C. E. Powell for the Broom and Clark and to C. C. Hargreaves for the Cock's Comb assay results appearing in this paper. He also wishes to express his appreciation to E. J. Hughes for his friendly criticism.

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A FURTHER STUDY OF THE ASSAY OF NITROGEN MONOXIDE.*[1]

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INTRODUCTION.

The tenth revision of the United States Pharmacopœia gave neither a purity rubric nor an assay for nitrogen monoxide. In a previous communication to this JOURNAL two of the authors with Reindollar (1) set forth a method of assay for nitrogen monoxide which depended upon the preferential solubility of the gas in water chilled to 0° C. Commercial samples were assayed and a standard sample of gas was assayed repeatedly. The probable error of a single determination of this series was 0.23 per cent. The method was made official in the eleventh revision of the Pharmacopœia and during the last two years has been subjected to careful scrutiny in the hands of many different analysts.

It is the purpose of this paper to present certain minor modifications of the method and further to compare it with the other procedures extant for assay of this gas.

EXPERIMENTAL.

Several samples of nitrogen monoxide of known N_2O content mixed with N_2 were prepared for us by Dr. Wardell of the Ohio Chemical Co. These gas mixtures were not compressed sufficiently so that a liquid and gaseous phase obtained in the cylinder as is present in the commercially

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* Scientific Section, A. PH. A., New York meeting, 1937.

[1] The expense of this investigation has been defrayed by a grant from the Board of Trustees of the United States Pharmacopœial Convention.

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