My thanks are due to Mr. C. T. Ichniowski, of this Department, and to Prof. E. G. Vanden Bosche of the Department of Physical Chemistry, for the p_H determinations included in these studies.

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FLUIDEXTRACT OF ERGOT.*

BY L. W. ROWE AND WILBUR L. SCOVILLE.

The physiological activity of Ergot having been established as being mainly due to alkaloids which are insoluble in water and soluble in alcohol, and these alkaloids having been shown to be instable, being easily oxidized, it seems reasonable to judge that more stable preparations of Ergot may result from the use of more strongly alcoholic menstrua and the use of reducing agents. This paper is designed to throw light on the subject from this angle.

The large amount of chemical and pharmacological work that has been done on ergot during recent years has disclosed three facts of pharmaceutical interest. We now believe that the physiological action of ergot is due almost entirely to its alkaloids; that these alkaloids, when free, are soluble in alcohol but almost insoluble in water, and that the alkaloids are instable, probably becoming oxidized easily and thus losing their potency. These facts help to suggest some ways of producing more stable preparations of ergot.

The following experiments were made with this end in view, the fluidextract being selected for study.

A quantity of ground and defatted Spanish ergot sufficient for all the preparations made was used, the results thus being comparative and the method of treatment being as nearly the same as was practicable in all cases.

The fluidextracts were all made by the general process directed for Fluidextract of Ergot in the Pharmacopœia, namely, percolation of the drug, concentration of the weaker portion of percolate by distillation under reduced pressure and solution of the concentrate in the reserved portion. Heat was thus used in making all samples. The fluidextracts varied in the alcoholic strengths of the menstruum used, the amount and character of the acid employed and in a preliminary sterilization of the drug, in some cases. Ergot being the result of a fungus blight may well be expected to contain several enzymes, perhaps among them an oxydase or peroxydase and the effect of sterilizing these is a part of the attempt to secure stability. The sterilizing was accomplished by moistening the drug thoroughly with 95 per cent (or in two cases with 77 per cent) alcohol and heating the mixture under a reflux condenser, the hot alcohol thus acting as the sterilizing agent. The alcohol so used afterward became a part of the menstruum for extraction, thus avoiding drying and loss.

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* Scientific Section, A. PH. A., Miami meeting, 1931.

The ergot used assayed fully 125% by the Cock's Comb Method.

No. 1 Fluidextract was made according to the U. S. P. formula and directions. It showed a p_H of 5.0 and assayed 125% of standard when fresh and 80% to 100% after 6 months, showing a loss of about 25%.

No. 2 corresponds to No. 1 except that the ergot was first sterilized. To 250 Gm. of the ground drug was added 125 cc. of alcohol and the uniformly mixed product was placed in a 500-cc. flask, attached to a reflux condenser and the mixture heated on a steam-bath for 30 minutes. It was then allowed to cool, 120 cc. of water and 5 cc. of hydrochloric acid added and thoroughly mixed, the wet drug transferred to a percolator, allowed to macerate 48 hours then extracted in the usual way with dilute alcohol. This fluidextract showed a p_H of 4.16 and assayed when fresh 200%. After 6 months it assayed 20%—showing a loss in this time of 90%.

No. 3 was also made with diluted alcohol, the drug being first sterilized by heating 250 Gm. with 125 cc. of alcohol, then after cooling 119 cc. of water and 6 cc. of 50% hypophosphorous acid to form the first portion of menstruum. In this case an acid having deoxidizing properties is used in place of hydrochloric acid. The fluidextract showed a p_H of 4.15 and assayed 125% when fresh, and 125% after aging six months. No loss during this time.

No. 4 was made with 77% alcohol in place of diluted alcohol and the drug was sterilized by heating with a portion of the menstruum. Hydrochloric acid provided the acidity in this sample. This sample showed a p_H of 4.90 and assayed 65% when fresh, and 20% after 6 months, showing a loss of about 70% in this time.

No. 5 was also made with 77% alcohol in a corresponding manner to that of No. 4, but hypophosphorous acid was used in place of hydrochloric. The p_H was found to be 4.68 and it assayed when fresh 125%. After 6 months it assayed below 20%, showing a loss of about 90% in this time.

No. 6 was made with 87% alcohol and without sterilization. It was acidulated with hydrochloric acid. The p_H was found to be 3.84 and it assayed 80% when fresh and 60% after 6 months, showing a loss of 25% in this time.

No. 7 corresponded to No. 6, being made with 87% alcohol, without sterilization but with hypophosphorous acid in place of hydrochloric. It showed a p_H of 3.75 and assayed 150% when fresh, and 100% after 6 months, showing a loss of about 30% in this time.

SUMMARY.

No definite conclusions can be drawn from these experiments, but there are some rather significant results.

On the question of the effect of sterilization there are no definite indications. One sample which was made from the sterilized drug showed no loss in 6 months and another showed a 90% loss—the two extremes. The average loss on three samples made without sterilization is about 30% and on four with sterilization is about 60% or twice as great. It does not look as though sterilization is of any benefit in securing stability, but some further experiments will be worth while.

The action of a reducing agent is more definite. Since the acidity as shown by p_H values is approximately the same in all cases we can compare the effects of hydrochloric and hypophosphorous acids. Four experiments containing hydrochloric acid showed an average loss of about 50%, and two of these averaged 80% loss.

With hypophosphorous acid three experiments showed an average loss of 40%, and two of the three showed an average loss of 60%. The differences are not marked enough to be conclusive, but some other factors may be eliminated in further experiments to help in a decision.

One factor which does not appear in these experiments but which other experience has developed is the effect of heat and aeration in making the fluidextract. We have good evidence that heat and exposure sometimes injure this preparation quite seriously, a loss of 50% being known in one instance due to concentration by distillation under reduced pressure.

In the series of seven fluidextracts designated in the preceding paragraphs which were made individually, the conditions of distillation were not as uniform as they should be and some were exposed to more heat and aeration than others. Some of the erratic results may, perhaps, be due to such exposures. At any rate we feel that the next series should be based on fluidextracts made without heat, *i. e.*, by the fractional percolation method. We also hope to have some coöperative assistance in this work and an invitation is hereby extended to any who may be willing to join in an effort to secure a more stable preparation of ergot.

We desire to acknowledge with thanks the assistance of Mr. J. A Sultzaberger who made the p_H determinations on the preparations above quoted.

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THE ASSAY OF PEPSIN.*, **

BY H. L. GREENBERG.[1]

INTRODUCTION.

The official assay for the proteolytic power of pepsin is a modified and improved procedure based upon the original test for the activity of Saccharated Pepsin which was made official in the pharmacopœial revision of 1880. The inability of experienced analysts, unfamiliar with the official method of assay, to secure concordant and reliable results using the official assay procedure led to the following critical study in an attempt to develop a more satisfactory method of measuring the proteolytic activity of pepsin.

DEVELOPMENT OF ASSAY METHODS.

According to Waldschmidt (1) the oldest method of quantitatively estimating peptic strength is that of Bidder and Schmidt (2) which dates back to 1852. Since that time there have been many methods worked out. Some of the more important of these may be grouped as follows:

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** From the laboratory of Glenn L. Jenkins, Professor of Pharmaceutical Chemistry, School of Pharmacy, University of Maryland. Abstracted from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.

[1] Alumni Research Scholar.