SECTIONS

BOARD OF REVIEW OF PAPERS (SCIENTIFIC SECTION).—*Chairman*, F. E. Bibbins; H. M. Burlage, W. G. Crockett, E. V. Lynn, C. O. Lee, L. W. Rising, L. W. Rowe, Heber W. Youngken, Ralph E. Terry, Carl J. Klemme.

CHANGES IN ERGOT WITH VARIOUS MOISTURE CONTENTS UNDER DIFFERENT CONDITIONS OF STORAGE.*,[1]

BY B. V. CHRISTENSEN[2] AND J. A. REESE.[3]

The purpose of this investigation was to study the changes taking place in ergot of various moisture contents under different conditions of storage. The U. S. P. XI requires that Ergot be preserved under all conditions of storage and transportation in water-proof and air-tight containers. These requirements have been subjected to severe criticism by importers and manufacturers of ergot preparations (1).

REVIEW OF THE LITERATURE.

Paulizky (2) reported in 1787 that some thirty years previously powdered ergot had been introduced into pharmacies under the name *pulvis ad partum;* that it was administered by midwives and physicians effectively, but it lost its action on keeping. This is probably the first reference to the deterioration of ergot.

Hirschberg (3), 1871, recommended that ergot be carefully dried and preserved in well-sealed vessels. Ficinus (4), 1873, suggested that powdered ergot, if deprived of fat by ether, would keep much better. Gerrard (5), 1875, suggested preserving ergot, when dry, by bottling it and fixing a piece of lime, tied in muslin, to the interior of the stopper. Mourrut (6), 1877, recommended the mixing of freshly powdered ergot with 5 per cent of powdered benzoin.

Zschiesing and Bombelon (7), 1881, and Perrett (8), 1882, suggested defatting as a means of preservation. Alpen (9), 1888, reported that ergot should be dried in thin layers, the last portion of moisture removed by exposure over lime or sulfuric acid in a desiccator, and stored in corked yellow bottles.

Grünfeld (10), 1892, alleged a rapid loss of activity for ergot (tested on the cock). He concluded that samples lose all their activity within eight months of the harvest.

Zanon (11), 1893, sealed ergot in jars in which there were alternate layers of perfectly dry sand and ergot. Keller (12), 1894, stated that in a properly-packed pulverized ergot the alkaloid will keep undecomposed for at least a year.

Meulenhoff (13), 1900, severely criticised Grünfeld's experiments; experimented with more cocks than Grünfeld and found hardly any loss of activity after two years.

Dahlin (14), 1912, concluded that when properly kept the alkaloidal content of ergot does not diminish. Tate (15), 1921, thought there was likelihood of a small increase in activity when ergot is stored. Forst (16), 1926, found that a sample of powdered ergot had lost 55 per cent of the alkaloids in six months. Burn and Ellis (17) found samples of old ergot very active.

Thompson (18), 1930, reported two samples of ergot that deteriorated more in air-tight containers than in paper bags in a dry place.

Pedersen (19), (20) reported that defatted ergot was more stable than ergot. Corran and Rymill (21), 1935, presented data to indicate that powdered ergot, in any form, is comparatively stable over periods of time varying from seven to eighteen months.

---

* Presented before the Scientific Section, A. PH. A., Minneapolis meeting, 1938.

[1] Abstract of a thesis presented to the Graduate Council, University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Director, School of Pharmacy, University of Florida.

[3] Assistant Professor of Pharmacognosy, Medical College of Virginia.

Rowe (22), 1937, found three old samples of ergot stored under ordinary conditions still equal in activity to the U. S. P. XI standard of potency. The moisture contents ranged from 3.91 per cent to 4.94 per cent.

EXPERIMENTAL.

Material.[1]—Twelve lots of ergot (35 Kg.) were used in this investigation. Four of these lots, A, B, C and D, had been examined by Christensen and Welch (23). Six lots, 1, 2, 3, 4, 5 and 6 (19 Kg.), were obtained in 1936. Five of these lots were labeled Portuguese Ergot, and one lot, Spanish Ergot. Two lots, 7 and 8 (14 Kg.), were obtained in 1937, and were labeled Portuguese Ergot. The authors were informed by the importer that these lots were from large shipments consisting of several tons; and that the majority of the Portuguese Ergot was of Spanish origin.

Storage.—Each lot of ergot was thoroughly mixed before preparing samples for storage. Samples of ergot, weighing from 60 Gm. to 500 Gm., were stored in completely filled, air-tight, wide-mouthed bottles; completely and half-filled, air-tight Mason jars; completely and half-filled, not sealed, Mason jars; completely and half-filled wide-mouthed bottles stoppered with a pledget of cotton; wide-mouthed bottles stoppered loosely with a cork; ground, glass-stoppered bottles; and in paper bags. The samples were kept at constant temperatures at 21, 27, 32 and 38° C., and at room temperature, for periods of time varying from three months to six years. The moisture contents were varied from 3.2 per cent to 12.8 per cent.

The moisture contents were adjusted by spreading the ergot in thin layers in an oven at 38° C., and by placing in humid chambers at a temperature not exceeding 38° C.

ASSAY PROCEDURE.

The U. S. P. XI Cock's Comb method and a modification of the colorimetric method of the British Pharmacopœia and Addenda were used in this investigation. The colorimetric method follows:

Assay: Extract 15 Gm. of ergot in No. 60 powder by percolation with Purified Petroleum Benzin until the fat is completely removed. Dry the extracted drug at a temperature not exceeding 40° C., transfer to a stoppered flask, add 150 cc. anesthetic ether and set aside for 10 minutes. Add 0.6 Gm. light magnesium oxide diffused in 25 cc. of water and shake the mixture at intervals during thirty minutes; add 1.5 Gm. of powdered tragacanth, shake vigorously, set aside ten minutes and filter through cotton into a separatory funnel 100 cc. of the ethereal solution, representing 10 Gm. of the ergot being assayed. Shake with 4 successive 10-cc. portions of a 1 per cent w/v solution of tartaric acid in water. A fifth extraction with a 5-cc. portion of the acid should give no precipitate with Mayer's reagent. If a precipitate is formed, continue the extraction with 5-cc. portions until a negative test is obtained. Mix the aqueous liquids, transfer to a porcelain dish, remove the dissolved ether by gentle warming on a water-bath in a current of air and add sufficient water to produce 40 cc. or other suitable volume. Mix 1 cc. with 2 cc. of the Allport-Cocking reagent (24) and let stand thirty minutes. In the same manner, mix 1 cc. of 0.012 per cent solution of Ergotoxine Ethanesulfonate in 1 per cent w/v tartaric acid in water (1 cc. of this solution contains the equivalent of 0.0001 Gm. of anhydrous ergotoxine) with 2 cc. of the Allport-Cocking reagent, and let stand for the same length of time. Determine the ratio of the color intensities by comparing them in a suitable colorimeter. The color produced by 1 cc. of 0.012 per cent solution of Ergotoxine Ethanesulfonate 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 color, produced during the test, does not differ by more than 20 per cent from that produced in the solution of Ergotoxine Ethanesulfonate.

Time may be saved by preparing dilutions of the acid solution of the alkaloids by mixing 1 part with 1, 2, 3, 4 and 5 parts of water, and mixing 1 cc. of each of these with 2 cc. of the Allport-Cocking reagent.

Allport and Cocking (24) have insisted on the use of pure anesthetic ether, to avoid oxidation of the alkaloids.

---

[1] Acknowledgment is made to the U. S. P. XI Revision Committee for furnishing the greater part of the ergot used in this investigation and the Ergotoxine Ethanesulfonate.

PART I.

REËXAMINATION OF OLD ERGOT.

Four lots of ergot, 2 Spanish and 2 Russian, which had been examined by Christensen and Welch (23) were reëxamined. These samples had been stored at room temperature in ground, glass-stoppered bottles for six years.

The moisture content of the samples had not been determined prior to storage. After storage the moisture content was determined using the U. S. P. XI Toluene method.

Fluidextracts of the drug had been examined by the U. S. P. X Cock's Comb method and by the Smith colorimetric method (25), using a standard Fluidextract of Ergot as a standard. The per cent of alkaloids in the drug itself had not been determined. The samples were reëxamined using the U. S. P. XI Cock's Comb method and the colorimetric method previously described. Ergotoxine Ethanesulfonate, supplied by the Committee of Revision of the U. S. P. XI, was used as a standard.

It is realized that the results given in Table I are not to be compared directly; the methods used before and after storage were different. The unstable fluidextract had been used for a standard before storage, and the alkaloids had been determined of a fluidextract prepared from the drug, and not of the drug itself. This was before the discovery of the alkaloids of ergot freely soluble in water and they probably had been removed. In addition, from data obtained in Part V of this investigation, it would appear that the fluidextract does not represent the alkaloidal content of the drug.

TABLE I.—ASSAY OF LOTS A, B, C AND D BEFORE AND AFTER STORAGE FOR SIX YEARS IN GROUND, GLASS-STOPPERED BOTTLES

Lot No.	Moisture after Storage Per Cent.	Assay, U. S. P. X before Storage Per Cent.	Assay, U. S. P. XI after Storage Per Cent.	Smith Assay of Fluidextract before Storage Alkaloid Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.
A	8.3	350	60	0.076	0.168
B	12.7	175	Less than 40	0.065	0.102
C	11.7	135	Less than 25	0.042	0.034
D	12.8	140	Less than 25	0.037	0.006

The physical appearance of lots B, C and D (those of the highest moisture contents) was much the same. The fracture of the ergot was flexible, and the color of the fracture was mostly yellow-brown. The color of the fracture of a few of the ergots was lavender. The appearance of the Spanish Ergot, lot A, was by far the best. About one-half had a short fracture, white to grayish white in color; the rest a flexible fracture of a yellow-brown color.

DISCUSSION.

The results of this part of the investigation seem to indicate that there is a relationship between physical condition and quality of the drug as measured by the colorimetric and U. S. P. XI Cock's Comb assay. This caused the authors to observe carefully the physical condition of all the ergot stored later. The results obtained later show far less correlation between physical condition and quality of the drug.

The results given in Table I show far more deterioration of ergot with moisture content from 11.7 to 12.8 per cent than of ergot with moisture content of 8.3 per cent. This deterioration was enough to cause the drug to be practically worthless. The relationship of moisture content and deterioration of the drug was carefully studied in succeeding portions of this work.

PART II.

CHANGES IN ERGOT WITH VARIOUS MOISTURE CONTENTS STORED IN AIR-TIGHT CONTAINERS AT DIFFERENT TEMPERATURES.

This part of the investigation was undertaken to ascertain the deterioration of ergot, if any, in air-tight containers with moisture content from 6.6 per cent to 12.6 per cent stored at 21, 27, 32 and 38° C., corresponding to 70, 80, 90 and 100° F. respectively. Some samples were also stored at room temperature.

EXAMINATION OF LOTS 1, 2, 3 AND 4.

Samples were prepared from lots 1, 2, 3 and 4 as given under storage in Part I. Thirty-two samples of 60 Gm. each were sealed (air-tight) in completely filled, wide-mouthed bottles and placed in uniform temperature control ovens. Six samples of 200 Gm. each were sealed (air-tight) in completely filled, wide-mouthed bottles and kept at room temperature and 38° C.

The moisture content was determined by the U. S. P. XI oven method; the average of two determinations was taken.

The per cent of alkaloids of each of the four lots was determined by the colorimetric method given in Part I. Lots 3 and 4 were also assayed by the U. S. P. XI Cock's Comb method.

The physical appearance of the samples was observed at the end of each week for ten weeks. No mold filament was present on any of the samples with moisture content below 8 per cent. Some of the samples with moisture content from 10.2 to 12.6 per cent had mold filament while others did not. Most of the samples had a thin grayish white coating which caused the ergot to appear as if it had been dusted lightly with a grayish white powder. From the results obtained later, when the containers were opened and the coating that was present examined under the microscope, the authors believe that a thin coating of mold was present on almost every sample during the later part of the observation period.

The four tables which follow summarize the physical condition of the four lots of ergot after storage for seventeen months.

TABLE II.—PHYSICAL CONDITION OF LOT 1 STORED IN AIR-TIGHT CONTAINERS FOR SEVENTEEN MONTHS.

Temperature ° C.	Moisture Per Cent.	Estimated Per Cent with Slight Mold.	Estimated Per Cent with Mold Filament.	Color of Fracture Estimated Per Cent White or Grayish White.	Color of Fracture Estimated Per Cent Yellow-Brown.
21	6.8	5	..	95	5
27	6.8	5	..	95	5
32	6.8	10	..	95	5
38	6.8	10	..	90	10
21	10.5	75	5	25	75
27	10.5	75	5	25	75
32	10.5	90	..	10	90
38	10.5	90	5	5	95

TABLE III.—PHYSICAL CONDITION OF LOT 2 STORED IN AIR-TIGHT CONTAINERS FOR SEVENTEEN MONTHS.

Temperature ° C.	Moisture Per Cent.	Estimated Per Cent with Slight Mold.	Estimated Per Cent with Mold Filament.	Color of Fracture Estimated Per Cent White or Grayish White.	Color of Fracture Estimated Per Cent Yellow-Brown.
21	7.4	2	..	95	5
27	7.4	2	..	95	5
32	7.4	5	..	90	10
38	7.4	5	..	90	10

21	10.5	50	25	10	90
27	10.5	50	25	10	90
32	10.5	90	..	5	95
38	10.5	65	10	10	90

TABLE IV.—PHYSICAL CONDITION OF LOT 3 STORED IN AIR-TIGHT CONTAINERS FOR SEVENTEEN MONTHS.

Temperature ° C.	Moisture Per Cent.	Estimated Per Cent with Slight Mold.	Estimated Per Cent with Mold Filament.	Color of Fracture Estimated Per Cent White or Grayish White.	Color of Fracture Estimated Per Cent Yellow-Brown.
21	6.6	..	..	100	...
27	6.6	..	..	95	5
32	6.6	5	..	90	10
38	6.6	10	..	95	5
21	12.6	50	..	...	100
27	12.6	75	..	...	100
32	12.6	80	..	...	100
38	12.6	50	..	...	100

TABLE V.—PHYSICAL CONDITION OF LOT 4 STORED IN AIR-TIGHT CONTAINERS FOR SEVENTEEN MONTHS.

Temperature ° C.	Moisture Per Cent.	Estimated Per Cent with Slight Mold.	Estimated Per Cent with Mold Filament.	Color of Fracture Estimated Per Cent White or Grayish White.	Color of Fracture Estimated Per Cent Yellow-Brown.
21	8	5	..	95	5
27	8	10	..	90	10
32	8	10	..	90	10
38	8	10	..	90	10
21	10.2	60	5	50	50
27	10.2	60	5	25	75
32	10.2	50	25	25	75
38	10.2	90	..	5	95

As a general rule, the higher the moisture content and the temperature the greater the tendency of the ergot to have a fracture of a yellow-brown color. The color of the fracture became darker as the moisture content and temperature increased, varying from a light yellow-brown to a dark yellow-brown. The fracture of the ergot, with a moisture content below 8 per cent, was short; of that above 8 per cent, slightly flexible.

The results of the assays before and after storage are given in the next five tables.

TABLE VI.—ASSAY OF LOT 1 BEFORE AND AFTER STORAGE FOR SEVENTEEN MONTHS IN AIR-TIGHT CONTAINERS.

Temperature* ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	Loss of Alkaloids Per Cent.
21	6.8	0.202	0.196	3
27	6.8	0.202	0.193	4.5
32	6.8	0.202	0.189	6.4
38	6.8	0.202	0.143	29.2
21	10.5	0.194	0.178	8.2
27	10.5	0.194	0.173	10.8
32	10.5	0.194	0.164	15.5
38	10.5	0.194	0.154	20.6

* Corresponding to 70, 80, 90 and 100° F., respectively.

TABLE VII.—ASSAY OF LOT 2 BEFORE AND AFTER STORAGE FOR SEVENTEEN MONTHS IN AIR-TIGHT CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	Loss of Alkaloids Per Cent.
21	7.4	0.223	0.218	2.2
27	7.4	0.223	0.216	3.1
32	7.4	0.223	0.201	9.9
38	7.4	0.223	0.192	13.9
21	10.5	0.216	0.191	11.6
27	10.5	0.216	0.187	13.4
32	10.5	0.216	0.183	15.3
38	10.5	0.216	0.161	25.5

TABLE VIII.—ASSAY OF LOT 3 BEFORE AND AFTER STORAGE FOR SEVENTEEN MONTHS IN AIR-TIGHT CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	Loss of Alkaloids Per Cent.
21	6.6	0.210	0.208	1
27	6.6	0.210	0.20	4.8
32	6.6	0.210	0.177	15.7
38	6.6	0.210	0.190	9.5
21	12.6	0.196	0.162	17.3
27	12.6	0.196	0.166	15.3
32	12.6	0.196	0.145	26.0
38	12.6	0.196	0.144	26.5

TABLE IX.—ASSAY OF LOT 4 BEFORE AND AFTER STORAGE FOR SEVENTEEN MONTHS IN AIR-TIGHT CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	Loss of Alkaloids Per Cent.
21	8	0.221	0.213	3.6
27	8	0.221	0.204	7.7
32	8	0.221	0.186	15.8
38	8	0.221	0.179	19.0
21	10.2	0.216	0.192	11.1
27	10.2	0.216	0.190	12.0
32	10.2	0.216	0.173	19.9
38	10.2	0.216	0.168	22.2

TABLE X.—COLORIMETRIC AND U. S. P. XI ASSAYS OF ERGOT BEFORE AND AFTER STORAGE FOR SEVENTEEN MONTHS.

Lot No.	Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
3	38	12.6	0.196	0.144	100	70
4	38	8.0	0.221	0.179	110	100
3	Room	6.6	0.210	0.20	100	100
4	Room	10.2	0.216	0.190	110	100
4	Room	8.0	0.221	0.205	110	110

A sample of lot 2, which was left in the original container for two years, was infested with bugs. The container was partially filled and air-tight. The sample still contained 0.18 per cent alkaloids.

A sample of lot 3, 12.6 per cent moisture, in a partially filled, loosely stoppered bottle at room temperature for fifteen months, was so molded that the ergot was almost beyond recognition. The sample had been in a moldy condition for more than a year. It contained 0.196 per cent alkaloids before storage; after storage, 0.106 per cent.

DISCUSSION.

The purpose of this investigation is not a study of assay methods; yet in order for the work to be of any value the methods of evaluation of the drug must be reliable. Examination of Table X shows that ergot assaying 100 to 110 per cent U. S. P. XI contained approximately 0.2 per cent total alkaloids. In order for a fluidextract of ergot to be U. S. P. XI it must contain active alkaloids corresponding to 0.05 per cent Ergotoxine Ethanesulfonate. Ergotoxine Ethanesulfonate contains 82 per cent ergotoxine. The fluidextract must then contain active alkaloids equivalent to 0.04 per cent anhydrous ergotoxine. Yet fluidextracts prepared from ergot assaying approximately 0.2 per cent alkaloids colorimetrically showed only 0.04 to 0.05 per cent alkaloid (as ergotoxine) by the U. S. P. XI Cock's Comb method. If the fluidextracts contained most of the alkaloids of ergot, then only 20 to 25 per cent of the alkaloids of ergot (as ergotoxine) were active in producing a bluing of the cock's comb.

Many investigators (26), (27), (28), (29), (30) have reported a fairly good agreement between the colorimetric and Cock's Comb assays of fluidextract of ergot. The Sub-Committee on ergot of the British Pharmacopœia (31) reached the conclusion that the results by the biological methods are lower than those obtained by chemical methods; the ratio of the average result is 2:1. Many workers have reported chemical assays of samples of ergot containing from 0.15 to 0.25 per cent alkaloids, but most of the chemical assays of fluidextracts of ergot reported are below 0.1 per cent. The authors reasoned from the preceding statements that the fluidextract does not represent the alkaloidal content of ergot and carried out experiments to find out if this is true.

Part V of this work tends to show that 40 to 65 per cent of the total alkaloids of ergot are as active in producing a bluing of the cock's comb as ergotoxine, and that the fluidextract contains approximately 50 per cent of the total alkaloids and approximately the same proportion of the ergonovine. With this in mind, Table X shows a fairly good agreement between the colorimetric and Cock's Comb assays.

Tables VI, VII, VIII, IX and X tend to show that ergot stored in air-tight containers with moisture content from 6.6 to 12.6 per cent deteriorates slowly. The deterioration with moisture content below 8 per cent at 21 and 27° C. was scarcely appreciable. Ergot with less than 8 per cent moisture at 32 and 38° C. deteriorated less than 16 per cent in all cases but one. No reason can be given to explain why one sample with 6.8 per cent moisture at 38° C. deteriorated more than the other samples at the same temperature. Ergot with moisture contents between 10.2 and 12.6 per cent deteriorated a maximum of 27 per cent as measured colorimetrically; a maximum of 30 per cent determined biologically. The amount of deterioration was generally proportional to the moisture content and the temperature.

From these five tables it can be seen that the deterioration of ergot in air-tight containers for seventeen months with moisture contents not greater than 12.6 per cent stored at 21, 27, 32 and 38° C. was usually too small to be measured biologically.

MOISTURE CONTENT OF ERGOT BEFORE AND AFTER STORAGE IN AIR-TIGHT CONTAINERS.

It has been inferred (1) that the moisture content of ergot stored in air-tight containers would increase as much as 3 per cent. In order to ascertain whether any such change would take place under the storage conditions employed in this investigation, the moisture content of a representative number of samples was redetermined. The difference in all cases was within the limit of experimental error. The results are given in Table XI.

TABLE XI.—MOISTURE CONTENT OF ERGOT BEFORE AND AFTER STORAGE IN AIR-TIGHT CONTAINERS.

Lot No.	Temperature ° C.	Period of Storage Months.	Moisture Content Before Storage.	Moisture Content After Storage.
2	21	17	7.4	7.6
2	27	17	7.4	7.6
2	32	17	7.4	7.5
2	38	17	7.4	7.5
1	21	17	10.5	11.0
1	27	17	10.5	10.6
1	32	17	10.5	11.0
1	38	17	10.5	10.5
6	27	7	10.2	10.1
6	32	7	10.2	10.3

PART III.

CHANGES IN ERGOT WITH VARIOUS MOISTURE CONTENTS STORED IN OPEN AND CLOSED CONTAINERS AT DIFFERENT TEMPERATURES.

This part of the investigation was undertaken to ascertain whether it is necessary to store ergot in air-tight containers. Ergot with moisture contents from 3.2 to 10.2 per cent was stored in open and closed containers at 21, 27, 32 and 38° C. and at room temperature.

EXAMINATION OF LOTS 5, 6, 7 AND 8.

Lots 5 and 6 were received in air-tight containers. These lots showed some sign of mold, and a very small quantity of the ergot was discarded. Lot 5 was in better condition than lot 6. Both lots were in fairly good condition, but, to prevent further mold before the ergot was stored, the two lots were spread in thin layers and exposed to the sun for three hours. After this treatment, lot 5 tested 9.2 per cent moisture; lot 6, 10.2 per cent.

Lots 7 and 8 were received in paper bags and were in excellent condition. Lot 7 had 9.2 per cent moisture; lot 8, 9.7 per cent.

The four lots were assayed by the colorimetric method given in Part I and by the U. S. P. XI Cock's Comb method.

Sixty-four samples were prepared and stored as given under storage in Part I. The weight of each sample was from 200 Gm. to 500 Gm.

The physical appearance of the samples of lots 5, 6, 7 and 8 was observed from time to time. The appearance as a rule was similar to lots 1, 2, 3 and 4. The samples with a moisture content greater than 7 per cent at 27, 32 and 38° C. in paper bags and loosely closed containers were in better condition than the samples at the same temperatures in air-tight containers. The difference in some cases was more marked than in others.

A sample of lot 7, stored in air-tight container at 27° C. with a moisture content of 9.6 per cent, had mold filament on more than half of the ergot, while another sample of the same moisture content and at the same temperature in loosely closed container had no mold filament and was in far better condition. The moisture content of the sample in loosely stoppered container decreased from 9.6 per cent to 8.5 per cent.

Some sign of mold was present in all the samples with a moisture content greater than 9 per cent regardless of the condition of storage. In some samples the mold was present as filaments readily recognizable, while in others only a thin grayish white coating was present on some of the ergots. No mold was present on any of the samples with a moisture content less than 6 per cent regardless of the storage condition.

The results before and after storage are given in Tables XII to VIII inclusive.

TABLE XII.—COLORIMETRIC AND U. S. P. XI ASSAY OF LOT 5 BEFORE AND AFTER STORAGE FOR SEVEN MONTHS IN HALF-FILLED, SEALED (AIR-TIGHT) CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	3.2	0.221	0.219	130	120
27	3.2	0.221	0.220	130	110
32	3.2	0.221	0.218	130	120
38	3.2	0.221	0.214	130	110
21	9.2	0.207	0.191	130	110
27	9.2	0.207	0.187	130	110
32	9.2	0.207	0.180	130	100
38	9.2	0.207	0.195	130	110

TABLE XIII.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 5 BEFORE AND AFTER STORAGE FOR SEVEN MONTHS IN LOOSELY-CLOSED MASON JARS.

Temperature ° C.	Moisture before Storage Per Cent.	Moisture after Storage Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	3.2	3.6	0.221	0.218	130	110
27	3.2	3.2	0.221	0.219	130	120
32	3.2	3.1	0.221	0.217	130	110
38	3.2	3.1	0.221	0.215	130	110
Room	3.2	4.3	0.221	0.218	130	120
21	9.2	8.9	0.207	0.197	130	100
27	9.2	8.1	0.207	0.199	130	110
32	9.2	7.8	0.207	0.196	130	120
38	9.2	6.9	0.207	0.193	130	100
Room	9.2	9.2	0.207	0.187	130	100

TABLE XIV.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 6 BEFORE AND AFTER STORAGE IN FILLED, SEALED (AIR-TIGHT) CONTAINERS FOR SEVEN MONTHS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	3.7	0.206	0.208	120	120
27	3.7	0.206	0.204	120	...
32	3.7	0.206	0.202	120	...
38	3.7	0.206	0.201	120	110
21	10.2	0.192	0.177	120	100
27	10.2	0.192	0.181	120	...
32	10.2	0.192	0.179	120	...
38	10.2	0.192	0.179	120	100

TABLE XV.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 6 BEFORE AND AFTER STORAGE FOR EIGHT MONTHS IN PAPER BAGS.

Temperature ° C.	Moisture before Storage Per Cent.	Moisture after Storage Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	3.7	3.8	0.206	0.204	120	120
27	3.7	..	0.206	0.209	120	...
32	3.7	..	0.206	0.204	120	...
38	3.7	..	0.206	0.203	120	110
Room	3.7	4.3	0.206	0.207	120	130
21	10.2	9.6	0.192	0.181	120	120
27	10.2	..	0.192	0.182	120	...
32	10.2	..	0.192	0.184	120	...
38	10.2	5.6	0.192	0.186	120	100
Room	10.2	10.1	0.192	0.180	120	110

TABLE XVI.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 7 BEFORE AND AFTER STORAGE FOR FOUR MONTHS IN FILLED, SEALED (AIR-TIGHT) CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	6.7	0.230	0.231	110	110
27	6.7	0.230	0.229	110	...
32	6.7	0.230	0.227	110	...
38	6.7	0.230	0.224	110	110
Room	6.7	0.230	0.228	110	120
21	9.6	0.223	0.216	110	100
27	9.6	0.223	0.203	110	...
32	9.2	0.224	0.216	110	...
38	9.2	0.224	0.212	110	110

TABLE XVII.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 7 BEFORE AND AFTER STORAGE FOR FOUR MONTHS IN LOOSELY-STOPPERED CONTAINERS.

Temperature ° C.	Moisture before Storage Per Cent.	Moisture after Storage Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	6.7	...	0.230	0.229	110	120
27	6.7	...	0.230	0.225	110	...
32	6.7	...	0.230	0.227	110	...
38	6.7	...	0.230	0.228	110	110
21	9.6	...	0.223	0.217	110	100
27	9.6	8.5	0.223	0.218	110	...
32	9.2	7.2	0.224	0.220	110	...
38	9.2	...	0.224	0.223	110	110

TABLE XVIII.—COLORIMETRIC AND U. S. P. XI ASSAYS OF LOT 8 BEFORE AND AFTER STORAGE FOR FIVE MONTHS IN FILLED, SEALED (AIR-TIGHT) CONTAINERS.

Temperature ° C.	Moisture Per Cent.	Alkaloids before Storage (Colorimetric) Per Cent.	Alkaloids after Storage (Colorimetric) Per Cent.	U. S. P. XI Assay before Storage Per Cent.	U. S. P. XI Assay after Storage Per Cent.
21	6.3	0.217	0.220	120	130
27	6.3	0.217	0.214	120	...
32	6.3	0.217	0.218	120	...
38	6.3	0.217	0.206	120	120
Room	6.3	0.217	0.217	120	...
21	9.7	0.209	0.203	120	100
27	9.7	0.209	0.205	120	...
32	9.7	0.209	0.20	120	...
38	9.7	0.209	0.195	120	...
Room	9.7	0.209	0.192	120	110

DISCUSSION.

The results recorded in the preceding tables tend to show that the deterioration of ergot with moisture content from 3.2 to 10.2 per cent stored in open and closed containers at 21, 27, 32 and 38° C. is very slight. The amount of deterioration was too small to be measured biologically. The differences in the results obtained by the U. S. P. XI Cock's Comb assay are within the limits of experimental error.

The deterioration of the alkaloids, determined colorimetrically, of the samples containing less than 7 per cent moisture, stored at 21, 27, 32 and 38° C. and at room temperature was negligible, regardless of the type of container used.

There was a slight deterioration of the alkaloids, determined colorimetrically, in the samples containing from 9.2 to 10.2 per cent moisture. The maximum deterioration of any sample was 13 per cent. The deterioration was generally proportional to the moisture content and the temperature.

This work tends to show that it is not necessary to store ergot in air-tight containers. At 27, 32 and 38° C., with moisture contents greater than 9 per cent, there was slightly less deterioration in loosely stoppered containers and paper bags than in air-tight containers. Since the moisture content of the samples in loosely stoppered containers and paper bags decreased, this probably explains why they deteriorated less than the samples in air-tight containers.

PART IV.

RELATIONSHIP OF ERGONOVINE TO THE TOTAL ALKALOIDS OF ERGOT AFTER STORAGE OF THE DRUG WITH VARIOUS MOISTURE CONTENTS AT DIFFERENT TEMPERATURES.

This part of the investigation resulted from the thought that possibly ergonovine may deteriorate faster than the other alkaloids of ergot. If this is true, neither the colorimetric determination of the total alkaloids nor the U. S. P. XI Cock's Comb assay will give any indication of this deterioration.

Unfortunately, the authors knew of no satisfactory method for the determination of ergonovine prior to storage. Since the deterioration of the samples of ergot at 21° C. containing less than 8 per cent moisture was almost negligible, it is hoped that the results are significant. Since the isolation of ergonovine (32), (33), (34), (35), (36), (37), (38), considerable interest has been manifested in this alkaloid, and it has been said that it is "the true active principle of ergot" (39). An isomer of this alkaloid has been reported by Smith and Timmis (40).

Trabucchi (41) reported a method for the determination of ergonovine in ergot preparations based on the principle that ergonovine is precipitated with picric acid only in strong concentrations, while the other alkaloids are precipitated completely in weak concentrations. This method was utilized in this work.

The tartaric acid solution of the alkaloids obtained in the colorimetric method described in Part I was treated with a saturated aqueous solution of picric acid (approximately 10 per cent of its volume) and filtered. The ergonovine was determined in the filtrate by the colorimetric method. Usually 1 cc. of the picric acid solution was added to 10 cc. of the alkaloidal solution; the precipitated alkaloids filtered off; 1 cc. of the filtrate mixed with 2 cc. of the Allport-Cocking reagent, and the color compared in a suitable colorimeter as described under the colorimetric method. If the color produced differed by more than 20 per cent from the standard, suitable dilutions were made of the Ergotoxine Ethanesulfonate solution. It was not found necessary to dilute the ergonovine solution. The ergonovine was calculated as anhydrous ergotoxine.

Samples from 6 of the lots of ergot, 1, 2, 3, 4, 5 and 6, were examined. The results are given in Table XIX.

TABLE XIX.—RELATIONSHIP OF ERGONOVINE TO THE TOTAL ALKALOIDS OF ERGOT AFTER STORAGE OF THE DRUG WITH VARIOUS MOISTURE CONTENTS AT DIFFERENT TEMPERATURES.

Lot No.	Temperature ° C.	Moisture Per Cent.	Period of Storage Months.	Total Alkaloids after Storage (Colorimetric) Per Cent.	Ergonovine (as Ergotoxine) after Storage (Colorimetric) Per Cent.	Ergonovine Per Cent of Total Alkaloids.
1	21	6.8	17	0.196	0.031	16
1	32	6.8	17	0.189	0.033	17
1	38	6.8	17	0.143	0.033	23
2	27	10.5	17	0.187	0.034	18
2	32	10.5	17	0.183	0.029	15
2	38	10.5	17	0.161	0.020	12
3	21	6.6	17	0.208	0.037	18
3	27	6.6	17	0.20	0.033	17
3	32	6.6	17	0.177	0.029	16
3	38	6.6	17	0.190	0.030	16
3	21	12.6	17	0.162	0.030	19
3	27	12.6	17	0.166	0.031	19
3	32	12.6	17	0.145	0.022	15
3	38	12.6	17	0.144	0.015	10
4	21	8.0	17	0.213	0.037	17
4	27	8.0	17	0.204	0.033	16
4	32	8.0	17	0.186	0.033	18
4	21	10.2	17	0.192	0.035	18
4	27	10.2	17	0.190	0.033	17
4	32	10.2	17	0.173	0.028	16
5	27	9.2	7	0.187	0.033	18
5	32	9.2	7	0.180	0.032	18
6	27	10.2	7	0.181	0.035	19
6	32	10.2	7	0.179	0.034	19

DISCUSSION.

The relationship of ergonovine to the total alkaloids remained remarkably constant, averaging 17 per cent of the total alkaloids (both calculated as ergotoxine). Only 3 samples deviated any appreciable amount from the average. These were the following: (1) a sample from lot 1, moisture content 6.7 per cent at 38° C., ergonovine 23 per cent of total; (2) a sample from lot 2, moisture content 10.5 at 38° C., ergonovine 12 per cent of total; (3) a sample from lot 3, moisture content 12.6 per cent at 38° C., ergonovine 10 per cent of total.

With the possible exception of two of the samples at 38° C. these results give no indication that ergonovine deteriorates any faster than the other alkaloids of ergot.

PART V.

RELATIONSHIP OF THE ALKALOIDS OF ERGOT TO THE COCK'S COMB ASSAY.

This part of the investigation resulted from apparent discrepancies between the colorimetric and Cock's Comb assays of ergot. This work was done in order to determine if there is any correlation between the two methods. There was also some doubt concerning the reliability of the U. S. P. XI method in determining the deterioration of ergot.

Many investigators (26), (27), (28), (29), (30) have reported a fairly good agreement between the colorimetric and the Cock's Comb assay of Fluidextract of Ergot. The Sub-Committee

on ergot of the British Pharmacopœia (31) reached the conclusion that the results by the biological method are lower than those obtained by chemical methods; the ratio of the average result is 2:1.

The authors observed during the early part of this investigation that samples of ergot which had around 0.2 per cent total alkaloids by the colorimetric method assayed only 100 to 130 per cent U. S. P. when a fluidextract was prepared according to the U. S. P. XI procedure. In order for a fluidextract of ergot to be U. S. P. XI it must contain active alkaloids corresponding to 0.05 per cent Ergotoxine Ethanesulfonate. Ergotoxine Ethanesulfonate contains 82 per cent ergotoxine. The fluidextract must then contain active alkaloids equivalent to 0.04 per cent anhydrous ergotoxine. Yet fluidextracts prepared from ergot assaying approximately 0.2 per cent colorimetrically showed only 0.04 to 0.06 per cent alkaloid (as ergotoxine) by the U. S. P. XI Cock's Comb method. If the fluidextracts contained most of the alkaloids of ergot, then only 20 to 35 per cent of the alkaloids of ergot (as ergotoxine) were active in producing a bluing of the cock's comb. Many investigators have reported chemical assays of samples of ergot containing from 0.15 to 0.25 per cent of alkaloids, but most of the chemical assays of fluidextract of ergot reported are below 0.1 per cent. The authors reasoned that the fluidextract does not represent the alkaloidal content of ergot, and experiments were performed to find out if this is true.

The total alkaloids were extracted from 4 fluidextracts, two prepared from samples from lot 5 and two prepared from samples from lot 6. The following process was used:

Five cubic centimeters of the fluidextract are diluted with 25 cc. of distilled water, rendered slightly alkaline with 3 per cent ammonia water and extracted with successive portions of 50, 35, 30 and 25 cc. of ether. The ethereal solution is extracted with 5 successive portions of 5 cc. each of a 1 per cent w/v solution of tartaric acid in water. A sixth extraction with a 4 cc. portion of the acid should give no precipitate with Mayer's reagent. Mix the aqueous liquids, transfer to a porcelain dish, remove the ether by gentle warming on a water-bath in a current of air and add sufficient water to produce 25 cc. or other suitable volume. The total alkaloids and the ergonovine content are then determined as described in Part I and Part IV.

The results obtained from the four fluidextracts are given in Table XX.

TABLE XX.—COMPARISON OF THE TOTAL ALKALOIDS OF ERGOT, TOTAL ALKALOIDS OF FLUIDEXTRACT OF ERGOT, ERGONOVINE CONTENT OF ERGOT AND ERGONOVINE CONTENT OF FLUIDEXTRACT OF ERGOT.

Lot No.	Total Alkaloids of Drug (Colorimetric) Per Cent.	Total Alkaloids of Fluidextract (Colorimetric) Per Cent.	Ergonovine (as Ergotoxine) from Drug (Colorimetric) Per Cent.	Ergonovine (as Ergotoxine) from Fluidextract (Colorimetric) Per Cent.	U. S. P. XI Assay of Fluidextract.
5	0.187	0.091	0.033	0.017	110
5	0.180	0.087	0.032	0.015	100
6	0.181	0.105	0.035	0.016	110
6	0.179	0.10	0.034	0.018	100

The results given in Table XX show that the fluidextracts of ergot contained approximately half of the alkaloids of ergot and about half of the ergonovine. Yet these fluidextracts assayed 100 to 110 per cent U. S. P. XI.

Experiments were then performed in order to obtain some comparison between the total alkaloids of ergot (colorimetrically) and the action of these alkaloids on the cock's comb.

The total alkaloids were extracted by the procedure used in the colorimetric method described in Part I. The tartaric acid solution of the total alkaloids was adjusted so that 1 cc. was equivalent to 0.5 mg. of ergotoxine as determined colorimetrically. When necessary the solution of the alkaloids was evaporated to suitable volume by gentle warming on a water-bath in a current of air.

Using cockerels standardized with a solution of Ergotoxine Ethanesulfonate containing 0.5 mg. per cc., the solutions of the total alkaloids were injected into these cocks and the potency of the total alkaloids determined.

The amount of Ergotoxine Ethanesulfonate necessary to produce a satisfactory bluing varied from 0.08 to 0.25 mg. per Kg. usually between 0.1 and 0.15 mg. per Kg. The quantity of total alkaloids necessary to produce the same effect as the standard was from 1.2 to 2 times the amount. These results were obtained from 6 lots of ergot using over 400 injections with 60 standardized

cockerels. These results tend to show that from 50 to 80 per cent of the total alkaloids of ergot are as active in producing a bluing of the cock's comb as Ergotoxine Ethanesulfonate. Therefore, 40 to 65 per cent of the total alkaloids are as active in producing a bluing of the cock's comb as ergotoxine.

The above results explain the difference between the colorimetric assay of the alkaloids of ergot and the U. S. P. XI assay of a fluidextract of ergot. When ergot was assayed by preparing a fluidextract and testing it on the cock's comb, the results were always less than half that obtained by assaying the total alkaloids of the drug by the same method. This is shown in Table XXI.

TABLE XXI.—RELATIONSHIP OF THE TOTAL ALKALOIDS TO THE COCK'S COMB ASSAY.

Average of Samples from Lot No.	Cock's Comb Assay of Total Alkaloids (Extracted from Drug) in Terms of U. S. P. XI Standard Per Cent.	Cock's Comb Assay of Fluidextract of Drug in Terms of U. S. P. XI Standard Per Cent.
3	210	100
4	240	110
5	300	130
6	300	120
7	250	110
8	260	120

The results of this part of the investigation tend to show that Fluidextract of Ergot prepared according to the U. S. P. XI procedure does not represent the alkaloidal content of ergot. The U. S. P. XI procedure seems to extract about half of the total alkaloids and about half of the ergonovine. The activity of the fluidextract on the cock's comb was less than half the activity of the total alkaloids of the drug when measured in the same manner and in comparable dilutions.

SUMMARY.

Twelve lots of ergot (35 Kg.) were used in this investigation. Samples of ergot were stored in completely filled, air-tight containers; half-filled, air-tight containers; ground, glass-stoppered bottles; completely and half-filled, not sealed, containers; wide-mouthed containers, stoppered loosely with cork and with pledget of cotton; and in paper bags. The samples were kept at constant temperatures ranging from 21° C. to 38° C., and at room temperature, for periods of time varying from three months to six years. The moisture contents were varied from 3.2 per cent to 12.8 per cent.

The results obtained in Part I show that ergot with moisture contents above 11 per cent stored at room temperature in ground, glass-stoppered bottles, deteriorated considerably in six years. This deterioration was in some cases enough to cause the drug to be worthless. One sample with moisture content of 8.3 per cent stored under the same conditions and for the same length of time was still fairly active, indicating that moisture content plays an important part in the keeping qualities of ergot.

The results obtained in Part II show that ergot stored in air-tight containers at 21, 27, 32 and 38° C. for seventeen months, with moisture contents from 6.6 to 12.6 per cent, deteriorated slowly. The amount of deterioration was generally proportional to the moisture content and the temperature. The amount of deterioration of the drug with moisture contents below 8 per cent at 21° and 27° C. was scarcely appreciable. Ergot with less than 8 per cent moisture at 32 and 38° C. for seventeen months deteriorated less than 16 per cent in all but one case. Ergot with moisture contents between 10.2 and 12.6 per cent deteriorated a maximum of 27 per cent as measured colorimetrically; a maximum of 30 per cent determined biologically.

The change in physical condition of ergot with moisture contents greater than 8 per cent was much more pronounced than the amount of deterioration as measured by the colorimetric or Cock's Comb assays. The samples with moisture contents greater than 10 per cent possessed a fracture that was flexible and mostly yellow-brown in color. The quantity showing this type of fracture was proportional to the per cent of moisture and the temperature. The color of the fracture was darker as the moisture content and temperature increased. The samples of the same lot showing more physical change also showed more deterioration of the alkaloids; yet the physical change was pronounced (all of the ergot of some samples possessed a fracture of a yellow-brown color), while the maximum deterioration of any sample studied during the seventeen month period was 27 per cent of the alkaloids (colorimetric) and 30 per cent of the activity of the Cock's Comb assay.

Ergot stored at 21, 27, 32 and 38° C. in air-tight containers with moisture contents greater than 6.6 per cent may show some sign of mold. The mold is of little importance unless the moisture content is greater than 8 per cent; close examination is necessary in order to discover the mold. A thin coating of mold, which causes the ergot to appear as if it had been dusted lightly with a grayish white powder, may be present. Ergot with moisture contents greater than 8 per cent may or may not have mold filaments. A sample with a higher moisture content and less mold may lose more alkaloids than a sample with a lower moisture content with more mold, if these samples are stored under the same conditions. The presence of a slight amount of mold is more objectionable from an esthetic viewpoint than from the standpoint of the deterioration of the alkaloids.

The moisture content of ergot stored in air-tight containers did not change. The difference was always within the limit of experimental error.

The results obtained in Part III tend to show that the deterioration of ergot with moisture contents from 3.2 to 10.2 per cent stored in open and closed containers at 21, 27, 32 and 38° C. and at room temperature for seven months is very slight. The amount of deterioration was too small to be measured biologically. The deterioration of the alkaloids, determined colorimetrically, of the samples containing less than 7 per cent moisture was negligible, regardless of the type of container. There was a slight deterioration of the alkaloids, determined colorimetrically, in the samples containing from 9.2 to 10.2 per cent moisture. The maximum deterioration of any sample in eight months was 13 per cent. The deterioration was generally proportional to the moisture content and the temperature.

This part of the investigation tends to show that it is not necessary to store ergot in air-tight containers. At 27, 32 and 38° C. with moisture contents greater than 9 per cent, there was slightly less deterioration in loosely stoppered containers and paper bags than in air-tight containers. Since the moisture content of the samples in loosely stoppered containers and paper bags decreased, this probably explains why they deteriorated less than the samples in air-tight containers.

The physical appearance of the samples with moisture contents greater than 7 per cent at 27, 32 and 38° C. in paper bags and loosely closed containers was better than the samples at the same temperature in air-tight containers. The difference in some cases was more marked than in others. This probably was due to the decrease in moisture content of the samples.

Some sign of mold was present in all the samples with a moisture content greater than 9 per cent regardless of the condition of storage. In some samples the mold was present as filaments readily recognizable, while in others only a thin grayish white coating was present on some of the ergots. No mold was present on any of the samples with a moisture content less than 6 per cent regardless of the storage condition.

Part IV of this investigation indicates that, with the possible exception of samples at 38° C., the ergonovine in ergot does not deteriorate any faster than the other alkaloids of the drug. The relationship of ergonovine to the total alkaloids remained remarkably constant, the ergonovine content averaging 17 per cent of the total alkaloids (both calculated as ergotoxine).

The results obtained in Part V indicate that Fluidextract of Ergot prepared according to the U. S. P. XI procedure does not represent the alkaloidal content of the drug. The U. S. P. XI procedure seems to extract about half of the total alkaloids and about half of the ergonovine. The activity of the fluidextract on the cock's comb was less than half the activity of the total alkaloids of the drug when measured in the same manner and in comparable dilutions.

Results obtained from 6 lots of ergot using over 400 injections with 60 standardized cockerels indicate that from 50 to 80 per cent of the total alkaloids of ergot are as active in producing a bluing of the cock's comb as Ergotoxine Ethanesulfonate; 40 to 65 per cent as active as ergotoxine.

The results of the investigation show that the colorimetric method is far more satisfactory for determining the amount of deterioration of ergot than the Cock's Comb method.

CONCLUSIONS.

1. Moisture content is the most important factor in the deterioration of ergot.

2. The results of this investigation indicate that it is not necessary to store ergot in air-tight containers.

3. The amount of deterioration of ergot in seventeen months, with moisture content below 8 per cent, is almost negligible if kept below 27° C.

4. The amount of deterioration of ergot in seven months at 21, 27, 32 and 38° C., with moisture content below 6 per cent, is negligible regardless of the type of container.

5. Ergot with moisture content from 8 per cent to 12.8 per cent deteriorates slowly at 21, 27, 32 and 38° C. The amount of deterioration is generally proportional to the moisture content and the temperature.

6. The change in physical condition of ergot with moisture content greater than 8 per cent is much more pronounced than the amount of deterioration as measured by the colorimetric or Cock's Comb assays.

7. The moisture content of ergot in air-tight containers does not change.

8. The ergonovine in ergot does not deteriorate any faster than the other alkaloids of the drug at 21, 27 and 32° C.

9. The U. S. P. XI procedure for Fluidextract of Ergot seems to extract about half of the total alkaloids and about half of the ergonovine. This should be further investigated.

10. The activity of Fluidextract of Ergot on the cock's comb is less than half the activity of the total alkaloids of the drug when measured in the same manner and in comparable dilutions.

11. Fifty to eighty per cent of the total alkaloids of ergot are as active in producing a bluing of the cock's comb as Ergotoxine Ethanesulfonate; 40 to 65 per cent as active as ergotoxine.

12. The colorimetric method is far more satisfactory for measuring the deterioration of ergot than the Cock's Comb method.

13. If it is desirable to keep ergot for several years with a minimum of deterioration, it is recommended that the moisture content be reduced to around 4 per cent and then stored in air-tight containers or in a dry place. The moisture content may be reduced to around 4 per cent if the drug is spread in a thin layer in a drying oven at 38° C. for about twenty-four hours.

14. If the drug is to be used in about a year a moisture content below 8 per cent will permit only a slight deterioration. If stored in a dry place the moisture content will decrease and there probably will be no deterioration.

15. There probably will be more deterioration in ergot with moisture content above 8 per cent in air-tight containers than in open containers; hence, with moisture content above 8 per cent, open containers are recommended.

ANNOTATED BIBLIOGRAPHY.

(1) Bott, W. J., Address at Official Meeting Am. Drug Manufacturers' Assoc., Washington, D. C., Dec. 5th to 8th, 1937.

(2) Paulizky, "Neues Magazin für Aerzte;" Barger, G., "Ergot and Ergotism" (1931).

(3) Hirschberg, A., *Arch. Pharm.*, April, 88, 89; *Am. J. Pharm.*, page 309 (1871).

(4) Ficinus, O., *Arch. Pharm.*, (Sept. 1873); *Am. J. Pharm.*, page 538 (1873).

(5) Gerrard, A. W., *Am. J. Pharm.*, page 331 (1875).

(6) Mourrut, *Repert. pharm.; J. therap; Am. J. Pharm.*, page 443 (1877).

(7) Zschiesing, G., *Pharm. Zeit.*, 49, 51; *Am. J. Pharm.*, page 457 (1881).

(8) Perrett, E., *Bull. gen. therap.*, 102, 202 (1882); Barger, G., "Ergot and Ergotism" (1931).

(9) Alpen, F., *Chem. Rep.*, 233 (1888); PROC. A. PH. A., 37, 430 (1889).

(10) Grünfeld, A., *Arb. pharm. Inst. Dorpat*, 8, 108–154 (1892); Barger, G., "Ergot and Ergotism" (1931).

(11) Zanon, *Nat. Drug.*, 139 (1893); PROC. A. PH. A., 42, 885 (1894).

(12) Keller, C. C., *Schweiz. Wochenschr. Chem. Pharm.* (1894); PROC. A. PH. A., 43, 545 (1895).

(13) Meulenhoff, J. S., *Nederland. Tijdschr. Pharm. Chem. Toxicol.*, 12, 225 and 257 (1900); Barger, G., "Ergot and Ergotism" (1931).

(14) Dahlin, T., *Apoth. Zeit.* No. 102 (1912); YEAR BOOK A. PH. A., 2, 160 (1913).

(15) Tate, G., *Pharm. J.*, 106, 485 (1921).

(16) Forst, A. W., *Arch exp. Path. Pharmakol.*, 114, 125 (1926).

(17) Burn, J. H., and Ellis, J. M., *Pharm. J.*, 118, 384 (1927).

(18) Thompson, M. R., JOUR. A. PH. A., 19, 436 (1930).

(19) Pedersen, O. Chr., *Norges Apotekerfor. Tids.*, 39, 49 (1931); *Chem. Abstr.*, 26, 3873 (1932).

(20) Pedersen, O. Chr., *Norges Apotekerfor. Tids.*, 40, 49 (1932); *Chem. Abstr.*, 26, 3873 (1932).

(21) Corran, P. F., and Rymill, F. E., *Quart. J. Pharm. Pharmacol.*, 8, 337 (1935).

(22) Rowe, L. W., JOUR. A. PH. A., 26, 312 (1937).

(23) Christensen, B. V., and Welch, A. D., *J. Pharmacol.*, 45, 183 (1931).

(24) Allport, N. L., and Cocking, T. T., *Quart. J. Pharm. Pharmacol.*, 5, 341 (1932).

(25) Smith, M. I., *Pub. Health Rept.*, 45, 1466 (1930).

(26) Smith, M. I., and Stohlman, E. F., *J. Pharmacol.*, 40, 77 (1930).
(27) Swanson, E. E., Powell, C. E., Stuart, E. H., Stevens, A. H., JOUR A. PH. A., 21, 229, 320 (1932).
(28) Swoop, D. F., Cartland, G. F., and Hart, M. C., JOUR. A. PH. A., 22, 8 (1933).
(29) Stevens, A. N., JOUR. A. PH. A., 22, 99 (1933).
(30) Smelt, E. M., *Quart. J. Pharm. Pharmacol.*, 6, 399 (1933).
(31) *Pharm. J.*, 127, 482 (1931).
(32) Thompson, M. R., Scientific Section, Washington Meeting, A. PH. A. (May 10, 1934).
(33) Dudley and Moir, *Brit. Med. J.*, 1, 520 (1935).
(34) Kharasch, M. S., and Legault, R. R., *Science*, 81, 388 (1935).
(35) Stoll, A., and Burckhart, *Compt. rend. acad. sci.*, 200, 1680 (1935); *Bull. sci. pharmacol.*, 42, 257 (1935); JOUR. A. PH. A., 24, 835 (1935).
(36) Jacobs and Craig, *Science*, 82, 16 (1935).
(37) *Science*, 83, 206 (1936).
(38) *Ibid.*, 83, 296 (1936).
(39) Thompson, M. R., JOUR. A. PH. A., 24, 748 (1935).
(40) Smith, S., and Timmis, G. M., *Nature*, 136, 259 (1935).
(41) Trabucchi, E., *Boll. soc. ital. biol. sper.*, 12, 232 (1937); *J. Soc. Chem. Ind.*, 56, 1132 (1937).

PHENYL AND NAPHTHYL URETHANES AND THE CORRESPONDING DI-SUBSTITUTED UREAS.*

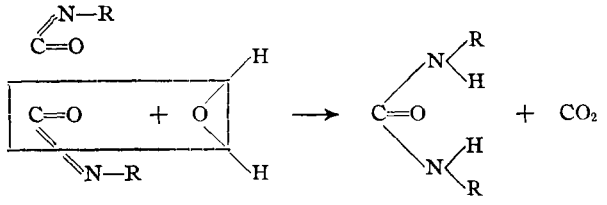
BY PAUL JANNKE.[1]

In a report on the study of *Gnaphalium obtusifolium* L., (1) it was stated that a phenyl urethane was prepared from a comparatively crude mixture of phenols. Though this derivative was easily prepared and readily separable from the reaction mixture, the naphthyl urethane of supposedly the same phenol was difficult to isolate from the unreacted substances and from di-α-naphthyl urea, a side reaction product.

Since the yield of di-α-naphthyl urea was so great when attempting to prepare the urethane of the unknown phenol, these questions arose:

(*a*) What precautions may be taken to prevent the formation of the di-substituted urea, and to assist the desired reaction? (*b*) What is the per cent of di-substituted urea contamination in the crystalline reaction product? (*c*) How might the urethane be separated from the di-substituted urea when the formation of the latter cannot be prevented?

It is a generally accepted fact that when an iso-cyanate comes into contact with moisture, the di-substituted urea is formed, and carbon dioxide is eliminated.



* Presented before the Scientific Section, A. PH. A., Minneapolis meeting, 1938.

[1] From the Laboratory of Edward Kremers.