

A CHEMICAL INVESTIGATION OF ERGOT OF RYE

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## INTRODUCTION

The published work relative to the phytochemistry of *Claviceps purpurea* is fairly voluminous. In recent years, however, no worker has undertaken a general chemical analysis of this drug with the view of gaining as much general information about it as possible. For this reason, it was considered that a summary of the literature together with experimental chemical work supplemented by pharmacological studies would be of value. Particularly, an effort has been made to confirm Stoll's method of isolating ergotamine from ergot as described in British Patent No. 125,396 of the Sandoz Chemical Co.

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## A CHEMICAL INVESTIGATION OF ERGOT OF RYE

### I. GENERAL

#### A. ORIGIN OF NAME

The name for the drug ergot of rye, possibly better known as simply ergot, is derived from the old French word "argot"<sup>(1)</sup>. This means both the spur of a cock, and smut, so that the fungus may have received its name either because it was like the spur of a cock or because it was something dirty. Other English names for it are spur, cockspur, cockspur rye, spurred or horned rye, eared rye, blasted rye, and others. The German names are likewise numerous; namely, Afterkorn, Mutterkorn, Schwarzkorn, Roggenkorn, Hahnensporn, Hungerkorn, and so on. The French names are also numerous, for example:- Bled avorte, bled cornu, clou de seigle, faux seigle, mere de seigle, seigle cornu, seigle corrompu, ergot de seigle. The Latin names are perhaps as numerous:- *Secale luxurians*, *Bauhin*, *Secalis mater*, *Thalius*, *Secale cornutum*, *Baldinger*, *Secale puerperale*, etc., and the Latinized name *ergota*.

Barger<sup>(2)</sup> lists several additional names. Italian: *Grano allogliato*, *grano cornuto*, *grano sprone*, *grano speronato*, *segala allogliata*, *segala cornuta*, *sperone di gallo*, *chiodo segalino*. Spanish: *Cornezuelo de centeno*. Portuguese: *Cravagem de centeio*. Rumanian: *Secara cornuta*. Danish: *Meldrøje*. Norwegian: *Meldrøie*. Swedish: *Mjöldryga*, *mjölöka*. Dutch: *Moederkoorn*. Russian: *Sporynia*.

B. BOTANY

The French botanist, Tulasne, was the first to clear up the botany of ergot<sup>(3)</sup> through his paper "Memorie sur L'Ergot des Glumacees" published in 1853. This work was extended and confirmed by Kühn<sup>(4)</sup>. Kirchhoff<sup>(5)</sup> has more recently confirmed this work by infection experiments with spores and by the cultivation of the fungus on artificial media.

According to Sharp<sup>(1)</sup>, ergot was looked upon as a rust or mildew as early as the times of Theophrastus of Lesbos (370 - 285 B.C.). Sharp states further: "Lonicer, Thalius, Caspar Bauhin, and John Ray all had some vague ideas about its botany. Others held ludicrous views, as, for example, Buffon (1707-1788) who said ergot was a collection of small animals like eels. Willdenow (1765-1812) in his 'Species Plantarum', showed that ergot could be produced by planting ergot in a rich moist soil. In his experimental work, De Candolle went further than Willdenow, for he showed that if the dust of ergot was sprinkled on a sound rye plant, the disease was produced. Fries regarded ergot as a fungus, which replaced the ovary of the rye, and Munchausen held much the same views." Quekett<sup>(6)</sup> in 1841 was the first to publish the results of a detailed study of ergot.

Due to the classical work of Tulasne and some of those following him however, there is no longer any doubt as to the life cycle of ergot, which is stated briefly as follows: The origin of the ergot sclerotium, developed on

the rye plant, is the fungus *Claviceps purpurea* which is parasitic during moist seasons on the ovary of grains, grasses and sedges of certain species. The development has three stages:

Stage 1. In the spring or early summer the spores of *Claviceps purpurea* are carried by the wind on to the flowers of various Gramineous plants. In the following, the rye plant only, is considered: Here the spores germinate and produce colorless hyphae, which cover the entire young ovary except its apex and penetrate the outer part of the pericarp. The covering material consists of a soft, white mass which gradually takes the place of the ovary and is known as the sphacelia. During this period a saccharine secretion, known as "honeydew", is produced by the hyphae and at the same time numbers of conidia (reproductive bodies) are formed, thus contributing to the further dissemination of the fungus by means of the insects attracted to the honeydew.

Stage 2. After the sphacelia has reached its full development the sclerotium is gradually produced at its base by the hyphae which now form a dense compact mass instead of a loose felt. The sclerotium grows and finally projects from the ear of rye (which has by now ripened), bearing on its apex the remains of the felt. In this compact form the fungus passes the winter.

Stage 3. The ergot falls to the ground and in the following spring sprouts into several long stalked globular heads called stromata or ascocarps. Each fruiting head or ascocarp has imbedded in its surface several flask-shaped cavities,

called perithecia, from the bases of which several sacs or asci develop from the ends of hyphae. Within each ascus are developed 8 threadlike spores (ascospores) which, when the ascus ruptures are discharged and are carried by the wind to the rye flowers which must be open to receive them. In this manner the cycle is completed.

### C. THERAPEUTICS

Sharp<sup>(1)</sup> states that the wise women of the Highlands of Scotland employed ergot (of bere or barley probably) from time immemorial. According to Flückiger and Hanbury<sup>(7)</sup> no undoubted reference to ergot has as yet been discovered in the writings of the classical authors. The first clear account of the therapeutic properties of ergot was given in 1582 by Adam Lonicer<sup>(8)</sup> who, in his Kreuterbuch, mentions its value in hastening labor. Lonicer's views seem not to have been generally known for many years later.

In 1747 Rathlaw<sup>(9)</sup>, a Dutch accoucheur, employed ergot as a means of rapidly ending lingering labor. He kept this drug a secret, although he admitted learning of the properties of the drug from midwives. It did not take long, however, for others to learn of the virtues of the drug. Levret, a Parisian accoucheur, and Lachapelle and Dupille<sup>(9)</sup>, Parisian midwives, are said to have used ergot and believed in its efficacy. Thirty years after Rathlaw's use of ergot, Desgranges<sup>(7)</sup> of Lyons prescribed it with success. The peculiar and important properties of ergot were hardly recognized until the beginning of the 19th century,

when due to the efforts of Stearns<sup>(10)</sup>, in the United States, its use quickly spread. In 1807 Dr. Stearns of Albany, New York wrote that he had for some time used a "pulvis parturiens", which quickly terminated lingering labor. Prescott, Bigelow, Chapman, Hosack, and others of his fellow practitioners, were not slow to profit by Stearns' hint, and very soon ergot was extensively tried on the American Continent<sup>(1)</sup>.

Ergot was admitted for the first time into an official publication when it was included among the substances of secondary importance, in the United States Pharmacopoeia of 1820. The admission of the drug into the first edition of this publication would thus seem to be due in great part at least to the efforts of the above-mentioned pioneers of the use of the drug in this country. Barger states that<sup>(2)</sup> "the drug was only admitted to the Pharmacopoeia of the London College of Physicians in 1836. According to the French Codex of 1839 ergot was required to be kept in all pharmacies, and Jourdan in his 'Pharmacopée universelle' of 1840 mentions that, besides being official in America, England and France, it had been included in the Pharmacopoeias of Schleswig-Holstein (Kiel 1831), Turin (1833) and Greece (1837). From the date of Stearns' publication it took about 30 years for ergot to become thoroughly established in Europe."

#### D. TOXICOLOGY

According to Barger<sup>(11)</sup> various authors have attempted to show that ergot and ergotism were known to the ancients. This, as he shows in the following quotation is open to question, however. "For an accurate description of undoubted ergotism we cannot

go back further than the second half of the 16th century, the same period which also gave us the first unmistakable descriptions of ergot itself." For a long time, cause and effect were not connected and epidemics occurred even as late as in the 19th century. Barger states<sup>(11)</sup> that the first undoubted outbreaks of ergotism took place in Germany in 1581, 1587, and 1596. The last is particularly famous because of its accurate description by the Medical Faculty of the University of Marburg<sup>(11)</sup> published in 1597.

Barger<sup>(11)</sup> attributed the absence of great epidemics after 1777 to the following causes:

- (1) Improvements in agriculture.
- (2) Introduction of the potato as an article of diet of the poor in the North.
- (3) Introduction of maize in the South.

To these reasons might be added the improvements in milling grain which have been developed.

Small epidemics of ergotism, however, occurred during the 19th century in Russia, Sweden, and Finland. Sporadic cases also occurred in France and Germany. The last large outbreak of ergotism in the United States occurred in New York in 1825<sup>(11)</sup>. Ehlers<sup>(12)</sup> mentions an epidemic at Tomsk, in Siberia, as late as 1883. A mild but extensive epidemic of ergotism in England was reported within the last 5 years by Morgan<sup>(13)</sup>. The victims were immigrants from Central Europe who lived on rye bread.

The toxicology of ergot is summarized<sup>(14),(15),(16)</sup> briefly as follows:

1. Types of Poisoning

a. Acute ergot poisoning

This occurs infrequently; usually in cases of attempted abortion. The symptoms are: Collapse, with a weak rapid pulse, tingling, itching, and coldness of the skin, unquenchable thirst, vomiting and diarrhoea, confusion or unconsciousness, hemorrhage from the uterus and occasional abortion; sometimes death from respiratory paralysis.

b. Chronic poisoning or ergotism

The symptoms of ergotism are of 2 kinds: Those of gangrene and those of nervous disorders.

(1) The gangrenous form

Appearance of pustules, due to defective circulation. In more severe cases the extremities are affected. The entire member may be affected in the gangrene, which has its line of demarcation: the finger, toe, or limb may slough away without bleeding.

(2) The spasmodic or neural form

The first symptoms are depression, weakness and drowsiness, often with headache and giddiness, painful cramps in the limbs and itching with pustulation of the skin. In severe cases paroxysmal convulsions set in, generally clonic, and often epileptiform, but leaving contractures

in the limbs or, less often, in the trunk muscles.

Treatment is hygienic and symptomatic.

## E. PHARMACOLOGY

### 1. Action of the Drug

#### a. On the Uterus

According to Meyer and Gottlieb<sup>(17)</sup> preparations of ergot along with pituitary extract and quinine, are considered the most important practical oxytocics, i.e., drugs to stimulate uterine movements. Ergot preparations stimulate the uterus especially when pregnant. In labor, the ordinary doses improve the uterine tone, and collect small irregular pains into larger effective contractions.<sup>(18)</sup> According to Sollmann<sup>(16)</sup> small doses may inhibit the contractions while excessive doses produce prolonged tonic contraction. He also states: "when proper doses are used during labor, with the os fully dilated and no obstruction, they tend to hasten normal delivery".

Really effective doses are rather dangerous, however, because the firm contraction may hinder delivery, and compress and asphyxiate the fetus.<sup>(16)</sup> McGuigan<sup>(18)</sup> states that because of the uncertainty of the drug's action, and because of the possibility of its causing prolonged tonic contractions of the uterus, ergot should only be used after the uterus has been emptied. It is then used in hastening the uterus to contract to its



normal size and to prevent hemorrhage. Ergot, McGuigan continues, has the disadvantage of producing severe uterine pains and is now much less used than formerly.

(1) Mechanism of Uterine Action

Sollmann<sup>(16)</sup> states that this is peripheral since it occurs also in the excised uterus.

(2) Relation to Tone and Calcium Salts in Excised Uterus

According to Sollmann<sup>(16)</sup> "Calcium excess, which depresses the tone of the virgin guinea pig uterus, increases the response to the contractor action of ergot".

b. On Blood Pressure

Cushny<sup>(14)</sup> and Sollmann<sup>(16)</sup> both indicate that when an ergot preparation (containing the usual proportion of active alkaloids and amines) is injected into a vein there occurs some fall in blood pressure followed by a rise above normal. A preparation containing but little of the specific alkaloids and a large proportion of histamine would naturally fail to increase the blood pressure because histamine dilates the capillaries.<sup>(14)</sup> In any case, unless the ergot preparation be injected into a vein, the blood pressure of man or animals will remain practically unchanged.<sup>(16)</sup>

c. Ergot as General Styptic

Bush<sup>(15)</sup> and Sollmann<sup>(16)</sup> state that ergot has been used as a general styptic. It is very unlikely that ergot in this capacity has any virtue for, as Bush points out, uterine

hemorrhage is checked by ergot because the uterine walls are made to so forcibly contract as to compress the leaking blood vessels and sinuses from the outside. Hemorrhages elsewhere would have to be checked by the action of ergot upon the walls of the blood vessels and since all blood vessels of the splanchnic area would be acted upon similarly, there would result a general rise in blood pressure which would cause an increase in hemorrhage.

d. Cyanosis of the Combs of Fowl

Ergot preparations administered to roosters cause the tips of the comb and wattles to become cold and blue. This effect may be preceded by temporary blanching. According to Sollmann<sup>(16)</sup> this effect is produced by ergotoxine and ergotamine. Histamine produces a similar but much prompter change, while the tyramine is ineffective.<sup>(16)</sup>

This reaction of ergot upon the comb and wattles of a certain type of rooster is made use of in a method of ergot assay described later.

e. Gangrene Action

If ergot is given repeatedly to roosters, the bluing persists in the tips of the comb, and gradually the entire comb and wattles are involved. If the doses are continued these parts may fall off.<sup>(16)</sup> A similar gangrene may attack the legs, tongue or wings.<sup>(14)</sup> According to Cushny<sup>(14)</sup> the gangrene of ergot poisoning arises from prolonged constriction of the vessels by the ergotoxine and ergotamine.

The susceptibility of different animals to the gangrene action of ergot varies.<sup>(16)</sup> Pigs react by pustular eruptions on the skin, and their ears are also particularly susceptible. Horses and cattle show similar changes, while dogs and rabbits are not affected.<sup>(16)</sup>

f. Action on the Alimentary Canal

In brief, Sollmann<sup>(16)</sup> describes this as follows: "Injection of ergot or its active alkaloids results in marked peristalsis throughout the whole alimentary tract. The sensitivity to distention is increased as is also the intestinal motor response to vagus stimulation. This produces violent diarrhea. The emesis, however, is already largely central."

g. Action on the Central Nervous System

Here Sollmann<sup>(16)</sup> summarizes as follows: The central nervous system is affected both directly and indirectly. Small doses may cause medullary stimulation; (vagus slowing; increased respiration; convulsions; etc.); moderate doses increase the flow through the cerebral vessels; large doses of ergot cause death by medullary paralysis.

Ergot has a narcotic effect on roosters.<sup>(16)</sup>

2. Action of the Constituents

The chief active constituents of ergot are the alkaloids ergotoxine and ergotamine and the non-specific amines histamine and tyramine. The base acetyl choline is also present but in negligible quantity. In addition to the above there are two

other alkaloids present which have a small fraction of the activity of ergotoxine and ergotamine; these comparatively inactive alkaloids are ergotinine and ergotaminine. A fifth alkaloid, psi-ergotinine, is also present, having just recently been isolated.

a. Ergotoxine and Ergotamine

These alkaloids are responsible for the characteristic action on the cock's comb and are concerned in the uterine and vascular effects.

Both have been shown to possess, for all practical purposes, identical pharmacological action, by Dale and Spiro<sup>(19)</sup> Burn and Ellis<sup>(20)</sup> also concluded that the two alkaloids have the same activity. Rothlin<sup>(21)</sup> found that the results of his experiments with ergotamine were similar to the results of Dale<sup>(22)</sup> with ergotoxine. The chief difference found by Rothlin was that ergotamine did not paralyze the respiratory center to the same extent as ergotoxine. In 1932 Moir<sup>(23)</sup> on the basis of comparative clinical investigations on the human uterus, arrived at the same general result as his predecessors did in the laboratory.

Pattee and Nelson<sup>(24)</sup> found a slight difference between the two alkaloids; 0.40 mgm. ergotoxine being equivalent to 0.45 mgm. of ergotamine. These results are substantiated by the work of Wokes and Crocker<sup>(25)</sup> who find "Biologically ergotamine appears to possess 0.8-0.9 the activity of ergotoxine, whilst ergotinine and ergotaminine possess about 0.1

this activity", and by the results of Swoap, Cartland and Hart<sup>(26)</sup> who find that 1 mgm. ergotoxine is equivalent to 1.25 mgm. ergotamine. Lozinski, Holden and Diver<sup>(27)</sup> go further and state that 1 mgm. of ergotoxine is equivalent to 1.66 mgm. of ergotamine. They also suggest this as the reason for discrepancies in cases where ergotamine or one of its salts was used as standard with the assumption that the strength of the standard was equal to a like amount of ergotoxine or a corresponding ergotoxine salt.

Rothlin<sup>(28)</sup> has recently (1933) brought forth evidence showing that there are other pharmacological differences between the two alkaloids. He states that ergotoxine injected intravenously or subcutaneously into the rabbit in suitable doses (0.5 to 1.5 mgm. per Kg.) regularly produces characteristic symptoms of excitement and appearance of convulsions accompanied by hyperthermia. Ergotamine produces in the same small doses either hypothermia or no change, the stronger doses, by intravenous injection, produce lesser hyperthermia and stimulation. In still larger doses, continues Rothlin, the same symptoms appear as with ergotoxine. From his experiments Rothlin concludes that on the average, ergotoxine is twice as powerful as ergotamine as far as fever-producing and convulsion-producing capacity is concerned. Rothlin also shows that orally or rectally, results of this type were negative except in doses of 10-20 mgm. per Kg.

b. Ergotinine and Ergotaminine

Both of these alkaloids are weak in their actions. Their activity relative to the two potent alkaloids, ergotoxine and ergotamine, is given in the table below as determined by Rothlin<sup>(29)</sup> in 1928.

c. Psi-Ergotinine

Has only recently been discovered<sup>(30)</sup> and its pharmacological activity has not been fully investigated.

<u>Alkaloid</u>	<u>Activity</u>
Ergotamine	1
Ergotaminine	1/10
<hr/>	
Ergotoxine	1
Ergotinine	1/200 - 1/300
<hr/>	

Table I.

The tartrates are shown in Table II.

<u>Salt</u>	<u>Activity</u>
Ergotamine tartrate	1
Ergotaminine tartrate	1/6
<hr/>	
Ergotoxine tartrate	1
Ergotinine tartrate	1/100
<hr/>	
Ergotamine tartrate	1
Ergotoxine tartrate	1
<hr/>	

Table II.

d. Histamine<sup>(31)</sup>

Lowers the blood pressure and powerfully stimulates the excised uterus. According to Thompson<sup>(32)</sup>, histamine is the only non-specific amine in ergot occurring in sufficient quantities to cause appreciable pharmacodynamic activity.

e. Tyramine

Has a pressor effect.<sup>(33)</sup> The amount present in liquid extracts of ergot is much too small to be of practical importance, however.

f. Acetyl-Choline

Lowers the blood pressure.<sup>(34)</sup> This compound is also usually present in negligible quantity.

3. Administration

According to Sollmann<sup>(16)</sup> the usual dosage is 2 to 5 cc., 1/2 to 1 drachm of the fluidextract, or the equivalent of the solid extract which is eight times as strong. The response to ergot is prompt even when taken by mouth. It appears within 15 to 30 minutes and lasts about one-half hour.

Burn<sup>(35)</sup> states that powdered defatted ergot, enclosed in capsules, may also be used medicinally to maintain a concentration of active alkaloid in the circulation during puerperium, an initial dose of the alkaloid having been injected under the skin after delivery of the placenta.

C. METHODS OF STANDARDIZATION

1. PHYSICAL

a. Spectrophotometrically<sup>(36), (37), (38)</sup>

This method is accurate quantitatively only if the alkaloid is present as an individual in the pure state. For routine purposes it is not of much use.

2. CHEMICAL

a. Gravimetric

(1) Keller-Fromme<sup>(39)</sup>

(2) Forst<sup>(40)</sup>

(3) Oettel-Forst<sup>(41)</sup>

b. Volumetric

(1) German Pharmacopoeia of 1926

c. Nephelometric

(1) Tukats<sup>(42)</sup>

d. Colorimetrically

(1) Smith<sup>(43)</sup>

e. Semi-quantitative

(1) Tschirch<sup>(44)</sup>

(2) Hering<sup>(45)</sup>

(3) Oettel<sup>(46)</sup>

(4) Küssner and Wolff<sup>(47)</sup>

All of the chemical assay methods determine the physiological potency of ergot in terms of the total alkaloids. In accordance with these procedures, the drug is usually extracted with ether to obtain the alkaloids which are then removed from the ether with acid. The oil present in the crude drug usually causes



troublesome emulsions unless the oil is first removed. The slight solubility of the alkaloids in mineral acids further complicates matters in some procedures.

In the gravimetric methods there is always the objection that the crude alkaloids when weighed, still contain impurities. Since there is no actual way of checking this and since the total alkaloids form such a small percentage of the drug, it is readily seen that even a very small amount of impurity could be responsible for a considerable margin of error.

In the titration of the alkaloids as in the current German Pharmacopoeial Method, ammonia is preferable to sodium carbonate as alkaloidal precipitant because, the deposited soda is not always completely washed out, and, in voluminous precipitates especially, traces of soda often remain.<sup>(40)</sup>,<sup>(41)</sup>

Wessel<sup>(48)</sup> proposes in this process that the alkaloids be weighed, rather than titrated, because the total amount of acid used is 0.5 cc. and this gives opportunity for greater unavoidable error than by weighing the crude alkaloids as in the gravimetric methods.

The nephelometric method<sup>(42)</sup> is based on the turbidity arising from the addition of Mayer's reagent to the alkaloidal salts in 0.5% tartaric acid solution. The method is said to give results agreeing closely with results obtained by the current German Pharmacopoeial Method. Only one gram of crude ergot is necessary for the evaluation.

The colorimetric method of Smith<sup>(43)</sup> is based on the color reaction of Van Urk<sup>(49)</sup>, which in turn is a modification

of the general reaction for indole derivatives. Smith's method evidently offers much that has been lacking in the way of a chemical test, if one may be permitted to judge by the numerous papers of various authors that have appeared concerning it, since the introduction of the method in 1930. This method, as do all the other chemical methods, estimates the potency of the ergot preparation in terms of total alkaloids.<sup>(50)</sup> Briefly, Smith's procedure<sup>(43)</sup> is as follows:

"Five cc. of the fluidextract are evaporated on the water-bath under a current of air or in vacuo to remove the alcohol. Excess heating should be avoided. The thick, syrupy residue is transferred quantitatively with the aid of about 50 cc. H<sub>2</sub>O to a separatory funnel. The aqueous suspension is rendered slightly alkaline with NH<sub>4</sub>OH to a distinct blue reaction with litmus. About 2 cc. of 1:10 concentrated NH<sub>4</sub>OH will generally suffice. The solution is then extracted with four successive portions of ether, using 40 cc. in the first, 25 cc. in the second and 15 to 20 cc. in the third and fourth. The ethers are then united, returned to the separatory funnel and washed two or three times with 25 cc. H<sub>2</sub>O and a few drops of NH<sub>4</sub>OH. This treatment removes most of the yellow alkali-soluble pigment which is present in greater or less amount in most ergots. One or two additional washings with water will remove the excess alkali. The washed ether is made up to 100 cc. and may be kept in this condition, if well stoppered and protected from light, for many weeks.

"To complete the determination, 50 cc. of the ether containing the alkaloids of 2.5 cc. of the fluidextract of ergot are extracted in a separatory funnel three times with an aqueous solution of one per cent tartaric acid, using 10, 10 and 5 cc., respectively. The acid solution is freed from ether by evaporating it on the water-bath under the electric fan to about 15 cc. and is made up to volume (20 cc.).

"Two tubes of 1 and 2 cc., respectively, of this solution are prepared and to the first 1 cc. H<sub>2</sub>O added. To both tubes 1 cc. of M/60 para-dimethyl-amino-benzaldehyde in concentrated H<sub>2</sub>SO<sub>4</sub> are added. The tubes are all exposed to direct sunlight for 30 minutes. They are then compared with standards containing 0.06, 0.08 and 0.10 mg. of ergotamine tartrate in a colorimeter. From these results the number of mg. per cc. of ergot alkaloids can be calculated."

This method in slightly modified form was recommended to the Pharmacopoeial Revision Committee of Great Britain by its Sub-Committee on Ergot and as a result, the modified form became official in the 1932 edition of the British Pharmacopoeia. The modification which they adopted, insofar as the actual colorimetric test is concerned, is one intended to remove the source of error which concentrated sulphuric acid introduces; namely, the development of excessive heat and subsequent destruction of alkaloid, that is, unless temperature control is provided. (50)

Specifically, M. I. Smith uses 1 cc. of a reagent consisting of a 0.25 per cent. W/V solution of p-dimethylaminobenzaldehyde in concentrated sulphuric acid, and 2 cc. of the alkaloidal solution. In the British Pharmacopoeial method, however, in order to avoid overheating, Smith's reagent has been diluted with an equal volume of water and 2 cc. of reagent is used in the test instead of 1 cc. The strength of the alkaloidal solution has been doubled and 1 cc. is used instead of 2 cc. In order to produce the maximum color on exposure to bright light, some rise in temperature is necessary, consequently a temperature of 45°C. was adopted, since it was the amount of heat which gave the best results.

The length of time necessary for the production of the desired blue-violet color varies, on a bright day, from ten minutes to two hours.<sup>(51)</sup> Thus it can be seen that in winter, difficulties would arise when one desired to make certain that the full color had developed. According to Allport and Cocking<sup>(51)</sup>, ordinary artificial light is not suited for the purpose, but a mercury vapor lamp or a carbon arc is a satisfactory source of light.

As a result of their experimental work on the colorimetric test, however, Allport and Cocking<sup>(51)</sup> have developed a modification of the British Pharmacopoeial procedure wherein light is not necessary for the development of the color. They accomplish this by making a reagent as follows: ".....0.125 per cent. W/V of p-dimethyl-amino-benzaldehyde in 65 per cent

sulphuric acid (V/V) to which 0.1 per cent(V/V) of a 5 per cent solution of ferric chloride is added (=0.005 per cent W/V FeCl<sub>3</sub>) . Two millilitres of this reagent was added to one millilitre of the alkaloidal solution....." These authors also show that the presence of as little as 4 parts per million of peroxide in the ether causes a decrease of about 5 per cent in the color value. Consequently they emphasize the importance of using pure anesthetic ether for the extraction of the alkaloids as specified in the British Pharmacopoeia. Allport and Cocking<sup>(51)</sup> obtain very close agreement between results obtained by their modification and that of the official method. Others<sup>(52),(53),(54),(27)</sup>, have also reported very favorably on this modification. Smelt<sup>(52)</sup> in addition to reporting favorably on the above modification also states that the temperature of 45°C., specified in the British Pharmacopoeia, is attained on mixing the liquids in the Allport-Cocking modification due to the increase in acid strength over that in the official process, and thereby requires no additional heat. Smelt recommends, however, that 15 minutes be allowed for the color to reach its maximum instead of one minute as stated by the originators of the modification.<sup>(51)</sup>

In some cases difficulty has been experienced in the British Pharmacopoeial assay due to emulsification. To remove this difficulty Smelt<sup>(52)</sup> proceeds as follows:

"Five millilitres of the liquid extract was diluted with 25 millilitres of distilled water, rendered slightly alkaline with about 0.7 millilitre of dilute solution of ammonia, and extracted with successive portions of 40, 35, 30, and 30 milli-

litres of ether. It was found advisable not to shake too vigorously during the first extraction with ether, but after this the mixtures could be shaken vigorously. The ether solution was washed as directed in the British Pharmacopoeia and then extracted 5 times with successive portions of 4 millilitres (or, for strong extracts, 5 millilitres) of 1 per cent. W/V solution of tartaric acid. After removal of the dissolved ether, the acid solution was made up to 20 millilitres (or, for strong liquid extracts, 25 millilitres)."

Swoap, Cartland, and Hart<sup>(26)</sup> make provision against emulsification by allowing the alcohol in the original 5 cc. of fluidextract to remain. This alcohol, they claim, is of advantage in the separation of emulsions.

Stevens<sup>(54)</sup> uses a Watkins extractor<sup>(55)</sup> to insure against emulsions.

Smelt<sup>(52)</sup> finds that dilution with water and comparison in Nessler tubes gives results comparable with those obtained by her with a Klett colorimeter. This would tend to simplify the apparatus necessary for the test if it is really of general application.

Upsher Smith<sup>(53)</sup> recommends the use of a copper solution as a "super-standard" in the use of the colorimetric test. He uses an ammoniacal cupric sulphate solution containing 0.67 per cent of chemically pure crystalline cupric sulphate, or the equivalent of the dessicated salt, to match a freshly prepared solution of ergotoxine ethanesulphonate containing 0.1 mgm. of anhydrous ergotoxine in 1 cc. He states that the

similarity in color of the copper solution to that of the standard is remarkably close, regardless of whether the British Pharmacopoeial or the Allport-Cocking reagent be used. It is best, however, to match the colors in daylight "using a Bausch and Lomb Duboscq colorimeter". In a series of experiments Smith<sup>(53)</sup> matches the ergot samples (a) against a freshly prepared British Pharmacopoeial (Hereinafter, B.P.) ergotoxine solution treated with the B.P. reagent; and (b) against an ammoniacal copper sulphate solution containing 0.67 per cent of chemically pure crystalline cupric sulphate. In another series of experiments, the B.P. reagent was replaced by the Allport-Cocking reagent (which requires no light for the development of the color), using the same samples of ergot. The two sets of experiments show a fairly close agreement and Smith suggests that the copper standard may have a useful function in that it serves to check the ergotoxine standard against a fixed standard, thus guarding against the use of an ergotoxine salt or solution that has deteriorated, and it provides an easily obtainable check of one's results.

Upsher Smith<sup>(53)</sup> also tried the use of a 2 per cent solution of vanillin in concentrated sulphuric acid (free from nitric acid) in place of the B.P. reagent as proposed by Freudweiler<sup>(56)</sup> Equimolecular proportions of ergotoxine, ergotamine, and ergotinine are stated to give the same degree of color.<sup>(56)</sup> Smith found that 65 per cent V/V sulphuric acid was more favorable because it developed less heat and with this substitution in Freudweiler's proposed test, Smith obtains results which are in fair agreement

with results obtained in the B.P. method and the Allport-Cocking modification. Upsher Smith states, however, that he prefers the Allport-Cocking modification because the Freudweiler reagent "produces a color which is hard to match in the Duboscq colorimeter, either by daylight or by artificial light, showing rose red, and lacking the bluish or purplish tint of the ergotoxine solution". Allport and Cocking<sup>(51)</sup> tried the effect of replacing the p-dimethyl-aminobenzaldehyde by other aldehydes, and it was observed that a large number of these produced a color with ergotoxine in the presence of a mineral acid when exposed to bright light. According to their observations the color would not develop in any case unless exposed to bright light. None of these aldehydes, among which were vanillin, piperonal, paraldehyde, benzaldehyde, etc., showed any advantage over the p-dimethylaminobenzaldehyde and, in fact, many were less sensitive.

In comparing results obtained on testing ergot by the Broom-Clark biological method, that is, the Broom-Clark method and its various modifications, with results obtained on testing the same sample of ergot by Smith's colorimetric method and its modifications, it is seen that practically all writers have reported favorably with respect to the use of the colorimetric method and its modifications as quantitative assay procedures. For example, the reports of Smith and Stohlman<sup>(57)</sup>, Swoap, Cartland, and Hart<sup>(26)</sup>, Stevens<sup>(54)</sup>, and the report of the Ergot Sub-Committee<sup>(50)</sup> of the British Pharmacopoeial Revision may be mentioned.



The latter report is of particular interest at this point. The committee believes from the evidence before it that 60 to 70 per cent of the total alkaloids consist of the active alkaloid ergotoxine. For this reason the committee recommended a total alkaloidal percentage of 0.05 which corresponds to an ergotoxine percentage of 0.03, the desired potency of the official extract as measured by the B.P. color test. The actual reasons for the committee's recommendation are readily apparent from the first 4 of their conclusions in the summary of their report:

"1. That ergotoxine is to be regarded as the active principle for which ergot preparations are administered, and that all preparations should be standardized from this viewpoint."

"2. That the colorimetric method carried out as recommended permits an accurate estimation of total alkaloid in ergot and in liquid extract of ergot."

"3. That the colorimetric method has advantages over gravimetric methods in that smaller amounts of material suffice and the assay is more quickly completed."

"4. That the biological methods are subject to a considerable margin of error and the evidence is that this margin is at least as great as that due to the variation in the relative proportion of ergotoxine and ergotinine in the total alkaloid."

SEMI-QUANTITATIVE METHODS;

Tschirch<sup>(44)</sup> was the first to propose one of this kind. His method proceeds essentially as follows: Extract 1 gram of drug with 20 cc. of ether, 20 cc. of water and 10 drops of ammonia. After gentle shaking and the elapse of 2 hours separate the ethereal solution and evaporate. Dissolve the residue in glacial acetic acid. Pour under the glacial acetic acid some sulphuric acid containing ferric chloride so as to form 2 layers. A blue coloration at the junction of the 2 layers shows a minimum of 0.02 per cent alkaloid.

Harmsma<sup>(36)</sup> reports favorably on this procedure, having found it more delicate than Tschirch indicated.

Hering<sup>(45)</sup> proposed a somewhat similar method: Prepare an infusion from 1 part of ergot powder (defatted) and 20 cc. of distilled water to which 1 drop of hydrochloric acid has been added. Filter off 4 cc. (= 0.2 g. powdered ergot), treat with 1 drop of aqueous ammonia and shake strongly with 10 cc. of ether. Pipette 5 cc. of the clear supernatant ether into about 3 cc. of pure sulphuric acid. This should produce a cornflower-blue color at the junction of the 2 layers.

Küssner and Wolff<sup>(47)</sup> published a volumetric method wherein the property of the alkaloids to form difficultly soluble crystalline salts with picric acid is made use of: Extract twenty-five grams of coarsely powdered drug by shaking for 1 hour with 120 grams of chloroform in a cork-stoppered 150 cc. flask. Filter. Evaporate the chloroform from 60 grams of the filtrate by placing on a water bath. Stir constantly. Cool, take up the

residue in 15 cc. of ether and filter into a glass cylinder graduated in tenths of a cubic centimeter. Wash dish and filter with sufficient ether to make 24 cc. Then add 1 cc. of a 5 per cent solution of picric acid in benzol. This makes a total volume of 25 cc. The quantity of precipitate formed is in definite proportion to the amount of alkaloid present. The potency of the drug is figured on the following basis: 1.6 cc. of precipitate corresponds approximately to the minimum requirement of the German Pharmacopoeia, that is, 1.6 cc. = 0.05% alkaloids, and 2 cc. = 0.06% etc.

Oettel<sup>(46)</sup> has proposed the following approximate method: Prepare a fluidextract from 5 grams of drug by percolation with 15 to 20 cc. of 50 per cent alcohol. Evaporate the liquid extract to 5 cc. below 40°C., and make alkaline with about 5 drops of 10 per cent sodium carbonate solution. Shake for 10 minutes with 20 cc. of ether. Wash the ethereal liquid three times with 10 cc. of water containing 1 drop of 10 per cent soda solution. Shake the ethereal solution with 10 cc. of 1 per cent tartaric acid solution. Free this of ether and treat with the soda solution, drop by drop. A flocculent, white precipitate must appear. Filter and take up with 2 cc. of glacial acetic acid. One drop of dilute ferric chloride solution is added to the acetic acid solution and to the mixture is added 2 cc. of sulphuric acid so as to form 2 layers. At the junction of the 2 liquids a blue-violet zone appears.

Peyer, Hensel, Jaschik, Breslau and Rosenthal<sup>(58)</sup> omit the Tschirch<sup>(44)</sup> test from their comparative study of approximate

methods of estimation of alkaloidal potency because they consider the Hering<sup>(45)</sup> method an improvement on the Tschirch procedure. In a series of tests with 20 samples of ergot they compare the results of the alkaloidal picrate method, the Hering method and the Oettel method with their results obtained on the same samples by the Keller-Fromme method. On the basis of these results they conclude as follows:

1. The alkaloidal picrate method is undesirable because of the excessive amount of material necessary and because of its lack of concordant results.

2. As approximate methods of assay, they recommend in order of naming: The Hering test and the Oettel test.

3. For practical purposes the Keller-Fromme method is a sufficiently accurate quantitative method of estimation.

### 3. BIOLOGICAL METHODS OF STANDARDIZATION

#### a. Straight Uterine Methods<sup>(59)</sup>

The isolated uterus exhibits pendulum-like movements and peristaltic contractions. In Locke's solution, if perfused with oxygen, these movements may be studied for hours.

In the straight uterine method of bioassay, the activity of the ergot preparation is observed directly upon the isolated uterine strips of cats or guinea-pigs and the resultant effect of the amines and alkaloids is observed; the individual constituents of both groups stimulating the tissue to contraction. These methods are of no quantitative

value because they measure the amines more than they measure the alkaloids. Thompson<sup>(60)</sup>, however, used this type of method as a basis in developing a suitable assay for the non-specific amine activity of ergot, using uteri of guinea pigs taken several weeks post-partem, the animals weighing from 500 to 800 Gms. By means of this assay it is possible to measure the amine activity of crude ergot and its galenicals, irrespective of the specific alkaloidal content.

b. Pressor Methods<sup>(61)</sup>

The pressor methods measure the resultant effect of all the active principles of ergot, and while the pressor reactions produced by the intravenous administration of the various constituents are characteristic, the appearance of these constituents in mixtures, such as usually exists in fluidextracts, practically precludes their application quantitatively.

c. Cock's Comb Method

According to Gittinger and Munch<sup>(62)</sup>, and Thompson<sup>(61)</sup> it is possible by careful attention to details, to satisfactorily assay ergot and its preparations by this method. This is true because a sample of crude ergot meeting the U.S.P. biological requirements always contains at least as much alkaloidal activity as is indicated by the cock's comb test, and if interfering quantities of amines are also present, the actual alkaloidal content will be greater than the amount indicated by the test. Thus even though amines, if present in appreciable quantities, do cause a lower appa-

rent alkaloidal potency in ergot or its preparations, they do not entirely destroy the value of the assay.

d. Vasomotor Reversal Methods

(1) Pressor<sup>(35)</sup>

This method depends on the antagonistic action, on the blood pressure, of epinephrine to the ergot alkaloids: while the normal epinephrine response is pressor, the pressor response after ergot alkaloid administration, is either reduced, abolished, or even reversed. The end point is the determination of the dose which just suffices to produce a reversal or abolition of the ordinary pressor effect of epinephrine. The method has never gained favor as a routine method of assay because of its lack of quantitative sensitivity.

(2) Smooth Muscle

The Broom-Clark<sup>(59)</sup> Method and its modifications<sup>(20)</sup>,<sup>(24)</sup>,<sup>(63)</sup>,<sup>(64)</sup>, also depend on the above mentioned antagonistic action of epinephrine to the ergot alkaloids. In this instance, however, the test is carried out on isolated smooth muscle (rabbit uterus). A similar method to this has been proposed by Thompson<sup>(60)</sup>, in which the test is carried out on similar strips of guinea pig uterus instead of similar strips of rabbit uterus. Histamine replaces epinephrine in this method. Thompson's modified procedure, however, requires greater skill in manipulation than the method based on the use of the isolated rabbit uterus.

In comparing the accuracy of the rabbit uterus method with the accuracy of the cock's comb method it has been generally agreed<sup>(59)</sup>,<sup>(24)</sup>,<sup>(63)</sup>,<sup>(32)</sup>, that the former affords somewhat greater precision than the latter, but the former is more difficult of technique and therefore requires more experience in its use.

## II. CHEMISTRY

### A. History

According to Barger<sup>(2)</sup> the earliest chemical investigations of ergot, in the eighteenth century, are naturally of little interest today since they were undertaken in the hope of ascertaining whether the material was a fungus and of determining the cause of the toxicity. This was the type of investigation carried out by Vauquelin in 1816. In 1831 Wiggers<sup>(66)</sup> traced the activity of ergot to a resin; he also examined the oil and discovered "cholestrene" and a sugar. By 1869 Ludwig<sup>(67)</sup> had already distinguished two sterols, one of which was ergosterol.

In 1864 alkaloids were shown to be present by Wenzell<sup>(68)</sup>. Others<sup>(69)</sup>,<sup>(67)</sup>,<sup>(70)</sup>,<sup>(71)</sup>, investigated further, but no alkaloids were obtained in a state of purity until Tanret<sup>(72)</sup> in 1875, crystallized ergotinine. Tanret thought that he had isolated an important active principle. As a matter of fact, he did obtain such an active principle from the mother-liquors of the crystalline alkaloid, but failed to recognize that it was a separate substance, and regarded it merely as

amorphous ergotinine. Dragendorff and Podwysotski<sup>(73)</sup>, Jacobj<sup>(74)</sup>, and other pharmacologists found ergotinine to be inert. For the recognition of the physiologically active alkaloid, however, their chemical methods were inadequate. The high molecular weight of this alkaloid, its amphoteric properties, and the fact that its salts are precipitated by electrolytes, caused it to remain adsorbed on acidic substances, which Kobert<sup>(75)</sup> and Jacobj<sup>(74)</sup> described as active principles.

These impure preparations, called "active principles" owed their activity to a single alkaloid, which was first isolated in the form of its crystalline salts by Barger and Carr<sup>(76)</sup> in 1906, and was named ergotoxine. Simultaneously Kraft<sup>(77)</sup> recognized this second alkaloid and showed that it could be formed from ergotinine and could be converted into the latter. He attributed this formation to hydration and called it hydro-ergotinine. Barger and Carr<sup>(76)</sup> showed by analysis that the two alkaloids actually differ by only a molecule of water.

In 1918, Stoll<sup>(78)</sup> discovered a third ergot alkaloid, ergotamine, which crystallizes readily and has a physiological activity like that of ergotoxine. It can be converted into an inactive isomeride, ergotaminine. Stoll's new alkaloids are evidently very closely related to the older ones. They occur only in certain specimens of ergot, and it has been questioned whether these are of the official variety<sup>(79)</sup>.



In 1930 Smith and Timmis<sup>(79)</sup> described the crystallization of ergotoxine, and in 1931 they also described a fifth alkaloid, psi-ergotinine<sup>(30)</sup>, which has not been fully investigated, but which is thought to be an isomer of ergotoxine.

Ergot contains a number of simple amines, usually formed as a result of bacterial action. It was through the investigation of ergot extracts that the remarkable properties of histamine were discovered.<sup>(37)</sup>

Ergosterol, first accurately studied as a constituent of ergot by Tanret<sup>(80)</sup> in 1889 and 1908, has been found widely distributed in nature and has acquired great biological importance. Ergothioneine, a base, was found in ergot by Tanret<sup>(81)</sup> in 1909.

Finally, it is interesting to note the relationship to ergotinine and its hydrate ergotoxine, of some of the substances in the drug reported as "active principles" and properly classified by Barger and Dale<sup>(82)</sup>.

Ecboline and ergotinine (Wenzell) = Mixtures of alkaloids containing choline (Meulenhoff).

Amorphous ergotinine (Tanret) = Impure mixture of ergotinine and ergotoxine.

Picrosclerotine (Dragendorff) = Ergotinine possibly mixed with ergotoxine.

Sclerocrystalline (Podwysotski) = Ergotinine.

Sphacelinic Acid (Kobert) = Inactive resin with adherent alkaloid.

Cornutine (Kobert) = Impure mixture of ergotinine with ergotoxine.

Chrysotoxin (Jacobj) = Inactive yellow coloring matter with a small proportion of adherent alkaloid.

Secalintoxin (Jacobj) = Mixture of ergotinine and ergotoxine.

Sphacelotoxin (Jacobj) = Impure ergotoxine.

Hydroergotinin (Kraft) = Synonym for ergotoxine.

#### 1. INORGANIC CONSTITUENTS

According to Dieterich<sup>(83)</sup>, fresh ergot contains from 4.4 to 4.8 per cent of water, and on keeping, the amount may increase to as much as 10 per cent. The ash content ranges from 2.2 to 7 per cent. In an ash analysis by Hermann<sup>(84)</sup>, the following results were obtained:

<u>Constituent</u>	<u>Amount Present</u>
NaCl	1.50
K <sub>2</sub> O	30.06
Na <sub>2</sub> O	0.65
CaO	1.38
MgO	4.88
Al <sub>2</sub> O <sub>3</sub>	0.59
Fe <sub>2</sub> O <sub>3</sub>	0.68
MnO	0.26
P <sub>2</sub> O <sub>5</sub>	45.12
SiO <sub>2</sub>	14.67
Total	99.97

TABLE III.

The chief constituent of the ash is potassium phosphate,  $\text{KH}_2\text{PO}_4$ .

## 2. ORGANIC CONSTITUENTS

### a. Carbohydrates

#### (1) Trehalose

According to Barger<sup>(2)</sup> the characteristic sugar of ergot and other fungi is trehalose  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ , present in variable amounts of the order of 0.1 per cent. It was discovered by Wiggers<sup>(66)</sup> in 1831. Mitscherlich<sup>(85)</sup> in one case obtained as much as 2 Gms. from 2 kilos of ergot, some samples of ergot however, contained no sugar. This disaccharide does not reduce, forms no osazone, and is hydrolyzed to 2 molecules of glucose. According to Buchheim<sup>(85)</sup>, trehalose may decompose producing lactic acid, in the process of its decomposition. This would account for the acid reaction and small sugar content of some ergots.

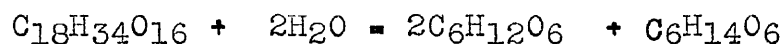
#### (2) Mannitol

This polyhydroxyl compound was found in ergot by Ludwig<sup>(67)</sup> and by later investigators such as Kraft<sup>(77)</sup>. It has the formula  $\text{C}_6\text{H}_{14}\text{O}_6$  and its melting point is  $166^\circ$ .

#### (3) Clavicepsin

This glucoside was discovered in ergot by Marino Zuco and Pasquero<sup>(87)</sup>. They used 95 per cent alcohol as their menstruum and by refluxing continually for

6 to 7 days, obtained yields which varied from 1.5 to 2 per cent. This compound is very soluble in water, slightly soluble in alcohol, and easily undergoes hydrolysis as follows:



One molecule of clavicepsin produces 2 molecules of glucose and one molecule of mannitol. The hydrated compound melts at  $91^\circ$ ; the anhydrous compound melts at  $198^\circ$ . Clavicepsin is strongly dextrorotatory:

$$[\alpha]_{\text{D}}^{20^\circ} = +142.27^\circ$$

#### (4) Mannan

This polysaccharide was obtained from ergot by Voswinkel<sup>(88)</sup> in 1891. On hydrolysis it produces mannose. Voswinkel first obtained it as a brown, amorphous, hygroscopic mass.

### b. Coloring Principles

#### (1) Sclererythrin

This reddish-violet substance was first investigated by Dragendorff and Podysotski<sup>(73)</sup> who gave it the above name. It is soluble in alcohol, alkalies, and in ether which contains sulphuric acid. The delicate chemical detection of ergot in flour is based on the recognition of this principle when extracted from the flour<sup>(89)</sup>. The characteristic absorption spectrum

of sclererythrin has also been frequently used for the detection of ergot<sup>(90)</sup>,<sup>(91)</sup>.

(2) Ergochrysin

This yellow principle, according to Barger<sup>(2)</sup>, was first accurately analyzed by Jacobj<sup>(74)</sup>, who assigned it the formula  $C_{21}H_{22}O_9$ . Ergochrysin, states Barger, is identical with the secalonic acid of Kraft<sup>(77)</sup> since the analyses fit the above formula almost as well as the one,  $C_{14}H_{14}O_6$ , assigned by Kraft himself. The substance forms lemon yellow needles which melt at  $244^{\circ}C$ . It is insoluble in water or gasoline, and slightly soluble in carbon disulphide, carbon tetrachloride, methyl alcohol, and ether. It is soluble in 160 parts of boiling alcohol, 200 parts of cold alcohol, 100 parts of boiling benzene and in 50 parts of boiling glacial acetic acid<sup>(2)</sup>. The alcoholic solution produces a reddish-brown color when treated with ferric chloride.

Kraft<sup>(77)</sup> assigned his secalonic acid the formula  $C_{14}H_{14}O_6$  which agreed very closely with his analyses. He adopted this formula, however, without benefit of molecular weight determinations. Recently Bergmann<sup>(92)</sup> determined the molecular weight of the substance as 556-557 and assigned it the formula  $C_{28}H_{28}O_{12}$  on the basis of his analyses. Bergmann found the melting point of the yellow leaflets, crystallized from chloroform,

to be 266°. On recrystallizing from a hot pyridine solution, containing alcohol, golden yellow needles were produced, having a melting point almost the same as Kraft's secalononic acid, that is, 242° to 244°. The composition of the latter appears to be  $C_{28}H_{12}O_{12} \cdot 1/2H_2O$ , however. According to Bergmann, boiling acetic anhydride produces about 10 per cent of a decaacetate, M.P. 240°. Methylation experiments produced no crystalline products.

(3) Ergoflavin

This yellow pigment,  $C_{15}H_{14}O_7$ , was found by Freeborn<sup>(93)</sup>, who separated ergoflavin from an evaporated ether extract of crude alkaloids, by means of sodium carbonate solution. Ergoflavin differs from ergochrysin in its melting point (338°), by its behavior to alkalies, and by its composition and molecular weight. By fusion with potash at 320° a volatile fatty acid is formed<sup>(93)</sup>. Dilute sodium hydroxide converts the substance into a colorless hydrate from which the anhydride is easily regenerated.

Bergmann<sup>(92)</sup> confirmed the formula for ergoflavin as given by Freeborn but assigned it a melting point of 344°. With acetic anhydride and a drop of concentrated sulphuric acid, Freeborn obtained the tetraacetate of a substance containing 1 molecule less of water than ergoflavin. Bergmann, however, both with

acetic anhydride in cold pyridine and with boiling acetic anhydride, always obtained a pentaacetate. He confirmed the pentaacetate by showing the presence of 5 hydroxyl groups according to his Zerevitinov determinations. By boiling a few minutes in dilute alcohol or alcoholic potassium hydroxide an orange colored solution was produced which was decolorized by acids with formation of a crystalline monobasic ergoflavonic acid, M.P. above  $330^{\circ}$ . An aqueous solution of this acid soon became yellow, when boiled, and regenerated ergoflavin. This points to the presence of a lactone group and thus the 7 oxygen atoms would be accounted for. Bergmann has stated further that when ergoflavin was fused with potash the following products were obtained:  $(\text{COOH})_2$ ,  $\text{CH}_3\text{COOH}$ ,  $3,5\text{-Me}(\text{HO})\text{C}_6\text{H}_3\text{COOH}$ , and a mixture of about 1 part  $m\text{-C}_6\text{H}_4(\text{OH})_2$  and 10 parts  $[2,4(\text{OH})_2\text{C}_6\text{H}_3]_2$ . With a large excess of nitric acid at room temperature, ergoflavin yielded a compound of the formula  $\text{C}_{16}\text{H}_{15}\text{O}_9\text{N}$ , M.P.  $260^{\circ}$ .

c. Glycerides and Sterols

Many of the earlier investigators knew of the large quantity of fatty oil present in ergot. Thus Herrmann<sup>(84)</sup>, Ludwig<sup>(67)</sup>, and Ganser<sup>(71)</sup> did some work on the oil around 1870. More recently the oil has been examined by MjØen<sup>(94)</sup>, Rathje<sup>(95)</sup>, Zellner<sup>(96)</sup>, Dieterle, Diester and Thimann<sup>(97)</sup>,

Matthes and Schutz<sup>(98)</sup>, Baughman and Jamieson<sup>(99)</sup>, and by Fiero<sup>(100)</sup>. The composition of the oil as well as the yield obtained depends upon the manner of its extraction: with gasoline Mjöen obtained 21 per cent, as did also Zellner; Rathje extracted with ether, and Dieterle, Diester and Thimann expressed the oil. According to Barger<sup>(2)</sup> the density at 20° varies from 0.9170 to 0.9259; the refractive index at 20° varies from 1.4685 to 1.4739; the amount of non-saponifiable matter varies from 0.35 to 1.037 per cent. Matthes and Schutz state that the oil is dextrorotatory, 10.5° to 10.7°, which is due to the hydroxy oleic acid present. The following data has been obtained:<sup>(2)</sup>

NUMBER	:Baughman: : and :Jamieson:	Matthes and Schutz	:Dieterle: :Diester : and :Thimann	: Rathje:	: Mjöen
Saponification	: 196.9	: 195.4	: 193	: 179.3	: 178.4
Iodine (of oil)	: 73.8	: 66.6-70.1	: 69.55	: 74.0	: 71.08
Iodine (of acids)	: 101.2	: ---	: ---	: ---	: 75.09
Reichert-Meissel	: 0.3	: ---	: 0.47	: 0.63	: 0.20
Hehner	: ---	: 96	: 96.1	: 96.25	: 96.3
Acid	: ---	: ---	: 13.19	: 11.38	: 4.95
Acetyl (of oil)	: 7.3	: ---	: 60.4	: 29.12	: 62.5
Acetyl (of acids)	: ---	: 73	: ---	: 77	: 75.1

TABLE IV



The iodine number shows that the oil belongs in the non-drying class. The Reichert-Meissel number indicates the presence of about 1 per cent of volatile fatty acid. The Hehner number is the percentage of insoluble fatty acids obtained. The acid number given above corresponds to about 6 per cent of free fatty acid, but, according to Barger<sup>(2)</sup>, fresh ergot oil may have as little as one-sixth this amount. The high acetyl number indicates the presence of some hydroxy acid. Matthes and Schütz<sup>(98)</sup> did obtain a large quantity of hydroxy-oleic acid, which is a characteristic constituent of the oil. The distribution of the fatty acids, according to Matthes and Schütz<sup>(98)</sup>, Baughman and Jamieson<sup>(99)</sup> and Fiero<sup>(100)</sup>, is as follows:

ACID	: Matthes : and : Schütz	: Baughman : and : Jamieson	: Fiero
Palmitic	: About 28%	: 21.5%	: 25%
Hydroxy Oleic	: 36%	: ---	: ---
Oleic	: 32.4%	: 62.5%	: 20.5%
Linoleic	: 3.6%	: 8.77%	: 13.2%
Myristic	: ---	: 0.3%	: 3.0%
Stearic	: ---	: 5.3%	: 2.1%
Arachic	: ---	: 0.7%	: ---
Ricinoleic	: ---	: ---	: 35.8%
Linolenic	: ---	: ---	: Traces

TABLE V

The non-saponifiable portion of the oil contains characteristic sterols. Ludwig<sup>(67)</sup>, in 1869, found two varieties, one melting at 141° and the other at 160°. Tanret<sup>(80)</sup>, in a later study of these, named the higher melting sterol, present in much larger amount, ergosterol,  $C_{27}H_{41}OH.H_2O$ . It crystallizes from alcohol and from ether, M.P. 162°. Tanret named the lower melting sterol, fungisterol,  $C_{25}H_{29}OH.H_2O$ , M.P. 144°. Both sterols are purified by means of their respective acetyl derivatives. With 90% sulphuric acid the two sterols are easily differentiated; fungisterol yields a ruby red color in about 10 seconds; ergosterol yields a dirty red color at the end of one minute.

Ergosterol is the more important of the 2 sterols because of the fact that on irradiation it yields vitamin D. This has brought it into great prominence and has led to the investigation of its adsorption spectrum, constitution and derivatives. The amount of ergosterol present in ergot is about 0.1 per cent.<sup>(77), (97)</sup>

#### d. Enzymes

According to Schindelmeiser<sup>(101)</sup> ergot contains at least two enzymes, one possessing diastatic, and the other fat-splitting properties. These enzymes lose their activity on prolonged standing or drying of the ergot.

##### (1) Diastatic

This activity was demonstrated by stirring fresh coarsely powdered ergot to a paste, addition of some

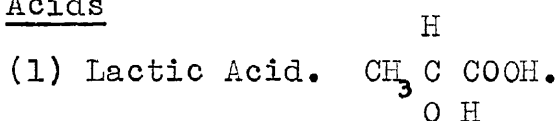
toluene-chloroform mixture as preservative, and shaking for 6 hours. The aqueous extract was separated and treated with 3 volumes of 90% alcohol. The precipitate was washed and 5 gms. of it was suspended in 200 gms. of 1% starch solution with preservative added. This was allowed to stand at 35° for 6 days. The contents were then boiled and filtered. A test for starch, with iodine solution, was negative. Schindelmeiser isolated glucose and maltose, as the respective osazones, proving the ability of the enzyme as a diastatic agent.

## (2) Fat-Splitting

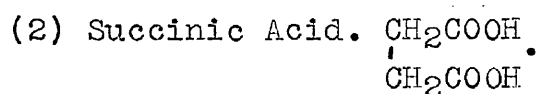
This activity was not shown as definitely as was the diastatic activity. To demonstrate this, Schindelmeiser in one experiment treated 5 gms. of non-defatted ergot powder with 20 gms. of olive oil. This mixture was allowed to stand for 12 days at 18-20° with daylight excluded. Ten gms. of the oil dissolved in ether-alcohol was titrated with tenth-normal alcoholic potassium hydroxide solution, using phenolphthalein as indicator. Thus the amount of fatty acid split off by the enzyme was estimated. The results were then calculated for 20 gms. of oil and were compared with the results obtained in like manner using defatted ergot. The difference, as Schindelmeiser pointed out was not very significant. For example, he obtained for the non-defatted ergot an amount of fatty acid

equivalent to: 6.3 cc., 7.8 cc., and 6.1 cc. n/10 alcoholic KOH. For the defatted ergot he found: 4.6 cc., 5.6 cc., and 4.9 cc. Schindelmeiser suggested that the fat-splitting enzyme was either dissolved in the oil or was in chemical combination with it.

e. Acids



According to Buchheim<sup>(86)</sup> this acid was found in ergot as a fermentation product of trehalose.



Engeland and Kutscher<sup>(102)</sup> found succinic acid in ergot. According to them it is derived from aspartic acid.

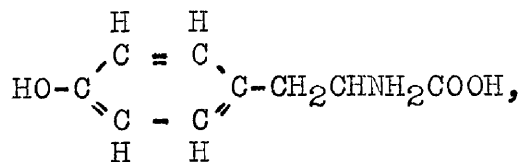
(3) Secale Amino Sulphonic.

Kraft<sup>(77)</sup> found this compound,  $\text{C}_{15}\text{H}_{26}\text{O}_{15}(\text{NH}_2)(\text{SO}_3\text{H})$ , M.P.  $200^\circ$ , and named it. In the isolation of the acid Kraft proceeded as follows: He exhausted the drug with chloroform and subsequently extracted with water. He then added an excess of basic lead acetate, freed the filtrate of excess lead, concentrated, and precipitated with potassium bismuth iodide. This produced a crystalline red precipitate which was decomposed with silver carbonate. The filtrate was acidulated with hydrochloric acid and evaporated over

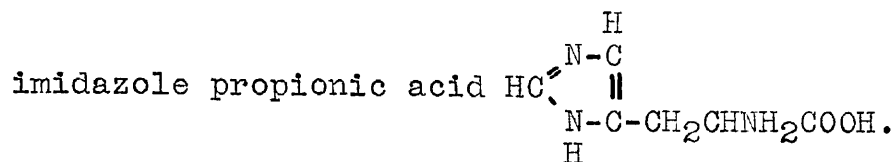
sulphuric acid. From this, betaine chloride crystallized. The remaining liquid was evaporated to dryness and extracted with absolute alcohol. On standing, the colorless crystals of secale amino sulphonic acid separated out. These were insoluble in absolute alcohol. The substance may be recrystallized either from dilute alcohol or from a small amount of water.

(4) Amino Acids

Leucine, alpha-amino alpha-isobutyl acetic acid,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH NH}_2\text{COOH}$ , was discovered in ergot by Buchheim<sup>(86)</sup>. Later, Vahlen<sup>(103)</sup> discovered clavin, an "active principle" of the formula  $\text{C}_{11}\text{H}_{22}\text{O}_4\text{N}_2$ . A molecular weight determination, however, did not support the above formula. Finally Vanslyke<sup>(104)</sup> separated Clavin into 39 per cent. leucine, 22 per cent isoleucine, which is ethyl methyl alpha-amino propionic acid,  $\begin{matrix} \text{C}_2\text{H}_5 \\ | \\ \text{C} \\ | \\ \text{H}_3 \end{matrix} > \text{CHCHNH}_2\text{COOH}$ , and 37 per cent valine, isopropyl alpha-amino acetic acid,  $(\text{CH}_3)_2\text{CHCHNH}_2\text{COOH}$ . Fränkel and Rainer<sup>(105)</sup> obtained a small quantity of crystalline tyrosine, which is beta-para-hydroxyphenyl, alpha-amino propionic acid,



and a small quantity of histidine, alpha-amino, beta-



According to Holtz and Müller<sup>(106)</sup>, the ergot fungus must form the amino acids from the rye protein since neither amino acids nor amines are contained as such in the rye grains.

f Amines

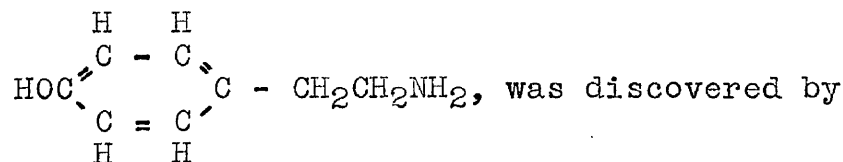
(1) Trimethyl amine,  $(\text{CH}_3)_3\text{N}$ , was found by Walz<sup>(107)</sup> in 1852. It was shown to be derived from choline by Brieger<sup>(108)</sup> in 1887.

(2) Putrescine, which is tetramethylenediamine,  $\text{NH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NH}_2$ , was found by Rielander<sup>(109)</sup> in 1908.

(3) Cadaverine, which is pentamethylenediamine,  $\text{NH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NH}_2$  was also found by Rielander<sup>(109)</sup>.

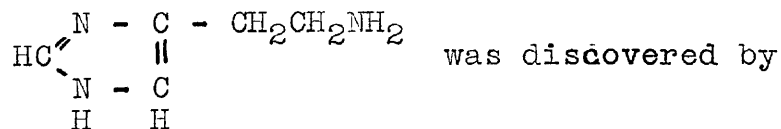
(4) Isoamylamine,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{NH}_2$ , which is present in traces, was found by Barger and Dale<sup>(31)</sup>.

(5) Tyramine, p-hydroxyphenylethylamine,



Barger and Dale<sup>(110)</sup> in 1909.

(6) Histamine, beta-Iminazolyethylamine,



Barger and Dale<sup>(31)</sup> in 1910.

(7) Agmatine, guanidobutylamine,  $\text{H}_2\text{N}-\overset{\text{H}}{\underset{\text{NH}}{\text{C}}}-\text{N}-(\text{CH}_2)_3\text{CH}_2\text{NH}_2$ ,  
 was found by Engeland and Kutscher<sup>(102)</sup> in 1910.

Thus it is seen that six amines have been obtained from ergot. According to Barger<sup>(2)</sup> these amines are formed from certain amino acids (leucine, tyrosine, histidine, lysine, and arginine) by decarboxylation, which is normally the result of bacterial action.

g. Additional Simple Bases

(1) Choline, trimethyl hydroxy ethyl ammonium hydroxide,  
 $(\text{CH}_3)_3\overset{\text{OH}}{\underset{\text{OH}}{\text{N}}}-\text{CH}_2\text{CH}_2\text{OH}$ , was isolated from ergot by

Brieger<sup>(108)</sup>,<sup>(77)</sup> in 1886.

(2) Acetyl-choline,  $(\text{CH}_3)_3\overset{\text{OH}}{\underset{\text{OH}}{\text{N}}}-\text{CH}_2\text{CH}_2\text{OOC CH}_3$ , was isolated

in the form of a platinum salt by Ewins<sup>(111)</sup> in 1914.

The correct formula for this compound was shown by Dudley<sup>(112)</sup> in 1929.

(3) Betaine, the anhydride of trimethyl amino-acetic acid,  
 $(\text{CH}_3)_3\overset{\text{O}}{\underset{\text{O}}{\text{N}}}-\text{CH}_2\text{C}=\text{O}$ , was isolated by Kraft<sup>(77)</sup> in 1906.

(4) Ergothioneine, the betaine of 2-thiol histidine,

$\text{HSC}=\overset{\text{H}}{\text{N}}-\overset{\text{H}}{\text{C}}(\text{CH}_3)_2-\overset{\text{O}}{\text{N}}-\text{O}$   
 $\text{N}-\text{C}-\text{CH}_2\text{CH}_2\text{CO}$  was isolated by Tanret<sup>(81)</sup>

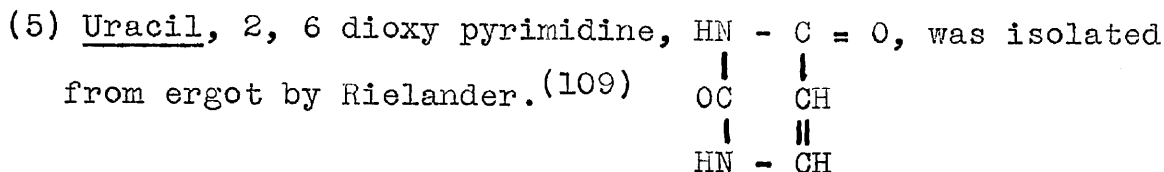
in 1909. To prepare this base the ergot was extracted with 90 per cent alcohol and after evaporation of the alcohol, the residue was filtered free of fat and resin.

Twenty per cent sulphuric acid was used to precipitate the coloring principles. The acid was neutralized with barium hydroxide solution and the filtrate was treated with basic lead acetate. After the resulting precipitate was removed by filtration, the excess lead was precipitated with sulphuric acid, the solution was made alkaline and was then extracted with chloroform to remove the ergot alkaloids. The solution was next acidified with acetic acid and treated with a warm 8 per cent solution of mercuric chloride. The mercury precipitate was filtered off, washed, suspended in a large volume of water and decomposed with hydrogen sulphide. After the mercuric sulphide was removed, the filtrate was evaporated, under reduced pressure, to a syrup. From this syrup ergothioneine chloride readily crystallizes, usually in a yield of about 0.1 per cent. The base was obtained by boiling the chloride with excess calcium carbonate. This was filtered, concentrated and treated with alcohol. The base may be recrystallized from boiling 60 per cent alcohol.

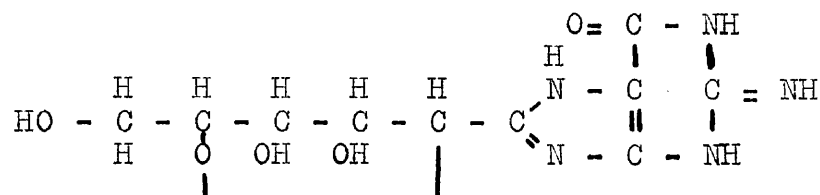
The base crystallizes in leaflets or in needles having the formula  $C_9H_{15}O_2N_3S \cdot 2H_2O$ . It is soluble in 8.6 parts of water at  $20^\circ$  and in about 1000 parts of boiling alcohol; it is insoluble in ether, chloroform or benzene.  $[\alpha]_D = 110^\circ$ ; M.P.  $290^\circ$ .



Ergothioneine has acquired considerable biochemical importance because it occurs in mammalian blood corpuscles and has reducing properties<sup>(113)</sup>.

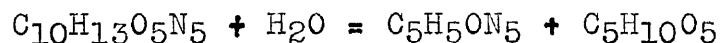


(6) Guanosine or Vernine, is a compound composed of a molecule of pentose with a molecule of guanine:



It was isolated from ergot by Schulze and Bosshard<sup>(114)</sup>.

Vernine is hydrolyzed to guanine and d-ribose:



#### h. Alkaloids

According to the literature up to the present time, ergot contains five well defined alkaloids: ergotoxine<sup>(76)</sup>, ergotinine<sup>(72)</sup>, psi-ergotinine<sup>(30)</sup>, ergotamine, and ergotaminine<sup>(78)</sup>.

Barger and Ewins<sup>(115)</sup> have shown that ergotoxine and ergotinine stand in the same relationship to each other as an acid does to its lactone or lactam ; in other words, ergotinine ( $\text{C}_{35}\text{H}_{39}\text{O}_5\text{N}_5$ ) is the anhydride of ergotoxine ( $\text{C}_{35}\text{H}_{41}\text{O}_6\text{N}_5$ ). The anhydride may be formed by boiling a concentrated solution of ergotoxine in methyl alcohol<sup>(77)</sup> or by boiling the ergotoxine with acetic anhydride for a few seconds.<sup>(76)</sup> Conversely, ergotoxine may be prepared from

ergotinine by the use of 3 per cent acetic acid or by the use of diluted phosphoric acid in alcohol. In accordance with the view that it contains a carboxyl group, ergotoxine is soluble in alkali; ergotinine is insoluble in alkali and this difference has been used as a means of separating the two. Psi-ergotinine is isomeric with ergotoxine<sup>(30)</sup> and is converted into ergotinine in the same manner as is ergotoxine, that is, by boiling with methyl alcohol. Psi-ergotinine may be converted into ergotoxine by boiling with alcohol and phosphoric acid.

Ergotoxine is easily soluble in alcohol and somewhat more difficultly soluble in ether. The alkaloid may be crystallized from hot benzene, yielding prisms containing 20 per cent of benzene<sup>(116)</sup>. In 0.6% chloroformic solution the crystals give  $[\alpha]_{5461}^{20} = -156^{\circ}$ . For the solvent-free

base in 0.6% chloroform solution,  $[\alpha]_{5461}^{20} = -195^{\circ}$ .

The alkaloid may also be crystallized from toluene, xylene or mesitylene. Ergotoxine is sparingly soluble in carbon disulphide and separates in prisms on spontaneous evaporation of the solvent. When an acetone solution is mixed with water, the base is precipitated in the amorphous state. It softens at 180° and melts indefinitely between 190 to 200°.

Ergotinine forms needle-like crystals, M.P. 219°, from alcohol,  $[\alpha]_D = +338^{\circ}$  in alcohol. Barger<sup>(2)</sup> gives the

following solubilities: At 15° it dissolves in about 400 parts by weight of ethyl alcohol, 1000 parts of dry ether,

90 parts of ethyl acetate, and 25 parts of acetone. In boiling solvents it is soluble in 77 parts of benzene, 52 parts of ethyl alcohol, and in 56 parts of methyl alcohol. It is very soluble in cold chloroform, moderately soluble in amyl alcohol and insoluble in petroleum ether. The salts of this alkaloid are amorphous.

Psi-ergotinine was obtained from crude ergotinine by fractional crystallization from dilute acetone. It was purified by solution in chloroform and fractional precipitation with ether,  $[\alpha]_{5461} = +459^{\circ}$  (chloroform). The

relative proportions of ergotinine and psi-ergotinine vary in different specimens of ergot, some specimens containing mainly ergotinine and others mainly psi-ergotinine as the subsidiary alkaloid, ergotoxine being invariably the predominant alkaloid<sup>(30)</sup>.

Ergotamine and Ergotaminine. These two isomeric alkaloids,  $C_{33}H_{35}O_5N_5$ , were isolated by Stoll in 1921. According to Smith and Timmis<sup>(79)</sup> they do not occur in all kinds of ergots.

Ergotamine crystallizes from aqueous acetone in plates,  $C_{33}H_{35}O_5N_5 \cdot 2H_2O \cdot 2C_3H_6O$ , decomposing at  $180^{\circ}$ .  $[\alpha]_D = +40^{\circ}$  in alcohol. Smith and Timmis<sup>(79)</sup>, found that the dry alkaloid placed in a bath at  $205^{\circ}$  and slowly heated, decomposes at  $213$  to  $214^{\circ}$ , corrected. This alkaloid crystallizes from the following, containing solvents of crystallization: methyl alcohol (pyramids), ethyl alcohol (needles) and

benzene (prisms). Ergotamine resembles ergotoxine in that it is a weak monacid base, the salts, however, are generally more soluble in water than those of ergotoxine<sup>(2)</sup>.

Ergotaminine, according to Stoll<sup>(78)</sup> is isomeric with ergotamine. Smith and Timmis<sup>(79)</sup> found its melting point (corrected) to be 252° when placed in a bath at 240° and slowly heated.  $[\alpha]_{D}^{20} = +381^{\circ}$  in chloroform. It crystallizes from alcohol in the form of plates resembling isocetes triangles. Ergotaminine is readily soluble in pyridine and glacial acetic acid. This alkaloid forms no crystalline salts and in this respect resembles ergotinine. The methods of interconversion that apply to ergotoxine and ergotinine apply also to ergotamine and ergotaminine<sup>(79)</sup>.

#### (1) Constitution of the Alkaloids

According to Barger<sup>(2)</sup> the price of these alkaloids and their high molecular weight make the investigation of their constitution very difficult. At the present time, little is known of the constitution of the alkaloids since comparatively few degradation products have been obtained. In 1910, Barger and Ewins<sup>(115)</sup> obtained isobutyryl formamide by the thermal decomposition of ergotoxine and ergotinine. Recently (1932), Soltys<sup>(117)</sup> obtained ammonia by the action of alcoholic sodium hydroxide, benzoic acid by oxidation with permanganate, and p-nitrobenzoic acid by treatment of these two alkaloids with nitric acid. Soltys further found that ergotoxine and ergotinine both contain 4 replaceable hydrogen

atoms since on warming with 4 equivalents of methyl magnesium iodide, both alkaloids react.

In 1932, Smith and Timmis<sup>(118)</sup> obtained a new base, ergine  $C_{17}H_{21}ON_3$ , from each of the alkaloids ergotoxine, ergotinine, ergotamine and ergotaminine. This base is crystalline and gives crystalline salts. From its formula the base can be seen to approximate roughly one-half of the alkaloidal molecule. Accordingly it was concluded that ergotoxine and ergotamine are similar with respect to this portion of the molecule. Ergine was obtained from the alkaloids by treatment with normal methyl alcoholic KOH in an atmosphere of nitrogen. The base contains one methylimino group. The compound crystallizes in colorless prisms from methyl alcohol with 1 molecule of solvent, decomposing at  $135^{\circ}$ . It also crystallizes readily from aqueous acetone in plates which decompose at  $115^{\circ}$ . Ergine is soluble in acetone, chloroform and ethyl acetate and has an optical rotation in acetone solution as follows:

$$[\alpha]_{20}^{20^{\circ}}_{\text{Hg-green}} = 514^{\circ}; \quad [\alpha]_{20}^{20^{\circ}}_{\text{Hg-yellow}} = 432^{\circ}.$$
 In chloroform solution:  $[\alpha]_{20}^{20^{\circ}}_{\text{Hg-green}} = 598^{\circ}; \quad [\alpha]_{20}^{20^{\circ}}_{\text{Hg-yellow}} = 503^{\circ}.$

The base resembles its parent alkaloids in giving the M. I. Smith<sup>(43)</sup> color reaction.

Jacobs and Craig<sup>(119)</sup> have recently published (1934), an account of a new degradation product (lysergic acid) obtained by them from crystalline ergotinine. They dissolved

the alkaloid in normal methyl alcoholic potassium hydroxide solution and removed the methyl alcohol at once by distillation under reduced pressure. The residue was treated with 8 per cent aqueous solution of potassium hydroxide on the steam bath for one hour. Basic volatile material was collected by means of a stream of nitrogen which passed through the reaction-flask into a solution of dilute hydrochloric acid. Ammonium chloride was thus obtained by cleavage of the amide group of ergotinine. Jacobs and Craig have assigned the formula  $C_{16}H_{16}O_2N_2$  to the new compound on the basis of their analytical data obtained from their investigation of the acid and its methyl ester. Lysergic acid, although sparingly soluble in water, is best recrystallized from water. The crystals separate with approximately 1 molecule of solvent which is held very tenaciously. The crystalline leaflets melt with decomposition at  $238^{\circ}$ .  $[\alpha]_D^{20} = 40^{\circ}$  (C = 0.500 in pyridine).

This compound gives the characteristic blue Keller test<sup>(2)</sup> of the ergot alkaloids as follows: The alkaloid dissolved in 2 or 3 cc. of glacial acetic acid is treated with a trace of ferric chloride, after which concentrated sulphuric acid is poured underneath, without mixing. At the junction of the two layers an intense cornflower blue appears at once; after a time the glacial acetic acid becomes violet, and the upper layers of the sulphuric acid, green. Lysergic acid behaves both as an acid and as a base. It is soluble in sodium hydroxide, ammonium hydroxide, sodium carbonate,

and hydrochloric acid. It is slightly soluble in dilute sulphuric acid and is sparingly soluble in the usual neutral organic solvents; it is quite soluble in pyridine. On titration with alkali, using phenolphthalein as indicator, lysergic acid does not give a sharp endpoint.

(2) Salts of the Alkaloids

Ergotinine and ergotaminine do not form crystalline salts. Many crystalline salts of ergotoxine and a lesser number for ergotamine have been reported.

(a) Ergotoxine methane sulphonate<sup>(120)</sup>,  $C_{35}H_{41}O_6N_5 \cdot CH_3SO_3H$ .  $2C_2H_5OH$ , M.P.  $214^\circ$  (corr.), crystallizes from alcohol in clusters of needles.

(b) Ergotoxine ethane sulphonate<sup>(120)</sup>,  $C_{35}H_{41}O_6N_5 \cdot C_2H_5SO_3H$ .  $2C_2H_5OH$ , M.P.  $209^\circ$  (corr.) crystallizes in the same manner as does the methane sulphonate. Both are formed by dissolving the base and acid together in alcohol and precipitation of the salts by cooling or by adding ether.

(c) Ergotoxine phosphate<sup>(76)</sup>,  $C_{35}H_{41}O_6N_5 \cdot H_3PO_4 \cdot H_2O$ , M.P.  $186-187^\circ$ , crystallizes from 50 parts of boiling 90 per cent alcohol in clusters of radiating needles.

(d) Ergotoxine hydrochloride<sup>(76)</sup>,  $C_{35}H_{41}O_6N_5 \cdot HCl$ , M.P.  $205^\circ$ , forms small diamond-shaped plates. It is crystallized by dissolving in 15 parts of warm 90 per cent alcohol and slowly adding ether.

(e) Ergotoxine hydrobromide<sup>(115)</sup>,  $C_{35}H_{41}O_6N_5 \cdot HBr$ , M.P.  $208^\circ$ , forms acicular prisms.

(f) Ergotoxine sulphate<sup>(121)</sup>,  $(C_{35}H_{41}O_6N_5)_2 \cdot H_2SO_4$ , was obtained by Kraft, and forms small crystals.

- (g) Ergotoxine acid sulphate<sup>(115)</sup>,  $C_{35}H_{41}O_6N_5 \cdot H_2SO_4$ ,  
M.P. 197<sup>o</sup>, forms prisms.
- (h) Ergotoxine nitrate<sup>(115)</sup>,  $C_{35}H_{41}O_6N_5 \cdot HNO_3$ , M.P. 197<sup>o</sup>,  
forms short broad prisms.
- (i) Ergotoxine picrate<sup>(115)</sup>,  $C_{35}H_{41}O_6N_5 \cdot C_6H_3O_7N_3$ ,  
M.P. 214 to 215<sup>o</sup>, pale yellow needles.
- (j) Ergotoxine oxalate<sup>(76)</sup>,  $(C_{35}H_{41}O_6N_5)_2 \cdot H_2C_2O_4$ ,  
M.P. 179<sup>o</sup>, from oxalic acid and excess of the base  
in ethereal solution.
- (k) Ergotoxine acid oxalate<sup>(76)</sup>,  $C_{35}H_{41}O_6N_5 \cdot H_2C_2O_4$ ,  
M.P. 179<sup>o</sup>, is formed by shaking a solution of ergo-  
toxine in xylene with excess of one per cent oxalic  
acid. Small prisms crystallize from alcohol con-  
taining acetone.
- (l) Salts of ergotamine

The sulphate, tartrate, phosphate, and normal oxalate are known. The salts of this base are, in general, more soluble in water than those of ergotoxine. Possibly the most widely used ergotamine salt is the tartrate, known commercially as "gynergin" and "femergin". Ergotamine does not form an acid oxalate but ergotoxine does.

The normal ergotamine oxalate is formed by using excess oxalic acid.



SOLUBILITY OF THE ERGOT ALKALOIDS

SOLVENT	ERGO-TININE	ERGO-TOXINE	ERGO-TAMINE	ERGO-TAMININE	PSI-ERGO-TININE
Ethyl Alcohol	1:400(15°)	Very soluble	Soluble	1:6400	More soluble than Ergotinine
Dry Ether	1:1000	Sparingly soluble	Less sol. than ergo-toxine		More soluble than Ergotinine
Ethyl Acetate	1:90	Very soluble			More soluble than Ergotinine
Acetone	1:25	Very soluble			More soluble than Ergotinine
Boiling Benzene	1:77				More soluble than Ergotinine
Boiling Ethyl Alcohol	1:52				More soluble than Ergotinine
Boiling Methyl Alcohol	1:56				More soluble than Ergotinine
Chloroform	(cold) extremely soluble	Very soluble	Less sol. than ergo-toxine	Slightly soluble	(cold) Extremely soluble
Light Petroleum	Insoluble	Insoluble	Insoluble		
Carbon Bisulphide		Sparingly Soluble			
Methyl Alcohol		Very Soluble	Soluble		
Nitro-benzene			Readily soluble	Slightly soluble	
Pyridine			Readily soluble	Readily soluble	
Benzene			Less sol. than ergo-toxine		
Glacial Acetic Acid				Readily soluble	

TABLE VI

The following constants have recently been redetermined by Smith and Timmis(79),(30):

SPECIFIC ROTATION OF THE ERGOT ALKALOIDS

ALKALOID	CHLOROFORM	ABSOLUTE ALCOHOL	ACETONE
Ergotinine	(c = 0.35) 'D line: 365° 'Hg-green line: 459°	(c = 0.15) 'D line: 348° 'Hg-green line: 439°	(c = 1.13) 'D line: 381° 'Hg-green line: 478°
Psi-ergotinine	(c = 0.353) 'D line: 410° 'Hg-green line: 513°		(c = 1.04) 'D line: 403° 'Hg-green line: 509°
Ergotoxine (Solution of crystals containing 21% benzene)	(c = 1); T = 19° 'Hg-green line: -179° 'Hg-yellow line: -156°		
Ergotamine	(c = 1); T = 20° 'Hg-green line: -181° 'Hg-yellow line: -159°		
Ergotaminine	(c = 0.5); T = 18° 'Hg-green line: 450° 'Hg-yellow line: 385°		

TABLE VII

THE MELTING POINTS OF THE ALKALOIDS ARE AS FOLLOWS:

ALKALOID	M. P. in degrees C. (corrected)
Ergotinine	: 239° (Smith & Timmis)
Ergotoxine	: Indefinite (190-200°)
Ergotamine	: 213-214° (Smith & Timmis)
Ergotaminine	: 252° (Smith & Timmis)
Psi-Ergotinine	:

TABLE VIII

(3) Methods of Alkaloidal Extraction

Tanret<sup>(72)</sup>, the first to isolate a pure ergot alkaloid, extracted the drug with 96 per cent alcohol, added enough sodium hydroxide to make alkaline, distilled off the alcohol and extracted the residue with ether. This solution was extracted with citric acid, the acid solution washed with ether, made alkaline with ammonia, and again extracted with ether. The ethereal extract was then concentrated sufficiently to cause crystallization of part of the alkaloid (ergotinine). The rest of the alkaloid was obtained in the amorphous state by adding petroleum ether.

Tanret's process gives rise to troublesome emulsions and in order to avoid this difficulty various modifications have been introduced. Keller<sup>(122)</sup> first removed the fatty oil with petroleum ether before extracting the alkaloid with ether. Kraft<sup>(77)</sup> extracted with ether, distilled off the solvent, and diluted the residual oily extract with 2 to 3 volumes of petroleum ether. The resulting precipitate consisted of alkaloids, yellow coloring principles and ergosterol. Kraft then treated the extract with ether and obtained the crude alkaloids by shaking the ethereal solution with 1/2 per cent tartaric acid solution.

Gehe & Co.<sup>(123)</sup>, in a patented process, first extracted the drug with ether, distilled off the solvent, and then mixed the residue with an ethereal solution of

a suitable organic acid (tartaric, citric, etc.) whereupon an alkaloidal precipitate results. This precipitate was then treated according to the known methods of alkaloidal separation.

Herold<sup>(124)</sup> proposed a method as follows: The drug was slightly moistened, rendered alkaline, and extracted with benzene. The solution was first washed repeatedly with slightly alkalized water and then with dilute citric or tartaric acid. The amorphous bases were precipitated by sodium hydroxide, care being taken not to exceed a pH of 7.7. The precipitate was filtered under reduced pressure, dissolved in ether, extracted with 1 per cent sulphanilic acid, and then reprecipitated with sodium carbonate. The product consisted chiefly of ergotoxine and ergotinine.

When working on a large scale, however, the slight solubility of the chloride, and especially that of the bromide, may be used to advantage. Barger and Carr<sup>(76)</sup> treated the residue, left on evaporation of the alcoholic solution, with petroleum ether to remove fatty matter, dissolved the alkaloidal residue in ethyl acetate, and shook with citric acid solution. Sodium bromide or hydrobromic acid was added and the precipitated hydrobromides collected. According to the above workers, a rough separation of ergotinine from ergotoxine was effected by repeated shaking of the solution of mixed hydrobromides, in dilute sodium hydroxide, with ether. This removed the ergotinine which was finally cry-

stallized from alcohol. The ergotoxine being more soluble in the alcohol than ergotinine, remained in solution.

In the extraction of ergotamine, Stoll<sup>(78)</sup> introduced an unusual method of procedure. This consisted essentially in treating the drug with a solution of a weakly acidic substance such as aluminum sulphate, ferrous sulphate, ferric chloride or copper sulphate, and separation of extractive matter from the acid material by treatment with a solvent such as benzene or ether. The alkaloid was then liberated by decomposition of its salt with an alkali, and then extracted with ether or benzene.

Stoll<sup>(78)</sup> laid stress on his "protective methods" of extraction and suggested that the failure of other investigators to obtain ergotamine may have been due to the use of ergots containing little or no ergotamine either because of age or deterioration, or because of poor original ergotamine content. He attributed the chief cause of the unsuccessful attempts at isolation of ergotamine to the use of insufficiently protective methods of extraction. Smith and Timmis<sup>(79)</sup> tried to obtain ergotamine from a great variety of samples of official ergot, but were unsuccessful. They examined three specimens of ergot growing on festuca grass, however, and, using the Kraft<sup>(77)</sup> method, were successful in each attempt. As a result of this investigation they have suggested that the isolation

of ergotamine and ergotaminine does not depend so much on the special methods of extraction which Stoll has stressed so much, but depends on the nature of the ergot.

Stoll has stated that he believes ergotaminine is only formed artificially by the chemical reagents used in some processes of extraction. This is supported somewhat by the fact that the use of strong alkalies in liberating ergotoxine from its crystalline salts leads to contamination with ergotinine and other impurities which prevent crystallization<sup>(79)</sup>. According to Thompson<sup>(125)</sup>, there is only one alkaloid in ergot which, depending on the method of extraction, may be isolated as ergotamine, ergotoxine or ergotinine. Indisputable proof, definitely showing that the alkaloid obtained is only a function of its method of extraction, as above, has not yet been brought forward.

(i) A New Constituent

According to the clinical results obtained by Moir<sup>(23)</sup> there is reason to believe that the characteristic and traditional effect of ergot is due to a substance as yet unidentified. Moir found that the effect of the drug comes on within four minutes of oral administration of the liquid extract (official in the 1914 edition of the British Pharmacopoeia), and that the character of the powerful uterine contractions was notably different from those of ergotoxine and ergotamine: "--they were frequent, regular, of fairly great excursion, and there was a rise in base line much greater than observed with any other drug." The official

solid extract and even defatted ergot powder also show great activity in stimulating contractions in the puerperal uterus. Moir states further: "--since histamine and tyramine were found to be inactive when given by mouth, either singly or in combination, it must be assumed that the oxytocic power of the liquid and solid extracts is due to a substance whose importance has hitherto been overlooked in the investigation of ergot." As yet no published confirmation of Moir's observations has appeared.

### III. EXPERIMENTAL

In order to carry out a general chemical analysis of ergot of rye, 50 pounds of the Spanish variety of drug was procured from S.B. Penick & Co. of New York City. The ergot on its arrival, was in good condition.

#### A. Preliminary Determinations.

##### 1. Ash

##### a. Total Ash

Four additional samples of ergot of rye were obtained, from Prof. M.R. Thompson of this institution, so as to form a basis for comparison of results obtained from the total ash and from the results obtained by qualitative determination of the elements present in the ash. In the following tables sample A was taken from the fifty pound lot of drug.

SAMPLE OF GROUND DRUG	TOTAL ASH		
	(1)	(2)	Average
A	2.99%	2.98%	2.99%
B	3.04%	2.97%	3.01%
C	3.03%	3.07%	3.05%
D	2.84%	2.82%	2.83%
E	2.89%	3.10%	3.00%

TABLE IX

b. Acid-insoluble Ash

This was determined only on the total ash of the sample from the fifty pound lot, that is, on sample A:

(1)	(2)
0.027%	0.023%

c. Acid-soluble Ash

This was calculated by subtraction of the acid-insoluble ash from the total ash:

(1)	(2)
2.963%	2.957%

d. Qualitative Determination of Elements

For the purpose of this determination 100 grams of each unground sample was separately ashed and then extracted with boiling dilute hydrochloric acid. This



acid solution was then tested according to routine qualitative analysis methods. The results are given in the following table:

ELEMENTS PRESENT				
A	B	C	D	E
Fe	Fe	Fe	Fe	Fe
Al	Al	Al	Al	Al
Mg	Mg	Mg	Mg	Mg
--	Mn	Mn	Mn	--
Na	Na	Na	Na	Na
K	K	K	K	K
Ca	Ca	--	--	Ca
--	--	Cu	--	--
Pb	--	--	--	--

TABLE X

Phosphorous was also found to be present in each of the above samples.

2. Preparation of Fluidextract

A fluidextract was prepared according to "Process C", United States Pharmacopoeia, Revision X, using a menstruum of dilute alcohol and 2 per cent hydrochloric acid. The drug was previously defatted with purified petroleum benzin,

and "Process C" was used in the extraction because this process eliminates the necessity of applying heat for the concentration of the liquid extract to proper volume. This is in accord with the work of Thompson<sup>(126)</sup> who has shown that a more potent and a more representative preparation is obtained by extraction according to "Process C" rather than by "Process B" which the Tenth Revision of the United States Pharmacopoeia (Hereinafter, U.S.P.X), directs. The test for exhaustion applied to the percolate was one of those recommended by Thompson<sup>(126)</sup> as follows:

"Approximately 4 cc. of this issuing percolate is diluted threefold with a saturated aqueous solution of sodium bicarbonate. Failure to develop precipitate turbidity or faint opalescence during one hour shows that extraction is complete for practical purposes."

"Process C" was followed according to the directions given on page 160, U.S.P.X:

"Divide 1000 Gm. of the powdered drug into three portions of 500 Gm., 300 Gm., and 200 Gm., respectively. Moisten the first portion of the drug (500 Gm.) with a sufficient quantity of the prescribed menstruum to render it evenly and distinctly damp and to so maintain it during maceration for six hours in a tightly-covered container. Then pack it in a cylindrical percolator, and add enough of the menstruum to saturate the powder and leave a stratum above it. When

the liquid begins to drop from the percolator, close the lower orifice, and, having closely covered the percolator, macerate for forty-eight hours, and then allow the percolation to proceed slowly, gradually adding more of the menstruum. Reserve the first 200 cc. of percolate, and continue the process until additional percolate measures 1500 cc., the latter being collected in successive portions of 300 cc."

"Moisten the second portion of the powdered drug (300 Gm.) with a sufficient quantity of the percolate collected in the preceding operation, immediately after the reserved portion, to render it evenly and distinctly damp and to so maintain it during maceration for six hours in a tightly-covered container. Then pack it in a cylindrical percolator, macerate, and percolate as directed for the first portion of the drug, using as menstruum the several portions of percolate from the preceding operation in the order in which they were collected, and, if this is insufficient, follow with more of the original menstruum. Reserve the first 300 cc. of percolate, and continue the process until the additional percolate measures 800 cc., the latter being collected in successive portions of 200 cc. each.

"Moisten the third portion of the powdered drug (200 Gm.), with a sufficient quantity of the percolate collected in the preceding operation, immediately after the reserved portion, to render it evenly and distinctly

damp and to so maintain it during maceration for six hours in a tightly-covered container. Then pack it in a cylindrical percolator, macerate, and percolate as before, using as menstruum the several portions of percolate from the preceding operation in the order in which they were collected, and if this is insufficient, follow with more of the original menstruum. Collect 500 cc. of percolate, and mix this with the two portions previously reserved to make 1000 cc. of finished fluidextract."

a. Determination of Potency of Fluidextract

In the determination of potency a modification of the Broom-Clark biological method was used. This modification in technique has been published in detail by Thompson<sup>(64)</sup>.

Each "reserved percolate" was tested separately by this biological method then mixed together and the mixture assayed, thus affording a check on the value obtained for the final assay. The results were as follows:

Reserve percolate I: greater than 200%

Reserve percolate II: greater than 200%

Reserve percolate III: less than 175%

Average: 192%

Roughly, the resulting mixture of percolates should show a potency around 190%. After mixing the portions, it was found that the potency of the preparation was 175% which may be accepted as a satisfactory agreement with respect to the assays.

b. Fatty Oil

After extraction of the ground drug with petroleum benzin, twenty per cent of fatty oil was obtained. The oil was light amber in color and had no appreciable odor. It was turned over to Mr. G. E. Cwalina for investigation.

3. Extraction of Alkaloids from Crude Drug

a. According to Stoll Method

Stoll used the following method<sup>(78)</sup> for the extraction of ergotamine and ergotaminine from the crude drug:

Two kilos of ergot were ground to grains of about the size of one cubic millimeter and thoroughly mixed with a solution of 60 Gm. of crystallized oxalic acid in half a liter of lukewarm water, the grinding being then resumed until the moist substance was reduced to a high degree of fineness, after which the drug was introduced into 5 liters of ether. After being allowed to stand for several hours with occasional stirring, the deep-orange colored liquid containing the principal part of the fatty oils and other neutral and acid constituents, was separated by suction and the residue was washed for another two hours with 3 or 4 liters of ether, in portions of half a liter at the time, further removal of fatty constituents being afterwards accomplished by percolation with 4 liters of ether. After a short suction of the ether from the fine, now more clear drug dust, the latter <sup>was</sup> again suspended in ether to which about 5 per cent ethyl alcohol had been

added, and to this a cooled solution of 200 to 220 Gm. of crystallized barium hydroxide in 3/4 liter of water was gradually added, the liquid and suspended matter being energetically agitated the while. The resulting solution gave a weakly alkaline reaction in which there was no free alkali liable to destroy the desired alkaloids. After two hours, the light yellowish extract in ether was separated by filtering under reduced pressure. With a good grade of ergot, Keller's iron chloride test, applied to one cc. of the filtrate gave an intense reaction. This reaction became gradually weaker after the drug had been washed with about 4 liters of the solvent, which was allowed to percolate slowly through the cellular tissue, under reduced pressure, for two or three hours, in portions of half a liter at a time. Finally, the reaction could only be observed plainly with large volumes of filtrate.

The extract of alkaloids in ether was concentrated at once to 1/2 to 3/4 liter by evaporation, and the concentrated liquid was decanted, leaving behind a residue which contained no alkaloids. The liquid obtained by this separation was evaporated to dryness in vacuo below 30°C. For the separation of further traces of fatty substances, and of phytosterols, the liquid yellow residue was treated with half a liter of petroleum ether, and, after filtering, the residue of crude alkaloids, dried in vacuum, was dissolved in ether. This produced

a light yellow solution which was concentrated to about 3/4 liter by evaporation, after which the base separated in groups of white crystals. The crystals obtained in the last stages of the crystallization, when the mother liquor was reduced by evaporation to a very small volume, consisted of yellowish globules containing only from 9-1/2 to 10 per cent of nitrogen, but by recrystallizing these with ether, they could be transformed into white particles containing also 11-1/2 to 12 per cent of nitrogen as in those crystals first obtained.

The method of Stoll, essentially as stated above, was applied in the attempted isolation of ergotamine and ergotaminine on 3 occasions, but in neither case was the attempt successful when applied to this sample of drug (lot #A, obtained from S.B. Penick & Co., New York City). As a result it was concluded that the drug originally contained very little, if any, of these alkaloids.

b. Extraction according to the Kraft Method

Three kilos of drug, Lot A, were then extracted as follows: The ergot powder was placed in a large flask and to it was added 800 cc. of water and 4-1/2 kilos of ether. This was shaken together for 1 hour, after which the ethereal liquid was filtered off and set aside. The remaining dampened powder was then treated ten times with 2-1/2 kilogram portions of ether (without addition of water) in the same manner as above. The 4-1/2 kilogram portion of extractive was left

untouched for the time being, but the succeeding portions were mixed and concentrated by distillation to the same weight as the first extract. All of the ethereal extracts were then combined and shaken out with portions of 1/2 to 1/4 liter of one-half per cent tartaric acid solution. The clear filtered shakings were then treated with powdered sodium bicarbonate until the liquid was alkaline to litmus. The precipitated alkaloids were gathered on a suction-filter, washed, and dried over sulphuric acid. About four grams of crude alkaloids were obtained.

(1) Extraction of Ergotoxine

These alkaloids were separated by means of their sulphates according to the following method of Kraft<sup>(77)</sup>. One part of dry crude alkaloid was dissolved without heat in three parts of glacial acetic acid and the solution diluted to 300 parts with water. The turbidity of the solution was removed by filtration, with the aid of a knifepoint of kieselguhr, on a suction filter. The filter was then washed with water until the filtrate measured 400 parts and this was treated with a filtered solution of one part of anhydrous sodium sulphate in 100 parts of water whereupon ergotoxine sulphate separated, while ergotinine sulphate remained in solution. After standing about two hours the precipitate was sharply sucked off. The damp sulphate was again stirred with water, ether abundantly added, and



the whole was shaken with the amount of sodium bicarbonate necessary to render the liquid alkaline to litmus, until the liberated alkaloid was dissolved in the ether. The ethereal solution was separated and dehydrated with anhydrous sodium sulphate. The ether was evaporated in vacuo and in this manner the amorphous ergotoxine was obtained. It was crystallized from hot benzene in accordance with the process of the Wellcome Foundation<sup>(116)</sup> wherein only weak alkalies such as sodium bicarbonate or borax are used in obtaining the base previous to its crystallization from benzene. Only a very small yield was obtained. The crystals softened at about 179° and melted indefinitely between 190 and 200°.

(2) Extraction of Ergotinine

The ergotinine sulphate present in the filtrate, obtained after precipitation of the ergotoxine sulphate, was precipitated with sodium bicarbonate, the alkaloid filtered by suction, washed, and dried over sulphuric acid. The alkaloid was crystallized from hot 70 per cent alcohol. The crystals obtained melted at 219°.

c. Extraction on Large Scale

For this purpose ten kilos of powdered ergot were treated according to the method of Stoll<sup>(78)</sup>, in an effort to learn whether any ergotamine or ergotaminine was present in the drug in small quantity. The attempted isolation in this case, however, was not carried out quickly enough because the alkaloids

known to have been present (ergotoxine and ergotinine) had decomposed, as indicated by negative Keller iron chloride tests, before the completion of the isolation process.

#### 4. Isolation of Ergochrysin

This substance was obtained by the method of Kraft<sup>(77)</sup>, that is, by extracting the defatted marc of the drug after extraction of its alkaloids by ether, with chloroform. The chloroform was distilled off under reduced pressure on a water bath and the residue then treated with petroleum benzin until a dry yellowish powder was obtained. This was rubbed to a thin consistency with glacial acetic acid and filtered and washed with small amounts of glacial acetic acid on the suction-filter. After this treatment, lemon-yellow needles of ergochrysin remained on the filter and were allowed to dry. The crystals were treated with boiling methyl alcohol to further separate any fatty material (ergosterin) and were crystallized several times from chloroform. M.P. 244<sup>o</sup>.

#### 5. Analysis of Hydro-alcoholic Extract

##### a. Alkaloidal Potency of "Fluidextract"

The marc left after extraction of the ten kilos of ergot with ether according to the Stoll process was tested for alkaloidal activity through the medium of a "fluidextract" prepared from a sample of the above marc by the U.S.P.X "Process C". This fluidextract was tested as follows:

(1) Van Urke-Smith color reaction<sup>(43)</sup>. Result:

Weakly positive.

(2) Cock's Comb Method of the U.S.P.X. Result:

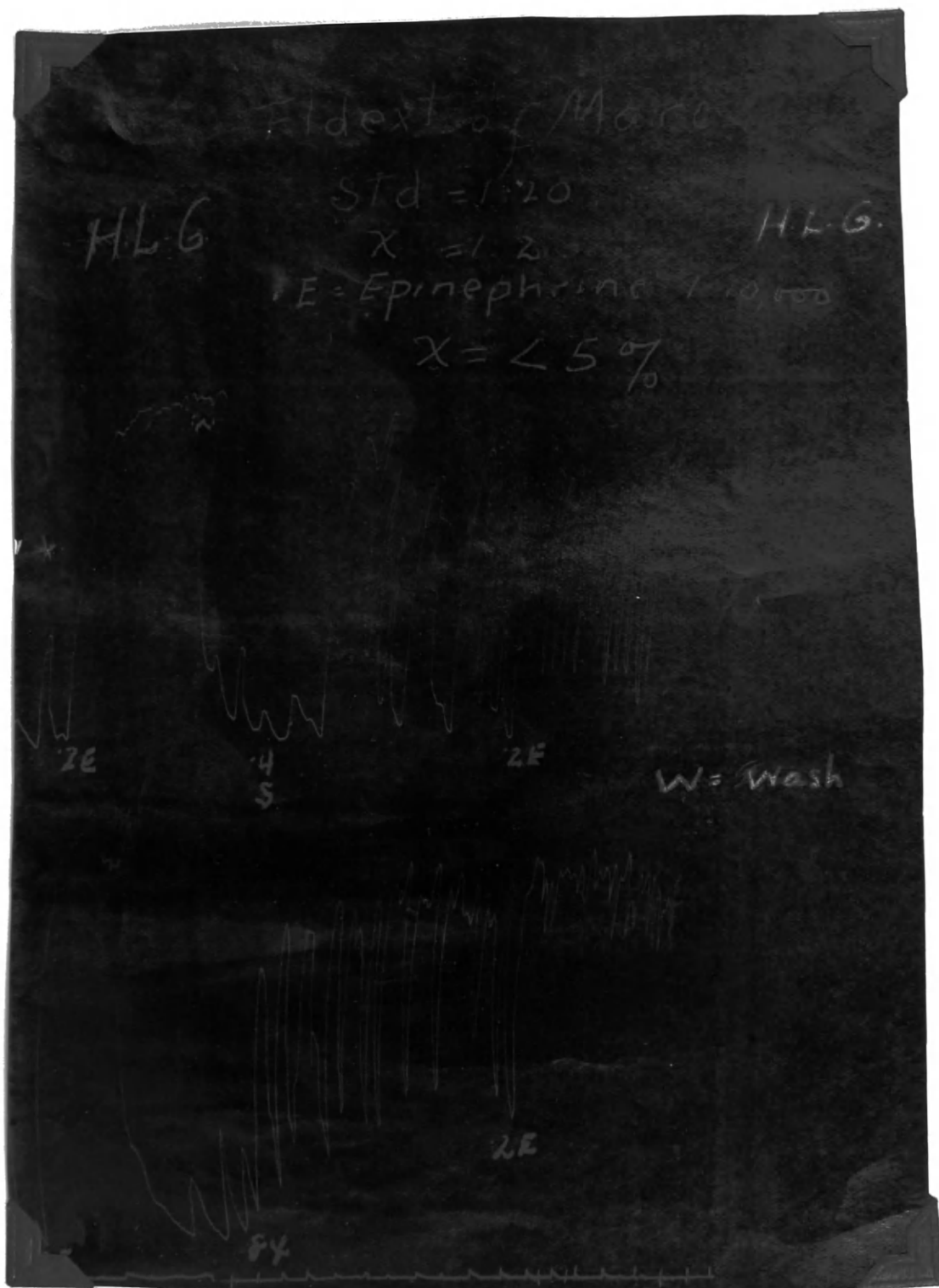


PLATE I

Potency considerably below 25 per cent.

(3) Broom-Clark Method using the Thompson technique<sup>(64)</sup>.

Result: Alkaloidal activity of less than 5 per cent  
(See plate I).

b. Activity of 25 per cent Alcoholic Extract

An aqueous-alcoholic extract was prepared from the marc left after the large scale extraction. This extract was tested for the type of activity found by Moir<sup>(23)</sup>. The experiment was carried out on virgin guinea pig uteri in the same manner as Pituitary Solution is assayed in the U.S.P.X. The type of reaction obtained seemed to be characteristic of histamine.

c. Treatment with Lloyd's Reagent

This experiment was carried out to ascertain the effect of treatment with Lloyd's reagent (hydrous aluminum silicate) as follows: One hundred cubic centimeters of the 25 per cent alcoholic extract was mixed with 5 cc. of dilute sulphuric acid and with 2 Gm. of Lloyd's reagent and the mixture agitated for 30 minutes. After the suspension had settled it was filtered.

(1) Comparative Studies

(a) On Guinea Pig Uterus

The filtrate was then tested on the uteri of virgin guinea pigs as before treatment of the extract with Lloyd's reagent. (See plate III).

This tracing shows that 0.25 cc. of the original solution (H) was equivalent to 0.3 cc. of the

$P = \text{Pituitary Soln. } 1:40,000$   
 $B = \text{Aq. Ext. (25\% Alc.) } 1:1$   
 $A = \text{Aq. Ext. (25\% Alc.) } 1:1$   
 $W = \text{Wash}$

H.L.G.

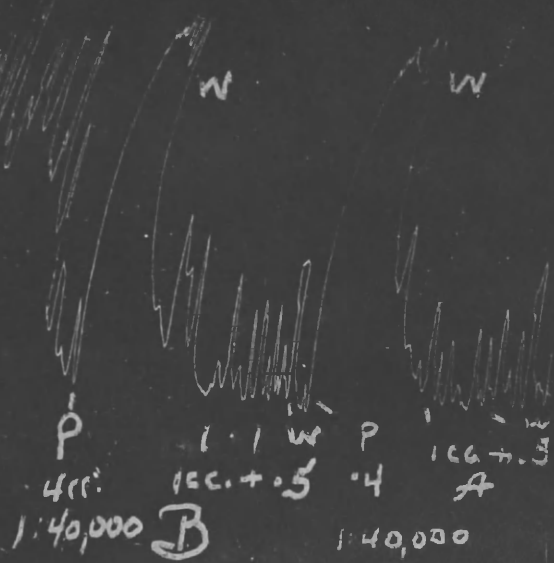


PLATE II

W = Wash

H = Original extract 1:10  
P = Pituitary Soln. 1:50,000

J = Filtrate 1:10

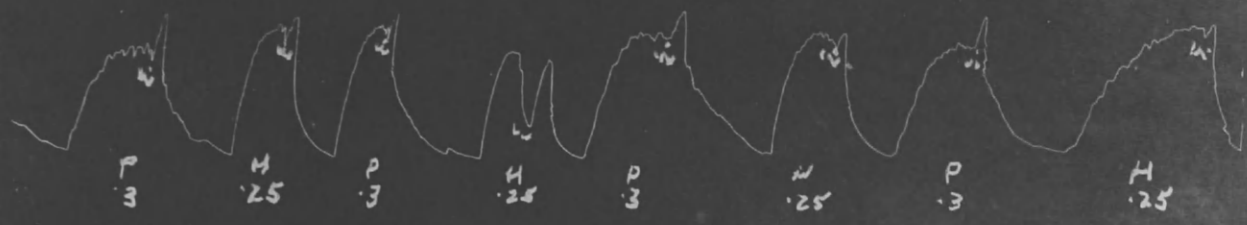


PLATE III

standard pituitary solution (P), and that 0.5 cc. of the filtrate (J) was equivalent to 0.25 cc. of "H".

or, 0.5 cc. of J = 0.25 cc. of H

therefore, 0.5 cc. J = 0.3 cc. P

In other words, one-half the activity of the original aqueous extract was removed by the treatment with Lloyd's reagent.

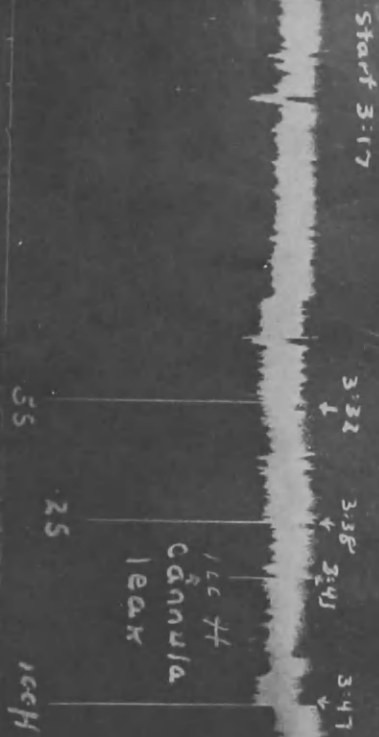
(b) On Blood Pressure

In order to preclude the possibility of the presence of the Moir principle in the extract, the liquids H and J were tested, by the usual blood pressure method, on a dog. (This experiment was necessary because histamine gives the same type of activity on the isolated uterine muscle of the guinea pig as does the Moir principle). The tracing is shown on plate IV.

In this experiment, the last four doses (0.4 cc. H, 0.2 cc. S, 1 cc. J, 1 cc. J), showing on a moving kymograph, the fall and prompt recovery from depressor effect removed all doubt that the constituent responsible for the activity in the 2 unknown solutions was histamine, or a body analagous to histamine in its action.

Thus the principle partly extracted by Lloyd's reagent, and responsible for the activity shown on the guinea pig uterus, was histamine or a body analagous to histamine in its action, and the principle showing an activity similar in nature to that activity clinically described by Moir<sup>(23)</sup> was apparently absent from these extracts.

Dog: Male  
 Wt: 23.5 Kgs.  
 Nembutol Anesthesia



S = Std Histamine Soln 1:10,000  
 H = Original Ag-Alc Ext. 1:10  
 J = Same ext. (1:10) after treating with Lloyd's Reagent  
 B = Blank = 1cc 25% Pic. to 9cc. Water  
 H.L. Greenberg



M = Histamine + Atrolal-free  
 Ag-Alc. Ext. - M.R.T.  
 (1cc = 0.5cc. Ergot)  
 R = Atrolal-free Ag-Alc. Ext. 1:1  
 Soln. - M.R.T.

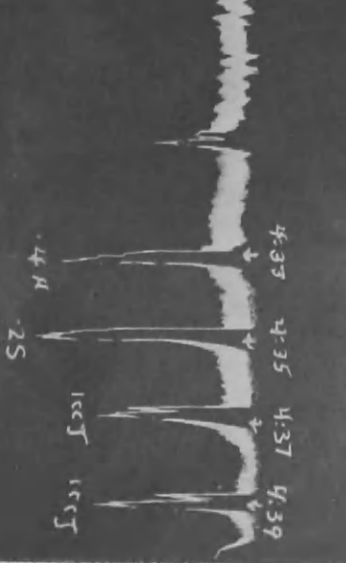


PLATE IV



It is believed that any clinically active substance such as described by Moir must necessarily be absent because of the fact that the extracts possessed no activity sufficiently persistent to be clinically significant. This point has been studied extensively by Thompson(127) and he has concluded that the activity, as measured in plate IV, is of a histamine-like character and exerts no significant uterine activity on the intact pregnant, non-pregnant or puerperal animal.

After the elapse of one year, liquid H was tested for amine activity in the laboratory of Professor M.R.Thompson by Mr.C. T.Ichniowski, using the blood pressure method. It was found, on assaying the amine content in terms of histamine, that the liquid extract contained less than 0.0025 mg. of amines per 0.15 cc.

As a result of this report no attempt was made to isolate amines from the extract because only an insignificant quantity of the substances appeared to be present.

d. Identification of Potassium and Magnesium Phosphates

Fifty cc. of the 25 per cent alcoholic extract was treated with 150 cc. of 95 per cent alcohol which resulted in the production of a fine precipitate. This was allowed to stand several hours, at the end of which a crystalline deposit was found. After treatment with charcoal and recrystallization, the phosphates were identified as follows:

(1) Potassium dihydrogen phosphate

By means of the flame test potassium was identified.

The phosphate radical was identified when on the addition of nitric acid and ammonium molybdate solution, a yellow precipitate resulted which was soluble in sodium hydroxide solution. The compound was identified as the dihydrogen phosphate through its solubility: Soluble in cold and hot water but insoluble in alkalies.

(2) Magnesium phosphate

This substance was identified when on the evaporation of its aqueous solution to about 2 cc., addition of ammonium chloride solution, addition of ammonium hydroxide until basic, and the addition of sodium dihydrogen phosphate solution, a white precipitate was produced as soon as the walls of the test tube were scratched with a glass rod. This precipitate indicated magnesium and since the phosphate radical was the only one found, the compound must have been a magnesium phosphate.

e. Identification of Glucose

The glycoside, Clavicepsin, reported as being a constituent in ergot by Marino-Zuco and Pasquero<sup>(87)</sup> was not expected to be present, as such, in this hydro-alcoholic extract. It was felt that Clavicepsin could not be present because at this time the extract was over a year old and was definitely acid to litmus. Since the glycoside is easily hydrolyzed by boiling with mineral acid<sup>(87)</sup>, it was thus thought that, although there had been no boiling, the length of time during which the Clavicepsin had stood in contact with the acid had provided for its hydrolysis. This view was supported by the fact

that the original extract already showing reducing properties towards Fehling's solution, did not show any appreciable increase in the degree of reducing power exhibited after heating with mineral acid. Consequently no attempt was made to isolate Clavicepsin from this extract. Instead, it was decided to try to isolate glucose as the phenyl osazone. The method employed was the usual method of procedure. Twenty cc. of extract was treated with 2 Gm. of a mixture consisting of 1 part phenyl hydrazine hydrochloride to 2 parts of sodium acetate. The test tube was placed in a boiling water bath and allowed to stand for 30 minutes. A yellow osazone was obtained and was recrystallized from boiling 60 per cent alcohol, M.P. 205°. Since it was observed that no white hydrazone was formed during the process of reaction, the possibility of the osazone having been formed from mannose was eliminated. The melting point and shape of the crystals viewed under the microscope limited the possibility to levulose and glucose as being the source of the osazone. In order to decide between the two, a Seliwanoff test for ketoses was run on the original extract. This reaction consists in the development of a deep red color followed by the appearance of a precipitate when a ketose, such as levulose, is heated with strong hydrochloric acid and resorcinol. The test was run as follows: Ten cc. of the reagent (0.5 Gm. resorcinol in 100 cc. hydrochloric acid diluted to 300 cc. with water)

was placed in a test tube and 2 cc. of the solution to be tested was added and immersed in boiling water. If a ketose is present, the solution will quickly turn red and a reddish precipitate will appear.

Three tests were prepared as follows:

1. Ten cc. of reagent and 2 cc. M/5 glucose + 2 cc. extract produced a negative result (no red).
2. Ten cc. of reagent + 2 cc. M/5 levulose + 2 cc. extract produced a red color and precipitate.
3. Ten cc. of reagent + 2 cc. extract produced no red.

Thus it is seen that the original extract must have contained glucose.

#### f. General Analysis

This was conducted on the 25 per cent alcoholic extract essentially according to the lead method outlined in Rosenthaler's<sup>(128)</sup>, "The Chemical Investigation of Plants". After heating 2-1/2 liters of the extract to boiling it was mixed with a saturated solution of lead acetate in slight excess. The lead precipitate (A) obtained, was washed by decantation, first with saturated lead acetate solution, and then with water until the washings were no longer acid to litmus. The filtrate, including the washings, concentrated by evaporation, was precipitated with basic lead acetate solution and washed with first this solution and then water, as above. The precipitate (B) was separated from liquid B in the same way as precipitate A from liquid A.

Precipitate A was then refluxed with 95 per cent alcohol for several hours, but only a negligible amount of extractive was obtained in this manner.

The residual precipitate A was then treated with ten per cent acetic acid. In order to ascertain the amount of material extracted by the 10 per cent acetic acid, basic lead acetate solution was added to some of the liquid extract. The absence of a precipitate, resulting on the addition of the basic lead acetate solution, indicated that practically no material had been extracted. The solution was then neutralized with sodium carbonate solution and then shaken with chloroform. No extractive was contained in the chloroform, as shown on evaporation. Ether likewise failed to extract anything from the neutral solution. Since it was thus shown that the original acetic acid extraction must have contained very little extractive matter, work on it was stopped.

The portion of precipitate A not dissolved by alcohol and acetic acid was suspended in water and decomposed with hydrogen sulphide. The lead sulphide was filtered off and set aside for investigation. The filtrate was treated as follows: A stream of carbon dioxide gas was passed through the liquid to remove the excess hydrogen sulphide. The liquid had a dark yellow color and pyroligneous odor. A test for tannins with ferric chloride solution was negative as was also a test for carbohydrates by the alpha-naphthol reaction.

The dark yellow liquid was shaken several times with ether and with chloroform. On concentrating the ether shakings, a small quantity of white needle-like crystals separated. The amount obtained, however, was too small for identification purposes. The chloroform extraction yielded nothing.

The yellow liquid was finally concentrated on a water bath and a small amount of dark syrupy residue remained.

The lead sulphide precipitate was next examined in order to see if it had carried down any substance with it on precipitation. The black precipitate was treated as follows:

The precipitate was extracted with portions of boiling water which yielded liquids similar in color and odor to the one obtained above. These liquid extracts were much darker at the beginning of the extraction than at the end. They were concentrated separately on a water bath, and in a beaker containing some of the lighter colored liquid, after its concentration, a minute quantity of whitish powdery substance was noted.

(1) Identification of Sulphur

The part of the lead sulphide precipitate not dissolved by the boiling water was next extracted with boiling alcohol. On allowing the hot solution to cool, after filtering, needle-like yellow crystals appeared.

These crystals were recrystallized from both alcohol and chloroform. They melted on ignition and at first, due to the present of impurity, they appeared to be organic in nature. After repeated recrystallization, however, it was seen that the crystals were inorganic. They melted at  $119^{\circ}$  and could be sublimed. They were soluble in chloroform and in hot 90 per cent alcohol; they were insoluble in water and cold alkalies. From these properties the substance was identified as monoclinic sulphur, present probably as a result of the use of hydrogen sulphide during the course of this procedure.

(2) Isolation of Mannitol

The precipitate (B), obtained by adding saturated solution of lead subacetate to the filtrate from (A), was divided into 2 parts. One part was extracted with boiling alcohol, but this procedure failed to extract any appreciable amount of material from the lead precipitate. The 2 parts of the lead precipitate were then combined, suspended in water, warmed on a water bath, and decomposed by introduction of hydrogen sulphide. The filtrate was evaporated to syrupy consistency under reduced pressure and then precipitated with alcohol. The precipitate was washed with alcohol and dried over sulphuric acid. The dry precipitate was then extracted by boiling with alcohol which on cooling gave rise to a white crystalline precipitate. This was purified by repeated solution in water and fractional precipitation. It was finally crystallized

from water. M.P.  $166^{\circ}$ . Since the above was essentially the process used by Kraft<sup>(77)</sup> in obtaining mannitol from erogt, the substance herein obtained was thought to be mannitol also. The addition of some mannitol to the white crystals did not lower the above melting point, consequently the crystalline substance was concluded to be pure mannitol.

The filtrate (B) obtained on separation of precipitate (B) was treated with hydrogen sulphide to decompose the lead subacetate present and filtered to remove the resulting lead sulphide precipitate. The filtrate, after being treated with carbon dioxide to expel the hydrogen sulphide, was divided into 3 parts. Part one was concentrated to syrupy consistency on a water bath. This syrup on being seeded with crystalline trehalose and after prolonged standing, failed to yield any trehalose. Since this was essentially Mitscherlich's<sup>(85)</sup> procedure in obtaining trehalose, it was concluded that the disaccharide, if originally present in the extract, had been hydrolyzed into its component molecules of glucose. Part two of liquid (B) was concentrated on a water bath and allowed to evaporate in vacuo over sulphuric acid.

The resulting mass remains to be investigated as do also part three of liquid (B) and the various lead sulphide precipitates obtained during the course of the procedure.



SUMMARY AND CONCLUSIONS

1. The total ash of the samples of drug examined varied from 2.83 per cent to 3.01 per cent.
2. The elements found to be present in the ash were: Iron, aluminum, magnesium, manganese, sodium, potassium, calcium, copper, lead, and phosphorous.
3. A sample of ergot, sample A, was found to have an alkaloidal potency of 175 per cent.

Three attempts to isolate ergotamine from this lot of drug, using a process essentially that of Stoll, were unsuccessful. It was, therefore, concluded that this drug originally contained little, if any, ergotamine.

4. Ergotoxine and ergotinine were obtained from lot A by means of the Kraft process.
5. Ergochrysin was obtained, by the Kraft process, from sample A.
6. A "fluidextract" prepared from the marc of a drug extracted with ether, showed an alkaloidal potency of less than 5 per cent.
7. On the basis of comparative studies made on uterine muscle and on blood pressure, it was found that about 50 per cent of a substance believed to be either histamine or a substance exhibiting an activity similar in nature to that of histamine, was extracted from a hydro-alcoholic solution by means of Lloyd's reagent.

8. The principle showing an activity of the type described by Moir was apparently absent from these extracts. It was concluded that any clinically active substance, such as described by Moir, must necessarily be absent because of the fact that the extracts possessed no activity sufficiently persistent to be clinically significant.
9. A precipitate, consisting partly of crystalline potassium dihydrogen phosphate and a magnesium phosphate, was obtained on addition of 3 volumes of 95 per cent alcohol to a 25 per cent alcoholic extract.
10. The acidity of an extract of this type (e.g. 25 per cent alcoholic extract) together with the time factor involved, resulted in the production of glucose through hydrolysis of either Clavicepsin or Trehalose, or both.
11. Crystals of sulphur were obtained during an analytical procedure which involved the use of hydrogen sulphide.
12. A substance believed to be mannitol was obtained from a 25 per cent alcoholic extract of a drug previously extracted with ether.

## - ADDENDUM -

Since completion of this paper, a new alkaloid, ergoclavine, has been reported. At present, however, only a brief description is available since the complete report has not been published.

Küssner<sup>(129)</sup> obtained ergoclavine in the following manner: The total alkaloids were extracted from solution in benzene or ether, by means of very dilute acid. By making alkaline with sodium hydroxide solution, the greater part of the ergotinine present, was obtained. After acidifying the alkaline liquid, ergotoxine was separated by renewed shaking with ether. By subsequent extraction of the liquid, made alkaline with soda, with trichlorethylene, a hitherto unknown alkaloid was obtained (ergoclavine).

Ergoclavine has the following properties: It crystallizes from 90% alcohol; after drying at about 20° and 30 mm. pressure, over phosphorous pentoxide, it has a melting point of 170-171° and an optical rotation in chloroform (1%) of +115°. This alkaloid, after drying as described, lost weight after heating for several hours at 80° and at a pressure of from 1 to 2 mm. The completely water-free or solvent-free, very hygroscopic substance melted at 177 to 178°,  $[\alpha]_D^{22} = +124^\circ$  (1% in chloroform). Incomplete analytical determinations, as reported by Küssner, have indicated the formula of the alkaloid to be  $C_{31}H_{39}N_5O_6$ .

According to Kreitmar<sup>(129)</sup> the pharmacologic activity both qualitatively and quantitatively was the same as that of ergotoxine.

The above constants of ergoclavine bear a resemblance to none of the previously discussed ergot alkaloids, but they do strikingly resemble those constants reported for the so-called "new alkaloid" sensibamine (found by Stoll to be impure ergotamine<sup>(130)</sup>) in British Patent No.388,529 of Chinoïn A.G. and Wolf. The difference between ergoclavine and sensibamine, as given by Küssner, was that the solubility of the former remained constant in solvents such as methyl and ethyl alcohols, acetone, and ethyl ether. When sensibamine was dissolved in any of these solvents, however, there was rapid transformation of the alkaloid into a substance insoluble in the solvent used.

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