REVIEW ARTICLE

THE ASSAY OF ERGOT AND ITS PREPARATIONS

BY G. E. FOSTER, B.Sc., Ph.D., F.R.I.C.

Chief Analyst, Wellcome Chemical Works, Dartford, Kent

OF the early reviews on ergot and its alkaloids those of the late Prof. Barger[1,2] are perhaps the best known, but the rapid advance in our knowledge of the alkaloids during the last 20 years has rendered out of date much of the information in these publications. Stoll[3,4], Wilkinson[5] and Glenn[6] have more recently reviewed the chemistry of the ergot alkaloids and it is the object of the present communication to give an account of the assay of ergot and its preparations, a field of investigation by no means exhausted and one in which much progress has been made in recent years. As an introduction to this subject a brief account of the alkaloids, already isolated from ergot, is desirable.

THE ERGOT ALKALOIDS

It is well known that the alkaloids of ergot may be separated into two main fractions generally described as the "water-soluble" and "water-insoluble" alkaloids respectively. Ergometrine, the most biologically important alkaloid, occurs in the water-soluble fraction. The alkaloids have the unusual property, characteristic of the group, of occurring in isomeric pairs, of which the lævorotatory member in each case is the more biologically active. Table I includes data by Stoll[4] for all the known alkaloids.

TABLE I

THE NATURAL ALKALOIDS OF ERGOT

Name	Formula	$[\alpha]_D^{20°\,C.}$ in $CHCl_3$	Discoverer
Ergotamine Ergotaminine	$C_{33}H_{35}O_5N_5$	−155° +385°	Stoll[7]
Ergosine Ergosinine	$C_{30}H_{37}O_5N_5$	−179° +420°	Smith and Timmis[8]
Ergocristine Ergocristinine	$C_{35}H_{39}O_5N_5$	−183° +366°	Stoll and Burckhardt[9]
Ergokryptine Ergokryptinine	$C_{32}H_{41}O_5N_5$	−187° +408°	Stoll and Hofmann[10]
Ergocornine Ergocorninine	$C_{31}H_{39}O_5N_5$	−188° +409°	Stoll and Hofmann[10]
Ergometrine Ergometrinine	$C_{19}H_{23}O_2N_3$	− 44° +414°	*Dudley and Moir[11] Smith and Timmis[12]

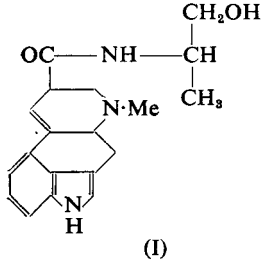
* See also Thomson[13], Kharasch and Legault[62] and Stoll and Burckhardt[63].

Much confusion in nomenclature was caused when Stoll and Hofmann[10] separated what had previously been known as ergotoxine[14] into 3 individual alkaloids, ergocristine, ergokryptine and ergocornine. Not all of these alkaloids and their corresponding dextrorotatory isomers were new

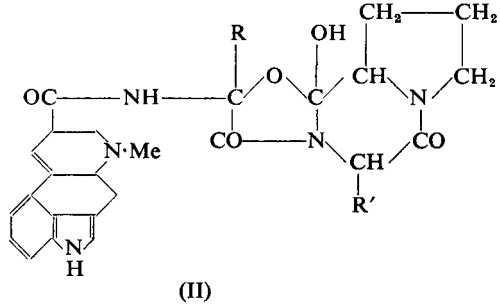
and some of them, in varying states of purity, had been described previously under other names. Smith[15] and his colleagues have correlated the old and new nomenclatures in the following scheme showing interconvertible pairs of alkaloids:—

(Ergocornine) Ergotoxine ⇌ ψ-Ergotinine[17] (Ergocorninine)
Ergocristine ⇌ Ergotinine[16] (Ergocristinine)

All the ergot alkaloids contain either lysergic acid or *iso*lysergic acid as the principal constituent of the molecule. The alkaloids of the "water-insoluble" group are polypeptides in which lysergic acid and *iso*-lysergic acid are joined to other amino-acids. The last pair of alkaloids in Table I, ergometrine and ergometrinine, are in a class of their own and have a simpler structure, the lysergic or *iso*lysergic acid being combined with an amino-alcohol. Within a few months of its isolation ergometrine was known to be the amide of D-lysergic acid and L-2-aminopropan-1-ol and its structure was later confirmed by partial synthesis[18]. The structural formulæ for ergometrine and the "water-insoluble" alkaloids[19] are shown in I and II respectively.



(I)



(II)

Detailed structures of the individual alkaloids represented by II are given by the following key:—

Alkaloid	R	R′
Ergotamine	CH_3	CH_2Ph
Ergosine	CH_3	$CH_2{\cdot}CHMe_2$
Ergocristine	$CH{\cdot}Me_2$	CH_2Ph
Ergokryptine	$CH{\cdot}Me_2$	CH_2CHMe_2
Ergocornine	$CHMe_2$	$CHMe_2$

The M. I. Smith Colour Reaction

At the stage in the long history of ergot when the paramount importance of the alkaloids in medicine became apparent, the need for a satisfactory chemical method for the assay of the drug and its preparations became of major importance. Forst[20] described a gravimetric assay process in which the alkaloids were isolated and weighed, while the German Pharmacopœia 6 included a process in which the alkaloids after extraction were titrated with standard acid. These assays required large samples (100 to 500 g.) of ergot for a determination and during the search for more sensitive methods attention was turned to colour reactions. The so-called Tanret-Keller reaction, first described by Tanret[16] for ergotinine

and later by Keller[21], was found unsatisfactory as no homogeneous colour of sufficient stability for colorimetric work could be obtained. Van Urk[22] modified the Tanret-Keller ring test by using Ehrlich's reagent, consisting of a solution of *p*-dimethylaminobenzaldehyde in ethanol, and thereby made it much more sensitive. In 1930 Smith[23] published his paper describing a modification of the Van Urk reaction and from this classic paper a colorimetric technique of assay emerged and gained world-wide acceptance.

Smith used a M/60 solution of *p*-dimethylaminobenzaldehyde in concentrated sulphuric acid and added 1 ml. of this reagent to 2 ml. of a solution of alkaloid in 0·5 to 1·0 per cent. w/v tartaric acid. On exposure to direct sunlight the reaction mixture developed a deep blue colour reaching maximum intensity in 10 to 15 minutes; in diffuse daylight of a cloudy day the full development of colour might take half an hour to 2 hours. Under appropriate conditions the colour intensity was proportional to the alkaloidal content of the solution tested. It was found particularly necessary that the tartaric acid solution should contain alkaloid, substantially free from non-alkaloidal matter, and this condition must be fulfilled whatever the source of the alkaloid. One interfering substance that affects the results when the reaction is applied to the estimation of the alkaloidal content of ergot is a yellow pigment, soluble in ether and alkali and probably identical with that isolated by Freeborn[24], and the assay process must be designed to exclude this substance from the final reaction mixture.

A report[25] was published in 1931 by the British Pharmacopœia Commission describing the work of its Sub-committee on Ergot and making recommendations regarding the standardisation of ergot and its preparations to be included in the B.P. 1932. The Sub-committee recommended the adoption of the M. I. Smith colour reaction for purpose of assay using as reagent a 0·25 per cent. w/v solution of *p*-dimethylaminobenzaldehyde in sulphuric acid. An alternative reagent consisting of a 0·1 per cent. w/v solution of *p*-dimethylaminobenzaldehyde in hydrochloric acid was also described and had the advantage of not producing heat when mixed with the test solution. The presence of traces of peroxide in the ether, used for alkaloidal extraction, vitiated the colour reaction when hydrochloric acid was used and as the sulphuric acid reagent was less affected it was preferred. It should be noted, however, that ether should always be tested to ensure the absence of peroxides before commencing an assay.

A most valuable contribution to the subject was made by Allport and Cocking[26] when they discovered that if a small amount of ferric chloride was added to the reagent the development of colour, obtained on mixing the reagent with a solution of ergot alkaloids, was greatly accelerated and that exposure of the reaction mixture to bright daylight was unnecessary. These authors recommended a reagent prepared by dissolving 0·125 g. of *p*-dimethylaminobenzaldehyde in a cooled mixture of 65 ml. of sulphuric acid and 35 ml. of water to which 0·1 ml. of 5 per cent. w/v ferric chloride solution is added. When carrying

out a determination they added 2 ml. of this reagent to 1 ml. of test solution.

The colour reaction may be used for quantitative work in two ways. A calibration curve may be constructed from colour readings obtained with dilutions of standard solution of pure ergot alkaloid, and the strength of the unknown solution may then be read from the curve after the colour developed with the reagent under controlled conditions has been measured. Alternatively, the test reaction mixture may be compared directly with a standard reaction mixture prepared independently for each determination. There is little doubt that the latter technique is the better for, in the author's experience, batches of reagent differ slightly in their colour producing properties and calibration curves cannot be relied upon for the most accurate work. Silber and Schulze[27] none the less have quite recently advocated the use of calibration curves and have recommended that reaction mixtures should be irradiated in the light of a mercury vapour lamp to ensure the full development of colour. This appears to be unnecessary, however, for when a test and a standard solution are compared together under identical conditions using the Allport–Cocking reagent the reaction mixtures may be matched with every confidence after allowing 5 minutes for colour development.

The lysergic or *iso*lysergic portion of the molecule is responsible for the Smith colour reaction which is given by both members of each isomeric pair of alkaloids. It must therefore be emphasised that a colorimetric estimation will determine the total alkaloidal content of the material under examination and will not necessarily estimate the biological potency in terms of the standard. Conclusions regarding potency can only be reached when the individual alkaloids have been suitably separated before the colorimetric assay is performed.

Reference Standards

The accuracy of many modern assays, both chemical and biological, is greatly enhanced when standard products are available for comparison. Ergotoxine ethanesulphonate and ergotamine tartrate were both available in a high state of purity when the colorimetric assay of ergot was developed and it was natural that standard solutions of these salts should be employed for assay purposes. Ergotoxine ethanesulphonate was adopted as a reference standard by the B.P. 1932 and the U.S.P. XI and has been mainly used in English-speaking countries, while ergotamine tartrate has been preferred by most European workers. It may be stated that both salts provide equally satisfactory standards.

The continued use of ergotoxine ethanesulphonate was rendered uncertain, however, by the work of Stoll and Hofmann[10] on the isolation of ergocristine, ergokriptine and ergocornine from some samples of ergotoxine. It was unfortunate that ergotoxine ethanesulphonate from one source consisted substantially of ergocornine ethanesulphonate, while the salt supplied by another maker contained a mixture of alkaloids. Foster[28] prepared a sample of purified ergocornine ethanesulphonate and compared it colorimetrically with what had previously been known

as ergotoxine ethanesulphonate. No difference could be detected with a visual colorimeter, while the Spekker absorptiometer indicated that the purified salt gave slightly the stronger colour.

The isolation of ergometrine and its recognition as the most important ergot alkaloid made it necessary to assay the drug for ergometrine, and this factor together with the uncertainty regarding the composition of ergotoxine ethanesulphonate caused the B.P. 1953 and the U.S.P. XIV to adopt ergometrine maleate as the standard of reference. A specification for pure ergometrine maleate is given in Appendix I of the B.P. 1953 and the U.S.P. organisation supplies samples of U.S.P. Reference Standard (Ergonovine Maleate).

The International Pharmacopoeia (1951, Vol. I) uses ergotamine tartrate as a standard of reference for the assay of ergot preparations. As the intensity of colour developed in the Smith test varies inversely as the molecular weight of the alkaloid, the content of water-soluble alkaloids calculated as ergotamine is multiplied by 0·559 in order to afford figures calculated as ergometrine.

In a recent publication Strong and Maurina[41] reported that ergometrine maleate deteriorated less rapidly on storage than does ergotoxine ethanesulphonate and, on this account, is to be preferred as a reference standard.

Spectrophotometric Assays

The alkaloids of ergot all exhibit in solution well-defined absorption in the ultra-violet region of the spectrum. Of early workers, Brustier[29] measured the absorption spectrum of ergotinine, and spectrophotometric data were used by Harmsma[30] and Van Itallie[31] for the assay of the alkaloids. The absorption curves which are similar for all the alkaloids have maxima at λ316–318 mμ and minima at λ272 mμ. Wokes and Crocker[32] carried out an extensive study of all the alkaloids available to them and published much spectrophotometric data. After the isolation of ergometrine Allport and Crews[33] carefully measured the ultra-violet absorption of this new alkaloid and found that although it was similar to that of the other alkaloids it exhibited greater intensity of absorption at the maximum. The B.P. 1953 (Appendix I) states for ergometrine maleate, to be used as a reference standard, that the extinction of a 1 cm. layer of a 0·0035 per cent. w/v solution in water at 312 mμ, calculated with reference to the substance dried to constant weight at 100° C. at a pressure not exceeding 5 mm. of mercury, is 0·635 to 0·645.

Like the Smith colour reaction the ultra-violet absorption of the alkaloids is characteristic of the lysergic or *iso*lysergic portion of the molecule and will therefore be proportional to the total alkaloidal content and not necessarily to the biological potency of the material under examination.

The blue colour produced in the Smith test has been examined spectroscopically by Wokes and Crocker[32] and by Allport and Crews[33]. Using the reagent proposed by Allport and Cocking[26] the colours produced by ergometrine and ergotoxine are the same and exhibit a maximum absorption at 550 mμ. With the original reagent of Smith, however,

the maximum absorption occurs at 580 mμ and the modified reagent containing ferric chloride therefore produces a colour different from that produced in the absence of the chemical catalyst. The accuracy of the colour test may be very considerably increased by employing a spectrophotometer and measuring the transmission of the reaction mixture at the wavelength of maximum absorption. Alternatively, a photoelectric colorimeter may be used together with an appropriate filter. Strong and Maurina[41] suggest measuring the absorption at 590 mμ.

A typical infra-red absorption spectrum for ergot alkaloids has been recorded[34] but the data were not employed in quantitative work.

Biological Assays

In view of the chemical complexity of ergot and its preparations it is not surprising that early workers should have regarded chemical standardisation with disfavour and resorted to pharmacological tests. For many years the cockscomb method suggested by Kobert[35] and described by Edmunds and Hale[36] was used and became the official method of the U.S.P. for the standardisation of ergot. In this assay a solution of the alkaloidal preparation is injected deeply into the breast muscles of a Leghorn cock and the characteristic darkening of the comb produced within 1 to 1½ hours is assessed in terms of a similar discoloration produced by a solution of reference standard consisting of fluid extract of ergot, ergotoxine ethanesulphonate or ergotamine tartrate. As experience was gained it became evident that the cockscomb method although satisfactory as a qualitative test, is insufficiently accurate for quantitative work.

Broom and Clark[37] in 1923 proposed a much more sensitive test based upon the well-known property possessed by ergotoxine of inhibiting the motor action of adrenaline on the plain muscle of the excised uterus of the rabbit. This method has been used in laboratories all over the world but opinion differs as to its precision. The B.P. sub-committee on ergot[25] concluded that no greater accuracy than ± 25 per cent. could be looked for by this method without a larger number of tests than could reasonably be carried out for the routine examination of a drug such as ergot.

Ergometrine, unlike ergotoxine, exhibits no antagonism to adrenaline and in fact shows some measure of resemblance to sympathomimetic drugs. Several procedures have been described for the biological assay of ergometrine but none so far has gained general acceptance by pharmacologists. The fall in body temperature of rabbits produced by ergometrine, together with its mydriatic action, has been used by de Beer and Tullar[38] who described an assay giving an accuracy of ± 20 per cent. using 16 rabbits. A more promising method was proposed by Vos[39] and depends upon the time for absorption of the drug on an isolated rabbit uterus. Foster and Stewart[40] employed this assay in their work on the stability of ergometrine preparations. In general terms the assay consists of adding the dilutions of test and standard preparations to an isolated organ bath, in which there is set up a segment of rabbit uterus, and

measuring the latent time before the uterus contracts. Concentrations of the standard and test preparations are adjusted to give latent periods of 1 to 3 minutes. The error of the assay is of the order of ± 14 per cent.

Owing to the difference between the pharmacological properties of the "water-soluble" and "water-insoluble" ergot alkaloids care must be taken to select an appropriate standard for biological work. The accepted standards of reference which are all equally satisfactory for colorimetric assays cannot be used indiscriminately in pharmacological determinations.

Chromatographic Methods

It has been known for the past 20 years that ergot alkaloids can be adsorbed on columns of alumina, magnesium oxide and other materials and subsequently eluted with suitable solvents. This technique was employed more especially in preparative work[42] and sometimes merely for the purpose of separating the alkaloids from coloured impurities. Brownlee[43] suggested the use of a column of alumina in the assay of liquid and soft extracts of ergot for removal of colouring matter before estimating the alkaloidal content.

In 1949 Foster, Macdonald and Jones[44] applied the technique of paper partition chromatography[45] to the separation and identification of ergot

TABLE II

ALKALOIDS CHARACTERISED BY STRUCTURES DERIVED FROM LYSERGIC OR *iso*LYSERGIC ACID, AMMONIA, A KETO-ACID, L-PROLINE AND ONE OTHER AMINO-ACID

Additional amino-acid	Ergotamine group (pyruvic acid group)	Ergotoxine group (dimethyl-pyruvic acid group)
L-phenylalanine	Ergotamine Ergotaminine $C_{33}H_{35}O_5N_5$	Ergocristine Ergocristinine $C_{35}H_{39}O_5N_5$
L-leucine	Ergosine Ergosinine $C_{30}H_{37}O_5N_5$	Ergokryptine Ergokryptinine $C_{32}H_{41}O_5N_5$
L-valine	—	Ergocornine Ergocorninine $C_{31}H_{39}O_5N_5$

alkaloids. Employing *n*-butanol-acetic acid-water mixture as solvent and detecting the alkaloids on the paper by means of their fluorescence in ultra-violet light it was immediately evident that ergometrine had been separated both from the "water insoluble" alkaloids and from the relatively biologically inactive ergometrinine. As a result of this work a specific physical chemical assay of ergot for ergometrine became available, for an approximate estimate of the ergometrine on the paper could be made by matching its fluorescence in ultra-violet light against a series of standards on the same paper. An accuracy of ± 20 per cent. was claimed by the authors.

Foster *et al.*[44] extended their work to the "water-insoluble" alkaloids, which are characterised by structures derived from lysergic and *iso*lysergic acids, ammonia, a keto-acid, L-proline and one other amino-acid. The relationships are summarised in Table II, embodying data by Stoll *et al*[46].

On hydrolysis the alkaloids afford amino-acids which may be separated and identified by paper partition chromatography, using the methods of

Consden, Gordon and Martin[45]. Table III includes some results obtained when samples of "pure" alkaloids were examined by this method. The technique is a valuable tool not only for identifying but also for assessing the purity of ergot alkaloids. For example, some of the alkaloids included in Table III are obviously contaminated with traces of other alkaloids.

TABLE III

Alkaloid	Amino-acids, identified chromatographically
Ergotamine	Proline, phenylalanine
Ergotaminine	Proline, phenylalanine
Ergocristine	Proline, phenylalanine and faint trace of valine
Ergocristinine (ergotinine)	Proline, phenylalanine and faint trace of valine
Ergocornine (ergotoxine)	Proline, valine and trace of phenylalanine

The methods described by Foster *et al.*[44] were quickly applied and extended in laboratories in many parts of the world and, indeed, so many papers have now appeared on the use of paper chromatography in the ergot field that it is impossible to refer to them all in the present review. It is therefore fortunate that Berg[47] has recently published a monograph on the subject in which he has included a very full list of references. Reference will be made, however, to some papers which appear to the present author of special interest.

Brindle, Carless and Woodhead[48] studied the separation of ergot alkaloids on paper, buffered to a *p*H range extending from 2 to 7, and using ether saturated with water as solvent. The effect of change of *p*H, type of buffer and solvent used, were recorded for the more readily available alkaloids. Within the *p*H range 2 to 5 the "water-soluble" alkaloids remained stationary at the top of the chromatogram while separation of the "water-insoluble" alkaloids occurred. For quantitative work the chromatograms were cut into sections carrying the individual alkaloids which were then eluted with 50 per cent. ethanol. The eluates were evaporated and the colour test applied.

Preliminary work using buffered cellulose columns was described[48] and later extended by Carless[49] for the purpose of carrying alkaloidal loads greater than could be handled on paper. Whatman ashless cellulose treated with citrate-phosphate buffer *p*H 3 was employed and anæsthetic ether, saturated with water, was used as developing solvent. Fractions of eluate were assayed colorimetrically for alkaloid and the results recorded graphically. From the resulting elution curves the percentage composition of any alkaloidal mixture under test was readily ascertained. After the removal of the "water-insoluble" alkaloids the column was made alkaline by passage of developing solvent containing diethylamine. This had the effect of releasing the "water-soluble" alkaloids, previously held at the top of the column, and they could then be eluted and estimated in the usual manner. Carless claimed that in quantitative work recoveries of 90 to 95 per cent. were achieved.

Most of the chromatographic techniques now available have been applied in the ergot field. Both ascending and descending development

have been employed while circular[50,51], and "reverse phase"[52], paper chromatographic methods have been used for qualitative separation of the alkaloids.

Berg[47] favours the circular paper disc method and has investigated the use of a number of solvents over a wide *p*H range. He considers the following solvents to be most useful: (1) benzene, with 10 per cent. of ethanol, saturated with water; and (2) *iso*propyl ether, with 10 per cent. of ethanol, saturated with water. The method is very simple and consists of placing a dry buffered filter paper, to which the test solution has been applied at the centre and the solvent allowed to evaporate, on to a glass plate covering a dish containing the developing solvent. The centre of the filter paper is placed over a small hole in the glass plate through which a thread, attached to the centre of the filter paper, passes into the solvent. A second glass plate is then placed over the filter paper to close the system. The solvent passes up the thread and uniformly spreads over the paper from the centre. On examining the developed chromatograms in ultra-violet light the alkaloids are revealed as a series of concentric circles. The main disadvantage of this method is the impossibility of using authentic tracers alongside unknown alkaloids and also the difficulty of assessing the intensity of fluorescent circles for quantitative work.

A 2 per cent. solution of *p*-dimethylaminobenzaldehyde in hydrochloric acid and a 1 per cent. solution of cinnamic aldehyde in methanol have recently been suggested[53] for detecting ergot alkaloids on paper chromatograms.

The Assay of Ergot

Many investigations have been carried out on the assay of ergot which presents the difficult problem of isolating the total alkaloids from the drug in a condition suitable for assay purposes. For biological assay a crude extract as employed by the U.S.P. XI is satisfactory, but for colorimetric work the alkaloids must be more completely purified. It has become generally accepted that it is better to defat the ergot with light petroleum before extraction of the alkaloids, and although many solvents have been tried for the extraction, ether and chloroform are now usually employed; at one time the use of acetone was advocated by some workers but owing to the amount of colouring matter which it extracts it is now looked upon with disfavour. It is proposed to refer to and comment upon a number of assay processes which are known to have been widely used.

B.P. 1932. The assay included in the B.P. 1932 was based upon the recommendations of the Sub-committee on Ergot[25] and the extraction procedure is in some respects similar to that of the German Pharmacopœia 6. Powdered defatted ergot is placed in a closed container with ether, water and magnesium oxide and the mixture shaken at intervals during 30 minutes. After addition of powdered tragacanth to absorb the water an aliquot of the ethereal extract is shaken with portions of 1 per cent. tartaric acid solution and the resulting acid solution containing

the alkaloids assayed colorimetrically. This method estimates the "water-insoluble" alkaloids together with some of the "water-soluble" alkaloids. It provides a rapid sorting test and approximate figures for the total alkaloidal content are obtained.

B.P. 1948 *and International Pharmacopoeia* 1951. After the isolation of ergometrine it became obvious that the assay procedure of the B.P. 1932 was inadequate and Hampshire and Page[54] were the first to describe a method designed to estimate the "water-soluble" alkaloids in ergot. This process was subsequently included both in the B.P. 1948 and in the International Pharmacopoeia 1951.

In this method the defatted powdered ergot is treated with a little dilute ammonia and then extracted with ether for 5 hours in a continuous extraction apparatus. The total alkaloids are then removed from the ethereal extract by shaking with 1 per cent. tartaric acid solution and the assay completed as in the B.P. 1932 process.

A portion of the tartaric acid solution of total alkaloids is then made alkaline with ammonia and the alkaloids extracted with ether. The ethereal extract is freed from "water-soluble" alkaloids by repeated washing with very dilute ammonia solution and the "water-insoluble" alkaloids, remaining in the ether, re-extracted with 1 per cent. tartaric acid solution and estimated colorimetrically. The difference between the contents of total alkaloids and of "water-insoluble" alkaloids gives an estimate of the "water-soluble" alkaloids present.

This process served a useful purpose, before more specific methods were available, as a means of controlling the quality of ergot purchased commercially. It has been criticised, however, on several grounds. The "water-soluble" alkaloids are estimated by difference and consequently any loss of alkaloid during the second part of the assay will be estimated as "water-soluble" alkaloid. The prolonged boiling with ether during the extraction brings about some decomposition of the alkaloids, as pointed out by Hampshire and Partridge[55], and some inter-conversion of the isomeric alkaloids also probably occurs. Foster *et al.*[44] found that boiling with ether brought about some hydrolysis of the alkaloids and that the resulting lysergic acid was estimated as "water-soluble" alkaloid. Another shortcoming of the process is that it does not differentiate between ergometrine and ergometrinine.

National Formulary IX. Work sponsored by the U.S.P. and the National Formulary Committee was published[56] in 1948 describing a collaborative investigation on the assay of ergot and, as a result, the method included in the N.F. IX was developed. In this assay process powered ergot is first macerated with chloroform, to which a little methanolic ammonia is added, the product is transferred to a small percolator and extracted with chloroform. After removing the bulk of the chloroform at low temperature, ether is added, the alkaloids are extracted by shaking with 0·2N sulphuric acid and a portion of the acid extract is assayed colorimetrically for total alkaloids. Another portion of the acid extract is made alkaline to phenolphthalein with ammonia and shaken with carbon tetrachloride in order to remove "water-insoluble"

alkaloids. The aqueous layer is then saturated with sodium chloride and the alkaloid extracted with ether. The "water-soluble" alkaloids are then transferred from the ethereal solution to 0·2N sulphuric acid and estimated colorimetrically.

The process, although it makes no separation of ergometrine and ergometrinine gives, in the present author's experience, results in close agreement with the actual amount of "water-soluble" alkaloids present in ergot. While individual workers often get good agreement between replicate assays, however, inter-laboratory agreement is less good. Strong and Maurina[41] have made a careful study of the N.F. IX assay and have published figures showing the deviation in results to be expected between independent laboratories. Agreement is far better between experienced workers and there are some pitfalls which should be brought to the notice of analysts having little experience in this field. Exposure of extracts to bright daylight should be avoided and contact of the alkaloids with alkalis reduced to a minimum. Only acid solutions of alkaloids should be allowed to stand overnight and then in a cool place, preferably below 10° C.

Other Methods of Assay. Foster *et al.*[44], in order to avoid contact with hot solvent and strong alkali, treated defatted powdered ergot with sodium bicarbonate and extracted the moistened mass by percolation with ether, containing 5 per cent. of ethanol. The alkaloids were then extracted from the ethereal solution with 5 per cent. lactic acid and the ergometrine determined chromatographically.

Carless[49] recommended percolation of defatted ergot, treated with sodium bicarbonate and moistened with a little water, with chloroform containing 5 per cent. of ethanol. The percolate was allowed to drip and evaporate on a portion of cellulose powder which was then transferred to a buffered cellulose column and the alkaloids separated chromatographically before colorimetric estimation.

Silber and Schulze[27], in work designed for the examination of single sclerotia, devised the following process: 0·1 g. of defatted powdered ergot is shaken for 2 hours with 6 ml. of ether and 0·1 ml. of 10 per cent. ammonia. The ether is separated from the ergot by centrifuging and the solution extracted successively with buffer *p*H 8 and with 2 per cent. tartaric acid solution. The buffer and tartaric acid extracts are then assayed colorimetrically for "water-soluble" and "water-insoluble" alkaloids respectively. The process, which does not distinguish between the individual alkaloids, has been used extensively by European workers studying the commercial cultivation of ergot.

The importance, both clinically and economically, of ergometrine has resulted in the chemical estimation of this alkaloid becoming of major interest. Chromatographic methods alone are specific for the individual alkaloids, although methods giving figures for "water-soluble" and "water-insoluble" alkaloid can be of value when ergots of the same variety, in which the ratio of the two alkaloidal fractions is reasonably constant, are compared. Table IV gives some typical results[44] obtained when samples of ergot were assayed by the process of the B.P. 1948, by that of the N.F. IX and chromatographically.

It will be seen that the N.F. IX and chromatographic methods gave results for the ergometrine content which were in fair agreement, but that the B.P. 1948 process afforded figures far in excess of the ergometrine present. This conclusion was confirmed when the analytical results were compared with the yields of ergometrine obtained during manufacturing operations.

TABLE IV

Sample of ergot	B.P. 1948 Process		National Formulary Process		Ergometrine determined chromatographically per cent.
	Total alkaloids expressed as ergotoxine per cent.	Water-soluble alkaloids expressed as ergometrine per cent.	Total alkaloids expressed as ergotoxine per cent.	Water-soluble alkaloids expressed as ergometrine per cent.	
1	0·22	0·043	0·215	0·023	0·019
2	0·10	0·0125	0·12	0·0086	0·006
3	0·19	0·038	0·195	0·025	0·026
4	0·16	0·040	0·195	0·020	0·016
5	0·20	0·045	0·21	0·022	0·024

Fluid and soft extracts of ergot may be assayed by simple modifications of the methods described for ergot. A fluid extract, for example, may be diluted with 1 per cent. lactic acid and examined by paper chromatography, when the colouring matter remains almost stationary at the top of the chromatogram.

Some Analytical Applications

The analytical procedures which have been reviewed are widely used commercially for the assay of ergot, its crude extracts and preparations containing the pure alkaloids. In particular, chromatographic methods have been of value for controlling the stages of manufacture of the alkaloids and in studying the stability of pharmaceutical preparations. For example, the optimum *p*H of an injection of ergometrine maleate in order to reduce formation of ergometrinine was established by use of paper chromatography. Analytical applications in the future, however, are likely to cover a wider field to meet the changing conditions of the ergot market.

We must not forget that ergot is a fungus infecting growing grain and is a pest which farmers have no wish to see on their crops. It is therefore not surprising that vigorous steps have been taken by agricultural authorities in countries where wild ergots are found, to bring infection of crops under control. This policy is reducing the available supplies of ergot resulting from natural infection and increasing attention is being given to cultivated ergots, upon which future supplies of the drug may well depend.

There are several stages in the life cycle of ergot and these are of importance when its cultivation is under consideration. The number of recorded *in vitro* attempts to cultivate *Claviceps purpurea* with a view to alkaloidal production is extremely small, and it is not possible to define the stage of growth at which alkaloid formation occurs. Schweizer[57] used as medium an emulsion of germinated rye grains sterilised

by use of organic antiseptics. Ascospores, obtained by germination of sclerotia in moist sand, were transferred to the rye emulsion. Germination was rapid and it was claimed that sclerotia were obtained. Other workers[58,59] have been less successful and in general found growth to proceed only to the production of mycelial tissues and conidia; no more than traces, if any, of alkaloid resulted. While future developments may enable sclerotia to be cultivated successfully *in vitro* artificial inoculation of growing rye is the only method at present offering commercial possibilities.

It is well known that conidia, produced by culture of ergot to the mycelium stage, may be used to inoculate ears of flowering rye and that normal development of sclerotia then occurs. Stoll[3] has described the application of this process on a commercial scale and there is little difficulty in carrying it out. The procedure has been successfully performed experimentally in many parts of the world, including Great Britain, where some large-scale field trials have been carried out in recent years.

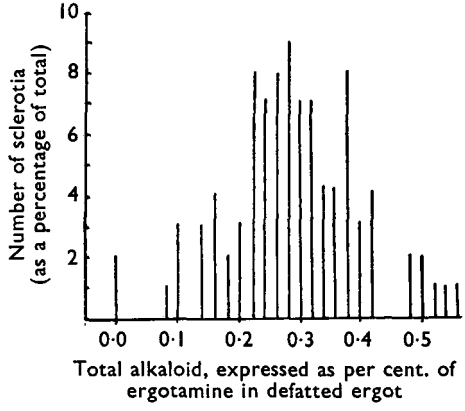


FIG. 1. Alkaloidal content of single sclerotia of Portuguese ergot.

The cultivation of ergot has opened a wide field for research of interest to botanists, chemists, mycologists, pharmacists and pharmacologists. Growth of strains of *Claviceps* on different hosts, cultivated under varying climatic conditions and on soils of differing composition, will provide an absorbing topic. By such means it may be possible to select strains affording maximal yields of ergometrine or ergotamine, commercially the most valuable alkaloids. In this work chemical analysis will play a part of paramount importance, for progress will be impossible without rapid and reliable methods capable of separating, identifying and estimating the alkaloids in the many samples to be examined.

Silber and Bischoff[60] have recently reported a very extensive study of the alkaloidal contents of strains of cultivated ergot. They investigated the alkaloidal distribution in different parts of the sclerotia, the alkaloidal content at different stages of development of the sclerotia and in ergots cultivated under different climatic and soil conditions. It was concluded

that the strain of ergot is the most important factor governing alkaloid formation. By examination of individual sclerotia it was found, as shown in Figure 1, that although the average total alkaloidal content of a strain of ergot is fairly constant there is a marked variation between sclerotia taken from the same crop.

Some interesting work has also been reported from America[53,61], where the effect of adding various compounds, representing structural moieties of lysergic acid, to media used for culture of *C. purpurea* has been studied.

The present author, when describing the first application of paper chromatography in the ergot field[44] to the 1949 meeting of the British Pharmaceutical Conference, referred to the already voluminous literature on ergot and stated that perhaps the paper he was reading might be the beginning of the end. It has actually proved to be more the end of the beginning and this review has been written more particularly for those who may be entering this fascinating field for the first time, in the hope that it may help to place in their hands the tools which will enable them to discover for themselves that abiding interest provided by the study of ergot.

REFERENCES

1. Barger, *Ergot and Ergotism*, Gurney and Jackson, London, 1931.
2. Barger, *Analyst*, 1937, **62**, 340.
3. Stoll, *Experientia*, 1945, **1**, 250.
4. Stoll, *Chem. Reviews*, 1950, **47**, 197.
5. Wilkinson, *Annual Reports on Applied Chemistry*, 1951, **36**, 175.
6. Glenn, *Quart. Reviews* (*Chem. Soc., London*), 1954, **8**, 192.
7. Stoll, Swiss Patent 79,879 (1918).
8. Smith and Timmis, *J. chem. Soc.*, 1937, 396; *Nature, Lond.*, 1936, **137**, 111, 1075.
9. Stoll and Burckhardt, *Z. physiol. Chem.*, 1937, **250**, 1.
10. Stoll and Hofmann, *Helv. chim. Acta*, 1943, **26**, 1570.
11. Dudley and Moir, *Brit. med. J.*, 1935, **1**, 520.
12. Smith and Timmis, *Nature, Lond.*, 1935, **136**, 259; *J. chem. Soc.*, 1936, 1166.
13. Thompson, *Science*, 1935, **81**, 636.
14. Smith and Timmis, *J. chem. Soc.*, 1930, 1390.
15. Foster, Smith and Timmis, *Pharm. J.*, 1946, **157**, 43.
16. Tanret, *C.R. Acad. Sci., Paris*, 1875, **81**, 896.
17. Smith and Timmis, *J. chem. Soc.*, 1931, 1888.
18. Stoll and Hofmann, *Z. physiol. Chem.*, 1938, **251**, 155.
19. Stoll, Hofmann and Petrzilka, *Helv. chim. Acta*, 1951, **34**, 1544.
20. Forst, *Arch. exp. Path. Pharmak.*, 1926, **114**, 125.
21. Keller, *Schw. Woch. Chem. Pharm.*, 1894, **32**, 121.
22. Van Urk, *Pharm. Weekbl.*, 1929, **66**, 473.
23. Smith, *U.S. Public Health Report*, 1930, **45**, 1466.
24. Freeborn, *Pharm. J.*, 1912, **88**, 568.
25. B.P. Commission, Reports of sub-committee No. 8, 1931.
26. Allport and Cocking, *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 341.
27. Silber and Schulze, *Pharmazie*, 1953, **8**, 675.
28. Foster, *Analyst*, 1945, **70**, 132.
29. Brustier, *Bull. Soc. Chim.*, 1926, **39**, 1538.
30. Harmsma, *Pharm. Weekbl.*, 1928, **65**, 1114.
31. Van Itallie, *Bulletin de la Fédération Internationale Pharmaceutique*, 1928, 237.
32. Wokes and Crocker, *Quart. J. Pharm. Pharmacol.*, 1931, **4**, 420.
33. Allport and Crews, *ibid.*, 1935, **8**, 447.
34. Svoboda, Shahovskoy, Oliver, Diller, Barton and Barnes, *J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 259.
35. Kobert, *Arch. exp. Path. Pharmak.*, 1884, **18**, 316.
36. Edmunds and Hale, *Hyg. Lab. Bull.*, 1911, No. 76.
37. Broom and Clark, *J. Pharmacol.*, 1923, **22**, 59.

38. de Beer and Tullar, *ibid.*, 1941, **71**, 256.
39. Vos, *J. Amer. pharm. Ass., Sci. Ed.*, 1943, **32**, 138.
40. Foster and Stewart, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 211.
41. Strong and Maurina, *J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 414.
42. Stoll, D. R. P., 627,027 (1936).
43. Brownlee, *Quart. J. Pharm. Pharmacol.*, 1945, **18**, 172.
44. Foster, Macdonald and Jones, *J. Pharm. Pharmacol.*, 1949, **1**, 802.
45. Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.
46. Stoll, Hofmann and Becker, *Helv. chim. Acta*, 1943, **26**, 1602.
47. Berg, Chromatographische Scheiding der Moederkoornalkaloiden en een Practische Toepassing Daarvan, Uitgeverij Excelsior, s'Gravenhage, 1953.
48. Brindle, Carless and Woodhead, *J. Pharm. Pharmacol.*, 1951, **3**, 793.
49. Carless, *ibid.*, 1953, **5**, 883.
50. Berg, *Pharm. Weekbl.*, 1951, **86**, 900.
51. Berg, *ibid.*, 1952, **87**, 282.
52. Tyler and Schwarting, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 354.
53. Paul, Kelleher and Schwarting, *ibid.*, 1954, **43**, 205.
54. Hampshire and Page, *Quart. J. Pharm. Pharmacol.*, 1936, **9**, 60.
55. Hampshire and Partridge, *ibid.*, 1941, **14**, 116.
56. *Bull. Nat. Form. Comm.*, 1948, **16**, 1.
57. Schweizer, *Phytopath. Z.*, 1941, **8**, 317–50, *cit. R.A.M.*, 1942, **21**, 70.
58. Bonns, *Amer. J. Bot.*, 1922, **9**, 339.
59. Berman and Youngken, *J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 200.
60. Silber and Bischoff, *Pharmazie*, 1954, **9**, 46.
61. Tyler and Schwarting, *J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 207.
62. Kharasch and Legault, *Science*, 1935, **81**, 388, 614.
63. Stoll and Burckhardt, *C.R. Acad. Sci., Paris*, 1935, **200**, 1680.