LABORATORY NOTES ON THE STABILIZATION OF FLUIDEXTRACT OF ERGOT.*

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In spite of the large number of published articles relating to the deterioration of Fluidextract of Ergot, the problem of a satisfactory method of stabilizing this preparation has not yet been solved. Because of this fact the laboratory notes herein reviewed are submitted with the hope that they may be of value to other workers interested in this problem. The authors wish to point out that many of the ideas are not original but have been gathered from the literature as suggestions worthy of investigation.

The various assay results reported in this paper were carried out according to one of three well-known methods of determining potency of ergot and its preparations. These methods were, *first*, a modification of the Broom and Clark method (1), *second*, the Cock's Comb method of the U. S. P. X and *third*, a modification of Smith's Colorimetric method (2).

Swanson in 1929 (3) pointed out that the control of the p_H of Fluidextract of Ergot is important. Our data, however, would indicate that the problem is more complicated than that of adjusting the p_H, by the addition of acid to the Fluidextract.

The various methods for determining p_H were investigated for a reliable method applicable to Fluidextract of Ergot and one which could be readily checked by laboratory chemists. The hydrogen electrode was discarded because the results are questionable in the presence of alcohol. The removal of the alcohol before determining the p_H so altered the Fluidextract that the figures obtained were of little value. The quinhydrone electrode [Coons (6)] gives p_H values which are readily duplicated when the following method is observed:

Add 0.1 Gm. quinhydrone (Eastman) to 5-cc. Fluidextract of Ergot. Adjust the temperature to 25° C. and read after allowing two or more minutes for the electrode to reach equilibrium. Before using the electrode it should be cleaned as Coons suggests by boiling for five minutes in 50% nitric acid solution, rinsing with distilled water, followed by boiling for five minutes in 10% sodium bisulphite solution and again rinsing with distilled water.

It has been the observation of the authors that some Fluidextracts of Ergot appear to be relatively stable while others made by the same method from another lot of ergot deteriorate rapidly. It is our opinion that some lots of crude ergot contain something that stabilizes the Fluidextract while other lots do not contain sufficient of this material to exercise the stabilizing influence for any appreciable time. In an attempt to answer this question the ash was determined from a number of Fluidextracts of Ergot in order to see if this factor had any relation to stability. The ash varied from 1.33 Gm. to 2.09 Gm. per 100 cc., but showed no relation to stability. The iron in the ash was next determined and was found to range from a trace up to 0.25 Gm. per 100 cc. calculated to ferric oxide. Again there was no relation to stability. Fluidextract of Ergot also contains traces of copper, aluminum and nickel. As a further check on the influence of metals, the percolation was carried out in glass, iron and monel percolators (see Table I). The results were negative.

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Since some Fluidextracts of Ergot are more stable than others, it occurred to the authors that perhaps this condition might be due to variation in the proportion of rye grains contained in different lots of ergot. This was checked by adding to the Fluidextract an extract of rye prepared in the same manner as the Fluidextract of Ergot. The result obtained was negative (see Table II).

TABLE I.—FLUIDEXTRACT OF ERGOT.

Drug No.		Sample No.	Additions per 500 Cc.	p_H	p_H after Aging. 6 Mos.	Assay. Broom and Clark. 1 Mo.	4 Mos.	8 Mos.	1 Yr.	U. S. P. X. 1 Mo.	3 Mos.	8 Mos.
A		P-22596	.....	4.5	4.75		140	90	55		120	25
	Glass percolator	P-22596 B	7.5 cc. 36% HCl	3.1	..			100				
		P-22596 D	15.0	2.2	..		160				115	
		P-22596 F	8.2	3.0	3.3	330	125	100	108	120	100	**50**
	Monel percolator	P-22597	.....	4.6	..		125					
		P-22597 B	7.5 cc. 36% HCl	3.1	..		150	100	97	300–320	300–320	25
		P-22597 C	8.25	3.0	..			100				100
		P-22597 D	10.0	2.9	..	160						
		P-22597 E	15.0	2.35	..		150				120	
B		P-22733	.....	4.7	..		40		30		25	
	Glass percolator	P-22733 C	8.25 cc. 36% HCl	3.1	..			15				15
		P-22733 D	10.0	2.9	..	100	50	20	30	100	50	**20**
											4 Mos.	
		P-22733 F	20.0	2.15	..		25				33	

TABLE II.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Additions per 500 Cc.	p_H	p_H after Aging.	Assay. Broom and Clark. 1 Mo.	4 Mos.	8 Mos.	1 Yr.	U. S. P. X. 1 Mo.	4 Mos.	8 Mos.
C	P22792	.....	4.6	4.8		120	75			200	50
	P22792 C	8.25 cc. 36% HCl	3.0	3.35	125	200	100	105	130	120	100
	P22792 E	15.0	2.2	2.7		100		80		50	
	P22792 F	20.0	1.65	..			100				**100**
D	P23803	.....	3.7	..	100	100			100		
	P23804	3.6 cc. 36% HCl	3.0	..	100–110	100–110			120	Colorimetric 8 Mos.	
	P23814	3.6 cc. 36% HCl 1% milk sugar	3.0	..		30	35	25	8 Mos. 40	15	
	P23815	3.6 cc. 36% HCl 1% cane sugar	3.0	..		66⅔	51	25	40	**35**	
	P23816	3.6 cc. 36% HCl 2.5% dextrose	3.0	..		100	100	62	80	65	
	P24151	3.6 cc. 36% HCl 2.5% rye extract	3.0	..				65			
	P24152	3.6 cc. 36% HCl 10 Gm. Hydroquinone	3.0	..				94			

The addition of cane sugar, milk sugar and dextrose to Fluidextract of Ergot resulted in increased deterioration (see Tables II and III). As an additional check on this point, two relatively stable and two unstable Fluidextracts of Ergot were tested for their reducing action of Fehling's solution (U. S. P. X, page 497) as follows:

Five-cc. Fluidextract of Ergot were evaporated so as to remove the alcohol, then 15 cc. each of Fehling's solution A and B added. The mixture was diluted to 100 cc., then boiled for two minutes and filtered through a tared Gooch crucible. The precipitate was washed with distilled water, followed by alcohol and by ether and then dried thirty minutes at 100° C. The blank was run in the same way omitting Fehling's solution A (copper sulphate solution).

Sample No. 1 (Stable) 1 cc. reduced 0.0214 Gm. CuO.
Sample No. 2 (Unstable) 1 cc. reduced 0.0116 Gm. CuO.
Sample No. 3 (Stable) 1 cc. reduced 0.0157 Gm. CuO.
Sample No. 4 (Unstable) 1 cc. reduced 0.0228 Gm. CuO.

TABLE III.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Method of Percolation. U. S. P. X Type A. Fluidextract.	Additions per 500 Cc.	p_H.	Assays. Broom and Clark. 1 Mo.	Broom and Clark. 2 Mos.	Colorimetric. 1 Mo.	Colorimetric. 2 Mos.
E	P24460	50% alcohol	6 cc. 36% HCl	3.0	110		100	
	P24461	50% alcohol plus 9.1 cc. 36% HCl per 500 Gm.		4.2	100		110	
	P24462	66⅔% alcohol	3.5 cc. 36% HCl	3.0	108		100	
	P24463	66⅔% alcohol plus 9.1 cc. 36% HCl per 500 Gm.		4.2	133		125	
	P24464	75% alcohol	2.5 cc. 36% HCl	3.0	106	110	110	
	P24465	75% alcohol plus 9.1 cc. 36% HCl per 500 Gm.		4.2	140		150	
	P24466	85% alcohol	3.0 cc. 36% HCl	3.0	130	90	140	
	P24467	85% alcohol plus 9.1 cc. 36% HCl per 500 Gm.		4.2	150		160	
	P24523	95% alcohol plus 9.1 cc. 36% HCl per 500 Gm.		4.4	177		120	
F	P24514	50% alcohol plus 9.1 cc.		4.6		167		130
	P24515	36% HCl per 500 Gm.	2.5% dextrose			109		95
	P24516		2.5% sodium thiosulphate			80		100
	P24517	85% alcohol plus 9.1 cc.		4.8		200	200	20
	P24518	36% HCl per 500 Gm.	2.5% dextrose			18		20
	P24519		2.5% sodium thiosulphate			30		20
	P24520	95% alcohol plus 9.1 cc.			135	115	140	100
	P24521	36% HCl per 500 Gm.	2.5% dextrose			62		80
	P24522		2.5% sodium thiosulphate			116		93

TABLE IV.—FLUIDEXTRACT OF ERGOT, U. S. P. X.

p_H Previously Adjusted to 3.0 with HCl.

Lot Fluidextract of Ergot.	Sample No.	Additions.	Assays. Broom and Clark, after Aging. 1 Mo.	Broom and Clark, after Aging. 4 Mos.	Broom and Clark, after Aging. 5 Mos.	U. S. P. X. 1 Mo.	Colorimetric. 1 Mo.
1	P24269	.....	100	90	84	97	100
	P24270	Aerated 48 hours	100			114	116
	P24271	0.5% petroleum benzin then aerated 48 hours	100	38			96
							4 Mos.
	P24272	¼ full bottle		38			45
							1 Mo.
2	P24274	.....	120	120	123		120
	P24275	Aerated 48 hours	90				110
	P24276	0.5% petroleum benzin then aerated 48 hours	100			120	100
	P24277	¼ full bottle		58			60
				2 Mos.			
1	P24716	.....		120			
	P24718	0.65% borneol		72.5			
	P24719	0.65% benzaldehyde		40			
	P24720	10% acetone		44			
	P24721	0.65% hydroxy methyl anethol		74			
	P24722	0.65% NaH_2PO_4		54			
	P24756	0.1% quinaldine		71			

Various substances that might function as antioxidants and inhibitors were added to the Fluidextract of Ergot, namely, hypophosphorous acid, linseed oil, vitamin A concentrate, hydroquinone, carotene, sodium thiosulphate, sodium hydrosulphite, ferrous sulphate, ergosterol, cholestrin, borneol, benzaldehyde, acetone, quinaldine and hydroxy methyl anethol. Of the foregoing compounds only the first four appeared to show any stabilizing influence on Fluidextract of Ergot (see Tables II, III, IV and V).

TABLE V.—FLUIDEXTRACT OF ERGOT, U. S. P. X.

p_H Previously Adjusted to 3.0 with HCl.

Lot Fluidextract of Ergot.	Sample No.	Additions.	Assay. Broom and Clark after 3 Wks. at 50° C.
3	P26417	0.025% carotene	73
	P26418	0.025% vitamin A concentrate	89
	P26419	.....	73
	P26625	0.5% ergosterol from ergot	55
	P26626	.....	51
	P26627	0.5% cholestrin	46
	P26628	0.5% vitamin A concentrate	95
4	P26634	0.5% ergosterol from ergot	63
	P26635	0.5% cholestrin	55
	P26636	0.5% vitamin A concentrate	50
	P26637	.....	41
5	P26910	0.5% vitamin A concentrate	40
	P26911	0.5% cod liver oil	45
	P26912	0.5% alcoholic extract cod liver oil	49
	P26913	.....	25
	P27028	0.5% cod liver oil	45
	P27029	0.5% linseed oil	60
	P27030	0.5% liquid petrolatum	50
	P27031	0.5% cottonseed oil	49
	P27032	0.5% ergot oil (petroleum benzin)	38
	P27033	.....	38
6	P27022	0.5% cod liver oil	50
	P27023	0.5% linseed oil	60
	P27024	0.5% liquid petrolatum	40
	P27025	0.5% cottonseed oil	47
	P27026	0.5% ergot oil	44
	P27027	.....	50

The method of the British Pharmacopœia, 1932, requires extraction of the ergot with fifty per cent alcohol containing one per cent of tartaric acid. The Fluidextract of Ergot made by this method (see Table VI) deteriorated within six months. This is about the same rate of deterioration as is observed when the U. S. P. X method of preparation is used. The addition of tartaric or of hydrochloric acids to the Fluidextract did not give a stable product as is shown by Tables VI, VII and VIII.

In Tables III, VI and IX the method of percolation was changed from the fractional percolation procedure of the U. S. P. X to the single percolation process which is described on page 159 of the Pharmacopœia as process Type A. This method of percolation yielded a Fluidextract of Ergot which is as active as the regular U. S. P. X method when made from the same lot of drug (compare Table VI).

Wokes and Elphick (4) found that defatting ergot increased the efficiency of the extraction with either neutral or acidified alcohol. Our results as shown in Table IX do not support this statement. As a matter of fact we have prepared a standard Fluidextract of Ergot by percolating the whole drug without having applied any preliminary treatment. Our attempt to answer the question as to whether the stability of the Fluidextract is influenced by the incomplete removal of the petroleum benzin from the drug before percolation is shown in Table IV. The addition of petroleum benzin to the Fluidextract apparently had no effect on the stability.

TABLE VI.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Method of Percolation.	Additions per 500 Cc.	p_H.	Assays. Broom and Clark. 1 Mo.	4 Mos.	6 Mos.	1 Yr.	U. S. P. X. 3 Mos.	6 Mos.
B	P22875 A	The 1932 British Pharmacopœia	3.7 Gm. tartaric	4.0		50			25	
	P22875 C		11.1 Gm. tartaric	3.1			50			80
	P22875 D	(1% tartaric in 50%	12.95 Gm.	3.0	160	100	25	25	50	33
	P22876 B	alcohol)	9.0 cc. 36% HCl	3.0			25	65	25	33
E	P23454	U. S. P. X	.....		50				33	
	P23454 D	Iron fillings mixed with ergot	20 Gm. tartaric				70			
	P23453	U. S. P. X		5.8	35				33	
	P23453 B	U. S. P. X	7.5 cc. 50% H_3PO_2	3.0			90			
	P23452	U. S. P. X	.....	5.4	100			40	70	
	P23452 B	Iron sulphate dried	3.0 cc. 36% HCl					70		
	P23452 D	powder mixed with ergot. 4 Gm. per 500 Gm.	20 Gm. tartaric				110			
	P23455 A	U. S. P. X type A	20 Gm. tartaric				100			
	P23455 B	Fluidextract 50% alcohol	5.0 Gm. $Fe_2(SO_4)_3$				105			

TABLE VII.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Additions per 500 Cc.	p_H	p_H after Aging.	Assays. Broom and Clark. 1 Mo.	4 Mos.	9 Mos.	2 Yrs.	U. S. P. X. 2 Mos.	5 Mos.	9 Mos
B	P27732 B	2.5 cc. 36% HCl	4.6			33⅓			100	50	25
				6 Mos.							
	P27732 D	7.5	3.0	3.2	40	33⅓	20	30	100	50	25
	P27732 E	10.0	2.5				15				
	P27732 F	12.5	1.9			18	12			25	
	P27732 M	5.55 Gm. tartaric acid	4.45			33⅓				30	
	P27732 N	7.40	4.3				20				25
	P27732 P	11.10	3.5				12				25
	P27732 Q	12.95	3.3			50				25	
C	P22791 A	3.70 Gm. tartaric	4.7			50				62	
	P22791 B	7.40	4.2				50				62
				8 Mos.							
	P22791 E	14.80	3.0	3.25			50	118			63
	P22791 F	16.65	3.0	3.20		100	100	100		120	100–120

In Table IV the effect of air upon Fluidextract of Ergot was observed by bubbling through the sample, for 48 hours, a current of air previously saturated with the menstruum by passing the air through diluted alcohol. No immediate loss of activity was observed, although, on aging the samples, the deterioration progressed more rapidly.

In 1930 Thompson (7) recommended that Fluidextract of Ergot be distributed in completely filled bottles in order to prevent undue exposure to air. Our findings (see Table IV) agree with his conclusions, since deterioration progressed more rapidly when stored in bottles that were one-fourth full.

Our results shown in Table III agree with Linnell and Randle (5) in that an acid alcohol menstruum is more efficient than alcohol alone. The table also indicates that the higher percentage alcohol extracts the activity more completely than does alcohol of lower concentration. All samples, however, deteriorated upon aging.

TABLE VIII.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Additions per 500 Cc.	p_H	p_H after Aging. 9 Mos.	Assays. Broom and Clark. 4 Mos.	9 Mos.	2 Yrs.	2 Mos.	U. S. P. X. 4 Mos.	9 Mos.
A	P-22595 A	.....	4.7		100	100		100	80	25
	P-22595 D	7.5 cc. 36% HCl	2.95	3.05	160	66	69	115–130	70	25
	P-22595 F	12.5 cc. 36% HCl	2.2		110	100			40–50	25
	P-22595 I	6.0 cc. 36% HCl	3.1		100	100				50
	P-22595 K	1.85 Gm. tartaric acid	4.45		130	90			5 Mos. 25	25
	P-22595 P	11.10 Gm. tartaric acid	3.15			50				
	P-22595 Q	12.95 Gm. tartaric acid	2.95		160				33	
C	P-22790		6.0							25
	P-22790 A	2.5 cc. 36% HCl	4.7		120	100	66⅔		62.5	
	P-22790 D	7.5	3.0			100	66⅔	80	100	100–110
	P-22790 E	10.0	2.5				33⅓			50
	P-22790 F	12.5	2.0			50			50–70	

TABLE IX.—FLUIDEXTRACT OF ERGOT.

Drug No.	Sample No.	Method of Percolation.	Additions per 500 Cc.	p_H	Assays. Broom and Clark. 1 Mo.	5 Mos.	U. S. P. X. 1 Yr., 4 Mos.	1 Mo.
E	P23500	U. S. P. X type A Fluid-	.....	4.6			85	
	P23500 A	extract menstruum—50%	5.0 cc. H_3PO_2	3.1	60	150	150	100
	P23500 B	alcohol plus 7 cc. 50%	7.5 cc. H_3PO_2				155	
	P23500 C	H_3PO_2 per 500 Gm. ergot	5 Gm. $Fe(SO_4)_3$			120	200	
	P23500 D		20 Gm. tartaric		90–100	150	112	100
	P23500 E		3.75 cc. HCl 36%	3.0		140	135	100
F	P24613	U. S. P. X except used whole ergot not defatted	.....	4.3	185		Colorimetric. 160	
	P24614	U. S. P. X except ergot not defatted	.....	4.65	180		120	
	P24615	U. S. P. X except used whole ergot	.....	4.45	130		160	
	P24616	U. S. P. X	.....	4.75	113		160	
	P24710	U. S. P. X except used whole ergot not defatted	.....	4.3	180		2 Mos. 160	

CONCLUSIONS.

1. Fluidextract of Ergot is not stabilized by adjusting the p_H by means of acids.
2. A reliable method for determining the p_H of Fluidextract of Ergot is outlined.
3. Some lots of crude ergot contain something which tends to stabilize the Fluidextract more than others.
4. The addition of rye extract to the Fluidextract of Ergot apparently does not influence the stabilization.
5. The addition of sugars increases the deterioration of Fluidextract of Ergot.
6. The power of Fluidextract of Ergot to reduce Fehling's solution bears no relation to stability.
7. Hypophosphorous acid linseed oil, vitamin A concentrate and hydroquinone favorably influence the stability of Fluidextract of Ergot.
8. Fluidextract of Ergot made by the method of the British Pharmacopœia deteriorates the same as that made by the U. S. P. X method.

9. The type of percolation is not important.

10. Defatting the drug before percolation is not important.

11. The activity of ergot is extracted more completely by acid alcohol than by neutral alcohol.

12. Higher percentage alcohol is more efficient for percolation of ergot than low percentage alcohol.

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THE TESTING OF ERGOT.

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In the course of investigating the breakdown rate of liquid preparations of ergot, we have in this laboratory been using all three usual methods of testing, *i. e.*, the Broome-Clarke rabbit uterus method, the Allport-Cocking color reaction, and the Cock's Comb method, with a distressing want of correlation, driving one to the verge of despair. We have experimented with a photographic modification of the Cock's Comb reaction that we think it worth drawing to the attention of other workers.

The method is to photograph the bird, before injection, by means of an appropriate light filter and red sensitive plates so that blue registers as black and red registers as white. The bird is then injected and after $1^1/_4$ hours is again photographed on the same plate, consequently the two photographs get the same development. The resultant prints although not necessarily good pictures of birds do pick up differences that are not visible to the unaided eye.

Before making an assay, one prepares two pairs of reference prints, one pair with a dose of some standard (in this case Ergotoxine ethanesulphonate solution $^1/_2$ mg. per cc.) of such size as to produce a minimum effect as in Fig. 1. Another reference photograph is made of the same bird with a larger dose and more pronounced effect as in Fig. 3. In assaying a sample marked "A" a first trial was made on the assumption that it was probably over-strength and a lesser dose of "A" was given than had been given to the same bird in Fig. 1 with the result shown