SCIENTIFIC SECTION

THE CHEMICAL AND BIOLOGICAL STANDARDIZATION OF OLEORESIN OF ASPIDIUM.*

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In 1830 Aspidium Filix-mas was introduced in both editions (Philadelphia and New York) of the U. S. Pharmacopœia, but was included in the Secondary List only. In 1860 it was transferred to the Primary List, and in 1880 it became official under its present title "Aspidium" (10). The crude drug itself has been employed very rarely in medicine since the introduction of the Oleoresin.

The constituents which are present in the crude drug are also presumably present in the Oleoresin (17). Although the literature shows little disagreement as to the constituents which are present in Aspidium, there is some debate as to which of these are the active anthelmintic agents.

Sollmann (18) states that the active constituents of Aspidium are *amorphous filicic acid* ($C_{33}H_{40}O_{13}$), *aspidinin, albaspidin, flavaspidic acid, aspidin.* He states further that the *amorphous filmaron* ($C_{47}H_{54}O_{13}$) is perhaps an impure filicic acid; and that the inactive constituents are crystalline *filicic acid* or *filicin* ($C_{35}H_{38}O_{11}$), and *aspidinol.* He suggests that the crystalline filicic acid is the lactone of the active amorphous acid, the two being readily converted into each other. Solis-Cohen (17) adds *filic acid* ($C_{35}H_{38}O_{12}$), filix-tannic acid, a green fixed oil, volatile oil, sugar, starch, resin and wax to the foregoing list. Amorphous filicic acid or filicin has been credited as the important anthelmintic constituent of Male Fern. However, this does not exclude the other constituents as possible anthelmintic agents or synergists, for some of them, at any rate are toxic (14). Shoemaker (16), quoting Kobert, makes the definite statement that the anthelmintic action of the Oleoresin of Aspidium does not depend solely on the filicic acid present, but also on the ethereal oils; while Kraft, according to Thorpe (21), believes that the anthelmintic action of the drug is due primarily to filmaron ($C_{47}H_{54}O_{16}$), an amorphous, brownish yellow acid which melts at 60° C., and marketed (7) as a 10% solution in Castor Oil called "Filmaron Oil."

Several different chemical formulas have been assigned to amorphous filicic acid. Solis-Cohen (17) gives the formula $C_8H_{10}O_5$; Thorpe (21), $C_{35}H_{42}O_{13}$ or $C_{35}H_{40}O_{13}$ for the active form, and quotes Poulsson's formula $C_{35}H_{40}O_{12}$, and Boehm's $C_{35}H_{38}O_{12}$ for the inactive. Sollmann (18) and Poulsson (13) agree with the formula $C_{35}H_{40}O_{13}$. Evers (5) concurs in both formulas for the active form as given by Thorpe. Fuller (7) quotes the following formulas: Luck, $C_{26}H_{30}O_9$; Grabowski, $C_{14}H_{18}O_5$; Gallas, $C_{18}H_{22}O_6$; and Daccoma, $C_{14}H_{16}O_5$. Hare (9) accepts the last formula. Green (8) places Aspidium and its constituents in the Phloroglucinol-Butyric Acid group or the Phloroglucinol group, because he found phloroglucinol and butyric acid among the decomposition products following acid or alkaline hydrolysis of an Aspidium extract. He points out by structural formulas the relationship between phloroglucinol and the constituents of Aspidium. On examin-

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ing these structural formulas it appears that aspidinol and filicic acid may be cleavage products of aspidin. The carbon bridge in his formula for filmaron is accepted as the reason for its instability. Green suggests the decomposition of filmaron during the manufacture of the Oleoresin as the possible reason for the presence of some of the other constituents of this drug.

THE STANDARDIZATION OF ASPIDIUM.

Until the appearance of the Tenth Revision of the U. S. Pharmacopœia (12) there was no official requirement as to the percentage of crude filicin present in the Oleoresin of Aspidium. Oleoresins were marketed which contained varying percentages of filicin. Berg (1) reported samples varying from 8 to 26 per cent of filicin. The report of the Committee on Drug Market of the AMERICAN PHARMACEUTICAL ASSOCIATION (22) in 1918 included samples which assayed only 8 per cent of crude filicin. Linke (26) found some 1-Gm. capsules which assayed from 5.45 to 14.22 per cent of crude filicin, and some commercial oleoresins which contained from 19 to 27 per cent.

The need for a carefully standardized product was generally recognized (3), especially in view of the fact that, when used in therapeutics, as large a dose is administered as the physician dares to employ, and the patient is possibly bordering on poisoning. The fact that a physiologically inactive drug cou'd meet the microscopic requirements given in the Ninth Revision of the U. S. Pharmacopœia was further evidence that an assay was needed (4). Accordingly, the Tenth Revision of the U. S. Pharmacopœia requires not less than 24 per cent of crude filicin to be present in the Oleoresin, and presents a chemical method for the determination of the filicin.

The consensus of opinion seems to be that chemical methods for the assay of Aspidium and of Oleoresin of Aspidium do not provide very satisfactory indices of the therapeutic worth of these agents (23). For purposes of standardization by chemical methods the active constituents of Aspidium has been assumed to be crude filicic acid. However, this is actually a mixture of complex composition, and no reliable methods have been devised to date by which each of the constituents may be accurately determined. A further claim is that crude filicic acid is accompanied by inert constituents in varying and unknown amounts.

Chemical assay methods usually involve, in the case of the crude drug, (*a*) the determination of the oleoresin, and (*b*) the determination of the crude filicic acid in the oleoresin thus obtained. In the case of the Oleoresin of Aspidium "(*b*)" alone is carried out. The U. S. P. X method for obtaining the oleoresin, and the U. S. P. X process for the assay of Oleoresin of Aspidium, in so far as the literature indicates, appear to be as satisfactory as any of the chemical methods which thus far have been proposed for the evaluation of this drug. However, there is one modification of the U. S. P. X assay of Oleoresin of Aspidium which will be referred to presently.

Many are of the opinion that biological methods for the evaluation of Aspidium and its Oleoresin give more trustworthy pictures of the therapeutic possibilities of these agents.

The Sub-committee on Biological Assays of the U. S. P. (XI) Revision Committee appointed a special Group Committee on Aspidium Assay. In an effort to obtain some working data which might be submitted to the members of this group

committee for collaborative study, this comparative study of the standardizing methods thus far used was carried out by the authors, with these objects in view: (*a*) possible improvements in the present official chemical assay procedure; (*b*) a more satisfactory, practical and simple biological assay; and (*c*) the relation, if any, which may exist between the percentage of filicin present in Oleoresin of Aspidium and the biological potency.

1. CHEMICAL METHODS.

Various chemical assays have been used for the quantitative determination of crude "filicin" in the Oleoresin of Aspidium.

The U. S. Pharmacopœial Method (12) is as follows:

"Warm the oleoresin on a water bath and stir until it is thoroughly mixed. Transfer about 5 Gm., accurately weighed, to a 200-cc. flask, dissolve in 40 Gm. of ether, and add 100 Gm. of an aqueous solution of barium hydroxide (3 to 100), and shake vigorously for 5 minutes. Allow the liquids to separate and filter off 86 Gm. of the aqueous fluid. Transfer this to a separator, add sufficient hydrochloric acid to produce a distinctly acid reaction, and extract with three successive portions of 30 cc., 20 cc. and 15 cc. of ether. Draw off and combine the ethereal solutions, filter, wash the filter with ether, evaporate, and dry the residue to a constant weight at 100° C. This residue weighs not less than 0.96 Gm., corresponding to not less than 24 per cent of crude filicin."

In the foregoing assay it is presumed that there are 1.25 Gm. of crude filicin in 5 Gm. of the Oleoresin, which is equivalent to a 25 per cent yield. Also, the aliquot portion, 86 Gm., is supposedly equivalent to 4 Gm. of Oleoresin, and contains 0.96 Gm. of crude filicin, which represents a 24 per cent yield. The aqueous layer contains *about* 6.25 Gm. of ether. Evidently, the aqueous layer may contain *more or less* than that amount. Accordingly, in weighing the aliquot portion there may be more or less than the equivalent of 4 Gm. of the Oleoresin, and consequently this element in the method Mr. Pabst suggests may lead to varying results.

The British Pharmacopœia Method (2) is almost identical with the U. S. P. method with only two minor differences. Instead of dissolving the 5 Gm. of Oleoresin in 40 Gm. of ether, 40 cc. of ether are used. In determining the percentage of crude filicin the statement appears that the residue should weigh not less than 0.8 Gm., which is equivalent to 20 per cent.

The Swiss Pharmacopœial Method (20) is carried out in the same manner as the two preceding methods with but two minor changes. The 5 Gm. of Oleoresin are dissolved in 30 Gm. of ether instead of 40 Gm., as directed in the U. S. Pharmacopœia, and 40 cc. in the British Pharmacopœia. The residue should weigh 1.04 to 1.12 Gm., corresponding to 26 to 28 per cent of crude filicin.

Fluck (6) recommends a slight change in the Swiss method. Instead of drying the residue to constant weight, he suggests drying for one hour only at 100° C. However, drying the residue for one hour only apparently fails to drive off all of the moisture, because continued heating causes the residue to lose, if so dried, considerable more weight. The results by this method are, of course, higher.

Lyons (11) suggests a modification of the Swiss method as follows:

"Dissolve 5 Gm. of the Oleoresin, accurately weighed, in 40 cc. of ether. Add 100 cc. of a 3% solution of barium hydroxide and shake vigorously for 5 minutes. Transfer to a separator, allow to stand for 10 minutes, filter and draw off 87 cc. To this add sufficient hydrochloric

acid to render it distinctly acid and shake out the precipitated filicic acid with three successive portions of 30 cc., 20 cc. and 15 cc. of ether. Filter the ether through cotton, evaporate and dry the residue to constant weight at 100° C."

Each of the foregoing methods is an aliquot portion method, and, if the objection suggested in the case of the U. S. P. method holds, then the same objection applies equally to each of the modified methods and related methods above.

2. BIOLOGICAL METHODS.

Sollmann's Earthworm Method (19) is as follows:

"The Oleoresin is shaken, and a very small drop (approximately 0.1 cc.) is placed on a small tared filter paper, weighed, and rubbed thoroughly with sand in a mortar, adding the BB solution (bilein, 0.04%; sodium bicarbonate, 1%; probably a 1% solution of sodium bicarbonate could be used as some comparative experiments show no essential difference), using 50 cc. of the solution for 0.01 Gm. of the Oleoresin. This is filtered, and represents 2 mg. of the Oleoresin per 10 cc. of the stock solution.

"The dilutions are made up in urine glasses as follows:

0.01 Gm. per 100 cc.—equals 50 cc. of the stock solution and water q. s. ad 100 cc.
0.005 Gm. per 100 cc.—equals 25 cc. of the stock solution and water q. s. ad 100 cc.
0.003 Gm. per 100 cc.—equals 15 cc. of the stock solution and water q. s. ad 100 cc.
0.002 Gm. per 100 cc.—equals 10 cc. of the stock solution and water q. s. ad 100 cc.
0.001 Gm. per 100 cc.—equals 5 cc. of the stock solution and water q. s. ad 100 cc.

"Five worms are placed in each glass and observed after 24 hours. A standard Oleoresin should kill the worms in 0.002 to 0.003%, differing probably a little with the season. Until this point is determined, it may be advisable to check the preparation to be tested against a preparation of known strength (*i. e.*, one that is fatal with 0.003%, and only depressant with 0.002% in cold weather)."

The major criticism of the Sollmann Earthworm Method is the length of time involved.

Zoltan's Method (25) is as follows:

"The ethereal extract of Male Fern is carefully warmed to 40° C. and well stirred. Four grams (4 Gm.) of the extract are then emulsified with powdered acacia and a 1% solution of sodium bicarbonate. This emulsion is diluted to 2000 cc. with tap water containing 1% of sodium bicarbonate; 500 cc. of the latter, equal to 1 Gm. of the extract, are again diluted with sodium bicarbonate solution to 2000 cc. This standard solution, containing 0.1 Gm. of the extract in 200 cc., can be used after 2 hours. The minimal lethal dose is fixed in several stages. A number of solutions are prepared, varying considerably in concentration; then two extreme values must be found, where either all of the animals are killed within 2 hours, or where they remain alive. At least six leeches, weighing between 3 and 5 Gm. must be used. In the second stage these two values must be brought so close that with the higher value the leeches just perish within two hours, while the lower value does not kill them. A third stage is employed to establish a biological average value, where about half the number of leeches are destroyed within two hours, the other half after the lapse of two hours. This value represents the minimal lethal dose. In 100 cc. of the standard extract, as above, the higher value was found to be 0.0036, the lower 0.002."

As far as the time element and the dilutions are concerned, this method appears to be satisfactory. However, leeches are difficult to keep as reserve specimens, and, added to this, is the difficulty in obtaining them.

Wasicky and Becker (24) recommend a biological assay in which fish are exposed to varying concentrations of the preparations. In a large number of experiments,

however, they found no parallel between the amounts of filicin obtained by chemical methods and the results by their biological procedure.

Schamelhout (15) recommends a biological assay with earthworms or small fish as the standardizing agent. The dilution of 0.002 parts to 100 parts is stated as the minimum concentration which will produce death. No time factor is introduced, however. Experimental work of this type with gold fish in the laboratories of the Division of Pharmacology of the University of Tennessee has shown that these animals do not react uniformly to drugs, and are not particularly adaptable to biological assay methods.

3. THE MATERIALS USED.

Two samples of powdered Aspidium and six samples of Oleoresin of Aspidium were purchased on the open market. Microscopic examinations (A. John Schwarz) demonstrated that the crude drugs were Aspidium Filix-mas with no adulterations. Chemical and physical examinations showed the absence of adulterants in the samples of Oleoresin.

Oleoresins were preparated from each of the powders, the method of the U. S. P. X being used.

One of the powders yielded but 0.7% of Oleoresin, and this was not used in any of the additional experimental work because the material was too limited. The second powder yielded 9.44% of Oleoresin.

The Oleoresins purchased were all of a dark green color, thick and with the characteristic odor and other common properties required in the official specifications. The specific gravities varied from 1.001 to 1.017.

4. SUGGESTED CHANGE IN U. S. P. X METHOD.

The following modified method was devised during the course of the study, and the results obtained apparently justify its proposal as a more reliable and more accurate chemical method.

The Procedure: Warm the Oleoresin on a water-bath and stir until it is thoroughly mixed. Transfer about 3 Gm., accurately weighed, to a 250-cc. flask, dissolve in 40 cc. of ether, add 75 cc. of a 3% aqueous solution of barium hydroxide and shake vigorously for 5 minutes. Transfer this mixture to a separator. Allow the liquids to completely separate, draw off and filter the barium hydroxide layer. Rinse the 250-cc. flask with two 25-cc. portions of a 3% aqueous barium hydroxide solution. After each rinsing, transfer the barium hydroxide solution to a separator, shake for one minute, allow the liquids to separate completely, draw off and filter the barium hydroxide layer. Transfer the combined filtered barium hydroxide solutions to a separator, make distinctly acid (to litmus) with concentrated hydrochloric acid and extract with three successive portions of 30 cc., 20 cc. and 15 cc. of ether. Draw off the combined ethereal solutions, filter, wash the filter with ether, evaporate and dry the residue to constant weight at 100° C.

The procedure was checked by blank tests. This modified method presents several possible advantages. The chances of error are apparently reduced, in view of the statements above, since an aliquot portion is not used, but instead the entire aqueous fraction. Less time is required in carrying out this method, since it is not necessary to determine the specific gravity, or to weigh the barium hydroxide, the ether and the portion of the aqueous layer. By using a smaller amount of Oleo-

resin, the residue is less, and, consequently, may be dried to constant weight more rapidly.

The method presents one possible disadvantage in the hands of the inexperienced worker. Occasionally, with some Oleoresins, an emulsion is formed which is quite stable. If the worker is careful, this may, however, be avoided.

5. COMPARATIVE RESULTS OBTAINED BY THE CHEMICAL METHODS.

The U. S. P. X Method, representative of the group discussed above, and the suggested modified method were employed in this portion of the comparative study. Three assays were carried out on each Oleoresin by each method. The results are tabulated below.

TABLE I.—CHEMICAL ASSAY OF OLEORESIN OF ASPIDIUM.

Method and Sample.	Per Cent of Filicin. Assay 1.	Assay 2.	Assay 3.	Average.	Variation.
Modified; Sample B	3.03	2.95	2.98	2.98	0.08
U. S. P. X; Sample B	3.12	2.94	2.85	3.00	0.27
Modified; Sample C	24.53	24.50	24.58	24.53	0.08
U. S. P. X; Sample C	25.60	25.32	25.51	25.47	0.28
Modified; Sample D	25.05	25.00	25.02	25.02	0.05
U. S. P. X; Sample D	26.20	25.87	26.01	26.02	0.33
Modified; Sample E	20.13	18.42	18.82	19.15	0.71
U. S. P. X; Sample E	20.50	21.02	20.41	20.64	0.61
Modified; Sample F	23.66	23.61	23.68	23.65	0.07
U. S. P. X; Sample F	24.90	25.13	25.02	25.01	0.23
Modified; Sample G	24.80	24.83	24.87	24.83	0.07
U. S. P. X; Sample G	26.01	26.24	25.82	26.01	0.42
Modified; Sample H	24.53	24.51	24.57	24.40	0.06
U. S. P. X; Sample H	23.82	24.35	24.11	24.09	0.53

The results tabulated above show that there is a greater variation in the percentages obtained by the U. S. P. X Method than by the Modified Method. Loss of ether by vaporization may explain in part the greater variation and the higher yield.

6. RESULTS OBTAINED BY A BIOLOGICAL METHOD, AND A COMPARISON WITH THE CHEMICAL METHOD.

A combination of the Sollmann and the Zoltan methods, somewhat modified by the workers, was studied, with the added object of determining whether or not any parallelism could be demonstrated. The thought presented itself that, since Ascarides obtained from hogs represented more closely the type of worms found in the intestinal tracts of the human and of the dog, this type of worm might lend itself more satisfactorily to a biological assay of this anthelmintic drug. Accordingly, supplies of this worm were obtained from the slaughter-house and used in this study.

A stock solution was made according to Zoltan's method, the dilution used representing 0.1 Gm. of the Oleoresin in 200 cc. of the emulsion (50 mg. per 100 cc.). Sollmann's method of diluting the foregoing stock solution was employed, a 1% solution of sodium bicarbonate being used, however, instead of tap water, in making up the dilutions. The dilutions used ranged from 1 mg. per 100 cc. to 10 mg. per 100 cc. (1, 2, 3, 5 and 10). Fresh supplies of worms, removed directly from the

intestine of the hog, were employed. Observations were made over a period of 48 hours with 42 series of worms. The records show that the worms in all cases but one lived through the end of the observation period. Death occurred in but one dilution, *viz.*, 1 mg. per 100 cc., and in this dilution the worms were alive for a period of 14 hours or more. Check worms, kept in physiological saline solution, remained normal over a period of four or five days. Similar checks and results were obtained with 1% sodium bicarbonate solution. The results showed that Ascarides are not uniformly susceptible to the Oleoresin. In view of these findings it is obvious that this type of worm does not lend itself to biological assays of Aspidium.

The experiments were then repeated using earthworms, which Sollmann states resemble intestinal worms in their reactions toward some anthelmintics. These worms are readily obtained and are easily kept. Preliminary experiments were carried out to determine what dilution might prove most satisfactory with this worm. Series were run with dilutions containing 1 mg., 2 mg., 3 mg., 5 mg., 10 mg. and 50 mg. per 100 cc. With three, *viz.*, the 5-, 10- and 50-mg. dilutions, the worms were killed within 12 hours, the other dilutions requiring a much longer time. The 50-mg. dilution killed the worms in 3 hours; the 10 mg., in 10 hours; and the 5 mg. in 12 hours. The 2-mg. dilution required 24 hours for death; and with the 1 mg. the worms were still alive at the end of 24 hours. Consequently, because of the convenient time factor, it was decided to use the 50-mg. dilution with six samples of the Oleoresin already studied chemically. The observations are recorded in the tables which follow.

TABLE II.—BIOLOGICAL ASSAY OF OLEORESIN B.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions: 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	38.
1 mg.	A*	A	A	A	A	A	A	A	I*	I	I
2 mg.	A	A	A	A	A	A	A	A	A	A	A
3 mg.	A	A	A	A	A	A	A	A	A	A	A
5 mg.	A	A	A	A	A	A	A	A	A	A	A
10 mg.	A	A	A	A	A	A	A	A	A	A	A
50 mg.	A	A	A	A	A	A	A	A	A	A	A

* Indicates: "A"—alive; "I"—paralyzed or dead.

In this series all the worms were alive up to a period of 34 hours. All were alive at the end of the experiments except in one concentration; *viz.*, the dilution of 1 mg. per 100 cc. at 34 hours.

TABLE III.—BIOLOGICAL ASSAY OF OLEORESIN C.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions. 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	48.
1 mg.	A	A	A	A	A	A	A	A	A	A	A
2 mg.	A	A	A	A	A	A	A	A	I	I	I
3 mg.	A	A	A	A	A	A	A	A	A	A	A
5 mg.	A	A	A	A	A	A	A	A	A	A	A
10 mg.	A	A	A	A	A	A	A	A	A	A	A
50 mg.	A	A	A	A	A	A	A	A	A	A	A

All of the worms treated with Oleoresin C were alive over a period of 34 hours. In only one case the worms died. In the dilution of 2 mg. per 100 cc. they were alive up to 34 hours.

TABLE IV.—BIOLOGICAL ASSAY OF OLEORESIN E.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions: 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	48.
1 mg.	A	A	A	I	I	I	I	I	I	I	I
2 mg.	A	A	A	I	I	I	I	I	I	I	I
3 mg.	A	A	A	I	I	I	I	I	I	I	I
5 mg.	A	A	A	A	A	I	I	I	I	I	I
10 mg.	A	A	A	A	A	A	A	A	A	A	A
50 mg.	A	A	A	A	A	A	A	A	A	A	A

The results in the above series show that death occurred in 24 hours in the dilutions of 1 mg. per 100 cc., 2 mg. per 100 cc. and 3 mg. per 100 cc.; and in 28 hours in 5 mg. per 100 cc. In the other two dilutions, 10 mg. per 100 cc. and 50 mg. per 100 cc. the worms were still alive at the end of the observations.

TABLE V.—BIOLOGICAL ASSAY OF OLEORESIN F.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions: 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	48.
1 mg.	A	A	A	A	A	A	I	I	I	I	I
2 mg.	A	A	A	I	I	I	I	I	I	I	I
3 mg.	A	A	A	A	A	A	A	A	I	I	I
5 mg.	A	A	A	A	A	A	A	A	A	A	A
10 mg.	A	A	A	A	A	A	A	A	I	I	I
50 mg.	A	A	A	A	A	A	A	A	A	A	A

Results reported in Table V show no relation between the death period and concentrations. Death occurred in the dilution of 1 mg. per 100 cc. at the 30th hour; in the 2 mg. per 100 cc. at the 24th hour; in the 3 mg. per 100 cc. and the 10 mg. per 100 cc. at the 34th hour, while in the other dilutions of 5 mg. per 100 cc., and 50 mg. per 100 cc., the worms remained alive until the end of the observations.

TABLE VI.—BIOLOGICAL ASSAY OF OLEORESIN G.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions: 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	48.
1 mg.	A	A	A	A	A	A	A	A	A	A	A
2 mg.	A	A	A	A	I	I	I	I	I	I	I
3 mg.	A	A	A	A	A	A	A	I	I	I	I
5 mg.	A	A	A	A	A	I	I	I	I	I	I
10 mg.	A	A	A	A	A	A	A	A	I	I	I
50 mg.	A	A	A	A	A	A	A	A	A	A	A

Death, in this series, occurred in the dilutions of 2 mg. per 100 cc. in 26 hours; 3 mg. per 100 cc. in 32 hours; 5 mg. per 100 cc. in 28 hours; and 10 mg. per 100 cc. in 34 hours, while in the weakest, 1 mg. per 100 cc. and the strongest preparations, 50 mg. per 100 cc., the worms lived until the end of the observations.

TABLE VII.—BIOLOGICAL ASSAY OF OLEORESIN H.

Dilution mg. per 100 cc.	Time in Hours after Placing Ascarides in Dilutions: 10.	12.	14.	24.	26.	28.	30.	32.	34.	36.	48.
1 mg.	A	A	A	I	I	I	I	I	I	I	I
2 mg.	A	A	A	A	A	A	A	A	A	A	A
3 mg.	A	A	A	A	A	A	A	A	A	A	A
5 mg.	A	A	A	A	A	A	A	A	A	A	A
10 mg.	A	A	A	A	A	A	A	A	A	A	A
50 mg.	A	A	A	A	A	A	A	A	A	A	A

The results in this series show that the worms in all cases but one lived until the end of the observations. Death occurred in only one dilution, 1 mg. per 100 cc., and in this dilution the worms were alive for a period of 14 hours or more.

TABLE VIII.—BIOLOGICAL ASSAY OF OLEORESIN F.

Dilution mg. per 100 cc.	Time in Hours after Placing Earthworms in Dilutions: 1.	2.	3.	4.	6.	7.	10.	12.	14.	24.
1 mg.	A	A	A	A	A	A	A	A	A	A
2 mg.	A	A	A	A	A	A	A	A	A	A
3 mg.	A	A	A	A	A	A	A	A	A	I
5 mg.	A	A	A	A	A	A	A	I	I	I
10 mg.	A	A	A	A	A	A	I	I	I	I
50 mg.	A	A	I	I	I	I	I	I	I	I

A study of the table above shows that out of the six preparations used only three—the 5 mg. per 100 cc., the 10 mg. per 100 cc. and the 50 mg. per 100 cc.—kill the worms within 12 hours, the others requiring a much longer period of time. These, therefore, appear to be the more satisfactory dilutions to use for biological assay purposes. A short assay is preferable for several reasons; *viz.*, less time is required to complete the experiment; the experimental animal is not subjected to abnormal conditions for so long a time, thus giving more accurate results; and, if a substance is to be used on man as an anthelmintic, it must prove efficacious in a relatively short time, since retention in the intestine may prove harmful to the host. Accordingly, the dilution, 50 mg. per 100 cc., was used in the following assay method.

Emulsions containing 50 mg. of the Oleoresin in each 100 cc. were prepared from Oleoresins C, D, E, F, G and H. Ten checks were carried out on each Oleoresin. A worm was placed in each of ten 150-cc. beakers, each containing 100 cc. of the emulsion. The results of these experiments follow.

TABLE IX.—BIOLOGICAL ASSAY OF OLEORESIN C.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	A	I	I	I	I	I	I	I	I
3 hours	I	I	I	I	I	I	I	I	I	I

TABLE X.—BIOLOGICAL ASSAY OF OLEORESIN D.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	A	I	I	I	I	I	I	I	I
3 hours	I	I	I	I	I	I	I	I	I	I

TABLE XI.—BIOLOGICAL ASSAY OF OLEORESIN E.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	A	A	A	A	A	A	A	A	A
3 hours	A	A	A	A	A	A	I	I	I	I
4 hours	A	A	A	A	A	I	I	I	I	I
5 hours	A	I	I	I	I	I	I	I	I	I
6 hours	I	I	I	I	I	I	I	I	I	I

TABLE XII.—BIOLOGICAL ASSAY OF OLEORESIN F.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	I	I	I	I	I	I	I	I	I
3 hours	I	I	I	I	I	I	I	I	I	I

TABLE XIII.—BIOLOGICAL ASSAY OF OLEORESIN G.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	A	A	I	I	I	I	I	I	I
3 hours	I	I	I	I	I	I	I	I	I	I

TABLE XIV.—BIOLOGICAL ASSAY OF OLEORESIN H.

Time after Placing Worms in Dilutions.	Earthworm: 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1 hour	A	A	A	A	A	A	A	A	A	A
2 hours	A	A	A	I	I	I	I	I	I	I
3 hours	I	I	I	I	I	I	I	I	I	I

In each series with each Oleoresin the 50-mg. dilution failed to kill the earthworm in 1 hour; from 60 to 90% of the earthworms were killed by this dilution in a 2-hour period with five of the six Oleoresins, 0% being killed in the case of the sixth sample; 100% of the earthworms were killed in the cases of 5 of the 6 samples in the 3-hour period, the sixth sample showing but 40% killed. Controls were carried out as with the other experiments.

In the cases of the 5 samples which, in the 50-mg. dilutions, killed 100% of the earthworms in 3 hours, chemical assays disclosed from 23.65 to 25.02% (average, 24.48%) of crude filicin. The sixth Oleoresin assayed chemically but 19.15%, and a period of six hours was required to kill all of the earthworms.

CONCLUSIONS.

1. A study of the published chemical assay methods shows them to be more or less unsatisfactory, since they apparently determine relative rather than absolute percentages of filicin present.

2. A comparative study of the Modified Method and the U. S. P. X Method, representative of practically all of the reported chemical methods, showed that the Modified Method is more accurate, more rapid and less complicated.

3. Powdered Aspidium and Oleoresin of Aspidium which fail to meet the U. S. P. requirements are sold on the open market.

4. Oleoresins prepared from powdered Aspidium which meets the U. S. P. requirements do not always assay chemically 24% of crude filicin.

5. Ascarides cannot be used for biological assay of this drug. Earthworms are satisfactory.

6. The biological assay study with earthworms shows that the percentage of crude filicin is a definite indication of the anthelmintic powers of Oleoresin of Aspidium.

NOTE: It is planned to carry on collaborative studies of the chemical and the biological assay procedures suggested in this paper with samples of (*a*) the Oleoresin itself, (*b*) of crude filicin, and (*c*) of the residue remaining after the extraction of the crude filicin.

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ARGENTINE PHARMACEUTICAL CONGRESS.

The second Argentine Pharmaceutical Congress will be held at Buenos Aires in August. One of the items on the program will be a discussion of pharmacy laws of various countries.