

REPORT ON PROXIMATE ANALYSIS OF EUPHRASIA.*

(Euphrasia Officinalis L. Order, Schrophulariaceæ, Eye-Bright.)

BY WILLIAM E. MELTON AND L. E. SAYRE.

Attention has been called recently to the value of *Euphrasia officinalis* (Eye-Bright) by one of the leading manufacturing houses in the East, stating that the medical profession is using the drug successfully in the treatment of certain catarrhal conditions. As there had been published no satisfactory or complete analysis of the drug, it was suggested that such an analysis would be of special interest. This gave the writer an incentive for the undertaking. Accordingly a quantity of the drug was obtained for this purpose. It may be mentioned, incidentally, that the literature pertaining to Euphrasia was deemed fairly well represented in King's American Dispensatory, third revision, pages 751, which is in substance as follows:

"Action and medicinal uses—slightly tonic and astringent the drug is used in infusion or poultice in catarrhal ophthalmia. It also is of service in mucous diseases attended with increased discharges; and in coughs, hoarseness, earache, and headache, which have supervened in catarrhal affections.

"It is also used in acute catarrh (fluent coryza) and to control inflammatory and catarrhal phases of the parts during or following an attack of measles. Also averts unpleasant after effects, as catarrhal conjunctivitis, nasal catarrh, catarrhal deafness, etc.

"Catarrhal diseases of the intestinal tract may also be treated with Euphrasia.

"In epilepsy four fluid ounces of the infusion taken in the morning on an empty stomach and at bed time is asserted to be successful in curing this ailment.

"Specific indications and uses are: Acute catarrhal diseases of the eyes, nose and ears; fluent coryza with copious discharge of watery mucous secretion of acrid mucous from the eyes and nose with heat and pain in frontal sinus. (Scudder.)

"The dose of specific Euphrasia is one to sixty drops while the dose of the infusion is two fluid drachms to four fluid ounces."

It should be understood that "specific" when applied to medicinal preparations denotes a class of preparations of such "standard" strength as to meet certain specific indications in treatment, therefore it is impossible to state the exact strength. The dose of such preparations is regulated and determined by the practitioner.

DESCRIPTION.

There are a number of species of Euphrasia common to the United States, but *Euphrasia officinalis* is described as:

"An elegant little annual plant, with a square downy, leafy stem, simple or branched, and from one to five inches in height. The leaves are very small, almost entirely opposite, ovate or cordate. The flowers are minute axillary, solitary, very abundant, and inodorous, with a brilliant variety of colors. The calyx is companulate and four-cleft. Stamens, four, fertile under the upper lip: Anthers violet, lower cells of the upper ones with a long spur. Pods oblong and flattened. The seeds are numerous, oblong, and grooved lengthwise."

Our supply of the plant is dependent upon European importation. The *indigenous* plants of this genus are characterized by very small flowers, some

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borne on compact leafy heads or short racemes, some species with white corolla, with lavender or purple veins and "yellow eye" and so forth. In the *Euphrasia Americana* the corolla is relatively large and showy. (Gray.)

The commercial powder of this drug, under the microscope, showed quite a quantity of sandy material, accounted for, doubtless, by the fact that the plant is very small being only from one to five inches in height and growing close to the ground, so that when collected it carries with it this inert material.

It may be mentioned in passing that according to King's Dispensatory, *Lobelia* was originally called "Eye-Bright," but the name properly belonged to *Euphrasia officinalis*. We are indebted to H. K. Mulford of Philadelphia and J. U. Lloyd of Cincinnati for samples of the drug, as obtainable in the market.

It will be seen from the accompanying illustration of the tissues as observed under the microscope, that the powder we have analyzed did not consist wholly of leaves but includes in addition, stems, flowers, and seeds. We are now endeavoring to import an authentic specimen of the whole plant in order to study it more closely and compare it with the commercial product we have obtained for analysis. From a dried specimen of the whole

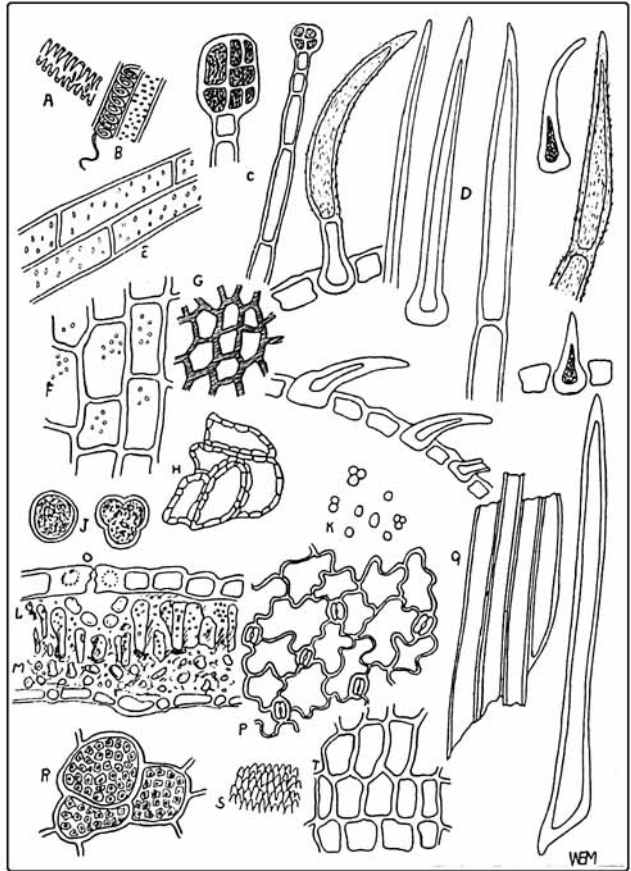


Plate No. 1. 200 Diameters.

Explanation of drawings: A, reticulate ducts; B, spiral and porous ducts; C, glandular trichomes; D, non-glandular trichomes; E, porous parenchyma; F, parenchyma tissue, containing chlorophyll grains; G, cork tissue; H, epidermal cells; J, pollen grains; K, starch grains; L, M, O, palisade cells, spongy tissue cells and stomata; P, epidermal tissue, showing stomata; Q, bast fibres; R, epidermis of seed coat (testa) vertical view; S, section of corolla; T, parenchyma cells.

plant, which was sent us by J. U. Lloyd, the powder of which we submitted to microscopical analysis shows more of the stem constituents, such as bast fibers, than was contained in the original sample and less trichomes and epidermal tissues from the leaves. This latter powder was of a much lighter color (very much less green) than the powder sent us for analysis. The phloroglucin and hydrochloric acid test

brings out this distinction quite markedly, showing more lignified tissues in this latter powder. In other words the powder furnished for analysis was not a powder from the whole plant.

ANALYSIS.

An analysis recorded in King's Dispensatory is substantially as follows:

"The recent leaves of the plant are commonly employed. Water extracts their virtues. Eng (1859), examining the recent plant, found it to contain mannit, grape sugar, volatile oil in small amount, an acrid bitter principle, cellulose, and other plant constituents, besides a number of acids of organic character, and tannin, the latter giving a deep green coloration with ferric compounds, and a bright light green reaction with the salts of lead."

ANALYTICAL PROCEDURE.

The powdered drug¹ was submitted to the action of various solvents as indicated in the accompanying report. From the analysis it would appear that one of the pronounced ingredients of the drug is of a very resinous character, which is partly soluble in ether and partly soluble in alcohol. From this it is assumed that there are different kinds of resinous products present as confirmed by experiment (XII).

In the subjoined table resinoids are noted under (III D), (IV A) and (XI A), the first being from the ethereal extractive of the drug while the second was from the alcoholic extractive. In (XI A) a larger amount of resinoid was obtained; this is accounted for by the fact that the resinoid was precipitated by adding the alcoholic solution to water acidulated with two per cent hydrochloric acid instead of water alone.

From a preliminary microscopic examination of the air-dried alcoholic extractive, the drug was found to contain a large number of oil globules which fact was verified by the results under (III B).

As will be seen from the report on alkaloids under (III E) positive results were obtained, though under (XV) in which the prollius fluid was examined the results were not satisfactory. While we believe that this drug contains a minute amount of alkaloid, more conclusive evidence should be obtained by a thorough analysis, time for which was not obtainable.

From the average of four determinations for nitrogen which gave 1.633 per cent of this element, the per cent of albumin was computed by using the factor 6.33. In the determination of the tannin, some trouble was experienced with the method used. An aqueous extract of the fat-free powder was precipitated with a two per cent solution of strychnine acetate;² the first precipitate which came down was filtered off after standing about an hour, and after drying and multiplying by the factor 0.6558 gave 1.841 per cent of tannin. The filtrate was allowed to stand several hours and as it was noticed that another precipitate formed, it too was collected and weighed giving 0.673 per cent. Subsequently more precipitate subsided but this latter was not used in computation.

¹ In making weighings for the different determinations reported some trouble was experienced from the fact that the air-dried powder was not constant in weight and changed considerably while on the balance.

² "Martindale, Extra Pharmacopœia" 17th Ed. p. 99.

The volatile matter from the drug powder was determined by steam distillation, the distillate was shaken with ether and the ether evaporated spontaneously as noted under (XIII A). This residue was dissolved in alcohol and poured into a small amount of distilled water. Upon putting a drop of this solution on the tongue a marked tingling sensation was produced.

It will be noted under (XIII B) that the drug seems to contain sublimable principles which for want of time were not investigated by extraction, etc. In Kraemer's "Applied and Economic Botany," page 173, several pages are devoted to this subject, stating that such substances as arbutin, a glucoside, had been found in a number of plants of the *Ereaceæ* by sublimation, as well as many other principles in such drugs as Cinchona, Rhamnus, Purshiana, Rheum, Hydrastis, Coca, and many other drugs. An extended study of the principles extracted by microsublimation as described in the above-mentioned references would more than likely prove very interesting.

The Hortvet Microsublimator will be used in this connection in further investigations.

LABORATORY DETERMINATIONS.

I.—Moisture Determination.

A definite weight of the finely powdered air-dried drug consisting of leaves of the plant was subjected to a temperature of 105° C. until the weight was constant, the produce was cooled in a desiccator and weighed.

II.—Ash Determinations.

The powdered drug, which was light and bulky, was incinerated and ashed in a porcelain crucible; the process was continued until a white ash was obtained; this was cooled in a desiccator to constant weight, and weighed.

The various fractions: those soluble in water in hydrochloric acid, and in sodium hydroxide, and the final insoluble residue, were obtained by the usual method in ash analysis. The various fractions obtained are stated in the subjoined table, which is the average of four determinations. (See tabular statement.)

III.—Ethereal Extract.

The powdered drug was percolated with ether in a continuous extractor (Soxhlet) for ten hours. The extract was evaporated to dryness and the weight of the residue recorded. This extract was treated as follows:

A. Volatile Oil.—The ethereal extract obtained above was treated with water, and evaporated to dryness at 100° C. and heated to 110° C. to drive off volatile matter. The difference between the weight of the residue and the original weight of the extract before heating is recorded as volatile oil.

B. Fixed Oil.—The dried extract from above was treated with small quantities of petroleum ether until all fatty substances were removed, after which the liquid was filtered off, and evaporated after drying, the residue was weighed and recorded as fixed oil.

C. Alcoholic Extract of Ethereal Residue.—The residual extract remaining after treatment with petroleum ether together with the filter, was macerated for ten hours in 80% alcohol. An aliquot portion was evaporated, dried, and weighed, and is recorded as Alcoholic extract from Ethereal Residue.

D. Resinoid from Ethereal Extractive.—The remaining portion of the alcoholic solution was concentrated to a small bulk and poured into twenty parts of distilled water. The precipitate was collected on a weighed filter, dried, weighed, and is recorded as Resinoid from Ether Extractive.

E. Waxy Residue.—The residual extractive remaining after treating the ethereal extractive with water, petroleum ether, and 80% alcohol, was dried and weighed and is reported as waxy residue. The physical properties being: dark green in color, practically no taste, insoluble in water, partly soluble in ether, and nearly entirely soluble in alcohol.

F. Alkaloid.—The filtrate from (*D*), alcoholic liquid from which resinoids were precipitated, was examined for alkaloids and gave the following results: A drop of the filtrate was put on a watch crystal, and acidulated with a small drop of dilute sulphuric acid, after which a drop of Mayer's reagent was drawn into it with a glass rod. A white precipitate appeared at once. The same procedure, using Wagner's reagent, gave an affirmative result.

A microscopic examination of the filtrate after concentration and filtering through cotton showed small scattered amorphous particles. Upon addition of Mayer's reagent these particles were increased in number, crystals of potassium iodide coming down later. The examination of the filtrate with Wagner's reagent gave an amorphous precipitate with the potassium iodide crystals coming down as the solution on the slide evaporated spontaneously.

When the above solution was tested it produced very benumbing effects which lasted for a half hour or more.

IV.—Alcoholic Extractive of Ether Marc.

The drugs remaining after treatment with ether were extracted with 80% alcohol by the Soxhlet continuous extraction method. An aliquot portion of the solution was evaporated to dryness on a water-bath, dried and weighed, being reported as alcoholic extractive of ether marc.

A. Resinoid.—The remaining alcoholic extract was evaporated on a water-bath to small bulk and poured into 20 parts of water, the precipitate collected, dried, weighed, and reported as resinoid from alcoholic extractive.

B. Vegetable Acids.—To the filtrate from (*A*) a solution of neutral lead acetate was added, the precipitate collected, washed, dried, and weighed. The precipitate was removed from the filter and the filter burned in a weighed crucible with a little ammonium nitrate to insure ignition and to prevent reduction of lead, the precipitate was then transferred to the crucible and incinerated at low heat, and finally ignited until the weight was constant. Upon deducting the weight of the residue from the weight of the precipitate the weight of the vegetable acids precipitated by lead was found.

C. Saccharine Matter (Glucose).—To an aliquot portion of the filtrate from (*B*) a solution of lead subacetate was added, the precipitate filtered off and the excess lead removed from the filtrate by acidulating it with hydrochloric acid and passing hydrogen sulphide into the warmed solution. The clear liquid was neutralized, made up to a definite volume and determined quantitatively for sugar with Fehling's solution.

The Munson and Walker Method III was used in this determination and the

per cent of glucose found from reference to the table attached to the above method.

D. Residual Extractive.—The remaining portion of the filtrate from (B) was treated with hydrogen sulphide as in (C) to remove the lead, evaporated to dryness on a water-bath, dried, and weighed. From this weight the weight of the glucose was subtracted and the remainder reported as Residual Extractive.

The tests for alkaloids with Mayer's and Wagner's Reagents were very unsatisfactory.

V.—Aqueous Extract.

The dregs remaining after treatment with ether and alcohol were macerated for twelve hours in 75 cc. of water, the liquid filtered off and made up to 100 cc. by pouring through the filter the required amount of water. The extractive in 50 cc. of this solution was determined and is reported as Aqueous Extractive from Ether and Alcoholic Dregs.

A. Gum.—The other 50 cc. of filtrate from (V) was concentrated on a water-bath to 25 cc. and to this was added 50 cc. of absolute alcohol, the mixture placed in a cool place for twenty-four hours to allow the gum to precipitate, which was filtered off, dried, weighed and reported as gum.

B. Albuminoid Extractive Matter.—The extractive remaining in the filtrate from (A) was determined by evaporation after which it was dried and weighed and is reported as Albuminoid Extractive matter.

VI.—Sulphuric Acid Extractive, from Ether, Alcohol and Water-extracted Dregs.

The dregs remaining after extraction with ether, alcohol and water, were dried and transferred to a flask provided with a reflux condenser and boiled for three hours with water containing 1% by volume of sulphuric acid (sp. gr. 1.84) using 100 cc. of this mixture for each gram of the residue. An aliquot portion of the filtrate obtained after filtering out the dregs was evaporated, dried and weighed and is reported as Sulphuric Acid Extractive.

A. Reducing Substances.—The remaining portion of the filtrate obtained from (VI) was tested for sugar, which should now be present through the hydrolysis of the starch. This hydrolysis having been brought about from boiling the dregs containing the unextracted starch with acidulated water in (VI). The process used was the Munson and Walker method as stated under (IV C) which depends upon the reduction of Fehling's solution and the weighing of the resulting copper oxide, after which this weight is multiplied by the factor 0.9 which should give the weight of starch and allied substances. From the microscopical examination of the drug very little starch was seen, we would say not over 0.5%, while from the above determination 9.684% was indicated, the difference being no doubt accounted for by some reducing substance contained in the filtrate from (VI). Consequently instead of reporting this as Starch and Allied substances we are reporting it as Reducing substances.

VII.—Sodium Hydroxide Extractive from the Ether, Alcohol, Water and Sulphuric Acid-extracted dregs.

The dregs remaining after extraction with the above were dried, powdered and boiled with water containing a 2% solution of Sodium hydroxide (2 Gms. of NaOH in 100 cc. of water) for one hour. From an aliquot portion of the filtrate the total extractive was obtained and is recorded as Sodium Hydroxide Extractive.

VIII.—Cellulose.

The dregs remaining after treatment with NaOH were washed with water, alcohol and ether, dried and weighed, after which they were ignited in a crucible, cooled in a desiccator and weighed again. The weight of the ash subtracted from the weight of the dregs is reported as cellulose.

A. Insoluble Residue (Sand).—The residue remaining after igniting the dregs in VIII is reported as Insoluble Residue (Sand). The discrepancy of 0.285% between insoluble residue here and that obtained in the ash determinations is probably due to the fact that in the ash determinations the 2% HCl used gave a soluble chloride which was not the case when 1% H₂SO₄ was used in the above determinations.

IX.—Nitrogen.

In the determination of nitrogen the Dyer modification of the Kjeldahl was used. The mean of four determinations is calculated to its equivalent in albuminoids by multiplying it by the factor 6.33 and is reported as Albumin.

X.—Tannin.

A definite weight of the powdered drug from which the fat had been removed with petroleum ether was macerated in water, the aqueous extract filtered off and the Tannin precipitated by the addition of a 2 per cent solution of strychnine acetate IV. The precipitate was collected, dried and weighed. The weight of the precipitate multiplied by 0.6558 gives the weight of Tannin.

XI.—Alcoholic Extract of the Drug.

100 Gms. of the drug was macerated with 500 cc. of 95 per cent alcohol for ten days with occasional agitation after which 10 cc. of the clear alcoholic liquid was drawn off with a pipette and allowed to evaporate in a petri dish, weighed and recorded as alcoholic extract of drug.

A. Resinoid (Precipitated with HCl).—The alcoholic extract after being weighed was redissolved in the least amount of alcohol required and this solution poured into 2 per cent hydrochloric acid in a small stream while stirring. The resulting precipitate was collected, dried, weighed and is reported as resinoid precipitated with HCl. The resinoid obtained was found to be very soluble in chloroform, partly soluble in ether, and partly soluble in alcohol. As the resinoid was dissolved out of the drug originally with alcohol, the fact that the resinoid is not now entirely soluble in it is accounted for by assuming that the resinoid was soluble in some constituent which was removed when it was precipitated and filtered.

B. Alcoholic Residue.—Upon evaporating the filtrate from (A) after the resinoid had been precipitated a black-brown sticky substance was obtained which was dried and weighed and found to be soluble in water, insoluble in chloroform, partly soluble in ether, and entirely soluble in alcohol.

XII.—Report on Resinoids.

The concentrated alcoholic solution of the drug was precipitated by adding this concentrate to water. An effort was made to fractionate the precipitate which was resinous, by adding successively different quantities of water to the alcoholic concentrate. After each successive addition of water the precipitate resulting therefrom was collected in four portions:

	Percentage of different resins.
First, precipitate when dried gave	1.3648
Second, precipitate when dried gave	0.7476
Third, precipitate when dried gave	0.2608
Fourth, precipitate when dried gave	0.2304
The total Resinoid precipitate	2.6

Characteristics of Fractional Resinous Precipitates.—First precipitate: Resinous, somewhat sticky, mass mostly soluble in ether and slightly insoluble in alcohol. A small portion of it only was soluble in alcohol. Second: Soluble in alcohol, slightly soluble in ether. Third and Fourth: Soluble in alcohol only.

XIII.—Volatile Matter.

Ten grams of the drug was steam distilled, by running steam into an aqueous mixture of the drug, for thirty minutes, collecting in that time forty-five cc. of the distillate which was shaken out with ether and the ethereal solution allowed to evaporate spontaneously on a tared watch crystal and weighed.

A. Sublimable Principles.—A few grams of the powdered drug were placed in a crucible and subjected to sublimation, a small funnel with a long neck was inverted and placed over the crucible. A light brown sticky substance which became crystalline when cold was deposited in the far end of the neck of the funnel. This deposit appeared to be soluble in water, alcohol, and chloroform but nearly insoluble in ether.

XIV.—Fat.

A definite weight of the drug was macerated in petroleum ether several days with occasional agitation, after which the liquid was filtered off and allowed to evaporate spontaneously, redissolved in the same menstruum and evaporated again, dried and weighed.

XV.—Test for Alkaloids.

A definite weight of the powdered drug was macerated in prollius fluid No. 1, which consisted of:

Chloroform	20 parts
Alcohol	76 parts
Ammonia Water	4 parts

for seven days, at which time the liquid was decanted off and allowed to evaporate. To the dregs was then added prollius fluid No. 2 which consisted of:

Ether	68 parts
Chloroform	22 parts
Alcohol	7 parts
Stronger Ammonia Water	10 parts

this being allowed to stand forty-eight hours after which the liquid was filtered off and added to prollius fluid No. 1 and the combined liquids evaporated to a syrupy consistency at 50° C., to remove the solvents, mainly alcohol. The syrupy liquid was re-dissolved in ether, the ethereal solution shaken in a separating funnel several times with small portions of 1% H₂SO₄ and the acid washings were drawn off and combined.

As the acid washings were more or less colored an attempt was made to remove this color so that it would not interfere with the alkaloidal tests by washing it with chloroform and then with ether but very little color was removed so the acid washings were made alkaline with ammonia and again shaken out with ether, the ethereal solution drawn off and shaken with small portions of 1% H₂SO₄ which appeared nearly colorless. This acid solution was tested for alkaloids with Mayer's reagent and gave the following results:

Mayer's Reagent.....1st test.....doubtful
After standing 24 hours.....doubtful

The acid solution was concentrated and again tested with Mayer's Reagent from which the following results are reported.

Mayer's Reagent on Concentrated Acid Solution
1st test.....very light precipitate
After standing 24 hours.....very little change.

TABLE OF CONSTITUENTS.

		Per cent.
Moisture, I.....		10.089
Ash, II.....		8.646
	Ash. Drug.	
Ash Soluble in Water.....	28.336	2.450
Ash Insoluble, Soluble in HCl.....	47.592	4.115
Ash Insoluble, HCl Soluble in NaOH.....	10.629	0.919
Ash Insoluble, Residue.....	13.443	1.162
	<u>100.000</u>	<u>8.646</u>
Ethereal Extractive (Soxhlet) III.....		10.120
	Per cent.	
Volatile Oil III, A.....	0.162	
Fixed Oil III, B.....	6.605	
Alcoholic Extractive from Ethereal Residue III, C.....	1.205	
Resinoid from Ethereal Extractive Residue III, D.....	0.515	
Waxy Residue III, E.....	0.872	
	<u>9.359</u>	
Alcoholic Extractive of Ether Marc IV.....		13.566
Resinoid from Alcoholic Extractive IV, A.....	0.504	
Vegetable Acids precipitated by Lead IV, B.....	4.131	
Saccharine Matter (Glucose) IV, C.....	0.866	
Residual Extractive IV, D.....	8.061	
	<u>13.562</u>	
Aqueous Extractive from Ether and Alcoholic Dregs V.....		9.923
Gum V, A.....	3.384	
Albuminoid Extractive Matter V, B.....	6.470	
	<u>9.854</u>	
Sulphuric Acid Extractive from Ether, Alcohol and Water extracted Dregs VI.....		20.446
Reducing Substances VI, A.....	9.684	
Sodium Hydroxide Extractive from previous extracted dregs VII.....		20.500
Cellulose VIII.....		14.090
Insoluble Ash (Sand) VIII, A.....		1.446
Nitrogen (calculated to Albumin) IX.....		10.348
Tannin X.....		1.841

Alcoholic Extract of the Drug XI.....		13.323
Resinoid (Precipitated with HCl) XI, A.....	4.300	
Alcoholic Residue XI, B.....	9.020	
	13.320	
XII, See above		
Volatile Matter (From steam distilling) XIII.....		0.024
Fat (From Petroleum ether extraction of the drug) XIV.....		0.644
XV, Alkaloids, See above		

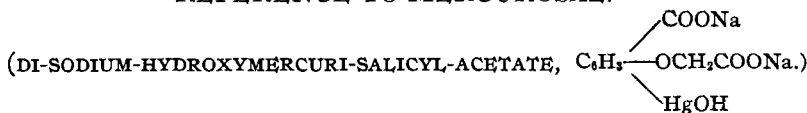
CONCLUSION.

The investigation, such as the writer has endeavored to make, has aimed to isolate the proximate principle or principles upon which the drug seems to depend for its alleged medicinal properties. The separation of the various constituents, as detailed in the accompanying outline, seems to indicate that the medicinal properties are contained in aromatic resinous bodies. The presence of volatile oil is evident as will be observed in the record of the analysis. Preparations of the drug for catarrhal conditions, such as a tincture or a syrup, are blended with other expectorants and sedatives affecting the irritated mucous membrane either locally or remotely. The tincture which has a deep olive-green color has a decided characteristic herby taste, having a rather soothing sensation to the mucous surfaces of the mouth and throat, leaving a slight tingling sensation not very unlike a mild local anaesthetic. It should be stated that the high percentage of fat and oil, it seems to the writer, can be accounted for only by the presence of fat soluble coloring matter and the presence of considerable seed in the powder.

Our investigation seems to indicate that the statement made in King's Dispensatory—"that the medicinal principles may be extracted with aqueous menstrua" should be corrected, as they seem to the writer to be misleading since the resinous bodies, in which the virtues seem to reside are only soluble in an alcoholic, hydroalcoholic, or similar menstrua.

UNIVERSITY OF KANSAS,
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THE ELIMINATION OF MERCURIALS WITH PARTICULAR REFERENCE TO MERCUROSAL.*



BY L. W. ROWE.

In a previous article¹ the relatively low toxicity of mercurosal was demonstrated by experiments upon animals. Its comparative freedom from corrosive action upon the wall of a vein was also determined by intravenous injections into animals.

The present article will deal first, with the study of the excretion of mercurosal both from a qualitative and quantitative standpoint, and second, with its

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