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CHEMICAL AND BIOLOGICAL ASSAY OF DRUGS. CASCARA SAGRADA.*

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PART I.

Color tests for the identification of Cascara Sagrada as outlined by the U. S. P. are indefinite and inadequate. Other drugs such as Frangula, Aloes, Rhubarb, Senna, and Ergot give very similar color reactions by these methods so that it seems impossible to make a positive identification of any of them by this test.

More attention should be paid to the color reactions of Cascara Sagrada for it seems that the coloring matter is a fairly reliable index of the activity of the drug.

In the present work, the method for identification of Cascara, as outlined in the U. S. P. was carried out on Frangula, Aloes, Rhubarb, and Senna. It was found that color variations were so inconstant that they would not suffice for identification; therefore, we have attempted other methods which are as follows:

ETHER EXTRACTION METHOD.

Fluidextracts of Cascara, Aloes, Frangula, and Rhubarb were prepared according to the U. S. P. method. Ten cc. each of the fluidextracts were completely extracted with ether, until a color was no longer imparted to the solvent and the solvent gave no color reaction with ammonia water. During the extraction, troublesome emulsions were often encountered. The aqueous residue left after

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extraction with ether was of a dark brown color and devoid of the bitter principle, and showed no color reacting constituent and when tested therapeutically on man was found to be entirely inactive. The ether extract was then concentrated to 200 cc. and the ammonia test applied. The following color reactions were obtained:

Aloes—Orange-red
Cascara—Orange-yellow
Frangula—Reddish purple
Rhubarb—Cherry-red

The ether extracts were then evaporated to dryness and gummy residues, varying from a bright yellow to an orange-red were obtained. When tested on man these gum-like substances were found to be therapeutically active, insoluble in water, and gave color reactions with ammonia as stated above. We found, when testing for the color reactions, that the depth of color was approximately proportional to the concentration; that the colors fade rapidly on exposure to light; and that concentration of the ammonia used gave a greater depth of color, but this is probably due to a greater concentration of the solvent because, upon dilution with water, the color approached more nearly the ammonia water controls. If the powdered drugs were not extracted completely, we found that the color depth varied with the fineness of the powder and temperature at which extraction was performed; however, there seemed to be no direct relation between color depth and temperature. The powdered drug extracted with water at 100° C. and at 70° C. showed an almost imperceptible color difference, while the same drug extracted in cold water gave a lighter color reaction, but not a color that would seem to indicate a direct relation between color depth and temperature.

We could reach no definite conclusions from the above experiments, therefore, we determined to use ethyl acetate and lead subacetate as a solvent and precipitant, respectively.

ETHYL ACETATE METHOD.

Fluidextracts of Cascara, Aloes, Frangula, and Rhubarb, ten cc. in amount, were completely extracted in the same manner as in the experiments already enumerated, with exception that ethyl acetate was used as a solvent. The residue left after treatment with ethyl acetate was of a dark brown color, was not therapeutically active, did not give a color reaction with ammonia and was devoid of a bitter principle. The ethyl acetate extract was pale yellow and gave the following color reactions with ammonia water:

Aloes—Precipitate and cherry-red upper strata
Cascara—Reddish pink
Frangula—Cherry-red
Rhubarb—Deep red color in ethyl acetate strata, while ammonia strata is colored light yellow.

The ethyl acetate extracts were evaporated to dryness and a yellowish gum-like residue was obtained. This gum-like substance was found to be therapeutically active. These acetate extracts when filtered through animal charcoal gave up completely their color reacting constituents.

LEAD SUBACETATE METHOD.

Ten cc. of the fluidextracts of Cascara, Frangula, Rhubarb, and Aloes were later treated with lead subacetate. There appeared a heavy flocculent brown

precipitate. The precipitate was collected and washed with distilled water until the washings ran through clear. The precipitate was then dissolved in ethyl acetate. H_2S was then passed through the ethyl acetate solution to determine if any lead were present. No precipitate occurred. H_2S was then passed through the brown aqueous solution. A heavy black precipitate appeared. H_2S was passed through the solution until the test for lead in the filtered solution was negative, whereupon, the solution was then concentrated to 200 cc. and tested with ammonia. The color reactions were negative, therapeutically the solutions were inactive and possessed no bitter principles. Tests upon the acetate solvents gave the following color reactions with ammonia.

Cascara—Reddish pink
Aloes—Orange-red
Rhubarb—Purplish red
Frangula—Light pink

CONCLUSIONS.

1. There seems to be no definite relation between the depth of color and the temperature at which extraction is carried out. This is especially true when the drug is not completely exhausted.
2. The depth of color varies directly with fineness of powdered drug when powder is not completely extracted.
3. The color of an ammonia-treated extract fades when exposed to light.
4. The active constituent, coloring matter, and bitter principle are soluble in ether, in ethyl acetate, and are precipitated by lead subacetate.
5. The coloring constituent is more soluble in ammonia than in ether; more soluble in ether than in ethyl acetate; more soluble in ethyl acetate than in water, and there appears to be one portion almost completely insoluble in water or at least very much less soluble than in ammonia, ether or ethyl acetate.
6. Color variations in tests for Cascara, Rhubarb, Frangula, and Aloes by the present methods and methods enumerated above are inadequate for a qualitative differentiation.
7. It seems probable that lead subacetate acts merely as a physical agent in causing precipitation, and that the animal charcoal acts similarly in removing the coloring constituent.

PART II.

From the above conclusions we were led to believe that Cascara Sagrada could be split into some of its constituent parts by a difference in solubility in immiscible solvents. We hoped to isolate or separate the active constituent, the coloring matter, and the bitter principle and, if possible, a quantitative determination by a colorometric method; therefore, we undertook the following experiment:

ETHER EXTRACTION METHOD.

One hundred grams of the powdered drug identified microscopically as Cascara Sagrada (1) was placed in a 3-liter flask and ether added. After shaking for a short time the ether became yellow, the depth of color depending somewhat upon the amount of agitation and the period of time the ether is in contact with the drug. The ether was then decanted and filtered from the powdered Cascara and placed in another flask to which 200 cc. of distilled water was added. The flask

containing the ether extract and water was then arranged for distilling. Heat in the form of a water-bath was then applied and the ether was distilled over (at a temperature not above 45° C.) and collected. The ether distillate was then again used as a solvent for the cascara, thus minimizing the amount of ether necessary for the extraction. This process was continued until the powdered drug was completely extracted as was shown by a failure to color the ether while in contact with the drug and a failure of the ether to give a color reaction with ammonia. After the powdered drug had been extracted, we prepared a fluid-extract from the powder by the U. S. P. method. We found the fluidextract much lighter in color than the normal extract, contained no bitter principle, gave no color reaction with ammonia and when tested therapeutically in excessively large doses showed an absence of the active cathartic principle.

When the ether extract containing its yellow coloring matter was added to the water in the distilling flask, the water remained practically colorless, thus showing ether to be the better solvent. Upon heating the flask, the yellow ether upper layer became progressively darker (probably due to concentration of the dissolved substances) and at the same time the aqueous substrata began to assume a darker yellow color, showing that at least a part of the precipitate being thrown out of solution by concentration of the ether solvent was being redissolved by the water. At the point of contact between the two immiscible solvents there appeared a black precipitate which remained floating on the surface of the water.

The ether was completely evaporated and the aqueous solution containing a soluble and insoluble substance was filtered. The insoluble substance collected on the filter paper was a deep brown or red gum-like substance, while the aqueous filtrate was of an orange yellow-color. The precipitate was then thoroughly washed with cold distilled water until the washings appeared clear, thus showing that the aqueous soluble portion had been removed. The precipitate was then thoroughly dried and in that condition took on the character somewhat of a powder, but upon addition of moisture it changed to its original state. This precipitate was a very deep dark red bordering on black; had a gum-like consistency when moist; had no odor; was devoid of bitter principle; gave a constant color reaction with ammonia, and when tested therapeutically was found to be active. A very small amount of this gum-like substance when tested with ammonia gave a deep cherry-red color, which did not assume a yellow hue when diluted.

The aqueous filtrate appeared as an orange-yellow, was extremely bitter and had an aromatic odor. This aqueous extract was then evaporated to dryness and a brown scaly residue was obtained. This residue could be powdered, was extremely bitter; was therapeutically active in small amounts and upon testing with ammonia gave a deep orange-brown color which upon dilution approached more closely a yellow, but with no indications of red.

1. From the 100 grams of powdered drug, we were able to isolate the following amounts from the various samples:

Sample.	Gum-like non-bitter principle.	Bitter principle.
I	6.35 Gm. per 100 Gm. of drug	2.89 Gm. per 100 Gm. of drug
II	7.10 Gm. per 100 Gm. of drug	3.06 Gm. per 100 Gm. of drug
III	5.94 Gm. per 100 Gm. of drug	2.63 Gm. per 100 Gm. of drug
IV	4.89 Gm. per 100 Gm. of drug	2.41 Gm. per 100 Gm. of drug

CONCLUSIONS.

1. It is necessary to amplify our first conclusions by the statement that there seems to be conclusive evidence that there are at least two color reacting constituents in Cascara Sagrada.

2. One color reacting constituent is practically insoluble in water and gives a deep cherry-red color with ammonia. The other color reacting constituent is soluble in water and gives an orange-brown color reaction with ammonia.

3. Both coloring constituents are soluble in ether.

4. It seems likely that the variation in the U. S. P. tests may in part be due to a difference in solubility of these two constituents.

5. It may be possible to derive a quantitative color test if these are the only two coloring constituents.

6. The bitter principle is soluble in water, accompanies or is the brown color reacting constituent and accompanies or is part of the therapeutically active principle, probably an anthraquinone.

7. The non-bitter principle is insoluble in water and is therapeutically active.

8. The bitter principle acts more slowly and produces marked griping, while the non-bitter principle acts more rapidly and with less griping.

Sample (1) was obtained from the Pharmacognosy Laboratory of the University of Illinois, College of Pharmacy, and was identified as Cascara microscopically by Prof. E. N. Gathercoal.

Sample(II) was obtained from the Pharmacy laboratory of the University of Illinois, College of Pharmacy.

Samples (III) and (IV) were obtained from a local drug store.

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THE STABILITY OF OFFICIAL PEPSIN PREPARATIONS.

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A. E. Taylor has stated[1] that "..... in general purine bodies tend to accelerate tryptic digestion." In 1922 Traut[2] observed that liquid medicinal pepsin preparations containing very small amounts of caffeine (a purine body) seemingly maintained their peptic activity more readily than without caffeine. It became of interest to determine whether other purine bodies, readily obtainable commercially and inexpensive could be used for this purpose in quantities so small as to be practically nontoxic. Purine derivatives available include the following: caffeine,

[1] *University of California Publications of Pathology*, I, p. 251 (1903–1907).

[2] November JOUR. A. PH. A., p. 686 (1922)—E. J. Traut and H. W. Vahlteich.