creased to $7.50 for *all* members for *all* publications, as proposed, many will resign and fewer new members will be gotten than could be otherwise.

The logic of the situation, therefore, suggests that the Association establish several classes of members, as follows:

(1) Members or Active Members who will pay $5.00 dues and receive the JOURNAL only.

(2) Contributing Members who will pay $7.50 dues and receive both the JOURNAL and the Year Book.

(3) Corporation Members who will pay $25.00 dues and receive special services in the way of information, reprints, etc. (similar to that offered by the American Chemical Society).

(4) Associate Members who will pay $3.00 dues and receive no publications; this could include drug clerks, soldier and sailor pharmacists, etc., who wish affiliation for prestige only.

Some such plan as this would be modern and business-like. It would mean a square deal both for the membership and the Association. Each member would get only what he wants and is willing to pay for and the Association would get what it pays for the service it renders to its members; and it would have a reasonable sum of money for "overhead expense" that would permit an expansion of its activities limited only by the size of its membership.

But, as you know, the whole question of annual dues, finances, membership, etc., is now in the hands of the Executive Committee for consideration and report to the Council and later to the Association, and I feel that I am but expressing the wishes of the Committee when I say that the latter will gladly welcome any and all suggestions reflecting the wishes of the membership to the end that the fullest light may be had on the subject and a satisfactory decision reached.

THE ASSAY OF CALABAR BEANS AND PREPARATIONS OF CALABAR BEANS.

BY GEORGE E. ÉWE.

The U. S. P. 7th did not prescribe the assay of calabar beans and its official preparations. Many manufacturers, however, standardized their output of these preparations.

Probably the most popular method of assay at that time was the ordinary gravimetric "shake out" method; using sodium bicarbonate and ether to extract the alkaloids from the drug or its preparations; extracting the alkaloids from the ether solution by means of dilute sulphuric acid; liberating the alkaloids again by means of sodium bicarbonate; extracting the liberated alkaloids with ether; evaporating the ether in a tared flask; drying the alkaloidal residue to constant weight and correcting this weight by dissolving the alkaloidal residue in dilute sulphuric acid, collecting and weighing the acid-insoluble matter and subtracting its weight from the original weight of the alkaloidal residue.

This method being applied to both the drug and the preparations made from the drug established uniformity in the alkaloidal content of the preparations.

It is a rule of drug assay laboratories to give preference to volumetric methods.

if possible, therefore when the U. S. P. 8th included a volumetric method of assay for calabar beans and preparations of calabar beans preference was given to that method. The U. S. P. 8th required calabar beans and preparations of calabar beans to be assayed by liberating the alkaloids with a solution of sodium bicarbonate in the presence of ether and extracting the alkaloids with ether; the ether solution of alkaloids then being extracted by very small portions of dilute acid; the alkaloids being liberated again by a solution of sodium bicarbonate and finally extracted with ether, which was evaporated to obtain the alkaloids. The alkaloids were finally titrated. This method was soon found to be open to the objection that the quantities of acid and ether specified for extraction of the alkaloids were entirely too small for the purpose and, as a consequence, low results were obtained.

The above-mentioned defects in the assay method of the U. S. P. 8th were ably pointed out also by A. H. Salway, D. Sc., Ph.D., in the *American Journal of Pharmacy* for February 1912. Salway found that the methods of obtaining the alkaloids from calabar beans in use by alkaloid manufacturers returned twice as much alkaloid as was indicated by the U. S. P. method of assay when applied to the crude drug. He also concluded that the defects were due to insufficient quantities of acid and ether used for extraction.

This laboratory was cognizant of these defects and followed the practice of using sufficient acid and ether in the extractions to obtain all of the alkaloids. The process was extremely tedious since at least 6 extractions were required for complete extraction.

Radical changes were made in the assay process for calabar beans when the U. S. P. 9th was issued.

The U. S. P. 9th process reads as follows:

Assay: Introduce 15 Gm. of Physostigma in No. 60 powder into a flask of about 250 mils capacity and add 150 mils of ether. Stopper the flask, shake it well and allow it to stand ten minutes, then add 10 mils of an aqueous solution of sodium bicarbonate (1 in 20) and shake the mixture vigorously at intervals during four hours. Now add 15 mils of distilled water, again shake the flask well, and, when the drug has settled, decant 100 mils of the ether solution representing 10 Gm. of Physostigma. Filter the solution through a pledget of purified cotton into a beaker and rinse the graduate and cotton with ether. Add 20 mils of tenth-normal sulphuric acid V. S. and evaporate off the ether, stirring during the evaporation with a rubber-tipped glass rod. After the resinous and fatty matter has agglutinated, pour off the acid solution through a wetted filter into a separator. Redissolve the residue in the beaker in about 15 mils of ether, add 2 mils of tenth-normal sulphuric acid V. S., evaporate off the ether with continued stirring as before and pour the acid solution on the filter. Repeat this operation until all of the alkaloid is extracted and then wash the filter with distilled water until it is free from alkaloids. Collect the solution and washings in a separator, add sufficient sodium bicarbonate to make the solution decidedly alkaline to litmus and completely extract the alkaloids by shaking it out repeatedly with ether. Wash the combined ether solutions with 10 mils of distilled water, separate the water completely and filter the ether solution, washing the container and filter with ether. Evaporate the ether solution to dryness, dissolve the alkaloids from the residue in exactly 5 mils of tenth-normal sulphuric acid V. S., and titrate the excess of acid with fiftieth normal potassium hydroxide V. S., using cochineal T. S. as indicator.

Each mil of tenth-normal sulphuric acid V. S. consumed corresponds to 27.52 milligrams of the alkaloids of Physostigma. (See Part II, Proximate assays, No. 15.)

This U. S. P. 9th method has not yielded satisfactory results in our hands, the results in all cases being extremely low. We have not traced the exact cause of the low results, but believe the loss to be due partly to incomplete extraction

and partly to decomposition of the alkaloids by the numerous manipulations and vigorous heating treatments prescribed and by the long exposure to light required in carrying out the process. This latter belief is supported by the fact that the aqueous alkaloid extractions both when acid and alkaline develop quite intense pink colors.

Very satisfactory results have been obtained, however, by the following extremely short method which was devised in these laboratories.

CALABAR BEANS.

Assay for alkaloids: Sample, 20 Gm. *finely powdered*. Place in dark, glass bottle, add 180 Cc ether, shake 10 minutes, add 10 Cc saturated solution sodium bicarbonate. Shake 4 hours. Allow to stand over night. Filter off through fluted filter paper as large an aliquot as obtainable. Place aliquot in an Erlenmeyer flask. Evaporate ether just to dryness. Dissolve residue of alkaloids in a mixture of 15 Cc of standard acid and 15 Cc water, using a little chloroform and heating to drive off the chloroform. Titrate back with standard alkali, using methyl red.

In using this method, there is no possibility of the ether extraction containing any sodium bicarbonate in solution and thereby being counted in as alkaloid as we have repeatedly run blank determinations which in no case used up any standard acid in the titration process. In a few cases a slight acidity has been indicated. This we ascribe to experimental error and, therefore, consider it advisable to run a blank in each assay in order to correct for variation due to this cause. In no case has the acidity gone above 0.07 Cc of $\frac{N}{10}$ acid.

The value of this method for the assay of calabar beans and preparations of calabar beans was proven by mixing physostigmine sulphate with oak sawdust and then assaying the mixture by this method, using the U. S. P. factors in the calculations.

The physostigmine sulphate employed was made by Hoffman-LaRoche and was the usual medicinal variety. It conformed to all of the requirements of the British Pharmacopoeia. It was prepared for the experiments by being dried to constant weight at 80° C in an air-oven, care being taken to protect it from the light by keeping its container enclosed by black paper at all times. A weighed portion of the anhydrous physostigmine sulphate was placed directly into a blue-glass bottle containing 10 Gm. of oak sawdust; 200 Cc of ether was then measured into the bottle, followed by 10 Cc of saturated solution of sodium bicarbonate. The rest of the process was conducted as outlined in the process mentioned above.

The following results indicate the value of this process for the assay of calabar beans and preparations of calabar beans:

Experiment. No.	Theoretical amt. of alkaloid in aliquot, Gm.	Amt. of alkaloid found upon titration. Gm.	Percentage of theoretical, %.
1	0.0679	0.0651	95.4
2	0.0595	0.0577	97.0
3	0.0720	0.0650	90.3
4	0.0631	0.0607	96.2
		Average	94.7

That this method yields more certain results than the method of the U. S. P. IX is shown by the following comparisons:

Experiment. No.	Sample.	U. S P. IX method, %.	Direct evap. of aliquot, %.
1.....	Drug	0.2	0.14
2.....	Drug	none	0.13
3.....	Drug	0.11	0.37
4.....	Fldext.	0.070	0.133
5.....	Fldext.	0.059	0.178
6.....	Fldext.	0.0821	0.150
7.....	Tincture	0.00218	0.00611
8.....	Tincture	0.00290	0.01305

Fluidextract of Calabar Bean is not official in the U. S. P. 9th and therefore no method of assay is prescribed. In the above-mentioned experiments the fluidextract was prepared for assay by placing it upon 10 Gm. of oak sawdust, drying the impregnated sawdust spontaneously in a darkened place and then assaying the impregnated sawdust as though it was crude drug.

As a result of these experiments the following methods are to be recommended as preferable to the method of the U. S. P. IX for the assay of calabar beans and preparations of calabar beans:

CALABAR BEANS.

Assay for alkaloids. Sample, 20 Gm., *finely powdered.* Place in a dark, glass bottle, add 180 Cc ether and 10 Cc of saturated solution of sodium bicarbonate. Shake 4 hours, allow to stand over night. Shake for 15 minutes. Allow to settle. Carefully filter off as much as possible and as quickly as possible through a fluted filter, collecting the filtrate in a 200 Cc graduated cylinder. Measure the aliquot and pour into an Erlenmeyer flask. Recover the ether on the steam bath. Remove the flask from the steam bath just as soon as all of the ether is off. Place 15 Cc of standard acid on the alkaloidal residue in the flask followed by 15 Cc of water and 3 Cc of chloroform. Boil off chloroform completely on the steam bath. Titrate back with standard alkali; use methyl red as indicator. Run a blank and make correction, if necessary.

1 Cc. $\frac{N}{10}$ acid = 0.02752 Gm. alkaloids.

FLUIDEXTRACT OF CALABAR BEAN.

Assay for alkaloids. Sample, 20 Cc. Evaporate spontaneously on 10 Gm. oak sawdust contained in an evaporating dish, in a darkened place. Treat impregnated sawdust like the drug, cleaning the evaporating dish with 10 Cc of saturated solution of sodium bicarbonate.

POWDERED AND SOLID EXTRACTS OF CALABAR BEAN.

Assay for alkaloids. Sample, 3 Gm. Mix with 15 Cc alcohol in an evaporating dish. Add 10 Gm. oak sawdust. Mix well. Evaporate spontaneously in a darkened place. Treat impregnated sawdust like the drug, cleaning the evaporating dish with 10 Cc of saturated solution of sodium bicarbonate.

TINCTURE OF CALABAR BEAN.

Assay for alkaloids. Sample, 200 Cc. Pour on 10 Gm. oak sawdust contained in an evaporating dish. Dry on a covered steam bath out of direct contact with the steam, mixing well occasionally. Treat impregnated sawdust like the drug, cleaning the evaporating dish with 10 Cc of saturated solution of sodium bicarbonate.

In the case of powdered and solid extracts occasionally the ether extract will contain so much colored matter that it is difficult to observe the end-point under the conditions of the method as stated above. Under these circumstances it is necessary to dilute the solution of the alkaloidal residue in standard acid to such a degree that the color is diminished to a point where it will not interfere with observation of the end reaction. This dilution treatment necessitates the use of a larger amount of methyl red indicator and the running of a blank using the same water and same volume of water as used in the assay.

LABORATORIES OF
H. K. MULFORD CO.