by the heat. It is quite clear that the lime has formed insoluble compounds with both the gummy bodies and the albuminoids, the latter forming calcium albuminates, probably similar to the better known copper albuminates. But the lime does not combine with all of these bodies in the juice, or if it does the compounds are soluble. The lower percentage of ash in the coagulum would indicate that if such soluble but coagulable compounds were formed, they were of a different nature, containing less lime. The albuminoids and gummy bodies in cane juice may then be divided into three classes: (*a*) Those which form insoluble compounds with lime, (*b*) those which are precipitated by heat, and (*c*) those which are not rendered insoluble by both lime and heat, the gummy bodies of class *c* being precipitable by nitrate of mercury and the albuminoids by copper hydroxide. Class *c* constitutes nearly one-half of these bodies in the cane juice. It is the further work of the sugar chemist to discover some cheap non-poisonous agent which will remove this class of bodies from the cane juice.

A NEW FORM OF PYKNOMETER.[1]

By J. C. Boot.[2]

Received November 9, 1896.

OF all the different forms of pyknometers, Fig. 1 represents the one that is probably most generally used.

Working with this form in a room where the temperature is much higher than the normal, there is one difficulty to contend with, which is, that in the time it takes to dry and weigh the pyknometer the liquid is continuously running out of the capillary tube in the stopper.

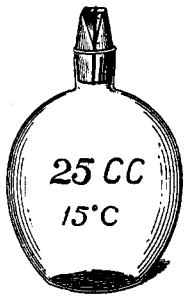

Fig. 1.

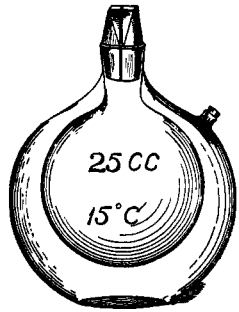

Fig. 2.

For the purpose of making accurate specific gravity determi-

[1] *Chem. Ztg.*, 1896, 20, 63.

[2] Read before the New York Section of the American Chemical Society, Nov. 6, 1896.

nations under such conditions, that is to say, when the temperature of the balance room is high, I have constructed a pyknometer with double walls, as shown in Fig. 2, in which the space between the two walls is carefully exhausted.

The apparatus is used in the following way:

Take for example a pyknometer of twenty-five cc. capacity and a normal temperature of 15° C.

Of the liquid, whose specific gravity is to be determined, we take about fifty cc. in a small flask and cool down to about 12° to 13° C.; a thermometer divided into $\frac{1}{10}$° or $\frac{1}{5}$° is used for noting the temperature.

After shaking the contents of the flask, wash out the pyknometer with about five cc. of the liquid, throw away these five cc. and then run in about twenty cc. The temperature of the liquid in the pyknometer will probably be about 14° C.

This twenty cc. are poured back into the flask and again about twenty cc. of the liquid are poured into the pyknometer. The temperature will now be about 14°.2 C.

This pouring of the twenty cc. into the pyknometer and then back into the flask is continued until the temperature of the liquid is 15° C.

We then fill up the pyknometer until it overflows and put in the stopper.

The apparatus can now be carefully dried and weighed without danger of any of the liquid running out of the capillary tube.

In this pyknometer, as shown in Fig. 1, it often happens that when drying, the simple pressure against the glass sides causes the liquid to run out through the capillary tube. Naturally this is impossible with my pyknometer.

The apparatus has proved very satisfactory. It weighs about thirty grams, and is made by Eimer & Amend, New York, and by Christ Kob & Co., Stützerbach, Germany.

THE CAFFEIN COMPOUND IN KOLA.[1]

By James W. T. Knox and Albert B. Prescott.

Received December 19, 1896.

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HISTORICAL.

Although the literature of kola extends back more than three hundred years, its chemical history is comparatively recent and may be briefly summarized as follows:

To Attfield[2] belongs the credit of proving that kola contains caffein; his work was done at the instance of Dr. Daniell,[3] who had made quite a study of the uses of kola among the natives of Western Africa, and who suspected from its physiological effects

[1] From the Proceedings of the American Pharmaceutical Association, 1896. Communicated by the authors.

[2] *Pharm. J. Trans.*, 1865, (2) 6, 457.

[3] *Pharm. J. Trans.*, 1865, (2) 6, 450.

that it contained a stimulating principle similar to that of coffee and tea. Their supply of material was limited and they seem to have carried their investigation into its chemistry no further.

In 1882 Heckel and Schlagdenhauffen[1] reported a more complete analysis of kola differing especially from that of the earlier workers in that it showed, in addition, the presence of theobromin and tannin. Heckel also noticed that a body was present which seemed to him to present considerable analogy to cinchona red, and he applied the name "kola red" (rouge de kola) to it. This, he afterwards found, gave a sublimate of caffein upon heating sufficiently, and he supposed the alkaloid to be retained mechanically, although he was unable to remove it by prolonged treatment with chloroform.

Knebel,[2] working in the Pharmaceutical Institute at Erlangen under the direction of Prof. Hilger (now at Munich), took up the investigation at this point in 1892, and isolated a tannin-like body to which he ascribed the formula $C_{14}H_{13}(OH)_5$ and which he named "kola red." This is not to be confounded with the kola red of Heckel, for Knebel considered Heckel's product an indefinite mixture, or rather an impure form of a glucoside which he (Knebel) believed to be present in kola, and which he believed to be composed of equal molecular proportions of caffein, glucose, and "kola red." This glucoside he called "kolanin," and, although he had only the dry seeds of kola to work upon, he inferred, from the proportions of caffein and glucose found therein, that in the fresh seeds no caffein was free. He inferred that the whole was combined in the glucoside, which under the influence of a diastatic ferment (which he isolated), with air and moisture, was partially decomposed, during the process of drying, into its component parts.

This hypothesis also seemed to explain why different workers, using different samples of the drug, had failed to get uniform results in the yield of caffein, for only the free alkaloids are removed by the immiscible solvents usually employed in assaying, combined alkaloids remaining behind. Different conditions in drying would give a variable extent of decomposition of this

[1] Heckel and Schlagdenhauffen, 1882 : *Repert. de Pharm.*, p. 163.

[2] E. Knebel, 1892 : *Apoth. Ztg.*, 7, 112.

(hypothetical) glucoside, and consequently afford discordant results in assaying.

Prof. Dr. Hilger[1] had noticed the apparent untrustworthiness of the usual assay methods in the examinations of cacao as well as of kola and was led by Knebel's results to investigate the chemistry of cacao, working at Erlangen. He succeeded in separating a glucoside from cacao analogous to the one Knebel supposed to be present in kola, and which yielded on hydrolysis with dilute mineral acids, theobromin, dextrose, and cacao red, $C_{17}H_{12}(OH)_{10}$, similar to kola red. The dextrose formed with phenylhydrazin a glucosazone of melting-point 204° C. Later,[2] at Munich, he applied the same and similar methods to kola, using the fresh seeds, and obtained a body, the "kolanin" of Knebel, yielding caffein, dextrose, and kola red on decomposition with dilute mineral acids, or diastatic ferments. He also describes in his paper his method for separating the ferment of kola, these details having been omitted from Knebel's original article.

Prof. Dr. Hilger has also kindly sent us the Inaugural-Dissertation[3] of Schweitzer for which we here make grateful acknowledgment. Schweitzer continued the work on kola and cacao, begun by Knebel and Hilger, verifying their results. His work on the (supposed) glucoside of kola indicates the combination of one molecule each of caffein and kola red, and three of dextrose, instead of one of each as claimed by Knebel. He proposes the formula $C_{40}H_{56}N_4O_{21}$ for "kolanin."

Another feature of his work on kola is, that while in the free alkaloids the proportion of caffein is to theobromin as 98.8 to 1.2, in the glucoside, he found it to be 80.3 of caffein to 19.7 of theobromin.

Of American workers, Schlotterbeck[4] and Topping[5] have done good service; Dohme and Engelhardt[6] have published reports of comparative assays of African and West Indian kolas. They found the African superior.

[1] A. Hilger, 1892: *Apoth. Ztg.*, 7, 469.

[2] A. Hilger, 1893: D'tsch. Vierteljahresschrift f. öffentl. Gesundheitspflege, 25, 559. Drittes heft.

[3] Carl Schweitzer, 1895: Inaugural-Dissertation zur Erlangung der Doktorwuerde, Munich.

[4] Kola: Monograph by J. O. Schlotterbeck, Detroit, 1894.

[5] C. O. Topping, 1894: *Proc. Am. Pharm. Ass.*, p. 178.

[6] Dohme and Engelhardt, 1896: *Am. Drugg.*, p. 12.

Other recent contributors are Kilmer,[1] whose paper is principally a compilation and Jean,[2] who examined a number of commercial preparations of kola, *i. e.*, its fluid extracts, tinctures, and wines.

A partial plant analysis of kola was made last year by one of us, with Prof. Schlotterbeck.[3]

THE SAMPLE.

Through the liberality of Messrs. Frederick Stearns & Co., Manufacturing Pharmacists of Detroit, Michigan, we were supplied with a large original package of kola nuts, just as imported by them from West Africa. The basket had not been opened, and the seeds were perfectly fresh, and were fine specimens. The best of these were selected, and as a means of preservation they were sealed up air-tight in clean and dry glass-stoppered jars. In about one-half the number of jars the air was displaced by dry carbon dioxide, and in a few others the red and white seeds were put up separately. That the carbon dioxide did not inhibit fungus growth, was shown by the appearance after five month's duration of a slight moldiness on some of the seeds put up in this atmosphere as well as on those preserved in air. This was not universally the case. At this time, after the expiration of nine months, we have several jars of the drug put up in exactly the same way, and at the same time in which the seeds are fresh and sound. Those preserved in carbon dioxide are somewhat paler, but internally are apparently unchanged, and a greater proportion of the seeds in this atmosphere, remained in prime condition, so that there would seem to be a slight advantage in its use. It is a rather remarkable fact that in those jars containing only red or only white seeds, there was no perceptible decomposition or fungus growth, and this regardless of the atmosphere used.

Action of Solvents.—When fresh kola seed is cut or bruised a chemical change immediately takes place, as shown by the rapid change of color of the cut surface from pink or cream-color to red-brown.

Desiring to avoid any decomposition of the drug in handling, a solvent was sought which would remove the active constituents

[1] F. B. Kilmer, 1896: *Am. J. Pharm.*, 68, 96.

[2] J. Jean, 1896: *Repert. de Pharm.*, (3), 7, 49.

[3] Knox and Schlotterbeck, 1895: Analysis of Kola, *Proc. Am. Pharm. Ass.*, p. 334.

without change. Water, absolute alcohol, ninety per cent. alcohol, fifty per cent. alcohol, ether, chloroform, benzene, acetone, ethyl acetate, dilute ammonia water, dilute potassium hydroxide solution, one per cent. hydrochloric acid, alcohol containing one per cent. of acetic acid, and glycerin, were the principal solvents tried. All were objectionable because the red coloration appeared almost immediately on contact of the cut surface of the drug with the liquid, coloring the solvent, if the coloring matter were soluble therein. Slicing the seeds under the surface of any of these liquids apparently neither retarded nor modified the formation of color.

Water was at first colored red, but gradually became turbid from precipitation of a portion of the matter at first dissolved; the alcohol of either strength gave a rich wine-red solution, which was permanent. The immiscible solvents removed only alkaloids and fat; both fixed and volatile alkalies developed an intense dark crimson color, changing to light scarlet on addition of excess of mineral acids, which change was accompanied by precipitation of a reddish flocculent substance; dilute acids heightened the color somewhat; glycerin seemed only to dehydrate the tissues without exerting solvent power.

Action of Heat.—This coloration has usually been ascribed to the activity of a diastatic ferment, and, therefore, recalling the action of heat on such bodies, it was decided to try the effect of sterilization on this drug. Accordingly a seed was sliced, the slices being received in boiling water. No coloration took place then or afterwards, a few seconds contact with the boiling water being sufficient to prevent its appearance. Further experimentation showed that up to 60° C. the heat augmented the production of color; from 60° to 65° it was somewhat retarded, and above 65° the slices retained their natural color.

On account of the starch present in kola, and for other reasons, the use of water was objectionable, so boiling alcohol was substituted successfully, and has been used exclusively by us for this purpose ever since. A temperature of 45° C. is sufficient when alcohol is employed to "coagulate the ferment," at least to prevent the coloration afterwards. Boiling chloroform and boiling acetone were each tried, but without successful results. Without

further discussion of the action of solvents, it may be remarked that, for pharmaceutical purposes and any others, when it is desirable to extract all the active constituents from kola with a single menstruum, our experience has shown that alcohol of not less strength than U. S. P. dilute alcohol is the most satisfactory agent.

Action of Cold.—It has been stated[1] that extreme cold seems to destroy the power of this ferment. An experiment, next described, seems to show that cold does not impair the potency of the ferment, but rather seems to preserve it. Two white kola seeds were sealed up in a dry bottle and immersed in a freezing mixture for three hours. On thawing, the production of the red-brown color was very rapid both upon cut and whole surfaces.

ASSAY OF KOLA.

Common Causes of Error.—Of the numerous methods proposed at various times by different workers for the assay of this drug, none has proven satisfactory and reliable. Many of these do not account completely for the free alkaloids of kola, and not at all for the combined alkaloids; others perhaps indicate pretty well the proportions of free alkaloids. Still, concordant results are not obtained, and the published assays of kola have a wide range of variation. Two reasons for these discrepancies have suggested themselves to us. First, insufficient knowledge of the properties of the combination bearing caffein has prevented the use of proper methods to secure complete liberation of the alkaloids from it; second, the caffein obtained is *weighed*, in a state of greater or less purity, varying with the method used and the care and judgment of the worker. It is not difficult to perceive how errors might thus be introduced.

We have worked out a plan of assay for kola especially intended to avoid these and some other common sources of error; it has been used by us in all the assays reported herein and in many others, and has given uniform and concordant results, higher than any we have yet seen. We offer it with a confidence based on experience.

Volumetric Method for Caffein Determination.—Before giving the details of manipulation for assay, it may be well to describe

[1] Kilmer, 1896: *Am. J. Pharm.*, 68, 104.

the volumetric method for the estimation of caffein which is substituted for gravimetric methods.

This new process, given by Gomberg[1] is based upon the very complete precipitation of caffein by Wagner's reagent from aqueous solutions when these are acidulated with mineral acids —a fact which seems to have been pretty generally overlooked by the text-books bearing upon the subject. This oversight is, no doubt, traceable to the fact that caffein, being soluble in water, with neutral reaction, and not forming salts permanent in water, is taken as free alkaloid for all tests in the wet way. Other alkaloids, for the most part, are taken in their salts, for reactions in solutions, their salts having the neutral reaction. Therefore the Wagner's reagent, like other reagents, was generally applied to the caffein solution without the presence of any acid, and under such conditions, it is true, as stated in the text-books, that "caffein is not precipitated by Wagner's reagent."

Gomberg recently investigated the subject at considerable length. Without entering into discussion of other features of his work, it may be stated briefly that he found that an aqueous solution of caffein, even so dilute as 1 to 8000, is precipitated by Wagner's reagent in the presence of mineral acid, and that the precipitate formed is constant in composition ($C_8H_{10}N_4O_2HI.I_4$) in varying conditions of formation.[2] Having established these facts, practical application of them was made for the volumetric estimation of caffein. A short outline of the principal features of this method follows.

A definite volume of Wagner's reagent of known strength is added in excess to the measured caffein solution slightly acidulated with sulphuric or hydrochloric acid, and the clear liquid is decanted off after the precipitate has settled or is filtered through asbestos after five minutes' standing. The excess of iodine is titrated with decinormal sodium thiosulphate solution, using an aliquot portion of the filtrate. From this the number of cubic centimeters of decinormal Wagner's reagent can be calculated, which number multiplied by 0.00485 equals the weight of the anhydrous caffein.

We have had considerable experience with this process for

[1] This Journal, 18, 331.
[2] This Journal, 18, 350.

estimating caffein, as one of us (K.) furnished the original analytical data for Dr. Gomberg's paper, and we have employed it almost exclusively in the work upon the caffein compound since that time.

The table of results (p. 71) is reproduced from Gomberg's article. By way of explanation it may be said that solutions of caffein of respectively 0.25, 0.50, 0.75, and 1.00 per cent. strength were used, the acidulation was with sulphuric acid, and Wagner's reagent was employed in varying proportions, using a very slight excess over the theoretical amount, one and one-third times the theoretical amount, twice the theoretical amount, and one-half the theoretical amount. The results show that the method accounts very accurately for the caffein, especially when the iodine solution is in considerable excess.

In Table No. 2 are given the results obtained where a considerable excess, eight to ten per cent., of sulphuric acid is used. The results are not very uniform and show that slight acidulation gives greater accuracy in the estimation.

The manipulations used by us are as follows: The caffein to be estimated is dissolved in water, acidulated with sulphuric or hydrochloric acid (preferably the latter[1]), avoiding an excess, and made up to a definite volume. The acid strength of this volume should be about one per cent. If the quantity of solution is not large, *i. e.*, not more than forty or fifty cc. the whole is taken; if large an aliquot portion is measured from a burette. Standard Wagner's reagent is run in from a burette, ten cc. at a time, shaking the solution after each addition and allowing it to stand a few moments so that the color of the supernatant liquid may be observed. When this liquid becomes wine-red the iodine solution is in sufficient excess, and the volume of Wagner's reagent added is read, at the same time noting the total volume of the mixed solutions. The caffein periodide is filtered out, after five minutes standing, with a dry asbestos filter, the filtrate collected in a clean dry vessel and transferred to the same clean and dry burette from which the caffein solution first, and later the iodine solution, were measured. (If calibrated burettes are used it is, of course, not necessary to employ the same one each time.)

[1] Caffein solution, 1 to 10000, acidulated by hydrochloric acid, is precipitated by Wagner's reagent, while 1 to 8000 is the limit if sulphuric acid be used.

TABLE NO. I.

Wagner's reagent employed.	I. Solution containing 0.25 per cent. of caffein.			II. Solution containing 0.50 per cent. of caffein.			III. Solution containing 0.75 per cent. of caffein.			IV. Solution containing 1.00 per cent. of caffein.			V. Solution containing 0.50 per cent. of caffein.		
	Taken.	Found.	Per cent. recovered.	Taken.	Found.	Per cent. recovered.	Taken.	Found.	Per cent. recovered.	Taken.	Found.	Per cent. recovered.	Taken.	Found.	Per cent. recovered.
Theoretical quantity plus 2 cc. ..	0.0600	0.0591 0.0589	98.33	0.1200	0.1175 0.1175	97.82	0.1500	0.1481 0.1471	98.40	0.1200	0.1182 0.1179	98.38	0.1200	0.1154 0.1165	96.78
$1\frac{1}{3}$ times the theoretical quantity	0.0750	0.0749 0.0749	99.88	0.1200	0.1191 0.1189	99 17	0.1500	0.1489 0.1485	99.13	0.1200	0.1191 0.1187	99.12	0.1200	0.1196 0.1197	99.75
Twice the theoretical quantity.....	0.0500	0.0502 0.0506	100.80	0.0800	0.0805 0.0789	99.63	0.1200	0.1184	98.67	0.1100	0.1091 0.1091	99.18	0.0800	0.0802 0.0791	99.63
One-half of the theoretical quantity.....	0.0750	0.0363 0.0363	48.40	0.1600	0.0794 0.0794	49.62	0.2250	0.1107	49.20	0.2000	0.1067 0.1067	53.35	0.1600	0.0791 0.0791	49.44

Columns I, II, III, and IV show the results obtained by decanting the clear liquid after the solutions had stood for one hour. In column V the results are those obtained by filtering the solution for titration through asbestos after five minutes standing.

TABLE NO. 2.

Wagner's reagent employed.	Caffein solution 0.25 per cent.			Caffein solution 0.50 per cent.		
	Taken.	Found.	Per cent. recovered.	Taken.	Found.	Per cent. recovered.
Theoretical quantity + 2 cc.....	0.0500	0.0487 0.0492	97.90	0.0500	0.0468 0.0479	94.70
One and one-third times the theoretical quantity	0.0200	0.0204 0.0198	100.50	0.0675	0.0642 0.0639	94.89
Theoretical quantity × 2.....	0.0250	0.0251 0.0245	99.20	0.0500	0.0480 0.0490	97.00
One-half of the theoretical quantity	0.0250	0.0119 0.0128	49.40	0.0600	0.0275 0.0281	46.33

From this an aliquot portion is measured into a porcelain dish and exactly neutralized with tenth-normal sodium thiosulphate. Each cc. of Wagner's reagent consumed = 0.00485 of caffein. The following example will illustrate the method of calculation:

"Unknown" caffein solution 30 cc.+ 30 cc. Wagner's reagent = 60 cc.

30 cc. filtrate = 6.2 sodium thiosulphate solution.

Whole solution (60 cc.) containing 30 cc. Wagner's reagent= 12.4 sodium thiosulphate solution.

30 cc. Wagner's reagent—12.4 cc. thiosulphate = 17.6 consumed by caffein.

17.6 cc. Wagner's reagent × 0.0485 = 0.08536 caffein.

The asbestos filters described are conveniently prepared by placing a small perforated platinum disk in a medium sized carbon tube; on this a layer of glass wool one-fourth of an inch in depth is placed. By aid of a good filter pump, finely divided asbestos pulp, previously acid-washed and suspended in water, is deposited in thin layers by pouring on in small portions and applying suction. This is continued until a layer of asbestos three-fourths to an inch in height is obtained. By keeping the asbestos mixture well stirred during the first part of the operation and allowing it to settle during the latter, the coarser particles will be deposited in the lower layers and the finer ones on the top. The filter is now washed, successively, with hot water, alcohol, and ether, and after drying twenty minutes at 80–100° C., is ready for use.

In the event that it is desired to decant the liquid for titration, instead of filtering, it is advisable to perform the precipitation in

a tall slender cylinder or test-tube on foot. After the precipitate has settled compactly, the end of a burette is immersed in the liquid to the desired depth, and the liquid drawn upward by means of suction produced by a rubber bulb fitted to the top of the burette.

The precaution is always taken, of course, to have the caffein free from any other substance that will affect the iodine solution, and to perform the precipitation in cold solutions.

By the exercise of ordinary care and judgment this method for the estimation of caffein will be found extremely accurate and satisfactory, while with a small amount of practice it admits of very rapid work. Having used it in more than one hundred estimations recently we feel qualified to recommend it.

MANIPULATIONS IN THE ASSAY OF KOLA.

Preparation of Sample.—Slice a sufficient quantity of the fresh seeds in thin papery slices, allowing them to fall in a beaker of boiling alcohol.[1] An ordinary potato slicer is a very convenient and effective instrument for this purpose. Remove the slices after a few moments' boiling, and allow them to dry spontaneously on clean glass plates. Distil the alcoholic solution, under reduced pressure,[2] to a syrupy consistence, and pour it on the sliced drug now being dried, rinsing the flask with successive small portions of alcohol. When dry remove the drug to a mortar, wash the glass plates with a few cc. of hot alcohol, and pour this on the drug which is to be finely powdered and preserved in dry, glass-stoppered jars. The powder prepared in this way corresponds closely to the original color of the seeds, being but slightly paler in each instance. There can hardly be any doubt that it represents correctly the fresh seeds, with subtraction of the water, and perhaps of a portion of the trifling amount of volatile oil they contain.

For free Alkaloids.—Weigh accurately five grams of the sample, transfer to a Soxhlet's extraction tube, and treat for six

[1] The boiling alcohol kills the ferment and also dries the drug quickly. While it will appear later that this ferment, contrary to the general opinion, has but little if any influence on the production of free caffein from the caffein compound, the use of alcohol has nevertheless been retained as an efficient drying agent, and one that preserves the drug apparently without allowing any chemical change whatever to take place.

[2] The object being to avoid the decomposition of the caffein-bearing combination by the hot water remaining after the alcohol is evaporated.

hours, or until exhaustion is complete, with chloroform. Evaporate the chloroform, and to the residue add thirty cc. of hot, one per cent. hydrochloric acid, and filter to remove fat, rinsing out the flask with several small portions of hot water, passing these through the filter, and washing the filter three or four times with hot water. The united filtrate and washings now amount to about seventy to seventy-five cc. Concentrate in a porcelain capsule on a water-bath to about ten or fifteen cc., transfer to a graduated cylinder, which has been carefully compared with the burette to be used for the Wagner's reagent, and rinse the capsule with three or four successive portions of hot water, making up the volume in the cylinder to thirty cc. after cooling. Now run in from the burette thirty cc. standard Wagner's reagent, and agitate well. Filter through asbestos after five minutes, and pour the filtrate into the same burette, previously washed and dried. About fifty-five cc. will be recovered. Run out an aliquot portion of the liquid (say thirty cc.), and neutralize the excess of iodine with decinormal sodium thiosulphate solution, then from the result calculate the number of cc. of iodine solution consumed. Multiply this number by 0.00485 to obtain the weight of the anhydrous caffein. With another aliquot portion of the filtrate make a duplicate titration.

It will be observed that the alkaloids are estimated only as caffein. Theobromin also is precipitated by Wagner's reagent but its proportion, 1.48 to 100.00 of total alkaloids, is so small that the error introduced by the difference in the factors of the alkaloids is not appreciable.

The Combined Alkaloids.—After the exhaustion with chloroform add alcohol of ninety per cent. to the drug, which is still contained in the extraction tube, and continue the treatment until exhaustion is complete, as shown by the absence of color in the portion of menstruum last siphoned over in the apparatus. Two or three hours are usually sufficient. This alcoholic solution may be treated in either of the following ways:

(*a*) Evaporate the solution to dryness in a tared porcelain capsule, and weigh. Take a small portion (0.200 or 0.300 gram), and determine the amount of nitrogen by combustion. This nitrogen is entirely alkaloidal, as proteid substances are not

extracted from the drug by the strong alcohol, and the total nitrogen can be calculated into caffein. From the amount of caffein found by combustion of the aliquot portion, calculate the total amount present in the whole extract.

(*b*) To the hot alcoholic solution add an excess of freshly precipitated lead hydroxide (litharge or lead carbonate will not answer), and digest on a water-bath for a few minutes, until the supernatant liquid is colorless. Then transfer to a porcelain capsule, rinse the flask with hot alcohol, mix with clean white sand, evaporate the mixture to dryness, and place the whole in the extraction tube. Treat with chloroform three or four hours, and determine the caffein volumetrically as previously directed.

We present herewith a table showing the results we have obtained by this plan of assay :

The sample.	Moisture, per cent.	Duplicates.	Fresh kola.			Dried kola.		
			Free alkaloids. Per cent.	Combined alkaloids. Per cent.	Total.	Free alkaloids. Per cent.	Combined alkaloids. Per cent.	Total.
No. 1. Dried kola. (Mixed.)	6.16	I.				1.859	1.783	3.642
		II.				1.828	1.836	3.664
		Av'ge.				1.843	1.809	3.652
						Calculated for "dry."		
No. 2. Fresh kola, red and white seeds.	53.9	I.	0.512	0.927	1.439	1.111	2.011	3.121
		II.	0.556	0.841	1.397	1.206	1.834	3.040
		Av'ge.	0.534	0.884	1.418	1.158	1.922	3.080
No. 3. Fresh kola, red and white seeds, very moldy.	53.9	I.	0.590	0.815	1.405	1.280	1.770	3.050
		II.	0.548	0.893	1.438	1.190	1.938	3.128
		Av'ge.	0.569	0.854	1.423	1.235	1.854	3.089
No. 4. Fresh kola, white seeds.	51.2	I.	0.595	1.029	1.624	1.220	2.110	3.330
		II.	0.562	1.006	1.568	1.153	2.060	3.213
		Av'ge.	0.578	1.018	1.596	1.186	2.085	3.271
No. 5. Fresh kola, red seeds.	57.3	I.	0.503	0.687	1.190	1.180	1.610	2.790
		II.	0.452	0.700	1.152	1.060	1.640	2.700
		Av'ge.	0.478	0.693	1.171	1.120	1.625	2.745

In explanation of the foregoing results, it should be stated that sample No. 1 is not of the same lot of drugs as the other four, but is one that was analyzed in this laboratory last year, and is also African kola obtained then from the house of Frederick Stearns & Co.

In order to facilitate comparison of the fresh and dry drug, the percentages of alkaloids obtained from the fresh have been also calculated for the corresponding amount of dry kola.

These results are much higher than those usually seen, the main difference being that our method accounts for both free and combined alkaloids. It will be seen that about one-half of the total alkaloids of dry kola exist in combination, and in the fresh seeds more than sixty per cent. of the alkaloids are "combined." It should also be noted that so far as the yield of alkaloids is concerned, the moldy kola does not differ from that in perfect preservation. Furthermore there is a difference between the red and the white seeds, as shown by the percentage of alkaloids and moisture.

Method of Dohme and Engelhardt.—Dohme and Engelhardt[1] have examined the method employed by Schlotterbeck and Knox[2] and have proposed another one in its stead which has in their hands, given higher results. Their process requires to boil the dried and powdered drug in thirty per cent. alcohol for two hours, filter, evaporate the filtrate to dryness with sand and magnesia and exhaust this residue with chloroform. The residue left after evaporation of the chloroform is dried at 100° C., and weighed as caffein. Using this method they have obtained 2.10 per cent. of caffein from African kola.

We have given their method a careful trial with the gravimetric results stated next below, the sample used being No. 1 of the foregoing table :

I. 2.04 per cent.
II. 1.93 " "

Sources of Error.—That the caffein thus obtained, although apparently pure, is not so in reality, is shown by the fact that when titrated with Wagner's reagent the per cent. is much lower.

I. 1.76 per cent.
II. 1.68 " "

There are still other objections to the method. The treatment with thirty per cent. alcohol does not remove all the free alkaloids, for the drug under assay after having been boiled with the alcohol and washed, as directed, was dried and treated with

[1] *Am. Drugg.*, 1896, p. 12.
[2] *Proc. Am. Pharm. Ass.*, 1895, p. 334.

chloroform in an extraction apparatus. The residue left after evaporation of the chloroform gave positive test for caffein. Nor does the diluted alcohol remove all the combined alkaloids, for after the treatment with chloroform just described, strong alcohol was added to the drug in the extraction tube, and the extraction continued. The wine-red solution obtained was treated with an excess of lead hydroxide, filtered, and the clear colorless solution evaporated to dryness. This residue also contained caffein.

It appears that the combined alkaloids removed by the thirty per cent. alcohol in Dohme's process and treated with magnesia and sand are not completely liberated by this treatment, and consequently are not removed by the chloroform. For after this magnesia-sand residue had been exhausted by chloroform as directed, strong alcohol was used as a menstruum, and it removed another portion of combined caffein, identified by the lead hydroxide treatment above mentioned. In our hands, with careful manipulation, the greatest amount of actual caffein obtained by this method is a little less than one-half of the total amount present, as shown by assay after the process described in this article.

Thanks are due to Mr. Robert J. Nisbet, Ph.C., for valuable assistance rendered in the assays and in the analyses by combustion.

GLUCOSIDE OF KOLA.

In the literature of kola is found frequent mention of a glucoside yielding caffein, glucose, and "kola red" on decomposition.[1] It has been stated that this glucoside is an extremely unstable body; that heat, moisture, dilute acids,[2] the ferment[3] of kola, or diastase is sufficient to re-solve it, partly or wholly, into its component parts.

It has even been suggested[4] that no alkaloids exist free in the fresh seeds, but they are wholly combined in this glucoside.

Methods for Separation.—The process used by Hilger,[5] and later by Schweitzer[6] for the isolation of this so-called glucoside, "kolanin," is as follows:

[1] E. Knebel, 1892: *Apoth. Ztg.*, 7, 112.
[2] A. Hilger, 1893: Dtsch. Vierteljahres. für Offent. Geshtspfl., 25, 559.
[3] C. Schweitzer, 1895: Inaugural-Dissertation, Munich.
[4] Knebel: *Loc. cit.*
[5] *Loc. cit.*
[6] See Schweitzer, 1895: Inaugural-Dissertation, Munich.

The drug is exhausted with alcohol, the extract evaporated to dryness, and the residue washed with water; then the insoluble portion is dissolved in weak alkali solution. To this, dilute mineral acid is added in slight excess, when the "kolanin" is precipitated. It is collected by filtration, washed with water, and dried. It is a red brown amorphous powder, containing 0.9 per cent. of ash. We have prepared a number of specimens from both the fresh and dried drug, by this process which we shall call Method No. 1. For purposes of comparison we have also employed other processes. In one of these, Method II,[1] the portion of alcoholic extract insoluble in water is dissolved in strong alcohol, and this solution then precipitated by the addition of about three volumes of ether. The precipitate is rapidly filtered at the pump, washed with ether, and dried in a vacuum desiccator over sulphuric acid. The product is a light impalpable powder, cream to light red in color, and is ash-free after three precipitations.

By another method (III) the residue left after washing the alcoholic extract with water was dried in a vacuum desiccator, powdered, and treated with chloroform to remove fat and whatever free alkaloids the water washing had left behind. Again dried and powdered, it furnished a product appearing much like that of Method I, but somewhat lighter colored.

We present herewith (p. 79) a table of results obtained by combustions of samples prepared from both the fresh and the dry seeds by the methods just described.

We do not at present enter upon interpretation of the above results for carbon, hydrogen, and nitrogen,[2] but leave their study until further work on this body, as now planned, shall have been finished. It seems to be largely a question of structure, and this must be settled before it can be positively stated whether the caffein compound in kola is a glucosidal body, or only a glucoside-bearing body. And, of these, the one may not differ from the other except in the order of its stages of decomposition. There is much evidence tending to show that this so-called glu-

[1] In Methods II and III, all distillations are carried on under reduced pressure.

[2] Following are the figures, respectively, of Schweitzer and of Hilger, for the cacao glucoside, the former by calculation from the yield of alkaloids and glucose, the latter by analysis (*Deutsch. Vierteljahr. Oeffentl. Gesundh.*, 1893, **25**. 559.)

	Calculated.		Found.											
			Method I.				Method II.				Method III.			
			From dry seeds.		From fresh seeds.		From dry seeds.		From fresh seeds.		From dry seeds.		From fresh seeds.	
Duplicates.	By Knebel:[1] $C_{14}H_{13}(OH)_5 + C_6H_{12}O_6 + C_8H_{10}O_2N_4$.	By Schweitzer:[2] $C_{40}H_{56}N_4O_{21}$.	I.	II.	I.	II.	I.	II.	I.	II.	I.	II.	I.	II.
C	52.50	51.71	62.11	61.91	61.82	61.70	61.39	61.71	62.16	61.96	62.06	62.22	61.43	61.26
H	6.25	6.03	7.06	7.12	6.98	7.16	6.62	6.79	7.05	7.16	6.80	6.96	6.87	6.98
N	8.75	6.03	8.04	7.74	6.04	5.97	5.89	5.67	6.23	5.95	6.92	6.74	6.13	5.94
O	32.50	36.23	22.79	23.23	25.16	25.17	26.10	25.83	24.56	24.93	24.22	24.08	25.57	25.82
Caffein by calculation from the N	30.31	20.88	27.85	26.81	20.92	20.68	20.40	19.64	21.58	20.61	23.97	23.34	21.23	20.57

[1] Assumed from the proportions of free caffein and glucose found in dry kola.

[2] Assumed from the respective yields of alkaloids and glucose by hydrolysis of the glucoside.

	Cacao glucoside.	
	Calc. by Schweitzer: $C_{60}H_{86}O_{15}N_4$.	Found by Hilger: $C_{68}H_{98}O_{36}N_4$.
C	65.34	52.85
H	7.80	6.22
N	5.08	3.63
O	21.78	37.30

coside is in reality a mixture of tannates of caffein and theobromin, and that the glucose obtained on hydrolysis is split off from the tannin. These evidences are given a little later on in this paper.

After combustion, the next step was to ascertain, if possible, whether the nitrogen was wholly that of the alkaloids or not.

ACTION OF DILUTE ACIDS.

It was attempted to recover the caffein, quantitatively, and for this purpose hydrolysis with dilute mineral acids was first resorted to.

Following carefully the methods of Schweitzer, *viz.*, to boil the "glucoside" for from four to six hours with twenty times its weight of five per cent. sulphuric acid, filter, neutralize the filtrate with barium carbonate, filter again, and estimate the caffein and glucose in the clear filtrate made up to a definite volume, we are unable to obtain results concordant with his. The method was also varied to admit of the removal of the dissolved tannin which remains in the clear filtrate after neutralization with barium carbonate, by precipitating it as lead tannate, filtering, removing excess of lead salt with hydrogen sulphide and boiling off the latter.

The strength of the acid used was varied from two to twenty per cent. and the time of boiling from two to ten hours. But in no case was the amount of caffein recovered equal to that indicated by the nitrogen percentage. In the samples used, the calculated proportion of caffein was, in round numbers, from 21.00 per cent. to 24.00 per cent. Yet 15.00 per cent. was the greatest

amount recovered, and the other amounts varied from that down to 4.76 per cent., the lowest.[1]

Hydrochloric acid diluted to the same strength was next tried with somewhat better but very unsatisfactory results.

It was now in order to institute a control analysis to determine whether or not all the caffein could be recovered after such treatment as has been described. To this end an experiment was made, as follows:

Duplicate samples of 0.300 gram pure caffein, and 0.500 gram pure kola tannin were boiled together with thirty cc. five per cent. sulphuric acid, after which the caffein was estimated in the way above described.

I. Taken 0.300. Recovered, 0.2635. Loss, 12.17 per cent.
II. " 0.300. " 0.2541. " 15.30 "

This loss suggested the possibility that some of the caffein might have combined with the tannin. Accordingly the reddish residue filtered out after boiling with acid was dried and exhausted with chloroform to remove all traces of free caffein. Then boiling alcohol was used as a menstruum, and gave a wine-red solution which was treated with lead hydroxide. The clear filtrate on evaporation gave positive evidence of caffein, both by appearance of the crystals and by chemical tests, showing that caffein tannate is actually *formed* during this process of treatment. Now if this is the case, it is hardly to be expected that the so-called "kolanin," a body very similar in properties to caffein tannate, would be quantitatively *decomposed* under exactly the same conditions. Indeed the experiments already described have shown that it is not. Nor is the statement[2] borne out that twenty per cent. sulphuric acid decomposes this natural combination of caffein completely, as the experiment described next below demonstrates.

[1] In this case the extremely low result is to be attributed in part to decomposition of the caffein in the course of the analysis. The tannin dissolved was removed in the way recommended by the United States Department of Agriculture, Division of Chemistry, Bulletin 46, p. 72, *i. e.*, by precipitation with lead acetate, and removal of excess of lead from the filtered liquid by the addition of sodium carbonate. The low result suggested the possibility of the decomposition of caffein by the solution of alkali carbonate, it being well known that alkali hydroxide solutions effect a decomposition into caffeidin, etc., on heating. So an experiment with a known quantity of pure caffein was tried: 0.110 gram caffein was boiled with ten per cent. sodium carbonate solution for four hours; and the caffein estimated volumetrically, 0.0593 gram being recovered, a loss of 46.1 per cent.

[2] Kilmer, 1896: *Am. J. Pharm.*, 96.

1.250 grams of the so-called glucoside were boiled vigorously with forty cc. of twenty per cent. sulphuric acid, and then filtered. The insoluble residue was examined in the way described in the preceding experiment, and "kolanin," apparently unchanged, was found, the evidence being quite positive.

Furthermore, it is not improbable that the hydrolysis of this substance is attended with incomplete recovery of the alkaloid liberated, for the four filtrations necessary, *viz.*, for removal successively of the insoluble red residue, barium sulphate, lead tannate, and lead sulphide are very likely to be accompanied by a loss of caffein from its adhesion to the moist, bulky precipitates.

Hydrolysis with dilute acids having been shown to be unsuited for the purpose of recovering completely the alkaloids from their natural combination, other and entirely different means were resorted to.

ACTION OF LEAD HYDROXIDE.

Recalling the fact that this so-called glucoside bears a close resemblance in properties to alkaloidal tannates, and in view of the action of lead hydroxide on this class of bodies, it was decided to try its effect upon "kolanin." Qualitative experiments to this end proving successful, it was next in order to ascertain whether or not the liberation of caffein was quantitative, and a full recovery possible. For this purpose 0.500 gram of the so-called "kolanin," whose average nitrogen content indicated 0.1052 gram caffein, was dissolved in twenty-five cc. hot ninety per cent. alcohol. To this, freshly precipitated lead hydroxide previously triturated with hot alcohol to a smooth cream was added, until, after a few moments' standing to allow subsidence of the precipitate, the liquid was clear and colorless. The mixture was then evaporated to dryness on a water bath, clean dry sand added to give the requisite volume, the whole then transferred to a Soxhlet's tube, and the beaker carefully rinsed with chloroform, the rinsings being added to the contents of the tube. Chloroform was then added, and extraction continued for two hours. The chloroform solution was then evaporated, and the caffein estimated volumetrically, 0.1040 gram being recovered.

This treatment does not decompose caffein, for 0.200 gram pure caffein dissolved in alcohol with 0.500 gram pure kola-

tannin and treated as above described yielded 0.1991 gram on volumetric estimation.

This simple and rapid process for liberating the caffein from the caffein compound affords a means for a very accurate determination of the combined alkaloids of kola, of which fact use has been made in the method of assay proposed and used by us, as previously described.

This reaction of kolanin with lead hydroxide indicates a tannate-like character for the body. There is reason to think that the glucose obtained by decomposing this so-called glucoside with mineral acids exists primarily in combination with the tannin-like body, for after chloroform had removed all the caffein from the mixture of alkaloids, lead salt, lead hydroxide and sand, described above in the experiments with "kolanin," treatment with water removed nothing further. The liberation of glucose therefore is not necessarily simultaneous with that of caffein, nor in consequence of it. This was further shown by decomposing the lead salt formed by the red coloring matter, through treatment with hydrogen sulphide, and thereby recovering the colored body previously combined with the caffein. This body so obtained, gives all tannin reactions toward iron salts, alkaloids, gelatin, etc., and has a pronounced astringent taste. On treating it with dilute mineral acid, in the manner directed by text-books,[1] very positive evidence of glucose was given, not only by its behavior with Fehling's solution, but with phenylhydrazin as well, of which mention is made later. The foregoing facts would seem to indicate that the so-called glucoside is a combination of caffein (and theobromin) with a glucoside tannin.

ARTIFICIAL KOLA-TANNATE OF CAFFEIN. METHOD OF PREPARATION.

By way of further investigation into this question we undertook to prepare artificially from kola-tannin and pure caffein a similar product which we proposed to compare with the natural compound. This was successfully accomplished as follows: An aqueous infusion of kola is poured into a ten per cent. solution of caffein acidulated with hydrochloric acid. The presence of

[1] Prescott: Organic Analysis, 467.

acid is necessary to obtain an aqueous caffein solution of sufficient concentration, and especially to avoid the re-solution of the tannate of caffein which takes place in the neutral solutions in the presence of an excess of either tannin or caffein. The precipitate, abundantly formed, is rapidly filtered at the pump, washed with cold water, and well drained. It is then dissolved in alcohol, and filtered to remove insoluble extraneous matter carried down in precipitation. The alcohol is then distilled off under reduced pressure until the solution has reached a syrupy consistence, and the evaporation continued to dryness over sulphuric acid in a vacuum desiccator.

PROPERTIES OF KOLANIN AND CAFFEIN KOLA-TANNATE COMPARED.

The product obtained is identical in appearance and sensible properties with the so-called kolanin. Both are insoluble in water, ether, chloroform, and cold dilute mineral acids; freely soluble in alcohol with dark wine color, from which solution they are re-precipitated by two or three volumes of ether; soluble in dilute acetic acid, more easily on warming; sparingly soluble in warm acetone; soluble in warm neutral caffein solution, and in warm kola-tannin solution; quite soluble in dilute alkali (both fixed and volatile) solution[1] with production of a very dark color, and at once reprecipitated therefrom by dilute mineral acids in slight excess, also by acetic acid, though the precipitate redissolves in an excess of the acetic acid on warming. They are decomposed in alcoholic solution by lead acetate and lead hydroxide. In all the ways tried both deport themselves alike and in a manner not inconsistent with the chemical behavior of an alkaloidal tannate.

Pure kola-tannin was also used for the preparation of the caffein salt, and yields a product identical in appearance and properties with that prepared from the impure kola-tannin of aqueous infusion of kola. But as the compound of the pure tannin does not apparently differ from the precipitate of caffein with kola infusion, the latter, less difficult of preparation, has been em-

[1] Considerable caffein may be removed from this alkaline solution by shaking out with chloroform. It is possible that the tannate is decomposed in part or wholly by the alkali and reformed upon addition of acid. The process is wasteful, so it is likely that the re-formation, if it occurs in this way, is complete.

ployed in the preparation of the several samples of caffein kola-tannate used for determining elementary composition, namely:

I. Prepared as described above. Reddish-brown powder, with astringent and bitterish taste.

II. In same way as Number I, the final product being dissolved in dilute alkali solution, and reprecipitated by dilute hydrochloric acid, filtered, washed, and dried. Reddish-brown powder, with astringent and bitterish taste.

III. In the same way as Number I, except that the final product was dissolved in alcohol and precipitated by ether, filtered, washed with ether, and dried. Lighter in color, but similar in taste to Number I.

IV. The clear filtrate after the precipitation of Number I gave, on twenty-four hours' standing, another copious precipitation of caffein kola-tannate, which was recovered in the usual way. Appearance and taste like that of Number I.

The following table shows the results obtained by duplicate combustion of each of these samples for carbon, hydrogen, and nitrogen:

CAFFEIN KOLA-TANNATE—ARTIFICIAL.

	Found.							
Method.	I.		II.		III.		IV.	
Duplicates.	1.	2.	1.	2.	1.	2.	1.	2.
C	59.27	59.41	60.11	59.89	59.64	59.35	59.94	60.22
H	6.21	6.02	6.18	6.07	5.96	6.10	6.08	6.24
N	6.20	5.96	5.30	5.54	5.61	5.45	5.15	5.27
O	28.32	28.61	28.41	28.50	28.79	29.10	28.83	28.27
Caffein calculated from the N	21.47	20.64	18.35	19.19	19.43	18.87	17.83	18.25

It will be seen by comparing these figures with those given for the natural form of combined caffein extracted from kola by physical solvents in our method, that the composition of the one does not differ very widely from that of the other, and that this artificial product has a fairly uniform and constant composition. It is, of course, a well-understood fact that the composition of alkaloidal tannates is by no means strictly constant, but that it varies according to temperature and concentration of solutions

used, and the mass of each used with respect to that of the other. The well-known variation in the composition of the same kind of tannin, obtained under different conditions, is also to be taken into account. The natural form of combined caffein, called kolanin, yields on an average about twenty-two per cent. of caffein, while that obtained artificially gives a slightly lower amount, about nineteen per cent. being the average. The difference may, perhaps, be due to the different conditions of formation, and not to any difference in the character of the bodies themselves. And, as previously stated, so far as reactions and physical properties are concerned, leaving a slight difference in elementary composition out of consideration, we have not thus far found any radical difference between the natural and the artificial products.

ACTION OF FERMENTS.

In order to ascertain whether or not diastase would liberate caffein from the so-called glucoside, the following test was made: 0.500 gram of the natural caffein compound of kola, and 0.0500 gram of pure diastase,[1] known to be 1 : 100, in twenty-five cc. distilled water, were kept at a temperature of 50°–53° for twenty-four hours, and for thirty-six hours at the ordinary temperature; the caffein liberated was then estimated. A control test was made at the same time, using the same amount of the caffein-bearing body, and the same volume of water, but no diastase. This was kept under exactly the same conditions of temperature and time as the first, and the caffein then estimated.

Sample with diastase yielded 0.0689 caffein, equal to 56.50 per cent. of the whole amount present.

Sample without diastase yielded 0.0706 caffein, corresponding to 58.83 per cent. of the total amount present.

This experiment was repeated on the artificial caffein kola-tannate with similar results.

These results indicate that the liberation of caffein is not due to the diastase, but to the water and heat used for its exhibition.

It was next in order to learn, if possible, the influence of the ferment of kola on the liberation of caffein from its combination

[1] Prepared by Mr. D. L. Davoll, Jr., Instructor in Organic Chemistry in the School of Pharmacy of this University, to whom our thanks are due.

existing in kola. For this purpose several perfectly fresh and sound red and white seeds were selected. One-half of the number were sliced into sixty cc. of water and kept for sixteen hours at 50°–55° and for twenty-four hours at ordinary temperature, then evaporated to dryness, powdered, and the free caffein estimated in duplicates in a weighed portion. The other half of the seeds were similarly treated, except that the slices were received in boiling water to destroy the ferment; and the whole was then kept at 50°–55°, the same length of time as those described above, then evaporated to dryness, powdered, and assayed.

Sample.	Per cent. Caffein.[1]	
	I.	II.
Not sterilized.....	0.644	0.639
Sterilized	0.629	0.662

It would seem from the above stated results that the kola ferment does not assist in the liberation of caffein from its natural combination, but that such liberation as takes place is rather to be attributed to the presence of moisture and warmth.

Moreover, it is of significance respecting the caffein compound, to observe that sterilizing the kola, which checks the formation of the colored body called kola-red, does not at the same time check the liberation of the alkaloid. In other words, it does not at all appear that caffein and kola-red are joint products of the one hydrolysis of a glucoside, though such has been the conclusion of previous investigators.

ESTIMATION OF THEOBROMIN.

This alkaloid forms such a small proportion of the total alkaloids that it is usually ignored in an assay, the whole being computed as caffein. As its ratio to the caffein present seems to be pretty constant, there appears no particular objection to this procedure unless a very precise analysis is desired.

We have found the gravimetric method proposed by Kunze,[2] with a few modifications, very satisfactory for the estimation of theobromin in the presence of caffein.

[1] Calculated for fresh seeds.

[2] W. E. Kunze, 1894: *Ztschr. anal. Chem.*, 24.

It is, however, necessary to purify the alkaloids by recrystallizing twice from water, or the traces of tannin and coloring matter adhering will reduce the silver nitrate and lead to erroneous results. After purification and drying at 100° C., 0.500 gram of the alkaloids of kola is dissolved in twenty-five cc. water, a few drops of ammonia added, and then five cc. silver nitrate solution (reagent), and the liquid heated on a water bath until the ammonia is entirely expelled. The silver theobromin is precipitated, and collected on a weighed asbestos filter, and washed with hot water until the washings no longer show the presence of a silver salt by the addition of hydrochloric acid. Hot dilute hydrochloric acid is now passed through the filter, followed by hot water until the washings are free from all traces of hydrochloric acid. The silver chloride remaining in the filter is now washed successively with alcohol and ether, dried twenty minutes at 85°–100° C. and the tube weighed. From the weight of the silver chloride we have the proportion:

Molecular weight of AgCl: Molecular weight of $C_7H_8N_4O_2$ :: Weight of AgCl: x = weight of the theobromin.

In total free alkaloids the proportion of theobromin was found to be 1.48 per cent., and in the total combined alkaloids, as would be expected, very nearly the same, in this case 1.51 per cent. Schweitzer,[1] however, found that it constituted 19.70 per cent. of the total combined alkaloids, and suggested that the increased amount of theobromin may be due to its formation from caffein by the prolonged boiling with the five per cent. sulphuric acid used by him for hydrolysis of the supposed glucoside.

But as a matter of fact, a methyl group is not so easily eliminated from caffein. That five per cent. sulphuric acid will not effect this change is shown by the results of the experiment next described. 0.3494 gram of caffein boiled for six hours with twenty-five cc. of five per cent. sulphuric acid showed at the end of that time not the slightest trace of theobromin by the silver nitrate test previously mentioned, and 0.3485 gram was recovered. Nor is caffein changed or colored by concentrated sulphuric acid even at 100° C.[2]

[1] 1895: Inaugural Dissertation, University Munich.

[2] Prescott's Organic Analysis, p. 82; Allen's Commercial Organic Analysis, vol. iii, pt. ii, 478.

Schmidt[1] found that by heating caffein with concentrated hydrochloric acid in a sealed tube for six to twelve hours, at 250° C., ammonia, sarcosin, methylamine, carbon dioxide, and traces of formic acid were formed, but no theobromin; he also found that concentrated hydrochloric acid has no action on caffein below 200°.

MELTING-POINT OF THE ALKALOIDS.

After repeated purification, the melting-point of the mixed free alkaloids of kola was taken, as was also that of the combined alkaloids; both were the same, 225°–227°, corresponding fairly well with that of pure caffein.

TANNIN.

(1) *Free Tannin.*—This was separated in various ways, the preferred method being as follows, taken in part from Allen:[2] The drug is exhausted with ninety-five per cent. alcohol, the alcoholic solution distilled *in vacuo* to a syrupy consistence, then washed with cold water. The insoluble matter is removed by decantation or filtration, and the clear wine-red solution fractionally precipitated with lead acetate (or lead hydroxide), the first and last portions of lead tannate being rejected. The lead tannate after being well washed is suspended in alcohol and decomposed with hydrogen sulphide. After filtration the alcoholic solution of tannin is distilled *in vacuo* to syrupy consistence and evaporation finished in a vacuum desiccator over sulphuric acid.

The tannin thus obtained is light-red to red-brown, having a faintly acidulous and decidedly astringent taste; deports itself as other tannins do towards iron salts ("iron greening"), gelatin, alkaloids, etc., etc. It is a glucosidal body, yielding on hydrolysis with mineral acids a dark-brown body. This was found to be insoluble in water or alcohol, and to give, on combustion, 69.20 per cent. of carbon, and 6.70 per cent. of hydrogen;[3] with the dark brown body was obtained glucose, identified by its action on Fehling's solution, and towards phenylhydrazin, with which latter it forms an osazone. We did not

[1] E. Schmidt: *Ann. Chem.* (Liebig), 217, 270.

[2] Allen's Commercial Organic Analysis, vol. iii, pt. i, p. 76.

[3] These figures do not correspond with those given by Knebel for kola red $C_{14}H_{12}(OH)_6$.

obtain a sufficient quantity of this osazone to take its melting-point, but its presence gave evidence of the glucosidal nature of the tannin.

(2) *Combined Tannin.*—The tannin existing in kola in combination with the caffein (as the so-called glucoside) was separated by means of lead hydroxide, following the above described manipulations. This "combined" tannin agrees in appearance and properties with the free tannin already described, being also a glucoside tannin.

The results obtained by combustion are also stated next below:

Duplicates.	Free tannin.		Combined tannin.	
	I.	II.	I.	II.
C.....	53.36	53.57	55.61	55.78
H.....	5.19	5.28	5.37	5.54
O.....	41.45	41.15	39.02	38.68

All calculations upon the composition of this body are reserved until after further work in its separation.

ANN ARBOR, MICH., JULY 6, 1896.

OBITUARY.

DR. HENRY A. MOTT, who has been an active member of the American Chemical Society since its organization in 1875, died at his home in New York City, on November 8, 1896. He was a grandson of the famous surgeon Dr. Valentine Mott, and was born at Clifton, Staten Island, New York, October 22, 1852. His primary education was obtained in the private schools of Rev. Mr. Tufts, at Munson, Mass., and of Prof. Berthet, on Broadway, near 18th street, in New York City. Later he entered the Academic Department of Columbia College, but finding the courses of study therein not wholly suited to his tastes and ambition, he applied for work leading to the degree of Doctor of Philosophy. On June 14, 1869, he entered the School of Mines in the course of mining engineering, and in 1873 he was graduated, receiving at the same time the degree of Bachelor of