THE CAFFEIN COMPOUND IN KOLA.
PART II. KOLATANNIN.[1]

BY JAMES W. T. KNOX AND ALBERT B. PRESCOTT.

THE investigation of the caffein compound in kola conducted by us last year[2] showed us that it was a kolatannate of caffein instead of a glucoside as formerly supposed. This question then arose: Is the tannin of this body identical with the free tannin existing in the kola nut? In the very brief examination made at that time it seemed that there was some difference, for combustions of both kinds of the tannin showed a difference in the respective amounts of carbon and hydrogen of each. No interpretation of the results was made at that time, for we considered the data insufficient, and the question was not settled.

It became necessary, in order that this point might be positively determined, to make a thorough comparative study of these two forms of kolatannin, and the research has been continued along this line with results which are stated in the following pages.

CLASSIFICATION OF TANNINS.

Several different means of classifying tannins have been proposed, such as "physiological" and "pathological"—referring, of course, to their origin in the plant; glucosidal and non-glucosidal; "iron-blueing" and "iron-greening;" tannins which are tanning agents and those which are not; those which yield a trihydroxyphenol by sublimation or by fusion with potassium hydroxide, and those which yield a dihydroxyphenol by sublimation, or the salt of a dihydroxybenzoic acid by gentle fusion with potassium hydroxide.[3]

Trimble[4] suggests a classification, not as a final one but which answers very well for the present, in view of the still very defective knowledge of this class of bodies. He proposes a division of them into "the gall tannin group" and "the oak tannin group." The tannins of the gall tannin group contain 50 to 52.5 per cent. of carbon and 3.10 to 4.50 per cent. of hydrogen; those of the oak tannin group contain, in round numbers, sixty

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[1] Presented by the Special Research Committee at the Forty-fifth Annual Meeting of the American Pharmaceutical Association, August, 1897.

[2] Knox and Prescott, 1896: This Journal, 19, 63.

[3] Prescott: Organic Analysis, p. 466.

[4] Trimble: The Tannins, II, p. 131.

per cent. carbon and five per cent. hydrogen. This classification holds good for the following reactions also :

	Gall tannin group.	Oak tannin group.
Ferric salts.	Blue color and precipitate	Green color and precipitate.
Calcium hydroxide	White precipitate becoming blue.	Light pink precipitate becoming red and then brown.
Bromine water.	No precipitate.	Yellow precipitate becoming brown.

Under this classification kolatannin is to be compared with the oak tannin group, for its compositions and reactions coincide more nearly with those of oak tannin than with those of gallotannic acid. We include herewith a brief summary of some important work done on oak bark tannins, not only for its historic interest, but because it has a bearing indirectly upon our own work on kolatannin.

RECENT LITERATURE OF OAK BARK TANNINS.

In February, 1880, Böttinger[1] published a lengthy article on "oak red," phlobaphene and tannin, which was followed about a month later by Etti's[2] publication of his own work on oak tannin—a valuable contribution to its literature. The methods used by them in the preparation of their working samples differed radically, the botanical source of their barks or extracts was unknown to them (or, if known, was not stated), the tannins obtained possessed properties different from each other, and it is not strange that the results obtained by these investigators were at variance in many particulars. If we may judge by their descriptions, it would seem that Etti's method of separation was much more likely to yield a pure product than that used by Böttinger, although by no means free from objections. Without doubt this work of Etti and that of Löwe,[3] dated in September of the same year, but which did not appear until the following year, added more to the knowledge of oak tannins than any previous contributions had done. The methods used by them are noteworthy, because they were radical departures from the old lead acetate methods formerly so much in vogue.

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[1] C. Böttinger, 1880; *Ann. Chem.*, (Liebig), **202**, 269.

[2] C. Etti, 1880; *Monatsh. Chem.*, 1, 262.

[3] J. Löwe, 1881: *Ztschr. anal. Chem.*, **20**, 208.

Etti extracted the oak bark with very dilute alcohol under gentle heat and added acetic ether and ordinary ether to the extract. After agitating, the faintly red-colored ethereal layer was separated, the ether recovered by distillation and used again for the same purpose. After distilling the ether, the alcoholic solution of tannin remaining behind was found to contain some crystals of ellagic acid, which were removed by filtration and the filtrate evaporated to dryness on a water-bath. The residue, a reddish-white powder, was oak tannin, mixed with a little phlobaphene, amorphous resin and gallic acid; the two last-named substances were separated by extracting the powder with non-alcoholic ether; it was next treated with a mixture of three parts acetic ether and one part ethyl ether, which removed the tannin and left the phlobaphene undissolved. After distilling off the solvent, the pure tannin remained behind as a reddish-white powder. Etti insists, too, that if the ethyl acetate be used again for a solvent it must be washed with sodium hydroxide solution and redistilled before use, as the tannin tends to decompose it slightly into acetic acid and alcohol, while the tannin is itself partially converted into its anhydride.

The method of Böttinger depends on the separation of the phlobaphene from tannin solutions by means of its comparative insolubility in water. The tanbark is extracted first with ether, then with alcohol, the alcohol solution is evaporated to dryness, and the residue extracted again with ether which removes the last traces of wax and fat. By warming the residue with water on a water-bath, a partial separation is effected of the insoluble phlobaphene from the soluble tannin. After filtration the tannin solution, still containing traces of phlobaphene, is repeatedly evaporated and redissolved, until upon diluting the liquid very largely and cooling it, no precipitation is had. On evaporating this solution, a light-colored residue is obtained which is soluble in water and which he considered the pure tannin.

Löwe extracted the oak bark with ninety per cent. alcohol, and distilled the extract *in vacuo* to syrupy consistency. Water was then added, which precipitated a large quantity of anhydride; after filtration common salt was added to saturation, which precipitated the remaining anhydrides completely; the liquid, after filtration, was agitated with ether repeatedly until this solvent would remove nothing further from the solution. The remaining ether was dissipated by gentle heat and after cooling the solution was shaken out with acetic ether. The acetic ether was removed by distillation and cold water was added to the remaining residue as long as precipitation ensued, after which it was again filtered and evaporated slowly in a vacuum desiccator to a dry mass, which is soluble in water and becomes cinnamon-brown on being pulverized.

It will be noticed that the methods of Etti and Löwe have much in common, the principal difference from the other methods being in the use of ethyl acetate, an immiscible solvent. It is difficult to say to whom credit should be given for the first application of this solvent to the preparation of tannin. In 1873,[1] however, Löwe mentions having used it in his work on sumach tannin, while in 1872[2] he also made use of it as a separative solvent for gallotannic acid.

Etti ascribed the formula $C_{17}H_{16}O_9$ to his tannin: a "first anhydride" $C_{34}H_{30}O_{17}$ was formed by heating the tannin at 140° C. He separated a ready-formed anhydride from the bark corresponding to this one, and made use of its barium salt $C_{34}H_{28}BaO_{17}$ to determine its formula. By boiling it with dilute hydrochloric or sulphuric acid, it loses another molecule of water, forming a second anhydride $C_{34}H_{28}O_{16}$. The third anhydride he prepared by boiling the pure tannin with dilute mineral acid, whereby two molecules of tannin lost three molecules of water, forming a body to which he ascribed the formula $C_{33}H_{26}O_{15}$. This anhydride corresponds, in Etti's opinion, to Oser's oak-red. Boiling the pure tannin with potassium hydroxide solution for some time, formed the first anhydride identical with the so-called oak bark phlobaphene; this was separated by acidulating the alkaline solution with mineral acid and filtering out the precipitated anhydride.

By various experiments, such as boiling the tannin with dilute acids, digesting it with ferments, etc., he arrived at the conclusion that oak tannin was not a glucoside but that such evidence of glucose as had been found by other investigators was due to the accidental presence of glucose in the sample.

He proposed a structural formula, the same as that of digallic

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[1] J. Löwe, 1873: *Ztschr. anal. Chem.*, 12, 128.

[2] J. Löwe, 1872: *Ztschr. anal. Chem.*, 11, 365.

acid with three hydroxyl groups replaced by three methyl groups, he having detected methyl chloride by burning the gas formed by heating the tannin with concentrated hydrochloric acid in a sealed tube.

Böttinger's work was principally on oak phlobaphene and oak-red, which he considered identical. The oak-red was formed by boiling the tannin with dilute mineral acid. He ascribed the formula $(C_{14}H_{10}O_8)_2.H_2O$ to it and believed oak tannin to be a glucoside.

Löwe proposed the formula $C_{28}H_{26}O_{14}$ for the tannin he prepared and $C_{28}H_{22}Pb_3O_{14}$ for the lead salt. He also prepared oak-red by the usual process of boiling the tannin with dilute acids and obtained in addition to the oak-red, $C_{28}H_{22}O_{11}$, a by-product of which he made a combustion, but did not identify. It was not sugar, however, and he states that oak tannin does not conduct itself as a glucoside. The oak phlobaphene is, according to his statements, a fourth anhydride of oak tannin of the formula $C_{28}H_{24}O_{12}$, and forms a lead salt $C_{28}H_{22}O_{12}Pb$.

A somewhat extended and not altogether good-natured controversy between Böttinger and Etti followed the publication of the latter's paper, Böttinger[1] insisting upon the correctness of his own work, and holding to his first statement that oak tannin was a glucoside, Etti,[2] on the other hand, contended that Böttinger's methods were faulty, that ready-formed glucose would not be separated from the tannin by Böttinger's process, and therefore sugar would of course be found in the solution after boiling the tannin with dilute acids. He performed a number of experiments tending to prove the correctness of his position, following Böttinger's methods also and criticising him severely. Böttinger[3] subsequently modified his position somewhat, but without conceding anything of importance.

In 1883 the next contribution appeared, in which Etti[4] again commented on Böttinger's work and quoted Löwe in confirmation of his own results. He reported a new tannin, of formula $C_{20}H_{20}O_9$, from *Quercus Pubescens*, while the other he had since

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[1] C. Böttinger, 1881: *Ber. d. chem. Ges.*, 14, 1598.

[2] C. Etti, 1881: *Ber. d. chem. Ges.*, 14, 1826.

[3] C. Böttinger, 1881: *Ber. d. chem. Ges.*, 14, 2390.

[4] C. Etti, 1883: *Monatshefte*, 4, 512.

learned was from *Quercus Robur.* This new tannin colored solutions of ferric salts green, while the first one produced a blue color with them. During this investigation the objection already noted to the use of ethyl acetate, *viz.*, its tendency to decompose the tannin, became so marked as to necessitate a different method of separation. The most important points of this new method are the use of alcoholic ether as a solvent for the tannin, phlobaphene and green resin. The resin is removed from the resulting product by benzene, and the phlobaphene by careful addition of lead acetate to the tannin solution. The precipitate of lead tannate is yellow, but if any anhydride (phlobaphene) be present, the precipitate will be colored more or less reddish-brown, depending on the proportion of it to the tannin. Lead acetate is added until the color of the precipitate indicates that all of the anhydride has been precipitated. After filtering, the liquid is again exhausted with alcoholic ether, the ether removed by distillation and the residual liquid evaporated to dryness on the water-bath. A rather remarkable peculiarity of Etti's work is that both of these tannins are nearly insoluble in water, 0.6 of tannin in 100 of water, while the experience of other workers has been quite generally that tannins are soluble.

Etti claimed to have formed four anhydrides of this tannin, $C_{20}H_{20}O_9$.

First anhydride,		$C_{40}H_{38}O_{17}$.	Oak phlobaphene, dried at 120°.
Second	"	$C_{40}H_{36}O_{16}$.	By boiling phlobaphene with 1 : 12 sulphuric acid and washing precipitate first with water, then with alcohol, and evaporating the alcoholic solution.
Third	"	$C_{40}H_{34}O_{15}$.	Dried at 125°. By boiling tannin with dilute sulphuric acid and collecting the precipitate.
Fourth	"	$C_{40}H_{32}O_{14}$.	By heating in 25 per cent. sulphuric acid containing 20 per cent. alcohol in sealed tube at 130° for four hours.

Etti believed also that four anhydrides were formed from the other tannin ($C_{17}H_{16}O_9$) and gave this view support by his own figures and some also of Oser, of Böttinger and Löwe. This would be apt to mislead the casual reader into attaching more importance to this theory of the four anhydrides, than is warranted by the actual results given by Etti. It would hardly seem that he was justified in making this use of the work of the other investigators. As a matter of fact he has selected figures here and there which gave support to his views, but ignored others of equal importance which did not harmonize with his ideas; *e.g.*, he quoted Löwe's figures for the analysis of oak-red, but did not mention his work with the lead salts of oak-red, which brought him to the conclusion that the formula of oak-red was $C_{28}H_{22}O_{11}$. There is no apparent reason for doubting that this work was equally as reliable as that which Etti quoted, and to omit it gives a careful reader the impression that deductions made from such peculiarly isolated facts would not necessarily be of great value. The chart is here appended:

		Calculated.	Found.			
Tannin $C_{17}H_{16}O_9$	Carbon	56.04	56.31	56.06		
	Hydrogen	4.40	4.63	4.69		
First anhydride "Phlobaphene," $C_{34}H_{30}O_{17}$ Grabowski's oak-red	Carbon	57.46	57.28	57.62		
	Hydrogen	4.22	4.64	4.33		
Second anhydride $C_{34}H_{28}O_{16}$	Carbon	58.96	58.76			
	Hydrogen	4.04	4.20			
			By Oser.	By Böttinger.		By Etti.
Third anhydride $C_{34}H_{26}O_{16}$ Oser's oak-red	Carbon	60.58	60.70	60.19	60.08	60.33
	Hydrogen	3.86	4.03	4.22	3.94	4.03
			Found by Löwe.			
Fourth anhydride $C_{34}H_{24}O_{14}$ Löwe's oak-red	Carbon	62.20	62.339	62.197	61.997	
	Hydrogen	3.66	4.154	4.015	4.056	

Böttinger[1] published in the same year, 1883, a piece of work done on the bromine derivatives of oak tannin. By direct addition of bromine to the watery extract of oak bark he formed a dibrom oak tannin, $C_{19}H_{14}Br_2O_{10}$, which was capable of forming a pentacetyl derivative. "By careful manipulation"—he does not describe it—he converted the dibrom into a tetrabromtannin, which also formed a pentacetyl compound. This seems to be the first work on bromine derivatives.

In 1884[2] he investigated hemlock tannins by means of their

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[1] C. Böttinger, 1883: *Ber. d. chem. Ges.*, 16, 2710.

[2] C. Böttinger, 1884: *Ber. d. chem. Ges.*, 7, 1041.

bromine compounds precipitated directly from their infusions by addition of bromine. Later[1] in the same year he prepared bromine compounds of a number of bark tannins by adding bromine directly to their infusions. Acetyl compounds of these were formed and analyzed.

Etti,[2] in a brief paper shortly afterward, discussed the points of difference between gallotannic acid and oak tannin. He also pointed out that by reason of its very hygroscopicity acetic anhydride was not a proper agent for determining the number of hydroxyl groups of tannins, as its tendency would be to form anhydrides which in turn would be acetylized and lead to incongruous results in the analysis.

Böttinger,[3] in 1887, published an article principally on oakwood tannin, which he separated by dissolving commercial oakwood extract in twenty parts of water and allowing it to stand. The clear liquid was evaporated to dryness and boiled with acetic anhydride, which formed an acetyl compound whose composition corresponded to formula $C_{15}H_7(C_3H_2O)_5O_9$. From this he recovered an anhydride of oakwood tannin and formed mono- and tetrabrom derivatives of it.

In 1889, Etti,[4] adhering to his previously published formulas for oak tannins, gave the results of an investigation of a tannin from the common Slavonian oak, to which he gave the formula $C_{16}H_{14}O_9$. It was nearly or quite insoluble in water. He formed several soluble magnesium salts with this tannin and suggested that it might exist in the plant in this form, thus accounting for its solubility in an aqueous menstruum when first extracted. Several anhydrides were formed.

Any résumé of the literature of oak tannins, however brief, would be incomplete without reference to the work of Trimble.[5] His monograph in two volumes contains a praiseworthy compilation of previous literature on the subject and full accounts of his own work. He has the credit of introducing acetone as an extractive menstruum for the oak tannins instead of alcohol. This solvent is said to act particularly well with oak bark. A

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[1] C. Böttinger, 1884: *Ber. d. chem. Ges.*, 17, 1123.

[2] C. Etti, 1884: *Ibid*, 17, 1820.

[3] C. Böttinger, 1887: *Ann. Chem.* (Liebig), **238**, 366.

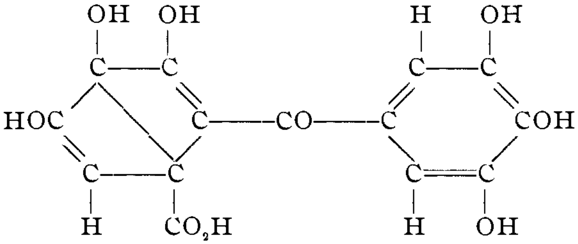
[4] C. Etti, 1889: *Monatshefte*, 10, 547.

[5] H. Trimble, 1894: "The Tannins." A monograph, J. B. Lippincott Co.

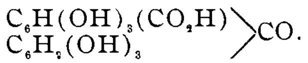
good bibliography concludes the work. He has confined himself thus far to analyses of the pure tannins and does not as yet propose formulas. In addition to the monograph just mentioned, he has been a frequent contributor on this subject in the periodical literature of chemistry.

LATE WORK OF SCHIFF ON DIGALLIC ACID.

In the present year, Hugo Schiff,[1] having already found that natural tannic acid is possessed of a slight optical activity, namely dextrorotatory, inquires as to its containing in some way asymmetric carbon, and infers a ketonic structure with six hydroxyl groups. He does not obtain a hydrazone from tannic acid. From experimental results he proposes this formula :



Irrespective of orientation, and for comparison of a kolatannin formula offered for consideration further on, the above formula may be held simply as



$$\left.\begin{array}{l}C_6H(OH)_3(CO_2H)\\C_6H_2(OH)_3\end{array}\right\rangle CO.$$

CAFFEOTANNIC AND CAFFEIC ACIDS.

The literature of caffeotannic acid, of the formula $C_{15}H_{18}O_8$, dates from Rochleder, in 1846.[2] Its production of caffeic acid (dihydroxyphenylacrylic acid), along with sugar, was set forth by Hlasiwetz in 1867.[3]

A few years ago, caffeic acid was obtained from the tannin of coffee, also from the tannin of the maté, by H. Kunz-Krause.[4] Very recently the same author has investigated caffeotannic

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[1] *Gazz. chim. ital.*, *1897*, 27, i, 90.

[2] *Ann. Chem.* (Liebig), 59, 300.

[3] *Ibid*, 142, 220.

[4] *Arch. Pharm.*, *1893*, 231, 613.

acid.[1] In his report he[2] finds confirmation of the composition of caffeic acid as a dihydroxyphenylacrylic acid. His analyses obtain the carbon dioxide given off when the caffeic acid is heated to 200° C., and are confined to this analytic method. A residue of a vinyl pyrocatechol is predicted. The author recognizes a class of bodies which he terms glucotannoids.

"FREE" KOLATANNIN.

PREPARATION OF THE SAMPLE.

The amorphous character of tannins, together with their extreme liability to decomposition, renders their separation and purification very difficult. The method used by us last year,[3] in the very brief examination of kolatannin, was based on that of Allen,[4] being fractional precipitation with lead acetate.

On taking up the work this year, it soon became evident that whatever may be said of lead-acetate methods as applied to other tannins, they certainly are unsuited to this one, for various reasons. The lead kolatannate, being bulky, is extremely slow and difficult of filtration, and the tannin in this state exhibits a marked tendency to pass into insoluble forms, principally anhydrides. During the washing, the lead salt becomes gradually darker, and this tendency is favored by the moisture present. If, on the other hand, the washing be less prolonged, the resulting product will be contaminated with various other constituents of kola extract, such as sugar, alkaloids, inorganic salts, coloring-matter, etc., etc. It is indeed very doubtful if any usual amount of washing will remove these impurities, hence the purity of a tannin, separated by precipitation with lead acetate, would be regarded with too much suspicion to be employed in analytical work, even if its sensible properties were not changed during the treatment. Kolatannin was at first supposed and since determined to be soluble in water. But on evaporating the solution of tannin after removing the lead from combination by hydrogen sulphide, the end-product was a red-brown

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[1] I am indebted to Prof. Kremers for bringing Kunz-Krause's article in the July *Berichte* to my attention, as I read this paper in the August meeting of the American Pharmaceutical Association.—A. B. P.

[2] *Ber. d. chem. Ges.*, *1897*, 1617.

[3] Knox and Prescott, *loc. cit.*

[4] Allen: Com. Org. Anal., **3**. pt. I., 76.

substance, insoluble in water, and possessing a not particularly astringent taste. So by the use of lead acetate a tannin is obtained whose purity is a matter of doubt, and whose properties have undergone radical changes during separation, both as to color and solubility. Furthermore, we desired to effect a perfect separation of the free tannin from that existing in combination with caffein (as caffein kolatannate) in order to determine by analysis whether they were identical tannins or not, the analysis of them last year having shown somewhat different values in each for carbon and hydrogen. Caffein kolatannate is sparingly soluble in water, more soluble in solutions of tannin and of caffein, so that the aqueous infusion of kola contains caffein kolatannate as well as free tannin, and the lead tannate obtained from it would be a mixture of the lead salts of both tannins, or both forms of tannin, and no delicate separation could be had in this way.

Plainly, another method was necessary. The objections just noted were for the most part neither new, nor limited to kolatannin. As previously stated, Löwe, in 1872, who had encountered many difficulties in the use of lead acetate for the separation of tannins, and found it necessary to employ other means to obtain a pure product, resorted to the use of ethyl acetate. This immiscible solvent, proving satisfactory in many cases, has since been used considerably, either alone or mixed with ether, in the separation of tannins for analytical purposes, and for some tannins at any rate is unquestionably the best separative solvent which has yet been given publicity as such. At the same time a single shaking out with ethyl acetate does not suffice for the preparation of a pure sample. More or less coloring-matter will be found in the residue left on evaporating the first portion of the solvent, so that several repetitions of the process are required. Care must be used not to carry the method of purification too far, for if this be done the decomposition of the tannin is apt to prove a greater obstacle than the original impurities. We find that four or five repetitions of the "shaking out" give the best results. In detail the manipulations are as follows, and the method has given satisfactory results:

The fresh kola nuts are sliced into boiling alcohol,[1] removed after a few moments boiling, and dried in a current of warm air, then ground up to a number twenty powder and packed firmly in a percolator. The alcohol so used in sterilizing the drug is diluted to about fifty per cent. strength, and employed as a menstruum, with addition of sufficient dilute alcohol to complete the extraction. The highly-colored extract of kola thus obtained is concentrated by distillation *in vacuo*, until the alcohol is all removed. This can be done with a good pump at between 18° and 20° C. The contents of the flask are then filtered, the insoluble portion being chiefly caffein kolatannate, while the solution contains caffein, kolatannin, caffein kolatannate, glucose, traces of fatty matter, and more or less colored derivatives of tannin according to whether the sterilization by boiling alcohol has been properly done. Common salt is then added to this filtrate to saturation and the caffein kolatannate is completely precipitated. It is filtered out and added to the first residue of caffein kolatannate. The reddish-colored filtrate is then transferred to a large separatory funnel and agitated with chloroform repeatedly to remove alkaloids and traces of fat. The dissolved chloroform is then separated by agitation with small portions of ether. Kolatannin is insoluble in chloroform, and only very sparingly soluble in ether, so this treatment does not remove any considerable amount of it. Ethyl acetate is now added to the liquid in the separatory funnel and the liquid extracted with it as long as any tannin is removed. Usually eight, ten, or even more successive portions are required for this. The united solutions of tannin in ethyl acetate are transferred to a distilling flask and concentrated to dryness under reduced pressure. To avoid loss of the ethyl acetate we use a condenser about a meter in length, and pack the receiver in ice. Sometimes also a condenser is placed between the pump and the receiver. With these precautions the waste is very slight. Before using the recovered ethyl acetate as a solvent again, however, it is washed with a weak solution of sodium carbonate and redistilled, to avoid the presence of any free acetic acid formed by decomposition of the ether. The statement of Etti already alluded to, concern-

[1] To prevent the formation of the colored body, which would otherwise appear.

ing the decomposition of ethyl acetate by tannin, has not been found to apply to kolatannin, but the washing was nevertheless adopted as a precautionary measure.

The tannin residue in the distilling flask is a porous, pinkish mass, very friable, and easily and completely soluble in water. It is redissolved in a convenient quantity of saturated salt solution, filtered if need be and again shaken out with ethyl acetate, which is distilled off in the way just described. The tannin residue is next dissolved in cold distilled water and shaken out as before, this process being repeated once or twice. The final tannin residue, after the ethyl acetate has been removed as far as possible by distillation, is treated in the flask with a small quantity of ether, which after thoroughly permeating the mass is removed by distillation, using only the partial force of the pump and gentle heat. This is repeated several times until the mass has no odor of ethyl acetate. Then transferring to a vacuum desiccator the tannin is kept for several days over sulfuric acid. If this does not remove the last traces of ether, gentle heat (60°–65°) will do so.[1]

PROPERTIES OF KOLATANNIN.

Kolatannin is a cream-colored powder with a slight pinkish tinge. It is freely and completely soluble in water, alcohol, acetone, ethyl acetate, sparingly soluble in ether, insoluble in chloroform and in petroleum benzin. The following tabulated comparative statement of its reactions will serve to point out some of the important differences between it and gallotannic acid, and to indicate the close resemblance between this tannin and that of oak bark.

[1] We desire to take this means of expressing our appreciation of the liberality and courtesy of the firm of Frederick Stearns & Company, Manufacturing Pharmacists, Detroit, Michigan, who supplied us with a large quantity of True African Kola nuts, for our research this year. The drug was quite fresh and in perfect preservation, very necessary conditions for work of this kind.

REACTIONS OF TANNINS.

	Kolatannin. Free.	Kolatannin. From caffein kolatannate.	Oak tannin. (Trimble.)	Gallotannic acid.
Ferric acetate	Green.	Green.	Green.	Blue-black.
Potassium dichromate	Dark brown ppt.	Dark brown ppt.	Brown ppt.	Brown ppt.
Chlorine	Pale ppt.	Pale ppt.		
Bromine	Light yellow ppt.	Light yellow ppt.	Yellow ppt.	No ppt.
Calcium hydroxide	Pink, then red, changing to ppt.	Pink, then red, changing to ppt.	Red, changing to ppt.	Darkens with bluish tinge, then ppt.
"Tartar Emetic"	No ppt.	No ppt.	White ppt.	White ppt.
Quinin Cinchonin Caffein	White ppt.	White ppt.	Ppt.	White ppt.
Albumen	Ppt.	Ppt.	Ppt.	Ppt.
Lead acetate	White ppt.	White ppt.	White ppt.	White ppt.
Ammoniacal potassium ferricyanide	Deep red.	Deep red.	....	Deep red.
Solution of formaldehyde with a condensing agent, as HCl	Pink ppt., becoming red.	Pink ppt., becoming red.		

COMBUSTIONS OF KOLATANNIN.

The tannin was analyzed by combustion with the following stated results :

	I.	II.	III.	Calculated for $C_{16}H_{20}O_8$.
Carbon	56.74	56.81	56.90	56.45
Hydrogen	5.71	5.62	5.76	5.90
Oxygen	37.55	37.57	37.34	37.65
	100.00	100.00	100.00	100.00

Having this satisfactory evidence of the preparation of a pure chemical compound, we next proceeded upon the task of determining its constitution, first by finding the number of hydroxyl groups in the molecule containing C_{16}.

PENTACETYL KOLATANNIN.

An acetyl derivative was next formed for the object above stated. The tannin was boiled with acetyl chloride for about one hour under a return condenser, then an equal volume of glacial acetic acid was added, after which the liquid was poured slowly into ice-cold water. The acetyl compound separated out as a voluminous whitish precipitate. This was rapidly filtered at the pump and well washed with cold water, then it was dried with bibulous paper and finally in a vacuum desiccator over sulphuric acid. It was a nearly white or gray-white powder, tasteless, having a faint odor of acetic acid, and was insoluble in water, sparingly soluble in ether, and freely and completely soluble in chloroform, in alcohol, and in glacial acetic acid.

Analysis of the body gave the following stated results:

		Found.		Calculated for
By combustion.	I.	II.	III.	$C_{16}H_{15}(C_2H_3O)_5O_8$.
Carbon	56.55	56.68	56.76	56.72
Hydrogen	5.39	5.36	5.24	5.47
Oxygen	38.06	37.96	38.00	37.81
By saponification.				
Acetyl	38.24	38.47	38.68	39.09

On comparing the values given by analysis with those calculated for the pentacetyl derivative, the agreement seems to be satisfactory.

The acetyl values in the analysis by saponification as detailed below are a little low, but it is quite possible that this is to be ascribed to defects in the method of estimation rather than to other causes. At any rate the acetyl content seems without doubt to indicate five hydroxyl groups in the C_{18} molecule rather than four or six.

The analyses by combustion were made in the ordinary way: the estimation of acetyl was conducted in the manner next described.

DETERMINATION OF ACETYL.

The acetyl compound, one-half to one gram, varying with the supposed acetyl content, is boiled with thirty to forty cc. of six per cent. sulphuric acid in a flask fitted with reflux condenser, for two hours. 100 to 150 cc. water are now added and without filtration the volatile contents of the flask distilled into a known quantity of decinormal potassium hydroxide solution, being careful to avoid a high enough temperature or sufficient concentration to cause distillation of the sulphuric acid. More water is now added to the distilling flask and the distillation repeated. The contents of the receiver should be kept alkaline and a few drops of phenol-phthalein solution added, so that any change in the reaction of the solution may be instantly noted. The distillation should be repeated several times until the last one hundred cc. of distillate contains no acetic acid. The excess of alkali is to be titrated with decinormal hydrochloric acid, and the amount of acetic acid calculated from the difference between the amount of alkali solution first taken, and the quantity of hydrochloric acid required to neutralize it. Barium chloride is now added, and if any precipitation takes place, the barium sulphate is collected and weighed, the sulphuric acid calculated and correction made for it. If a halogen be present in the acetyl compound, a sufficient excess of silver sulphate is added to the contents of the distilling flask before distillation, to precipitate it completely as silver salt.

The above was occasionally deviated from by heating the acetyl compound with the dilute acid in sealed tubes two or three hours at 130°, then cooling, opening, transferring to a flask and distilling as before described.

The following described method was used a few times, but without apparent advantage over the others: The sample is boiled with twenty times its weight of alcoholic solution of potassium hydroxide, of five to eight per cent. alkali strength, in a flask under return condenser. Phosphoric acid is added in excess and the contents of the flask distilled with steam into a receiver containing a known quantity of decinormal alkali solution. To drive the acetic acid over completely requires distillation of about a liter of water. The same precautions are observed as in the preceding methods if halogens are present.

BROMINE DERIVATIVES OF KOLATANNIN.

The bromine compounds of this tannin have been investigated at considerable length, for the purpose of obtaining further data concerning its constitution.

TRIBROM KOLATANNIN.

When bromine water is added to a water solution of kolatannin, a dark-colored body is precipitated, and with continued addition of the reagent the precipitation goes on until complete, during which the precipitate formed becomes lighter-colored until at the end, in the presence of an excess of bromine, it is pale brownish-yellow and further addition of bromine water produces no change. It is quite voluminous and cannot well be filtered at the pump, but is collected by ordinary filtration and well washed with water until the washings remain clear when treated with silver nitrate solution. It is dried first on porous plates, then in a vacuum desiccator to constant weight.

During the process of drying it gradually changes color until it becomes reddish-brown. It is odorless and nearly tasteless, insoluble in water, ether, chloroform, and benzene, but is readily soluble in alcohol and in acetone.

In the clear filtrate, after the bromine compound has been removed, hydrobromic acid is easily identified, and for that reason the change is believed to be one of substitution. It may be questioned perhaps whether this is not caused by the action of bromine on the water; the bromine water, however, was freshly prepared, and the hydrobromic acid formed under these conditions would be very little. But in preparing twenty-five or thirty grams of this bromine compound, after its precipitation, the filtrate was agitated with carbon disulphide to remove the excess of bromine, then separated and the aqueous portion concentrated to small volume, which had an acid taste and reaction and in which hydrobromic acid was easily identified.

The bromine compound was analyzed with the following stated results :

		Found.		Calculated for
By combustion.	I.	II.	III.	$C_{16}H_{17}Br_3O_8$.
Carbon	32.91	33.12	....	33.28
Hydrogen	2.69	2.58	....	2.95
Oxygen	22.86	22.63	....	22.19
As silver salt.				
Bromine	41.54	41.67	41.62	41.58

The percentage of carbon in this body, when compared with that of the pure tannin from which this was prepared, seemed to indicate the substitution of three hydrogen atoms by that number of bromine atoms.

PENTACETYL TRIBROM KOLATANNIN.

(From Tribrom Kolatannin.)

For the purpose of obtaining further data, an acetyl compound of the bromine derivative was formed : The bromine compound is boiled with acetyl chloride for about forty-five minutes, under a reflux condenser. An equal volume of glacial acetic acid is added to the clear red solution and the liquid well cooled by freezing-mixture. Then it is poured slowly into twelve or fifteen times its volume of ice-cold water, which is constantly stirred. A thermometer should be kept in the beaker containing the precipitate, and if the temperature rises above 10°, it is best to cool the contents by placing the beaker in a freezing-mixture, resuming addition of the acetyl chloride on cooling, until all has been used. The acetyl compound separates as a flocculent yellow precipitate, is quickly filtered, well washed and dried, first on porous plates, then in a vacuum desiccator.

It is a tasteless, golden-yellow powder, having a faint odor of acetic acid. It is insoluble in water, nearly insoluble in ether, but soluble in alcohol, in acetone, and in chloroform. It loses acetic acid when gently heated, and both bromine and acetic acid on stronger heating. It was analyzed with the following stated results :

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{12}Br_3.(C_2H_3O)_5O_8$.
Carbon	39.43	39.55	39.65
Hydrogen	3.44	3.34	3.44
Oxygen	26.57	26.65	26.43
As silver salt.			
Bromine	30.56	30.46	30.48
By saponification.			
Acetyl	26.91	27.12	27.32

The results obtained agree very closely with the calculated formula and give additional confirmation to the formulas assumed for the pure tannin and its tribrom compound.

HALOGEN DETERMINATION.

For the halogen determinations in the bromine compounds of kolatannin, the well-known process of heating the halogen derivative with fuming nitric acid and silver nitrate in a sealed tube for two hours at 175°, was followed. Experience taught us, however, to first make a preliminary determination with a considerable excess of silver nitrate to ascertain the approximate amount of halogen present. For example, where in one case the preliminary determination indicated 40.88 per cent. of bromine, the following ones, in triplicate with only a slight excess of silver nitrate, were respectively 41.54 per cent., 41.67 per cent., 41.62 per cent. And as a rule the preliminary determinations have given low results, but are necessary to furnish data for the second and more exact estimations.

Having learned the approximate amount of halogen present, the following details of manipulation were followed: The size of the sample taken is adjusted so that the weight of the resulting silver salt shall be 0.15 to 0.20 gram. Six or eight per cent. more than the theoretical quantity of silver nitrate is now weighed and placed with the sample in a hard glass tube with six cc. fuming nitric acid, after which the tube is sealed and heated at 175°–180° for two hours. It is then opened and the contents are transferred to a beaker with about 100 cc. water and heated on the water-bath for one-half hour. The silver bromide is then collected on weighed asbestos filters connected with the pump, washed successively with hot water, alcohol and ether, and dried twenty or thirty minutes at 80°–85° to constant weight.

In some cases the excess of silver nitrate in the filtrate was determined gravimetrically as silver chloride for a check on the results.

Our thanks are due to Professor Edward D. Campbell, of this University, for many valuable suggestions on halogen and acetyl determinations.

TRIBROM PENTACETYL KOLATANNIN.

(From Pentacetyl Kolatannin.)

Having acetylized the tribrom compound of tannin and obtained a pentacetyl derivative of it, we decided to brominate the pentacetyl compound and determine by analysis whether or not the resulting product was identical, in other words to ascertain whether the order in which the substitutions were made affected the result.

This bromination was accomplished by dissolving the pentacetyl tannin in strictly pure, anhydrous chloroform, free from alcohol, and adding a slight excess of bromine. The solvent was then distilled off in partial vacuum, while the brominated acetyl compound remained behind as a golden-yellow mass. This was treated with ether several times and redistilled, using the pump to remove the traces of chloroform, and finally transferred to a vacuum desiccator and left until completely dry.

In color, taste, and solubilities this body is identical with that obtained by acetylizing the tribrom derivative. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{12}Br_3(C_2H_3O)_5O_8$.
Carbon	39.49	39.62	39.65
Hydrogen	3.39	3.47	3.44
Oxygen	26.61	26.34	26.43
As silver salt.			
Bromine	30.51	30.57	30.48
By saponification.			
Acetyl	26.84	26.98	27.32

Inasmuch as the results of the analysis of this body coincide quite as closely with the theoretical figures as in the preceding case, it may be safely assumed that the bodies are identical and that in the preparation of a pentacetyl tribrom kolatannin it makes no difference whether the bromine or acetyl is introduced first.

TETRABROM KOLATANNIN.

We next wished to ascertain whether this tannin was capable of taking more than three bromine atoms into combination in each molecule. Nothing but the tribrom derivative was formed by treating an aqueous solution of the tannin with bromine water in excess, so another solvent was tried successfully. Taking a weighed quantity of tannin, it was dissolved in alcohol and bromine was added gradually to the alcoholic solution in slight excess of the theoretical quantity necessary to form a tetrabrom derivative. The liquid remained clear, all the bromine compounds of kolatannin that we have yet prepared being easily soluble in alcohol. The bromine compound was precipitated by pouring the brominated alcoholic solution into twelve or fifteen volumes of ice-cold water; being insoluble in water, it was separated as a voluminous reddish-brown precipitate, which was filtered, washed and dried in the way already described under the method of preparation of tribrom kolatannin.

This body agrees in solubilities with the tribrom compound; it is slightly darker, however, and has a faint odor of bromine. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	1.	II.	$C_{16}H_{16}Br_4O_8$.
Carbon	29.59	29.46	29.27
Hydrogen	2.46	2.38	2.44
Oxygen	18.97	18.88	19.52
As silver salts.			
Bromine	48.98	49.28	48.77

This, then, is a tetrabrom derivative of kolatannin. From it an acetyl compound was formed, following the manipulations already described.

PENTACETYL TETRABROM KOLATANNIN.

This acetyl compound was yellow and but slightly darker than that of the tribrom derivative. In other physical properties it appeared identical with that body.

It was analyzed with the following stated results:

By combustion.	Found. I.	II.	Calculated for $C_{16}H_{11}Br_4(C_2H_3O)_5O_8$.
Carbon	35.82	35.97	36.03
Hydrogen	3.02	2.95	3.01
Oxygen	24.02	24.14	24.02
As silver salt.			
Bromine	37.14	36.94	36.94
By saponification.			
Acetyl	24.42	24.59	24.83

From the above it is seen that the tetrabrom derivative forms a pentacetyl compound equally as easily as the tribrom derivative.

PENTABROM KOLATANNIN.

This derivative was formed while trying to prepare a hexabrom substitution product, but by reason of an insufficiency of bromine the resulting product yielded analytical results corresponding fairly well with those calculated for pentabrom kolatannin.

By combustion.	Found. I.	II.	Calculated for $C_{16}H_{15}Br_5O_8$.
Carbon	25.87	25.99	26.13
Hydrogen	2.07	1.86	2.04
Oxygen	17.35	17.31	17.42
As silver salt.			
Bromine	54.71	54.84	54.41

This body may be a mixture of the hexa- and tetrabrom tannins. It is somewhat difficult of formation, however, and we have not always been able to obtain it in a manner analogous to the methods of preparation of the other bromine compounds. We do not consider that all conditions necessary to its formation are yet understood. There is no good evidence that this is a mixture of the other derivatives, however, and for the present there is really no sufficient reason for mentioning it as other than a pentabrom tannin. It is soluble in the same agents as the other bromine compounds, and is less stable than the tetrabrom compound, the odor of bromine being much more distinct. It appears to be characteristic of these bodies, that with increasing percentage of bromine, there is decreasing stability (that is to say, a greater tendency to lose bromine), and darker color.

PENTACETYL PENTABROM KOLATANNIN.

(From Pentabrom Kolatannin.)

This was prepared by acetylizing pentabrom kolatannin in the usual way. In properties and appearance it closely resembles the other bromacetyl derivatives, except that its color is somewhat darker yellow. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{10}Br_5(C_2H_8O)_5O_8$.
Carbon	33.54	33.39	33.02
Hydrogen	2.77	2.65	2.65
Oxygen	20.97	21.37	22.01
As silver salt.			
Bromine	42.72	42.59	42.32
By saponification.			
Acetyl	22.15	22.36	22.75

This body was also prepared from pentacetyl kolatannin, as stated next below.

PENTABROM PENTACETYL KOLATANNIN.

(From Pentacetyl Kolatannin.)

Having ascertained by previous experiment that addition of a considerable excess of bromide to an alcoholic solution of kolatannin, resulted in the formation of a hexabrom derivative, we wished to prepare a hexabrom pentacetyl derivative by brominating the pentacetyl derivative in the same manner.

Analysis of the resulting product showed, however, that contrary to our expectations, not a hexabrom, but a pentabrom, derivative was formed, identical with the one described in the preceding section. The analytical results obtained were as follows:

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{10}Br_5(C_2H_3O)_5O_8$.
Carbon	33.19	32.97	33.02
Hydrogen	2.60	2.71	2.65
Oxygen	21.90	21.95	22.01
By silver salt.			
Bromine	42.31	42.37	42.32
By saponification.			
Acetyl	22.31	22.47	22.75

HEXABROM KOLATANNIN.

By treating an alcoholic solution of kolatannin with bromine in considerable excess a hexabrom compound is formed, which is separated in the same way as the tetra- and pentabrom derivatives. It is darker colored than either of the others, has a very distinct odor of bromine, and is soluble in the same media as the other bromine compounds. It was analyzed with results stated next below:

By combustion.	Found. I.	Found. II.	Calculated for $C_{16}H_{14}Br_6O_8$.
Carbon	23.48	23.57	23.59
Hydrogen	1.68	1.79	1.72
Oxygen	15.73	15.38	15.73
As silver salt.			
Bromine	59.11	59.26	58.96

TETRACETYL HEXABROM KOLATANNIN.

(From Hexabrom Kolatannin).

This acetyl compound was prepared in the usual way, by boiling with acetyl chloride and was supposed, before analysis, to be a pentacetyl derivative. It had a dark yellow color, a faintly pungent taste and a slight odor of bromine and acetic acid. Soluble in chloroform and in alcohol, insoluble in water. The analytical results which are next stated show it to be a tetracetyl hexabrom tannin:

By combustion.	Found. I.	Found. II.	Calculated for $C_{16}H_{10}Br_6(C_2H_3O)_4O_8$.
Carbon	29.57	29.39	29.33
Hydrogen	2.21	2.32	2.25
Oxygen	19.41	19.21	19.55
As silver salt.			
Bromine	48.81	49.08	48.87
By saponification.			
Acetyl	16.86	17.19	17.52

INDICATIONS OF MOLECULAR CONSTITUTION.

From the analytical results of the pentacetyl pentabrom tannin and of the hexabrom compound with its tetracetyl derivative, it appears that the total number of groups or atoms which may be substituted in the way described is ten; that if five of acetyl be introduced first, only five bromine atoms may be substituted for hydrogen, but that if the bromine be introduced first six bromine atoms can be introduced by carrying bromination to the limit, and in this hexabrom compound only four hydroxyl groups remain. This appears to indicate that the sixth bromine atom introduced bears a relation to the molecule in some respect different from that of any other bromine atom. But it might be expected that a limit would be reached in the total number of negative groups or atoms which can be retained in the molecule. The greatest number of acetyl groups probably indicates the entire number of hydroxyls. And a balance of evidence seems to favor the conclusion that there are six atoms of hydrogen directly united with the carbon of a benzene chain. These numbers are all in ratio to sixteen atoms of carbon. That the molecule contains C_{16}, or some multiple of this, appears from the quantitative results. We attempted to find the molecular weight of kolatannin, tribrom kolatannin and pentacetyl tribrom kolatannin by the cryóscopic method, but the results so far obtained are utterly meaningless. Further indications of moleculer constitution may be drawn from a study of the anhydrides, account of which next follows, also from the results of the action of acids, and especially from the products by fusion with alkali, all stated further on in this paper.

THE ANHYDRIDES OF KOLATANNIN AND THEIR DERIVATIVES.

By judiciously heating kolatannin several anhydrides may be prepared, as described below, the amounts of water driven off varying with the temperature and the duration of its application. The first anhydride of the series, that in which two molecules of tannin lose one molecule of water, and whose empirical formula is $(C_{16}H_{19}O_7)_2O$ is formed by heating kolatannin at 107°–110° to constant weight. This anhydride is darker colored than the tannin from which it is prepared and is less soluble in water. The second anhydride, by an oversight, we did not attempt to prepare. If we may judge by the temperature required for the preparation of the first, third and fourth members of the series, this one would result from heating the tannin a sufficient time at 120°–125°. Its theoretical formula is $C_{16}H_{18}O_7$. The third anhydride, $(C_{16}H_{17}O_6)_2O$, results from heating the tannin at 135°–140°. It is dark reddish-brown and nearly or quite insoluble in water. The fourth anhydride, $C_{16}H_{16}O_6$, is dark brown, insoluble in water, and is the result of heating kolatannin two hours at 155°–160°.

These anhydrides are all soluble in alcohol, and in solution of kolatannin of sufficient concentration.

FIRST ANHYDRIDE.

The method of preparation has been described in the preceding section. This is a yellowish-red body, soluble in water, in alcohol, and in dilute alkali solutions. The calculated loss of weight of the tannin in preparing this anhydride was 2.65 per cent. The observed loss of weight was 2.77 per cent. The body was subjected to combustion with the following stated results:

	Found.		Calculated for
	I.	II.	$(C_{16}H_{19}O_7)_2O$.
Carbon	58.27	58.39	58.18
Hydrogen	5.38	5.61	5.45
Oxygen	36.35	36.00	36.37

FIRST ANHYDRIDE OF TRIBROM KOLATANNIN.

The first anhydride of kolatannin was dissolved in water and precipitated by adding an excess of bromine, collecting the precipitate by filtration, and drying exactly as described in the method of preparation of tribrom kolatannin. It is soluble in the same media as tribrom kolatannin, is darker colored, is odorless and tasteless. Analysis of it proved to be an anhydride of tribrom kolatannin.

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{16}Br_3O_7)_2O$.
Carbon	33.61	33.80	33.86
Hydrogen	2.92	2.76	2.65
Oxygen	21.31	21.17	21.16
As silver salt.			
Bromine	42.16	42.27	42.33

FIRST ANHYDRIDE OF TETRABROM KOLATANNIN.

Prepared in the same way as tetrabrom kolatannin, using in this case the first anhydride of kolatannin. It is hardly to be distinguished from the other tetrabrom compounds in appearance or other sensible properties. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{15}Br_4O_7)_2O$.
Carbon	29.47	29.66	29.72
Hydrogen	2.37	2.21	2.17
Oxygen	18.57	18.42	18.58
As silver salt.			
Bromine	49.59	49.71	49.53

FIRST ANHYDRIDE OF HEXABROM KOLATANNIN.

This was prepared by adding a considerable excess of bromine to the alcoholic solution of the first anhydride of kolatannin, and separating the product in the usual way. It is dark red, tasteless, and has a faint odor of bromine; is insoluble in water, ether, and chloroform; soluble in alcohol and in dilute alkali solutions. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{13}Br_6O_7)_2O$.
Carbon	23.96	24.15	23.88
Hydrogen	1.64	1.69	1.49
Oxygen	14.61	14.57	14.93
As silver salt.			
Bromine	59.79	59.59	59.70

THIRD ANHYDRIDE OF KOLATANNIN.

The method of preparation has been described. The calculated loss of weight on heating the tannin was 7.96 per cent.; the observed loss was 8.16 per cent. It is a red-brown powder, odorless, nearly tasteless, but somewhat astringent. Soluble in alcohol and in alkali solutions, insoluble in water, ether, and chloroform. It was analyzed with the following stated results:

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{17}O_6)_2O$.
Carbon	61.72	61.80	61.54
Hydrogen	5.29	5.46	5.13
Oxygen	32.99	32.74	33.33

The bromine derivative of this anhydride and those of the fourth anhydride to be described further on in these pages, were prepared in a manner strictly analogous to those already described.

THIRD ANHYDRIDE OF TETRABROM KOLATANNIN.

A dark-brown, tasteless powder having a faint odor of bromine.

Insoluble in water, ether, or chloroform ; soluble in alcohol and in alkali solutions. The analytical results were :

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{13}Br_4O_6)_2O$.
Carbon	30.59	30.73	30.57
Hydrogen	1.99	1.81	1.91
Oxygen	16.32	16.19	16.56
As silver salt.			
Bromine	51.10	51.27	50.96

THIRD ANHYDRIDE OF HEXABROM KOLATANNIN.

A dark-brown, tasteless powder having a distinct odor of bromine. It is insoluble in water, ether, and chloroform ; soluble in alcohol and in alkali solutions.

The analytical results were :

	Found.		Calculated for
By combustion.	I.	II.	$(C_{16}H_{11}Br_6O_6)_2O$.
Carbon	24.04	24.39	24.43
Hydrogen	1.52	1.38	1.27
Oxygen	13.22	12.88	13.23
As silver salt.			
Bromine	61.22	61.35	61.07

FOURTH ANHYDRIDE OF KOLATANNIN.

This was prepared in the way previously described. The calculated loss of weight attendant upon its preparation from kolatannin is 10.61 per cent. The observed loss is 10.78 per cent. It is a dark-brown, odorless, tasteless powder, soluble in alcohol and in alkali solutions ; insoluble in water, in ether, and in chloroform. It was analyzed with the following stated results :

	Found.		Calculated for
	I.	II.	$C_{16}H_{16}O_6$.
Carbon	63.20	62.98	63.37
Hydrogen	5.09	5.16	4.95
Oxygen	31.71	31.86	31.68

FOURTH ANHYDRIDE OF TETRABROM KOLATANNIN.

A dark-brown, tasteless powder having a faint odor of bromine. Soluble in alcohol and in alkali solutions ; insoluble in water, in ether, and in chloroform. It was analyzed with the following stated results :

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{12}Br_4O_6$.
Carbon	30.91	30.68	31.02
Hydrogen	1.95	1.86	1.78
Oxygen	15.40	15.58	15.51
As silver salt.			
Bromine	51.74	51.88	51.69

FOURTH ANHYDRIDE OF HEXABROM KOLATANNIN.

A very dark-brown powder having a distinct odor of bromine. Soluble in the same media as the previously described bromine compounds. The analytical results are :

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{10}Br_6O_6$.
Carbon	24.96	24.79	24.71
Hydrogen	1.39	1.21	1.16
Oxygen	11.70	12.01	12.35
As silver salt.			
Bromine	61.95	61.89	61.78

PRODUCTS OF THE ACTION OF DILUTE ACIDS UPON KOLATANNIN.

When kolatannin is boiled with dilute sulphuric or dilute hydrochloric acid, it first passes into solution, and on continued boiling a reddish precipitate is formed, which becomes gradually darker the longer the heating is kept up. This precipitate is collected by filtration and well washed with water, in which it is insoluble. It is also insoluble in alcohol, and only partially soluble in alkali solution. Combustions of this substance, while giving fair duplicates for the same sample, indicate that its composition is not uniform and constant. Samples Nos. I and IV were prepared by boiling kolatannin with six per cent. sulphuric acid, in a flask fitted with a return condenser ; No. II by heating the tannin and dilute acid in a sealed tube ; No. III by boiling the tannin and dilute acid in a beaker with occasional addition of water to compensate for evaporation ; No. V by boiling caffein tannate with dilute acid in a flask fitted with return condenser ; No, VI is the result of combustion of a similar substance made and reported last year.[1]

	I.	II.	III.	IV.	V.	VI.
	Av. of 2.	Av. of 2.	Av. of 2.	Av. of 2.	Av. of 2.	Av. of 2.
Carbon	53.60	50.45	51.27	56.38	54.49	69.20
Hydrogen.	5.34	4.85	5.24	5.69	5.87	6.70
Oxygen	41.06	44.70	43.49	37.93	39.64	24 10

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[1] Knox and Prescott, 1896 : This Journal, 19, 89.

The aqueous filtrate was shaken out with ether, the residue after evaporation of the ethereal solution was redissolved in water and boiled a short time, which caused precipitation of the coloring-matter. Then filtering, it was again extracted with ether, and the operation repeated several times. At length the ethereal solution left a nearly colorless residue on evaporation, which responded to the tests for protocatechuic acid. Oak bark tannin also, when subjected to similar treatment with dilute mineral acids, yields protocatechuic acid.

Etti, Böttinger, and Löwe each mention the insoluble red body formed from oak tannin on boiling it with dilute acids and consider it an anhydride. They do not agree on which of the anhydrides it is, however. In fact, as already mentioned, there are many points in their work on tannins on which they were at variance. Etti[1] found gallic acid in the acid filtrate. Löwe[2] does not mention having examined the liquid for bodies other than sugar. Trimble,[3] however, whose work is the most recent, found protocatechuic acid as a by-product; in every case. He does not commit himself on the question of whether the dark brown, amorphous, insoluble substance, separated from tannin by the action of hot dilute acids, is an anhydride.

Whatever may be the relation of the body separated in this way from oak bark tannin to the original tannin, there does not as yet seem to us to be any good reason for considering the corresponding substance precipitated from acid solutions of kolatannin, an anhydride of kolatannin. The evidence thus far is against it. Boiling with dilute mineral acids is in general a means of hydrolysis, rather than anhydrolysis; we would therefore expect saponification instead of anhydride formation; as a matter of fact an aromatic acid—protocatechuic—has been found in the acid filtrate after collecting the insoluble red substance in question, while if the reaction were one of simple anhydride formation, no by-product should be present. If the red-brown substance were an anhydride of kolatannin, we would expect it to have a higher percentage of carbon than the original tannin, but in most cases combustion indicates a lower percentage of

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[1] C. Etti, 1884: *Ber. d. chem. Ges.*, 17, 1820.

[2] J. Löwe, 1881: *Ztschr. anal. Chem.*, 20, 208.

[3] H. Trimble: "The Tannins," II, p. 92.

carbon. We would also expect to find the body of uniform composition when prepared by similar means, but the analyses just mentioned show that it is very variable in content of carbon and hydrogen, while its appearance also varies from red to dark brown. At present no more can be said of this body than we have already said; *viz.*, that it is red or dark brown, amorphous, insoluble in water, in alcohol, in ether and in chloroform, only partially soluble in dilute alkali, and that it is a decomposition product obtained by boiling kolatannin, or caffein kolatannate, with dilute mineral acids.

Knebel's[1] "kolaroth" is a body obtained in the same way, except that he prepared it from caffein kolatannate, or as he supposed "kolanin," which was the alleged "caffein-bearing glucoside" of kola. Inasmuch as the free tannin of kola and that combined with caffein are identical, as will appear further on in these pages, it makes no difference which kind of it is used for this purpose. Knebel, however, worked with caffein tannate from the dried drug, while our work has been entirely on that from the fresh drug. He fails to give the figures of his combustion, but Hilger[2] reports the following stated results:

	Found.		Calculated for
	I.	II.	$C_{14}H_{13}(OH)_5$.
Carbon	63.09	62.65	63.16
Hydrogen	6.85	6.50	6.75
Oxygen	30.06	30.85	30.09

This calculated formula does not differ widely in carbon content from that calculated for the fourth anhydride of kolatannin, $C_{16}H_{16}O_6$.

	Calculated for $C_{16}H_{16}O_6$.
Carbon	63.37
Hydrogen	4.95
Oxygen	31.68

The hydrogen is rather high, however, which makes the oxygen correspondingly low. The results of an analysis of a body like the one Hilger analyzed might have been construed to indicate it as a member of the anhydride series, and inasmuch as he found a substance yielding analytical figures nearly coinci-

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

[2] A. Hilger, 1893: *Vierteljahr. f. öffentl. Gesundpflg.*, 25, 559.

ding with those just given for the fourth anhydride, it is perfectly reasonable to suppose that if we had made combustions of a larger number of samples, we might have obtained some results which could be used in support of the idea that these decomposition products of tannins are simple anhydrides of them. Our experience has taught us that the composition of this product is by no means constant with the means used in its preparation, and we saw no good reason for selecting the figures of one combustion from those of a dozen others presumably as near correct, merely to give support to a theory.

It is somewhat puzzling that Knebel and Hilger have found this body of uniform composition. As a matter of fact Knebel reports no figures in support of his claim, and Hilger reports only two combustions; still it would be supposed that before assigning a formula to a body of this kind a larger number of combustions would have been made, and with different samples. We do not feel justified in accepting the formula $C_{14}H_{18}(OH)_6$ proposed by them for it, nor do we at present possess sufficient data for a formula. We do not even know that it is not a mixture of two or more substances; and although we have not made any separation of them, it seems to us more probable than otherwise that it is a mixture in varying proportions of certain products of decomposition of kolatannin.

The suggestion of Knebel that the tannin of kola is an oxidation product of "kolaroth" according to the equation

$$\underset{\text{Kola red.}}{C_{14}H_{18}(OH)_6} + 4O_2 = \underset{\text{Gallotannic acid.}}{C_{14}H_{10}O_9} + 4H_2O$$

is untenable for the two-fold reason that we do not find kolatannin or this decomposition product of it to be so readily oxidizable as stated by him, and furthermore, the kolatannin is not identical with gallotannic acid, the formula of which he has given in the above equation as the formula of kolatannin, nor have we at any time found any gallotannic acid present in kola.

Knebel claims to have prepared a pure product of his kolaroth from the acetyl compound of it; and he further claims that the exact separation of kolaroth may be had by boiling his so-called "kolanin" (caffein kolatannate) with acetyl chloride, whereby an acetyl compound of "kola red" is formed, and is separated by pouring the mixture of acetyl chloride and caffein kolatannate into water. The acetyl compound of kola red is thus precipitated and the glucose and caffein pass into solution.

Let us examine this statement in the light of the recent knowledge of the chemistry of this interesting drug. First, as conclusively proven by us last year, the body called "kolanin" is not glucoside composed of caffein, glucose and "kola red," but is a combination of caffein with the tannin of kola, $C_{16}H_{20}O_8$ a and with anhydrides of that tannin, in varying proportions, depending largely on the method used to separate it from the drug. And it contains no glucose whatever, for we have since learned that the evidence of sugar found in it, and in free kolatannin last year, and reported in our paper, was due to the accidental presence of glucose from a faulty method of separation. Moreover, acetyl chloride does not effect an exact separation of the caffein from this combination. We prepared an acetyl derivative of caffein kolatannate exactly as done with the other acetyl products and analyzed it, with the following results :

Carbon	58.30
Hydrogen	4.85
Nitrogen	2.54
Oxygen	34.31

The nitrogen content corresponds to 8.79 per cent. of caffein. The caffein kolatannate from which this acetyl compound was made contained 6.53 per cent. nitrogen, corresponding to 22.6 per cent. caffein. Caffein was also found in the liquid from which the acetyl compound was filtered. The separation then is not complete and the acetyl compound is not a pure acetyl compound of caffein kolatannate, nor was it expected to be ; for as caffein kolatannate contains kolatannin and some of its anhydrides, both capable of forming acetyl compounds, it naturally follows that any acetyl derivative prepared will not be a simple derivative of one of the bodies, as stated by Knebel, but will be a mixture of acetyl derivatives of the tannin and its anhydrides, probably in the proportions in which they existed prior to the operation. With this understanding of it, it is difficult to see how Knebel could have prepared the pure and simple acetyl compound in the way stated by him.

THE QUESTION OF SUGAR PRODUCTION.

Results were obtained in our work last year which seemed to indicate that kolatannin was a glucosidal body. We wished to investigate this question more fully, and have done so in the following described way:

Kolatannin was dissolved in water and precipitated by lead acetate, and the collected lead salt washed with water for several hours; the lead salt was then decomposed by dilute sulphuric acid, the lead sulphate filtered out, and the acid filtrate boiled for four hours in a flask fitted with a return condenser. The acid strength was supposed to be about five to six per cent. The insoluble matter was filtered out, the acid removed by addition of barium carbonate, and the liquid again filtered; the coloring-matter was precipitated by lead acetate and the precipitate removed by filtration, after which the remaining lead acetate was removed by hydrogen sulphide. The filtrate was boiled to remove all the hydrogen sulphide, and made alkaline with a little sodium hydroxide solution. The liquid was still slightly colored. It was tested with Fehling's solution, and gave a red precipitate. A portion of it, made slightly acid with acetic acid, gave negative results when the osazone test was applied, and a control test made at the same time, using a little solution of grape sugar in addition, gave the well-known yellow precipitate. This showed that there was no interference with the phenylhydrazin test. Basic lead acetate solution was added, which precipitated the traces of coloring-matter in the solution, and, after removing the excess of lead, the solution was tested with Fehling's solution again, with negative results this time.

This experiment was repeated in a slightly different way, as follows: The well-washed lead salt of about one gram kolatannin was heated for two hours at 110° C. in a sealed tube with thirty cc. hydrochloric acid, the tube then opened and the contents filtered to remove lead chloride and the amorphous red decomposition product of kolatannin. The filtrate was red-colored and was shaken out several times with ether. The ethereal solutions were set aside for further examination. The filtrate was then boiled to remove the traces of ether, and exactly neutralized with sodium hydroxide, after which basic lead acetate solution was added until no further precipitation occurred, the precipitate removed by filtration, and the excess of lead salt precipitated as lead sulphate by cautious addition of dilute sulphuric acid. The solution was again filtered, made slightly alkaline with sodium hydroxide solution, and tested with Fehling's solution with negative results.

The importance of using basic lead acetate to precipitate the coloring is shown by the fact that if only normal acetate be used a small amount of coloring-matter is left in solution and gives a precipitate with Fehling's solution, while if basic lead acetate be employed, all the coloring-matter is removed and the copper solution is not affected.

In the ethereal solution, protocatechuic acid in small quantity was found, as stated in another part of this paper.

The experiments described having given negative results, the interpretation is made that kolatannin is not a glucoside and that the evidence we had to the contrary last year was caused by the incomplete removal of the glucose of the plant, due to a faulty method of separation for the tannin, since discarded by us.

It is significant that this result places kolatannin in a category and gives it a character essentially different from the character of caffeotannic acid, as understood by the chemical world from the report of Hlasiwetz.

ACTION OF FUSED ALKALI.

Fifteen grams kolatannin were fused for two hours with potassium hydroxide, the fused mass cooled and acidulated with sulphuric acid, after dissolving it in water. Without filtering the mixture, it was shaken out with ether, the successive portions of this solvent united and evaporated. The residue was distinctly crystalline, the crystals assuming an arborescent form for the most part. This residue was purified several times by recrystallization, after which it was dissolved in water and submitted to tests. Protocatechuic acid and phloroglucin were found to be present.

ACTION OF HEATED GLYCERINE.

One gram kolatannin was heated with three cc. glycerine in a porcelain capsule for twenty minutes at 195°–200°. Fifty cc.

water were then added and the mixture was shaken out with ether. The residue left on evaporating the ethereal extract was found to contain protocatechuic acid, by chemical tests.

KOLATANNIN FROM CAFFEIN KOLATANNATE.

It has thus far not been found practicable to substitute other means of separation of this tannin from its caffein salt for the treatment by lead hydroxide, although the disadvantages of this agent are fully appreciated .It is, however, much to be preferred to lead acetate in this particular case, as lead acetate precipitates some caffein tannate along with the lead tannate, and the resulting tannin is apt to be contaminated accordingly.

PREPARATION OF THE SAMPLE.

The caffein tannate is dried on porous plates and finely powdered and sifted ; it is then washed with a mixture of ether and chloroform to remove any traces of adhering fat, and dried again. It is then washed with ice-cold water by agitation, drained and washed again until the washings give no precipitate of silver chloride on being treated with solution of silver nitrate acidulated with nitric acid. The caffein kolatannate is now dissolved in dilute alcohol, making a very concentrated solution ; this solution is filtered and poured into ten or twelve times its volume of ice-cold water, whereupon the caffein compound is reprecipitated. It is collected by filtration, drained well and redissolved in dilute alcohol, using gentle heat on the water-bath. Freshly precipitated lead hydroxide triturated to a smooth paste with warm alcohol is now added in excess and the mixture warmed on the water-bath for a few minutes, with stirring. The precipitate is now allowed to subside, and if sufficient lead hydroxide has been added the supernatant liquid will be colorless or faintly straw-colored, while the precipitate of lead kolatannate mixed with lead hydroxide forms a compact magma at the bottom of the container. The liquid, which contains kola alkaloids, is poured off and the precipitate is washed repeatedly with dilute alcohol, first by decantation and then by filtration, until several cc. of the filtrate evaporated in a porcelain dish leave no perceptible residue and give no test for caffein. The precipitate is compact and is much more readily washed than that given by lead acetate. It is then suspended in dilute alcohol and treated with hydrogen sulphide until the tannin is all liberated. The lead sulphide is then filtered out and washed with dilute alcohol until the washings are colorless. The filtrate is concentrated by distillation *in vacuo* to small volume, and eight or ten volumes of water are added. This precipitates a reddish mass, a mixture of certain anhydrides of kolatannin, which is filtered out and set aside. The filtrate containing tannin together with dissolved anhydrides is now shaken with ether once or twice, and the washings thrown away. Then ethyl acetate is added and the tannin separated and purified in the way already described, under the method of preparation of kolatannin.

This "combined" kolatannin, although not quite as light-colored as the "free" tannin, shares all its reactions and solubilities.

COMBUSTION OF "COMBINED" KOLATANNIN.

It was analyzed by combustion and the following results obtained, which coincide very closely with those resulting from combustion of the "free" tannin:

	Found. I.	 II.	 III.	Calculated for $C_{16}H_{20}O_8$.
Carbon	56.53	56.78	56.42	56.45
Hydrogen	5.68	5.79	5.74	5.90
Oxygen	37.79	37.43	37.84	37.65

THE PENTACETYL COMPOUND.

This was prepared in the same manner as the other acetyl derivatives and is identical with the pentacetyl compound of "free" kolatannin, heretofore described.

The analytical results are as follows:

By combustion.	Found. I.	 II.	 III.	Calculated for $C_{16}H_{15}(C_2H_3O)_5O_8$.
Carbon	56.75	56.44	56.61	56.72
Hydrogen	5.54	5.41	5.31	5.47
Oxygen	37.71	38.15	38.08	37.81
By saponification.				
Acetyl	38.27	38.53	38.68	39.09

THE BROMINE COMPOUNDS.

These were prepared in identically the same way as the corresponding ones of the free tannin. In appearance and properties there seems to be no difference between these and those of free kolatannin, and for the sake of brevity we shall omit further description and confine ourselves merely to the statement of analytical results in each case.

TRIBROM DERIVATIVE.

By combustion.	I.	Found. II.	III.	Calculated for $C_{18}H_{17}Br_3O_8$.
Carbon	33.09	33.25	....	33.28
Hydrogen	2.84	2.98	....	2.95
Oxygen	22.50	22.13	....	22.19
As silver salt.				
Bromine	41.57	41.64	41.74	41.58

PENTACETYL TRIBROM DERIVATIVE.

(Formed by acetylizing the tribrom derivative.)

By combustion.	I.	Found. II.	III.	Calculated for $C_{18}H_{12}Br_3(C_2H_3O)_5O_8$.
Carbon	39.64	39.82	....	39.65
Hydrogen	3.47	3.61	....	3.44
Oxygen	26.44	25.94	....	26.43
As silver salt.				
Bromine	30.45	30.63	30.71	30.48
By saponification.				
Acetyl	26.66	26.97	....	27.32

TRIBROM PENTACETYL DERIVATIVE.

(Formed by brominating the pentacetyl derivative.)

By combustion.	I.	Found. II.	III.	Calculated for $C_{18}H_{12}Br_3(C_2H_3O)_5O_8$.
Carbon	39.88	40.15	....	39.65
Hydrogen	3.26	3.45	....	3.44
Oxygen	26.34	25.74	....	26.43
As silver salt.				
Bromine	30.52	30.66	30.57	30.48
By saponification.				
Acetyl	26.78	27.04	....	27.32

TETRABROM DERIVATIVE.

By combustion.	Found. I.	II.	Calculated for $C_{18}H_{16}Br_4O_8$.
Carbon	29.13	29.35	29.27
Hydrogen	2.29	2.46	2.44
Oxygen	19.86	19 30	19.52
As silver salt.			
Bromine	48.72	48.89	48.77

PENTACETYL TETRABROM DERIVATIVE.

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{11}Br_4(C_2H_3O)_5O_8$.
Carbon	36.12	36.33	36.03
Hydrogen	2.78	2.96	3.01
Oxygen	24.33	23.78	24.02
As silver salt.			
Bromine	36.77	36.93	36.94
By saponification.			
Acetyl	24.64	24.78	24.83

PENTABROM PENTACETYL DERIVATIVE.

(Formed by brominating the pentacetyl derivative.)

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{10}Br_5(C_2H_3O)_5O_8$.
Carbon	33.23	32.98	33.02
Hydrogen	2.62	2.72	2.65
Oxygen	21.71	21.78	22.01
As silver salt.			
Bromine	42.44	42.52	42.32
By saponification.			
Acetyl	22.49	22.26	22.75

HEXABROM DERIVATIVE.

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{14}Br_6O_8$.
Carbon	23.85	23.68	23.59
Hydrogen	1.77	1.83	1.72
Oxygen	14.76	14.72	15.73
As silver salt.			
Bromine	59.62	59.77	58.96

TETRACETYL HEXABROM DERIVATIVE.

	Found.		Calculated for
By combustion.	I.	II.	$C_{16}H_{10}Br_6(C_2H_3O)_4O_8$.
Carbon	29.66	29.92	29.33
Hydrogen	2.46	2.29	2.25
Oxygen	18.73	18.55	19.55
As silver salt.			
Bromine	49.15	49.24	48.87
By saponification.			
Acetyl	16.48	16.73	17.52

KOLATANNIN ANHYDRIDES OBTAINED FROM CAFFEIN KOLATANNATE.

The insoluble, reddish-brown precipitate, formed on addition of cold water to the tannin solution after the separation of the tannin from its lead salt, as mentioned in the method of preparation of "combined" kolatannin, was well washed and dried at 100°. Combustion of this showed a carbon percentage intermediate between that of the second and third anhydrides of kolatannin.

As the solubilities of the anhydrides appear to be about the same, we were unable to effect a separation of them, and for this reason we heated the (supposed) mixture at 135°–140° to constant weight, in order to convert it all if possible into the third anhydride. It was then subjected to another combustion with the following results:

	Found.		Calculated for
	I.	II.	$(C_{18}H_{17}O_6)_2O$.
Carbon	61.27	61.46	61.54
Hydrogen	5.28	5.43	5.13
Oxygen	33.45	33.11	33.33

These results agree very well with the calculated formula and with those obtained by combustion of the third anhydride of so-called "free" tannin of kola.

The identity of the tannin of caffein tannate with the free tannin having been positively settled by the analyses already given, and by one or two evidences yet to be stated, it was deemed unnecessary to make a further comparative study of its bromine derivatives.

ACTION OF DILUTE ACIDS.

The experiments described as having been performed on "free" kolatannin, were repeated on this kind of kolatannin also with parallel results. The same is to be said of the products of fusion with potassium hydroxide and of heating with glycerine.

FURTHER INDICATION OF MOLECULAR CONSTITUTION.

Whether there be a carboxyl in kolatannin or not, remains open to question and to further determinations. Its moderate acid character is not inconsistent with the negative polarity of a polyhydric phenol, reinforced by the oxygen of an anhydride group, yet destitute of entire carboxyl. Our numerous analyses of kolatannin and its several derivatives as described in this paper, are in close agreement with a polyhydric phenol anhydride carrying two methyl groups and one methoxy group, but standing altogether as a di-addition benzene derivative, two such benzene nuclei pairing together in an ester-like anhydride. On this supposition, a monomethyl dihydrogen trihydroxybenzoic acid has paired ester-wise with a monomethyl methoxydihydrogen trivalent phenol. The di-additive benzene constitution is not improbable among plant constituents of this class, finding parallel in the additive structure of the terpenes. If we admit the hypothesis of the molecular formula above indicated, we must predicate that one of the six hydrogen atoms joined to benzene-carbon is in position somewhat unfavorable to its bromine substitution, and when brominated quite preventing the acetylization of one of the hydroxyls. Finally, there remains no proof as to the distribution of the hydroxyl and methoxyl groups between the two benzene nuclei.

$$\begin{array}{lr} C_6H_3(CH_3)(OH)_3 & CO \\ & | \\ C_6H_3(CH_3)(OCH_3)(OH)_2 & O \end{array} = C_{16}H_{20}O_8.$$

AS TO CHEMICAL BEARINGS UPON PHARMACOLOGY.

Finally, it must be admitted that the stable caffein compound of an acid of such marked chemical individuality as that of kola-tannin can by no means be assumed to have the same physiological effects as free caffein. And this subject is one of pharmacological importance for the caffein of all the beverage plants.

We are under many obligations to Mr. James Heggie, B.S., for his very efficient assistance in preparing and analyzing the various derivatives of tannin reported in the preceding pages.

DISCUSSION OF RECENT ASSAY METHODS.

Within the past year two processes for the assay of kola have been offered, which seem to us not to be free from objections. We give below the essential points of these methods without entering into unimportant details.

METHOD OF JEAN.[1]

The author ascribes his plan of procedure in part to MM. Chodat and Chuit. He boils the dried and powdered drug with "milk of lime," dries the whole in an oven and powders it again. This powder is exhausted with chloroform, which

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

removes the free alkaloids. The chloroform is evaporated to dryness, the residue dissolved in hot water and filtered. The filtrate is evaporated to dryness and weighed as caffein.

For "kolanin:" the drug, after treatment with chloroform, is extracted with alcohol, the alcohol evaporated from the percolate and the soft extract remaining is dissolved in boiling water, which solution, after cooling, is filtered. The insoluble matter remaining on the filter is dried in an oven and weighed as "kolanin."

The most serious defects in the above-described method are (1) the boiling with "milk of lime," (2) the prolonged heating necessary to dry the drug after that treatment, (3) weighing the caffein instead of estimating it with Wagner's reagent volumetrically, (4) the means employed to separate so-called "kolanin," (5) estimating caffein kolatannate ("kolanin") by weight as such, instead of by its caffein content.

(1) Boiling with "milk of lime," or other aqueous alkalies, tends to decompose caffein—a fact too well known to require further comment. This treatment also tends to liberate caffein from its tannate, and thus prevents an exact determination of the proportion of "free" to "combined" alkaloid that originally existed in the drug. The starch of kola, amounting to nearly forty per cent.,[1] causes the drug to become of a jelly-like consistence when boiled with aqueous liquids, and when dried the mass is very hard to powder, and even when powdered the condition of it is well calculated to resist the penetrating action of the solvent, and thus cause incomplete extraction.

(2) The gelatinous condition of the drug after boiling with "lime water" renders it hard to dry completely, and as very prolonged heating is necessary, there is danger of loss of caffein by sublimation.

(3) We do not consider that the alkaloids of kola are sufficiently pure when removed in this, the usual manner, to be weighed as such; Gomberg's[2] volumetric method gives more accurate results.

(4) If any caffein kolatannate escapes decomposition during the first part of the assay, which will occasionally happen, it is

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[1] Knox and Schlotterbeck, 1895: *Proc. Am. Pharm. Assoc.*, 334.

[2] M. Gomberg, 1896: This Journal, 18, 331.

removed by the extraction with alcohol. But as it is somewhat soluble in water and more soluble in aqueous solutions containing tannin, the directions to collect and weigh the portion left undissolved by water will generally be found superfluous, for the small amount of it present will generally pass into and remain in solution. Dieterich[1] had this experience, and met with nothing but disappointment in trying to estimate caffein kolatannate by this method.

(5) Caffein kolatannate has been shown to be a body of somewhat variable composition, with a caffein content ranging from nineteen to twenty-five per cent. Inasmuch as the value of kola as a stimulant probably depends primarily on its percentage of caffein, and as the weight of its caffein compound indicates only approximately the amount of its combined caffein, there would not seem to be any reason for attaching much importance to the weight of this compound, if a very precise valuation of the drug is desired. We hold that a direct estimation of the caffein of this compound is preferable and at least as expeditious.

METHOD OF CARLES.[2]

Ten grams kola, one gram calcium hydroxide, and twenty grams eighty per cent. alcohol, are mixed together and dried on the water-bath until the weight is reduced to fourteen grams. The mixture is then powdered and transferred to a 100 cc. flask containing thirty-five cc. of a mixture of 100 parts chloroform and twenty parts alcohol, and heated one hour on a water-bath. After filtration the residue is extracted next with twenty cc. of the same solvent, and finally with ten cc. The united extracts are evaporated to dryness, and the residue taken up with ten cc. boiling water containing four or five drops of one per cent. sulphuric acid, then with six cc., and finally with five cc. The solutions are united, filtered, evaporated to constant weight, and weighed as caffein.

For "kolanin," which the author recognizes as caffein kolatannate, another sample of the drug is taken and extracted with water to remove the caffein and other soluble constituents. The drug is next extracted with seventy per cent. alcohol, the extract evaporated to dryness, transferred to a filter, washed

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[1] Dieterich, 1896: Chem. Centrbl., 2, 675.

[2] P. Carles, 1896: *J. Pharm. Chim.*, 16, 104.

with cold water, then dried at gentle heat and weighed. If it be desired to estimate the alkaloids of this compound, one gram "kolanin," one gram calcium hydroxide, and three grams chalk with a little seventy per cent. alcohol are mixed together and evaporated on the water-bath to about six grams, and extracted with alcoholic chloroform in the manner already described.

The objections to this method are in part among those already mentioned in the discussion of Jean's method. The use of lime or other alkalies in the assay of a caffein-bearing drug is to be deprecated. The solvent used is not a proper one for the reason that sufficient alcohol is present to extract other constituents in addition to the alkaloids, which are not removed from the caffein during the subsequent treatment of the residue, and which, when weighed with the caffein, lead to erroneous results. Moreover, the manner of applying the menstruum is inconvenient, does not insure complete extraction, and is in no way preferable to the ordinary extraction by the use of Soxhlet's apparatus. The addition of the sulphuric acid is unnecessary and does not add to the purity of the final product, which is dark-colored and very plainly impure. The objection to weighing a final residue as caffein, finds especial application in this method.

As the properties of caffein kolatannate had not been made known very generally at the time of publication of these methods, there is some excuse for the assumption of both these writers that it is wholly insoluble. Carles has proceeded on this hypothesis in directing the drug to be extracted with cold water to remove the water-soluble constituents before exhausting it with alcohol to remove the caffein compound, but inasmuch as caffein kolatannate is not only somewhat soluble in water, but considerably more soluble in solutions of caffein and of tannin, the extraction of kola by water will remove a considerable amount of it. The same is to be said of the final washing of the caffein compound with water, which is quite inadmissible in quantitative work. Carles seems to have recognized the uncertain value of gravimetric determinations of caffein kolatannate, and is to be commended for offering an alternate method providing for its valuation according to the amount of its alkaloids.

At our request, Mr. James W. Cobb, Ph.C., has assayed a sample of dried kola by each of the foregoing methods and by the method adopted by us last year,[1] after thoroughly familiarizing himself with them by preliminary work.

	Method of Jean. I.	Method of Jean. II.	Method of Carles. I.	Method of Carles. II.	Knox and Prescott. I.	Knox and Prescott. II.
Caffein by weight	1.93	1.79	2.16	2.33	....	....
The same titrated	1.83	1.71	1.87	1.98	1.96	1.88
"Kolanin"	none	none	2.89	3.17	....	....
Combined alkaloids	....	....	....	....	1.62	1.61
Alkaloids of "kolanin"	....	....	0.59	0.69	....	....
Total alkaloids	1.83	1.71	2.46	2.67	3.58	3.49

It will be seen that the impurity of caffein separated by Jean's method amounts to 4.84 per cent., and that of the caffein by Carles' method amounts to 14.25 per cent., which was determined by titrating the caffein with Wagner's reagent, after Gomberg's method.

Both give very low results as compared with those obtained by our own. They are tedious and otherwise unsatisfactory, in addition.

Further notes on the assay of kola are in preparation.

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ANN ARBOR, MICH., AUGUST, 1897.

NOTE.

A Correction.—In the September number of this Journal the author, together with Mr. F. Thompson, published an article entitled "A Preliminary Thermochemical Study of Iron and Steel," in which they give the results obtained on a number of samples of iron and steel of different composition and which had been subjected to varying heat treatments. On taking up the work again this fall and reviewing critically the results of last year's work, I find that there are at least two serious errors in the results that I wish to explain now in order to prevent anyone else from being misled by the conclusions drawn from the results as stated in the article. The statement that the loss by radia-

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[1] Knox and Prescott, 1896: This Journal, 19, 73.