

THE ASTRINGENT PRINCIPLE OF MATÉ.*

BY JOSIAH C. AND BERTHA L. DR.G. PEACOCK.

The article commonly known in the commerce of this country as maté or yerba maté consists of the leaf and leaf stalk of *Ilex Paraguayensis*, a species of holly indigenous to a great part of that section of South America, east of the Andes, south from the Amazon to below the mouth of the LaPlata.

The material itself is called yerba or caa by the natives, while the vessel in which the infusion of it is prepared is known as maté; and because of its use as an article of diet, the material of commerce is frequently spoken of as Paraguay tea. But the names yerba maté and maté for the leaf seem to have been generally adopted outside of its habitat.

On account of the presence of caffeine in maté, for over a hundred years past it has attracted the attention of chemists and pharmacists the world over. The names of many well-known plant analysts are associated with the early consideration of maté as a possible article of diet and use, as in the cases of tea and coffee; especially has this been true in European countries. In that part of the continent where it grows, it is the table and social beverage of the rural districts. It is not so generally consumed in the large cities of its native land; but the comparative consumption as relates to tea is many times in favor of maté.

In the work of the first investigators effort was directed toward a study of the tannin or astringent principle; but no examination of this constituent has been made in the last fifty years that would warrant a greater credence than the statements of the times preceding these. With all possible deference to those who have reported on the tannin of maté, it must be said that their conclusions are scarcely justified by their work, for the application of reagents to this principle surrounded as it is in infusion by a cordon of closely adherent natives of the leaf is a method of examination too archaic for present-day demands. So that while much has been written practically nothing has been done during the last fifty years to carefully isolate and purify this principle. But the literature of the subject has been freely copied and endorsed. Most of the analyses of maté were published prior to 1887, although the last of general quotation was contributed by Kuntz-Krause in 1893. Attempts were made by some to extract the tannin, but the majority of those who have examined it contented themselves with results from reagents applied to the decoction of the leaf. From 1836, when Trommsdorff reported the presence of tannin and of the other substances he found, this astringent principle has been playing hide and seek with all who have pursued it, or they with it, for most have unwittingly discarded or destroyed it while they followed the phantom of hope that it was still ahead and yet to be overtaken. Indeed it is difficult to understand how one could be earnestly concerned with work on this substance without acquiring a better impression of it than is commonly recorded. In the article published in *Archiv der Pharmacie*, 1893, page 613, Kuntz-Krause has splendidly compiled the bibliography of maté. And he has done one other valuable service, even though involuntarily, for in substantiating in the main the results of the investigators who preceded him (especially the conclusions of Arata as referred to later) he has clearly shown that what he assumed to be maté tannin was not that substance. Among the later writings which have cited Kuntz-Krause and thus given his results

* Read before Pennsylvania State Pharmaceutical Association meeting, 1922.

extended authenticity is a paper by McKendrick and Harris in the *Pharmaceutical Journal* (British) July 16, 1898. But the theme of this text partakes more of preparation for a psychological acceptance of certain properties attributed, though rather contradictorily, to maté with the view to its adoption for beverage purposes than it does of any thought of adding definite knowledge.

The statement by Arata that "maté tannin requires 73.66 volumes of alcohol" to dissolve it proves beyond all doubt that he never had the substance which he sought, for it instantly dissolves in any amount of alcohol; and, likewise, does it invalidate his belief that (since he found the material upon which he worked to yield on dry distillation pyrocatechin and resorcin) the substance he thought was maté tannin "must be classed in the group of oxyphenylpropionic acid," while the abandon with which Kuntz-Krause acquiesced in this and in practically everything else which had been previously said about maté tannin demonstrates most effectively that his results cannot be accepted as dependable.

A fairly concise statement of the constituents which have been reported for maté is given in Maisch's "Organic Materia Medica" as follows: "Tannin, 10-16%; caffeine, 0.2-1.6%; little volatile oil and stearopten, wax, glucoside; proteids about 8%; ash 4 to 8%." Cellular and ligneous matter, resinous extractive and chlorophyl, the last in abundance, should be mentioned as known constituents, while carbohydrates of the sugar groups have been reported as present by some of the investigators. The statement by Maisch appears to be based on an analysis by Robbins (*Amer. Jour. Pharm.*, 1878, p. 273); while this work was of practical value as a contribution to the knowledge of the composition of maté, the process by which he determined the tannin would fail to give a pure product, and, therefore, not an accurate result.

Very elaborate plant analyses of maté were published by Peckolt in *Zeit. d. allg. Öster. Apot. Verein* and abstracted in the *Pharmaceutical Journal* (British) for August 18, 1883, and none more clearly show the mental attitude of the plant investigator of 1860-1865 (the period wherein they were made) toward the results to which they addressed themselves. In the *Pharmaceutical Journal* for May 29, 1886, Dauber reports a proximate analysis of maté and states that in an estimation of the tannin he found 5.22%; the determination was by the Procter (hide powder) method. From the effect in the mouth when maté is chewed, this proportion would appear to be nearer correct than 10-16% as given by Robbins or 12.5% as reported by Kuntz-Krause.

The vagaries of all this literature led to the work which we have undertaken. The isolation of the tannin for positive identification has been our sole object; little thought or effort has been put on any other constituent.

The result of this work has clearly shown that the astringent principle of maté is a phlobaphene which enters solution in water because of its existence in the leaf in combination with caffeine or allied substances, or by formation of such soluble effect upon treatment with water in preparing the decoction. That this principle actually goes into solution in water has of course been known as long as maté has been known, but why it does so was learned for certainty only after this work had been completed.

Boiled with water maté yields a dark amber or dirty greenish, fluorescent decoction, which upon concentration very materially deepens or darkens in color, as

though from the formation of a tarry substance that rises as a scum and appears related to and to contain chlorophyl originally present or as a product of some progressive change during the application of heat. Much deposition takes place in the form of an impalpable precipitate. The development of this black and black-green color and substance was prevalent throughout the course of this work, when a watery solution was evaporated; some features of a similar nature will be noted as we proceed.

A filtered decoction, which has been evaporated to about one-fourth of its original bulk, is a dark amber liquid possessing all of the peculiar odor and taste of maté. The taste is primarily aromatic with subsequent sensation of astringency; the properties are marked when the leaf itself is chewed. The effect of the decoction on blue litmus paper was strange in that it destroyed the color as though by a bleaching action. Egg albumen produced a considerable precipitate in the cold decoction; and within an hour the mixture was observed to be taking on a gradually deepening color of emerald-green shade that in twenty-four hours was extremely advanced. This was a highly interesting behavior, especially so since during the time it was progressing a similar effect had been observed in the use of hide powder as hereinafter detailed.

The decoction of maté was not precipitated on addition of acetic acid, but was so disturbed by hydrochloric and sulphuric acids; in the case of hydrochloric acid the effect was to separate the tannin-like substance from its caffeine association with consequent deposition of the former; while with sulphuric acid not only did all of this occur but the calcium salts in solution were also thrown out as insoluble sulphate, thereby increasing this precipitate in quantity compared to that from hydrochloric acid; the same effect was observed when alum was added to note its action in connection with the gelatin test. Indeed, acetic acid actually cleared up whatever slight opalescence might be present in the decoction. Gelatin renders maté decoction turbid, but only so on long standing. If however a few drops of acetic acid are added a dense turbidity instantly appears. McKendrick and Harris (*loc. cit.*) state that maté tannin neither tans hides nor precipitates gelatin, to which believed features they attribute much importance for their purpose. Ferrous salts produce slight yellowish green colors in the decoction and a greenish white precipitate characteristic of such insoluble ferrous formations. The cold decoction gave with ferric chloride a dark slate-green colored precipitate; with lead acetate a heavy granular, lemon-yellow precipitate. As to indications of permanency for the decoction, even upon standing for several days but little change was observable.

When hide powder was macerated in the clear decoction, it was found to almost completely remove all color, taste and odor, and did remove all cause of the peculiar effect on blue litmus paper; the resulting liquid had only the faintest reaction with ferric and lead salts. This behavior is well worthy of careful notice. It was in connection with this treatment of the decoction with hide powder that an unusual phenomenon was observed—the nearly white ball of hide powder from which the detannated liquid had been expressed was found after a few hours to have acquired a decidedly green color on its surface, the same developing inwardly with an orderly progression until at the end of a day's time the entire mass had become so colored.

The decoction yielded the tannin-like substance to acetic ether when shaken with that solvent. Both acetone and acetic ether applied directly to maté extracted the astringent principle. Separate portions of the tannin-like substance were removed by the use of each of these solvents in addition to the lots extracted with water. In each instance the particular lot of the substance was carried through the processes of purification both for its final identification and to compare the value of methods. It is doubtful if any of the methods of extraction or of solvents in such use, with the view of obtaining the substance for complete purification, have any advantage over the treatment with hot water, as in making the decoction; certainly none when care and cost of solvents are considered. The water exhausted material displays to the taste and sight as complete removal of desirable constituents as does that from treatment with any other solvent. The maté after extraction with any solvent looks but little different than it did before such treatment (as evinced by the samples* shown herewith) but the taste and smell will at once detect the absence of the peculiar constituents.

If the name maté were conjured into a fancy of inseparable comradeship of the native and the vessel in which daily he so frequently prepares his beverage, this fidelity of companionship could full well be continued as the experience of the constituents during his or others' attempts to separate them; for after all soluble matter appeared to be removed from a given lot of maté with a certain solvent, the effect of the next solvent was as though the door of another room with identical furnishings had been opened. A notable instance of this behavior was when a lot had been exhausted with chloroform, the latter removed from the marc, and acetic ether applied, when afresh came what was believed to have been taken out; while water subsequently applied extracted a considerable amount of color and astringency. This experience brought to mind the manner of the native in preparing the infusion to be drunk; and, so far as the slow extraction of the soluble matter is concerned (but no further), justifies his custom—he makes three or four such treatments of the leaf with water in the vessel, and these he offers his guests in turn through the same tube (called *bombilla*) to their evident gratification of both appetite and propriety.

The writers secured by use of each of the solvents mentioned material that indicated tannin, but time and again this substance was mysteriously lost to well tried methods of purification, and of which treatments solution in water has always been demanded as of first importance in proof of purity. Failure succeeded failure until at last it was thought there might be a very closely adhering matter which enveloped the tannin and prevented its solution in water; but this was not found to be the reason for its strange conduct, which later was understood. However the tenacity with which the caffeine accompanied the astringent principle was not only noteworthy because troublesome, but gave rise to the thought disposed of in the preceding sentence. What in its absence would have given a handsome puffed product of the tannin-like substance in a vacuum flask, in the presence of even a minute trace of caffeine refused to rise above a varnish film. In all such cases caffeine crystals were plainly visible in the mixed residue when the acetic ether by which the tannin had been removed from a water solution was recovered;

* Samples were shown by the authors at the meeting.

and this caffeine, as we later learned, was the reason for the presence of any tannin in any aqueous liquid. To free the residue of these traces of caffeine it was mixed with small pebbles or sand as a means of effecting greater surface contact for such solvents as would normally dissolve caffeine but not tannin; chloroform was then applied in numerous successive portions with thorough trituration. After this treatment, the chloroform adhering to the residue was vaporized, and the tannin-like substance taken out by solution in acetic ether or in a mixture of alcohol and ether; both of which upon removal (in vacuo) left the tannin-like substance in the form in which it is here shown.* Several repetitions of this treatment were required to completely eliminate the caffeine, wax, chlorophyll, resinous extractive and other constituents which so persistently adhered. From the time the tannin-like substance had been removed from the decoction by acetic ether, up to this stage, indications of an eventually pure product had been gradually growing better. But the substance in its now isolated form had not been subjected to the presence of water, so the real surprise of the work was yet in store both as surprise and work.

The writers had never known the fluffy, porous product invariably obtained at this juncture in the past to be other than almost entirely soluble in water, although their experiences with tannins during the last few years has inclined them more and more to be prepared for the unusual, an attitude which here stood them well for in this instance the exact reverse was to be true; the tannin from maté was found to be apparently totally insoluble in water, hot or cold. However, the expectation of obtaining a water-soluble tannin still seemed positively warranted by the circumstance that the substance had been originally extracted from maté by water. This behavior was therefore at once accepted as the best evidence that the tannin was still contaminated with resinous or waxy matter, or both, which served to water-proof it; and much effort was put forth to remove these interferences. Over and over the substance was dissolved in alcohol and this solution attenuated with ether to the limit of dilution where the mixture of solvents would retain most known tannins (but precipitate certain vague extractive substances), but all of this was to no avail in changing the disposition of the tannin towards its solubility in water. The precipitates referred to in the preceding sentence were very closely scrutinized to prevent possible discard of the principle, and it is proper to say now that they were indeed infinitesimal in their total quantity; they were undoubtedly the result of some change in the tannin-like substance. These treatments failing to yield a water-soluble tannin, repeated separate triturations of the solid material in succession with ether, chloroform and benzol were made to remove what they might find as vitiators of the looked-for solubility. But the unusual in tannin had again been found, and a little deliberation, to which we now felt entitled, followed by tests proved the completion of the task before us, in this way; pure caffeine and the tannin-like substance were together dissolved in alcohol, the solution evaporated to dryness, and the residue treated with water, in which it was found to be readily and speedily soluble. While to try out the tannin in the most natural of its situations, this test was repeated using, instead of extraneous purified caffeine, the crudely improved article of this nature that had been separated in the work, when again the tannin dissolved both under the conditions de-

* Exhibited by authors at meeting.

tailed for the tests and also by contact with this crude caffeine in the presence of water without the intervention of alcohol. And from these solutions acidification with hydrochloric and sulphuric acids caused immediate separation of the tannin-like substance. Here then was the reason for solubility at the start and insolubility after perfect purification. It might here be recalled that gallotannic acid at first precipitates caffeine from its solution, but on continued addition redissolves the precipitate. By incineration of a portion in a platinum crucible the substance was proven free from all inorganic matter.

In appearance the substance so nearly resembles the official gallotannic acid of the United States Pharmacopoeia, that it would easily be mistaken for it; but it has that strikingly characteristic difference of insolubility in water; 1000 parts of water boiled with one part of the substance for half an hour failed to dissolve this minute proportion beyond the degree required to barely tint the liquid with the slightest yellowish tinge. It is readily and very freely soluble in alcohol (much more freely than the proportionate volume of 73.66 reported by Arata, indeed it requires no more than 0.7366); soluble in mixtures of alcohol and water, even those weak in the former agent. As inferable from preceding statements, it is insoluble in chloroform, strong ether and benzol, likewise in petroleum benzin; but soluble in mixtures of alcohol and ether, even to as low as ten percent or less of alcohol. Also, soluble in acetic acid, 36%, acetone and glycerin, without the need of heat. The odor of the substance, while tannin-like, suggests its origin, and though insoluble in water it strangely dissolves sufficiently in the saliva to thus again give indication of its source. It has pronounced sialagogue properties, as has the leaf. Its solution in alcohol gives with ferric chloride a dark green color and precipitate, without any evidence of blue whatever; with lead acetate a heavy canary-yellow granular precipitate. Though insoluble in water, in the presence of this medium acidulated with sulphuric acid the substance quickly and abundantly reduces potassium permanganate. Boiled with water both under reduced pressure and under that increased from confined steam, no appreciable change was effected in either the solubility or other observable features. The action of water acidulated with sulphuric acid is particularly interesting since such treatment of known tannins soluble in water results in the formation of other substances; no such change occurs here, however, for even under high magnification the substance insoluble from the start is found unaltered after hours of active boiling. The substance is already what this treatment usually produces.

Sodium carbonate solution instantly dissolves the substance with production of a golden yellow liquid; bicarbonate of soda solution showed but slow action towards dissolving the substance, which under the influence of its surroundings was rapidly altered in color as though by action of atmospheric oxygen; but when warmed (and thereby converted into carbonate) the solution dissolved such portion of the material as had not been altered as described. Sodium hydroxide solution ($\frac{N}{10}$ and weaker) immediately dissolved it. It is soluble in the weakest ammonia solution, giving a yellow color to the liquid, which was both decolorized and precipitated by excess of hydrochloric acid. Baryta water and lime water slowly dissolved it, but not without the alteration referred to and its attendant loss of material. All of these solutions displayed the yellow color given by sodium carbonate; all of them were reprecipitable by acidification with mineral acids,

and these precipitates under high magnification revealed the peculiar granular structure of the original substance. Lime water acted much more satisfactorily as a solvent than did baryta water; in the case of the former but little change took place before solution was effected, while with baryta water alteration seemed to have priority and preference. This should not be taken as indicative of its entire relation to barium oxide, however, for by rubbing the substance with barium carbonate until intimately mixed the substance slowly reacts with it in the presence of cold water, and rapidly with warmth, to form a soluble barium compound; and from the yellowish solution of which, hydrochloric acid again precipitated the tannin-like substance, while the color of this solution disappeared as did that of every other similar solution under this circumstance. Truly this substance is comparable to an acid anhydride of an inorganic salt.

Returning to further consideration of the green color obtained in the reaction with albumen, it was found that the purified astringent principle produced the same effect with albumen; and that these changes would occur both in daylight and in darkness. From casual observation it is believed that there may be a definite relation demanded between the agents involved, or that in very dilute solutions the appearance of the color is much slower. Within a few days, the green color and precipitate had turned to a dirty brick-red shade suggestive of this characteristic of the phlobaphenes; the liquid had no odor indicative of putrefactive changes. The disposition to form this green color was also shown in a medium of saliva. As manifestation of possible biologic relations, these behaviors are deserving of further study.

This substance has the physical and chemical properties of the so-called phlobaphenes in the highest degree of purification and permanence ever met with by us. Indeed these features so closely approximate those of the water-soluble tannins that the isolation of this substance in such pure form may well be regarded as a distinct advance in the knowledge of these elusive principles; and, especially should the presence of the subject of this paper as it occurs in maté in conjunction with caffeine serve to better assure our understanding of similar astringent drugs. It would be idle to speculate on the relation of this substance to water-soluble tannins, until more is learned of all of them, but its isolation proves again the need of constant caution in accepting the well intentioned and often laboriously produced work and results of the early times in plant analyses.

Knowing as we now do that the tannin of maté is combined or similarly associated with caffeine, we would, in any attempt to extract it for further examination, at once acidify the decoction with acetic acid and proceed to remove the substance by means of acetic ether.

If it should be named let its designation be the title of this paper.
