

The Question of Tannin in Maté

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INTRODUCTION.—In South America and associated countries a beverage is made from maté, the dried leaves of various species of *Ilex* (Fam. *Aquifoliaceae*)—shrubs indigenous to Brazil and Argentina. It is usual to take the drink as an aqueous infusion, prepared in a silver cup or gourd, from which the name “maté” is derived.

Owing to the discovery of the shrub in Paraguay by early Jesuit missionaries in the seventeenth century, all varieties of maté, irrespective of species, have come to be known as *Ilex paraguayensis*. The genus *Ilex*, however, contains two-hundred-and-eighty species, and Wehmer¹ gives the names of nine different varieties of *Ilex* leaves sold under the name of maté. To-day, the finest variety obtainable is derived from *Ilex paraguariensis* (St. Hilaire), and in Parana it is forbidden by law to export any other.

The process for curing maté is distinct from that used for common “tea”; moreover, it is unlikely to produce any appreciable change in the phenolic

constituents of the plant. Tea is rolled and fired, maté is not. In several publications it is stated that maté is dried by means of smokeless heat.*

According to the descriptions given in abstracts from this literature and elsewhere the modern curing process may be summarised roughly as follows:— The twigs and leaves are collected between May and September, this period being fixed by law to avoid damage to the shrubs. First, there is a preliminary toasting and drying by means of hot air; direct heat is carefully avoided, because smoke would give the finished product an undesirable taste and odour. Secondly, after a definite period of storage, the leaves and twigs are separated by means of a special threshing process. Lastly, the leaves are subjected to a final drying by means of hot air conveyed through pipes or tunnels. The final product should be green, hard and resistant to fermentation and deterioration.

The presence in maté of so-called tannin, either identical with, or analogous to, that in tea, has been definitely reported by several chemists, but it should be pointed out that little or no work has been done on the phenolic constituents of the drug since 1922. This was before the advent of such specific tests as that requiring the use of goldbeaters' skin, and we have therefore made a critical examination of the results of earlier work. The more important references are as follows:— Rochleder and Hlasiwetz² concluded that the main phenolic constituent of maté was identical with the caffetannin of coffee. Arata³ found the plant phenol to be analogous to, but not identical with, that of coffee. Peckolt⁴ published a confirmation of Arata's work. Kunz-Krauze⁵ described the main constituent as a glucoside of either caffeic or chlorogenic acid containing an unknown optically inactive hexose. Peacock and Peacock⁶ concluded, on very slender evidence, that maté contained an astringent principle which they isolated and identified as a phlobaphene.

At the suggestion of the Society's Analytical Investigation Scheme Committee we have made a further investigation, dealing primarily with the question of the presence of genuine tannin; secondary considerations were the establishment of a difference between the plant phenols of maté and common "tea" and an attempt to separate colouring matter from the main plant phenol in maté, with the object of obtaining data relating to their constitution and structure.

PRELIMINARY EXAMINATION.—Thirty individual samples of cured maté were examined, including imports dating as far back as 1920 and 1927, as well as current imports from wholesale houses and purchases from London stores. A cursory botanical examination showed that all the samples were typical of the genus *Ilex*, although there was undoubtedly some variation in species.

A small and limited supply of each kind of maté for comparison purposes was obtained by courtesy of the authorities at Kew. Coffee beans (*Coffea arabica*) and Asiatic tea leaves (*Thea chinensis*) were also compared with maté, with a view to bringing out essential similarities and differences. Except in certain instances, aqueous extracts (1 in 20) were used for the tests. The extracts were prepared by decocting the comminuted material with water for fifteen minutes; when necessary,

* "Maté: An Important Brazilian Product," by C. R. Cameron (*Bulletin of the Pan-American Union*, Oct., 1929, pp. 988-1005); "Il Mate o Tè del Paraguay," by Dr. C. Micastro (*L'Agricoltura Coloniale*, Nov., 1928, to Aug., 1929); "A Exploracao do Mate" (*Boletim do Ministerio da Agricultura, Industria e Commercio*, Brazil, for April and May, 1929).

chlorophyll, caffeine, fats, waxes, resins, etc., were removed by treatment with organic solvents.

It will be realised by those familiar with the analysis of tannins that the available tests capable of giving specific results are very few. In this work, particular emphasis has been laid on the results obtained with goldbeaters' skin,⁷ gelatin solution, and certain tests devised by Ware, all of which, with certain exceptions, are described in *Allen's Commercial Organic Analysis*, Volume V, under the section dealing with tannins.⁸ It was found necessary to employ a limited number of colour reactions, but only those were used with which it was considered possible to carry out control tests.

Aqueous extracts of maté had the following general characteristics:—Yellowish-brown in colour, giving an acid reaction to litmus (pH 5 to 6); they gave precipitates with solutions of cinchonine and quinine sulphates and a copious yellow precipitate with basic lead acetate; ferric iron (with increasing pH) gave a grass-green colour, whereas ferrous iron (with carefully controlled decreasing pH) gave green, violet, purple and deep brown colours; aqueous ammonia (10 per cent.) gave a beautiful emerald-green which deepened rapidly on shaking or allowing the mixture to stand in contact with air.

The reactions with iron salts were particularly interesting, especially when considered in conjunction with the results of tests for genuine tannin detailed in the next section of this paper. The results given with ferrous iron (Mitchell's ferrous tartrate reagent)⁹ have special significance when interpreted according to Ware.¹⁰ Ware¹¹ classified plant phenols by the colours and precipitates obtained with the ferrous tartrate reagent in the presence of an appropriate quantity of $N/50$ sodium bicarbonate solution or a weak solution of ammonia; if no colour is obtained, the phenol is provisionally assigned to class A; a violet colour indicates probably a phenol of class B, and a deep brown colour probably a phenol of class C.

Maté extracts gave results typical of classes B and C; the more important phenols belonging to class B are pyrocatechol, protocatechuic acid, the catechinols (catechins), chlorogenic acid, caffetannin, ipecacuanhic acid, adrenalin and catechol tannins; class C (containing most of the pyrone and quinonoid phenols, and some benzophenone phenols) is represented chiefly by the anthoxanins, such as the flavone, luteolin, and the flavónols morin, quercetin, quercetrin, rutin, myricetin, etc.

It is not practicable to set out here in full all the tests used to show the absence of various phenols from maté; it is sufficient to state that the following substances were thoroughly tested for, particular attention being paid to the possible presence, in the free state, of the first two in the list:—Gallic acid, catechol, protocatechuic acid, resorcinol and phloroglucinol. There was no evidence indicating the presence, in the free state, of any of these substances.

Another qualitative reaction of extracts of maté is the beautiful emerald-green colour obtained with ammonia. We believe this reaction to be characteristic of maté, and probably of caffetannin; the colour is very intense, persisting for several hours and slowly changing, on long contact with air, to a deep brown. This is undoubtedly an oxidation reaction, because the colour can also be obtained with acid permanganate, and, conversely, can be completely and rapidly destroyed

by reduction with zinc and hydrochloric acid. Aqueous extracts of coffee beans gave a similar reaction, but the colour was very much less intense.

It is well known that, under suitable conditions, many phenols (notably gallic acid, gallotannin, catechol, protocatechuic acid and resorcinol) will give a green colour with ammonia or other alkali, but the colour is usually very transient and much less intense than that given by maté extractives; moreover, it must be recalled that four of the above-mentioned substances have been shown by many tests to be absent from maté.

There has been a general tendency in the past to associate chlorogenic acid with this peculiar green colour given by various extracts with ammonia solution; furthermore, it has been customary to identify caffetannin with chlorogenic acid, in spite of the fact that the evidence for their identity is very slender. Nierenstein,¹² dealing with hydrolysable tannins, gives good reasons for the conclusion that caffetannin and chlorogenic acid are not identical. Gorter,¹³ describing the reactions of chlorogenic acid isolated from coffee, makes it clear that it gives with ammonia not a green colour, but a definite yellow which, on standing, acquires a reddish tint.

The preliminary examination of maté extracts indicated the presence of two plant phenols, *viz.* an iron-greening anthoxanin (probably a flavone or flavonol derivative) which was responsible for the deep brown colour given with Mitchell's reagent in the presence of sufficient alkali; and secondly, what is here described as a pseudo-tannin (probably a caffetannin), which gave the violet colour with Mitchell's reagent, also in the presence of sufficient alkali; appreciable quantities of these two phenols undoubtedly exist in maté. As will be seen, the results recorded at a later stage of this work tend to substantiate this suggestion.

EXAMINATION OF FRESH AND CURED MATÉ FOR TANNIN.—The more important results are given below in tabular form, the tests used being all fairly well known, with, perhaps, the exception of the antipyrine test devised by Ware.¹⁴ This routine test for tannins, used in conjunction with a suitable phosphate buffer gave excellent results with controls containing very small amounts of gallotannin.

TABLE I

| Test | Observation | Inference |
|---|--|--|
| <i>Goldbeaters' skin</i> | | |
| (a) Treatment for tanning | No opacity | Tannin absent |
| (b) Staining with ferrous sulphate | Greyish-green stain | Indication of iron-greening anthoxanin |
| (c) Decolorisation with acid | No final stain | Phlobaphen absent |
| Gelatin solution | Complete absence of turbidity or precipitate | Tannin absent |
| Ware's antipyrine test | No turbidity or precipitate | Tannin absent |
| Ware's iron and ammonium citrate test | No precipitate | Tannin absent |
| Ware's modification of Stiasny's reaction | No characteristic precipitate Iron-greening and iron-browning filtrate No blue colour produced | Phlobatannin absent Gallotannins absent |
| Ware's modification of Mitchell's ferrous tartrate test | No blue or violet colour produced | Gallotannin absent |

In addition to the tests described above, a hide-powder test, kindly made by Dr. E. W. Merry of The British Leather Manufacturers' Research Association, gave noteworthy results. The comminuted material (25 g.) was extracted with 1 l. of water in a Procter extractor at a temperature not exceeding 60° C. The sample contained 33.2 per cent. of water-soluble substances, of which 12 per cent. was absorbed by the hide powder, as used in the official method of tanning analysis; the remaining 21.2 per cent. was not absorbed. Lack of sufficient fresh material made it impossible to make a control test; but since hide powder will absorb substances other than tannins, including natural colouring matters and non-volatile organic acids, we suggest that the absorption in the case of maté was due to the presence of one or both of these constituents in the leaves of the plant.

The results of sensitive spot-tests on filter-paper, devised by Ware,¹⁵ confirmed the foregoing conclusions; the same author's tests with iodine and ammonia were also confirmatory.

It is noteworthy that the goldbeaters' skin test, the most specific test known for detecting genuine tannin, gave a negative result with each of the thirty samples of maté examined. We conclude that there is not, and never has been, any genuine tannin in maté, for the following reasons:

- (a) First and foremost there is no phlobaphen present.
- (b) There is no gallic acid present.
- (c) The modern process by which the leaf is cured is unlikely to produce tannin or to destroy it.

A COMPARISON OF FRESH MATÉ WITH FRESH TEA.—These tests bring out certain pronounced differences between maté and tea. Little can be said about the plant phenols in tea, because the chemistry of tea-tannin is a mass of contradictions and requires further elucidation. Comparisons were also made with fermented tea, but the results were so similar to those given in Table II that there was no point in recording them. The only noticeable difference between fresh and fermented tea was that the cured material gave strong positive results for phlobaphen, whereas the fresh tea did not.

It is clear from these results that, so far as qualitative tests go, there is little similarity between maté and tea. Tea undoubtedly contains genuine tannin, but maté does not, and this constitutes the chief and most far-reaching difference between the two.

A COMPARISON OF FRESH MATÉ WITH FRESH COFFEE.—These tests bring out differences and similarities between maté and coffee. A comparison of the cured materials also gave very similar results, except that with coffee the reactions were much less intense; this was probably due to destruction of plant phenols during roasting.

Coffee beans are known to contain caffetannin and chlorogenic, caffeic, and gallic acids. From the comparison given on p. 140 it seems probable that a little genuine tannin is also present; this would be quite feasible, since it is rare to find gallic acid unassociated with tannin in a plant. It is noteworthy that the remarkable reaction with ammonia (p. 137) is also shared by extracts of coffee.

TABLE II

| Test | Observation | Inference |
|--|---|--|
| Goldbeaters' skin | Maté: Already described. Tea: Definite opacity with bluish-green stain | — Tannin present; gallotannin, gallic acid or catechol tannin present |
| Gelatin solution | Maté: Already described Tea: Characteristic precipitate | — Confirmation of presence of tannin |
| Ware's modification of Stiasny's reaction | Maté: Already described Tea: Characteristic precipitate, iron-blueing and iron-greening filtrate | — Phlobatannin present; gallotannin, gallic acid or catechol tannin present |
| Osmium tetroxide solution (1 per cent.) + appropriate buffer | Maté: Reddish-brown, no violet or blue Tea: Purplish-violet | Confirmed absence of gallic acid Probable presence of a pyrogallol tannin |
| Bromine water | Maté: No precipitate on standing Tea: Precipitate on standing | Absence of catechol tannin Probable presence of catechol tannin |
| Nierenstein's test (0.5 per cent. diazobenzene chloride) | Maté: No precipitate on standing Tea: Precipitate on standing | Absence of catechins or catechol tannin Presence of one, or both, of these substances |

SEPARATION OF THE PLANT PHENOLS IN MATÉ.—The preliminary examination of maté extracts indicated the presence of two plant phenols: one classified as a pseudotannin, and the other as a member of the large family of natural yellow colouring matters. Perkin¹⁶ has called attention to the fact that many natural colouring matters and tannins associated in plants often show a marked resemblance to each other in constitution and chemical reactions. This seemed probable with

TABLE III

| Test | Observation | Inference |
|--|--|---|
| Goldbeaters' skin | Maté: Already described Coffee: Slight opacity, bluish-green stain | — Indication of a little tannin; gallotannin, gallic acid or a catechol tannin present |
| Solution of gelatin | Maté: . Already described Coffee: Slight turbidity | — Confirmation of above result |
| Ware's modification of Stiasny's reaction | Maté: Already described Coffee: Very slight precipitate, iron-greening and iron-blueing filtrate | — Confirmation of above results |
| Ammonia solution, 10 per cent. | Maté: Beautiful emerald-green intensified on shaking Coffee: Very similar to maté only less intense | Caffetannin present Caffetannin present |
| Osmium tetroxide solution (1 per cent.) + appropriate buffer | Maté: Already described Coffee: Same as maté, but tinged with violet | — Probably a little gallic acid or tannin present |
| Nierenstein's test (0.5 per cent. diazobenzene chloride) | Maté: Already described Coffee: No precipitate on standing | — Absence of catechins and catechol tannin |

maté, since the reactions of one phenol nearly always tended to be masked by those of the other.

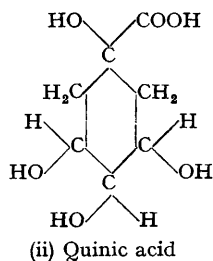
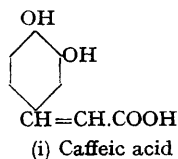
For this reason attempts were made to separate the two constituents. Fractional precipitation with lead gave poor results; various attempts to remove the colouring matter by adsorption or oxidation were equally unsuccessful. Extraction with different organic solvents yielded, towards the end of the work, some measure of success. It was noticed that neutral acetone extracted some colouring matter from maté, but very little or no pseudotannin; alkaline acetone extracted a fair amount of colouring matter, but again, little or no pseudotannin; acidified acetone extracted both plant phenols in sufficient amount to afford hope of a good separation when the extract was treated with dry ammonia gas for a definite period. The process actually used was briefly as follows:

One kg. of dried and powdered maté was allowed to stand in contact with dry chloroform for six hours with occasional shaking. The chloroform was then decanted, and the leaves were pressed; washing with several portions of ether and a final pressing of the leaves completed the removal of caffeine, chlorophyll, fats, resins, waxes, etc. It goes without saying that the plant phenols in maté had previously been found to be almost insoluble in chloroform and ether. Next, the pressed material was spread out on a tray and dried at a temperature not exceeding 60° C. Treatment with acidified acetone followed (pH 3 to 4, adjusted by means of concentrated sulphuric acid), sufficient solvent being added to cover the leaves completely, and twenty-four hours being allowed for the extraction. The solution was filtered through a Buchner funnel, and a fairly rapid current of dry ammonia gas was passed through the filtrate for about two minutes. The pinkish-red precipitate obtained was quickly washed with neutral acetone (several times), and then transferred to a desiccator to dry. The filtrate, containing colouring matter, was again treated with ammonia gas, any precipitate obtained being neglected; after re-filtering, if necessary, the new filtrate was evaporated to dryness under reduced pressure, and the residue was transferred to a desiccator.

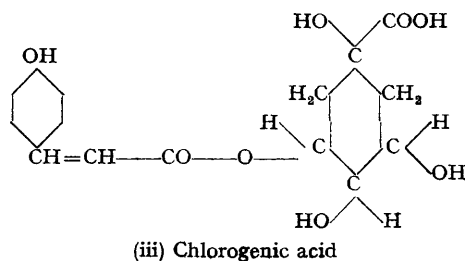
This represents a rough attempt to separate the colouring matter from the plant phenol or pseudotannin, but the method will probably need some re-adjustment before being applicable on a large scale.

EXAMINATION OF THE PRECIPITATE.—The precipitate obtained by the method described had the following general characteristics:—It was readily soluble in water, forming a clear reddish-brown solution; the reaction to litmus was markedly acid, and the colour given with ammonia was the deep emerald-green already described; ferric iron (with increasing pH) gave a grass-green colour, whereas ferrous iron (with decreasing pH) gave green, violet-purple, and finally a deep wine-red colour, but no brown colour was noticed. According to Ware,¹¹ this reaction is typical of pseudotannins in general. Precipitates were obtained with cinchonine and quinine sulphates, heating by itself yielded catechol, and fusion with alkali gave catechol together with a volatile acid not yet identified. It is worth noting that the preliminary reactions for colouring matter, and also others described later in detail, gave negative results when applied to an aqueous solution of the above precipitate.

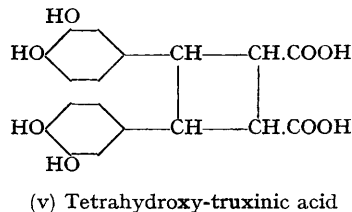
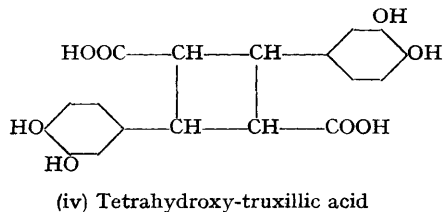
Caffetannins give, on hydrolysis, (i) caffeic and (ii) quinic acid, and a residue not yet fully investigated.



Nierenstein (*loc. cit.*) is in favour of regarding caffetannin as a condensation product of (iii) chlorogenic acid, the established formula of which is given below.



If this view be accepted, the residue obtained on hydrolysis would be either (iv) tetrahydroxy-truxillic acid or (v) tetrahydroxy-truxinic acid.



At present little is known of either of these acids; moreover, Nierenstein¹² points out that in accepting the suggested formula for caffetannin it must be realised that the production of caffeic acid would be entirely due to the presence of some unchanged chlorogenic acid in the caffetannin. Finally, he agrees with Gorter,¹³ who is of the opinion that caffetannin is a mixture of several substances, including chlorogenic acid.

An attempt was made to produce caffeic acid from the precipitate obtained from acidified acetone. The precipitate was treated with a slight excess of 10 per cent. aqueous potassium hydroxide solution and hydrolysed under reduced pressure for thirty minutes; after cooling, the brown solution was acidified with dilute sulphuric acid and then shaken out with several portions of ether. The ethereal layers were mixed, the solvent evaporated, and the residue was taken up in a little water. This aqueous solution gave positive results in the following tests devised by Gorter¹³ for caffeic acid obtained by him from coffee beans: a grass-green colour with weak ferric iron solution; a lemon-yellow colour with

lead acetate solution and baryta water; ready reduction of silver nitrate, and a yellow colour with ammonia solution, changing to a reddish tint on standing. This last reaction is also shown by chlorogenic acid.

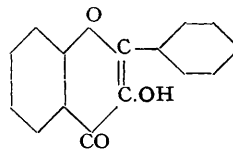
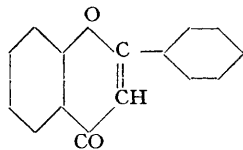
The same tests were applied to a residue obtained by a method proposed by Griebel¹⁷ for determining chlorogenic acid in coffee. The method depends on the conversion of chlorogenic acid into caffeic acid by alkaline hydrolysis; the results were positive for caffeic acid.

An attempt to produce quinic acid from the precipitate under discussion was based on the knowledge that this substance, when distilled with sulphuric acid and manganese dioxide, yields quinone. The precipitate was hydrolysed with excess of 30 per cent. aqueous potassium hydroxide solution for one hour; subsequent distillation with concentrated sulphuric acid and manganese dioxide, gave, on standing, a yellow deposit which was collected and identified as quinone.

We fully realise that the results just described need further confirmation; nevertheless, we maintain that there is, at this stage of the work, evidence for concluding that caffetannin, or some closely allied substance, is present in maté. As previously mentioned, the search for a practical method of separating the two phenols in maté has only recently been successful, and work is still in progress with the object of obtaining the pseudotannin and colouring matter in the crystalline state. Attempts were made to crystallise the pinkish-red precipitate described above, but the final product was invariably a hard brittle mass. This may have been due to impurity, or, on the other hand, possibly the precipitate is a mixture of substances; if so, this would be another point in favour of caffetannin being present.

Another possibility is that chlorogenic acid exists in the free state in maté. The tests described by Charaux¹⁸ and by Gorter,¹⁹ depending on colour reactions with iron salts after acid hydrolysis of fresh extracts, gave results indicating the presence of chlorogenic acid. Hoepfner²⁰ describes a colorimetric method for determining chlorogenic acid in coffee; this was tested with maté, coffee being used as a control, and the result was positive. In spite of this, we think that it is difficult to state definitely whether the reactions just described would be given by chlorogenic acid existing in the free state in maté, or by the same acid existing as a component of a mixture such as caffetannin may be. This must be left for further investigation.

EXAMINATION OF THE FILTRATE.—The presence of an iron-greening anthoxanin, probably a (vi) flavone or (vii) flavonol derivative, was indicated by the results obtained in certain tests in the preliminary examination.



Attention has been called to the fact that the reactions of one phenol interfered with those of the other; owing to this confusion, it was thought at one time

that the colouring matter in maté was almost certainly a flavonol derivative, but the separation process has made it possible to correct this error.

The filtrate from the acid acetone precipitate should contain the colouring matter; a residue obtained from such a filtrate in the manner already described, gave the following general reactions: It dissolved in water to give a pale yellow solution, being slightly acid to litmus and giving a copious yellow precipitate with lead acetate; with ferric iron (increasing pH) a green colour was obtained; with ferrous iron (decreasing pH) there was a green colour, which, on adding sufficient $N/50$ sodium bicarbonate solution, became deep brown; no violet or purple colour was seen, thus indicating, according to Ware,¹¹ that phenols belonging to class B had been eliminated; in other words, plant colouring matter had been separated from pseudotannin. When heated alone, the dry residue gave catechol; when fused with alkali, it gave protocatechuic acid and phloroglucinol.

The following results, presented in tabular form, sum up the more important reactions of the colouring matter in maté.

TABLE IV

| Test | Observation | Inference |
|---|--|---|
| Calico or wool mordanted with aluminium | Bright yellow—no reddish tint—fairly fast to light; fluoresced in ultra-violet light | Flavone or flavonol present; probably a flavone |
| Ware's iron and ammonium citrate test | Production of a heavy brown precipitate | Presence of a catechol, phloroglucinol, flavone or flavonol |
| Willstätter's reduction reaction | Complete absence of any colour | Flavonols absent; flavone derivative probably present |
| Perkin's test (air-oxidation of an alkaline solution, followed by acid precipitation) | Definite precipitation after acidification | Flavone derivative present |

All natural hydroxyflavones dye fabrics mordanted with aluminium a yellow shade, the intensity of which is dependent upon the position of their hydroxyl groups. The yellow given by flavonols to mordanted calico is usually slightly tinged with red, which serves as a useful distinction from flavones. Willstätter's reduction test with zinc and hydrochloric acid is another useful reaction for distinguishing flavones from flavonols. A third reaction, based on Perkin's observation that hydroxyflavones are not, as a rule, readily oxidised in alkaline solution, serves as a useful confirmatory test. The tests for colouring matter in maté were controlled with oak-bark containing quercetin and onion-skin containing apigenin.

We thus consider that there is good evidence pointing to the existence in maté of a flavone derivative. In further work it is hoped to obtain confirmation of this by preparing the colouring matter in the crystalline state and investigating its chemical constitution.

CONCLUSIONS.—It has been shown that maté is completely free from genuine tannin. This is a matter of considerable importance, in view of the effects of ordinary tea upon the digestion.

It has also been shown that maté contains an appreciable amount of a natural

yellow plant colouring matter; it is reasonably certain that this colouring matter is a derivative of flavone.

Certain evidence has been obtained pointing to the presence in maté of caffetannin or some closely allied compound.

Comparisons have been made with coffee and tea, and attention has been drawn to important differences and similarities.

We wish to express our thanks to Dr. C. A. Mitchell and Dr. H. E. Cox for their help and for many useful suggestions received during the course of the work. Thanks are also due to Dr. M. Nierenstein and Mr. Alan Ware for confirming certain results, and to Prof. O. L. V. de Wesselow, Director of the Medical Unit Laboratory, St. Thomas's Hospital, for kindly providing facilities for the work to be done.

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DISCUSSION

Dr. H. E. Cox congratulated the authors on adding so valuable a chapter to the study of maté. He drew attention to Capt. T. A. Joyce's lecture on maté (*Nature*, 1934, **134**, 724), from which it appeared, among much interesting matter, that the first chapter had been written in 1617 by Cornejo, and now Mr. Woodard and his colleague had added one more. It was particularly important to note that the authors had established the absence of true tannin, as in commercial circles it was still asserted that about 7 per cent. of tannin was present. He thought it was a matter for congratulation that the authors had established the nature of the chlorogenic acid and the absence of tannins by definite chemical study, and had not depended solely on colour reactions which were known to be somewhat erratic. It was significant that the hydrolysis yielded caffeic acid, which was known to be a product of caffetannin, and he hoped that they would succeed in establishing the identity of the hydrolysis products of the chlorogenic acid, and also that they would continue their good work, so that we might get to know something of the real constitution of the substances present.

Mr. WOODARD replied that they had been able to produce quinic acid on distillation with sulphuric acid. They had also been able to show that caffeic acid was present.

Dr. C. A. MITCHELL said that the authors had made a valuable addition to the long series of papers that had been brought before the Society by way of the Analytical Investigation Scheme. When considering the conflicting statements upon the presence or absence of tannin in products such as maté, it was necessary to have a clear conception of what was meant by "tannin." Definitions for tannin were notoriously vague, but it seemed reasonable to regard a tannin as a substance that would "tan," and for this reason great importance attached to the results of the goldbeaters' skin test, which was essentially a "tanning" reaction. If this test gave negative results, as it did with maté, there could be no true tannin present, but it would be interesting to learn whether the authors had ascertained the nature of the substances that were adsorbed from the extract of maté by hide-powder. In view of the results obtained with goldbeaters' skin, they were evidently not tannins.

Mr. R. L. COLLETT asked whether the green colour obtained with ammonia was also obtained with other alkalis.

Mr. WOODARD, replying, said that he very much appreciated the remarks made by Dr. COX. In the first place he would like to emphasise the fact that the work dealt primarily with the question of genuine tannin in maté, and that most of the time at their disposal had been devoted to this purpose. Also, he was of opinion that before any useful knowledge bearing on the chemistry of the plant phenols in maté could be gained, it would be necessary first to separate colouring matter from essential plant phenols and then to purify the products so obtained. This was not an easy matter, although he was pleased to say that reasonable success had been attained in making a separation of the two phenols, and some evidence was given in support of their chemical identity. The green colour given by maté extracts with ammonia was also obtained with solutions of sodium and potassium hydroxide, although the colour was much less intense. He had observed that the green colour was destroyed by acids alone, as well as by reduction with zinc and hydrochloric acid. With regard to the points raised by Dr. MITCHELL, he had not considered it necessary to make a full investigation of the substances adsorbed by hide-powder. Since all tests for genuine tannin gave negative results with maté, and also since hide-powder was known to adsorb both colouring matter and non-volatile organic acids, he considered the adsorption in this case to be due to the presence of one or both of these constituents in the leaves. The green colour given by alkalis with aqueous maté extracts was similar in many respects to the bluish-green colour given by coffee extracts under identical conditions. This reaction was commonly attributed to viridic acid, but he thought that this conclusion was a little too hasty. Viridic acid was a substance of unknown constitution, since, according to Vlaaderen and Nhulder (*J. prakt. Chem.*, 1858, **67**, 261), there were at least six different kinds of viridic acid in coffee beans. His own observations led him to regard this interesting colour reaction as an indication of the presence of caffetannin or some closely allied compound.
