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THE ESTIMATION OF THEOBROMINE IN COCOA AND ITS PRODUCTS.

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THE methods available at present for the estimation of theobromine are all, for various reasons, far from being satisfactory, yet they are so numerous that the introduction of another seems to necessitate an apology. As Whymper remarks (Allen, "Comm. Org. Anal.," vol. vi.), "The recorded values of theobromine are very variable and untrustworthy, arising from the lack of any very accurate and reliable method of estimation." It is this unreliability that has urged the author to search for some method which will be simple, efficient, and rapid, and at the same time give concordant results in the hands of different workers. At the present time, with most of the methods suggested, agreement between different workers is difficult to obtain. The process described below is given in the belief that it overcomes these difficulties.

PREVIOUS METHODS.—The methods suggested up to the present day may be roughly divided into four groups:

A. Those based on the precipitation of the alkaloid from the acid extract of cocoa by phospho-tungstic or phospho-molybdic acids, and the estimation of the

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alkaloid in the precipitate, by the nitrogen content (Weigmann), by ignition (Wolfram and Legler), or by solution in a solvent (Kunze, Monthulé).

B. The cocoa, with or without a previous acid treatment, is extracted with water and an alkali; MgO in the method of Mulder, Bell, Savini, Trojanowski, Frommes, Beckurts, and Dekker; CaO in the method of Zipperer; Ba(OH)₂ in the method of Eminger; PbO in the method of Süß; and the alkaloid extracted by chloroform from the dried filtrate (Dekker, Frommes, Savini, Zipperer, Süß, Beckurts), or from the residue obtained by evaporation of the whole to dryness (Mulder, Bell, Eminger, Trojanowski). In the methods of Trojanowski and Bell the solvent used is alcohol.

C. The alkaloid is extracted directly with a solvent, such as chloroform (Diesing), or chloral alcoholate (Kreutz) from the dry material.

D. The alkaloid is extracted from the *wet* material by a solvent, phenol-chloroform (Maupy and Débourdeaux).

CRITICISM.—A criticism of most of these methods has been made by W. E. Kunze (ANALYST, 1894, 11, 194), but there are other criticisms which the author would like to offer—criticisms which the grouping of the methods facilitates.

A. The precipitation by phospho-tungstic or phospho-molybdic acids has been stated to be incomplete, and certainly in the hands of some operators it gives low results. The precipitate itself is one difficult to free from impurities owing to the nature of the materials precipitated together with the alkaloid, this operation taking a whole day to perform. The estimation of the theobromine by the nitrogen content of the precipitate obviously gives high results, as does the ignition of the aqueous or amylic-alcohol extract. The extraction of the alkaloid by chloroform has the drawbacks of low solubility, etc., mentioned below (under B.), though the actual alkaloid obtained is generally purer than that obtained by the methods of the second group.

B. The solution of the alkaloid in water, as before, but the precipitation of objectionable substances by alkalis, as magnesia, lime, baryta, or lead, has the disadvantage again of poor filtration, and also of incomplete precipitation. It has therefore been thought by some (Mulder, Bell, Eminger, Beckurts, Trojanowski) that filtration is unnecessary. In either case the result is much the same, other bodies besides the alkaloid being extracted by the solvents, as is apparent from the colour of the resulting alkaloid and the colour of its solution in water. The solubility of theobromine in chloroform, as has been previously pointed out by the author (ANALYST 1920, 45, 133), is very low; consequently a large quantity of solvent or long extraction is necessary, and so even slightly soluble bodies have every chance of being dissolved with the alkaloid. In addition to this, the extraction from a perfectly *dry* material is very slow, and in the author's opinion never complete. This seems to have been recognised by Savini and Frommes, who employed small quantities of ammonia in the solvent. Alcohol, as used by Bell and Trojanowski, which contains the small quantity of water found necessary for the extraction, is a better solvent for the alkaloid, but also has the great disadvantage of dissolving more foreign bodies than chloroform. Kunze obtained about four times more extract (theobromine?) by this than by any other method.

Many authors (Frommes, Kreutz, Eminger, etc.) have insisted on a treatment with acid being necessary as a preliminary to the estimation of theobromine,

suggesting that a compound is present from which the alkaloid cannot be obtained without this previous treatment. The compound is generally regarded as a glucoside. Kreutz (*ANALYST*, 1909, **34**, 20) remarks that "it is impossible to separate the free theobromine from that portion of the alkaloid existing as a glucoside." Fritsch ("Fabr. du Chocolat," p. 264) suggests a combination of theobromine with tannin. If Fritsch's assumption be correct, and this compound be formed or present, an acid treatment would be of no help, as this compound is not decomposed by acids. It is, however, readily soluble in boiling water, and easily decomposed by magnesia, baryta, lime, or lead by the precipitation of the tannin. The author has never been able to obtain an increase in the yield of theobromine by this acid treatment; in fact, combining this with Dekker's method, usually from 0.1 to 0.25 per cent. lower yield was obtained, but the alkaloid was obviously purer, being much whiter in colour. Any compound present—and a definite proof of its existence is still wanting—is decomposed by water and the compounds mentioned above. Even unfermented beans do not give a higher result after the acid treatment. The only way a higher result can be obtained is by a breaking down of the cellular tissue of the material by the acid, in cases where very fine powders are not used, or where no water is used, as with the methods of Kreutz and Diesing.

It has been stated (Diesing and Süß) that lime, and to a less extent magnesia, decomposes some of the theobromine on prolonged heating, and it is accepted that in the case of Zipperer's low results this was due to decomposition of the alkaloid by the lime. With magnesia the author finds a slight decomposition, reducing the percentage on prolonged heating at 100° C. by 0.1–0.2 per cent., but with short heating the result is not affected. Thus in the second group it is fairly certain that the small reduction in the percentage is brought about by the long treatment with alkalies. In the author's hands the acid treatments have never shown any decomposition of the alkaloid.

C. A direct and complete extraction of the alkaloid with a solvent from the dry cocoa is impossible. The quantity of material other than alkaloid extracted is very considerable, amounting, in the case of fermented cocoas, to 50 per cent. of the total extract, whilst the actual theobromine extracted is seldom more than half that really present in the material. The alkaloid is only slowly given up. After 80 hours' extraction the author found between 0.06 and 0.10 per cent. again extracted in 24 hours, though after 120 hours no more could be extracted. Again, the low solubility of the alkaloid in chloroform is a drawback, and, as Maupy has shown, water is absolutely necessary to the complete extraction. To extract the remainder of the theobromine, Kreutz, after a direct extraction with chloroform or chloral alcoholate and ether, gives an acid treatment, neutralises, dries, and extracts the alkaloid again with chloroform. This second extraction has the drawbacks mentioned above, and the method as a whole gives high results.

D. To overcome these difficulties, Maupy (*ANALYST*, 1897, **22**, 191) suggested and worked out a method, using phenol-chloroform as the solvent, and mixing the solvent to be extracted with water. Without water this method gives much the same results as Diesing's. Phenol-chloroform is a good solvent, but inconvenient to work with, and, unfortunately, dissolves out other bodies which precipitate with the

theobromine, thus giving high results. The amount of other matter depends upon the cocoa used, and varies between 0.2 and 0.5 per cent. With the object of purifying the product, Débourdeaux (*J. Soc. Chem. Ind.*, 1917, 612) added to the process a long and tedious method of purification. Dekker objected to the method on the ground that the alkaloid was not completely precipitated from solution. This only occurs on those rare occasions when a *very large* amount of foreign matter has come through, and in those cases precipitation is complete after standing overnight.

It is obvious from the above review that none of the methods as yet suggested gives satisfactory results. Even the methods most commonly in use (Dekker and Kunze) have very serious drawbacks. Cocos of different origin have been found to give very variable amounts of foreign matter in solution with the alkaloid, particularly in the case of fermented and unfermented cocos of the same origin. This, perhaps, accounts for the higher results obtained by some workers with fermented cocos, a result which led them to suggest the formation of theobromine during the process of fermentation. The method described below gives a pure extract in all cases, and is rapid and easy to work.

The solvent used is tetrachlorethane (acetylene tetrachloride), which, as has been previously pointed out (*ANALYST*, 1920, 45, 133), is one of the few good organic solvents for theobromine. Used on the damp cocoa, it has just the same drawbacks as phenol-chloroform, but with a previous treatment with magnesia this difficulty is overcome, and the complete extraction of the pure alkaloid is obtained.

NEW METHOD.—If the cocoa bean is to be extracted, it is necessary to remove the fat with petroleum spirit (of b.-pt. below 80° C.). If, however, cocoa or cocoa shell is being used, this preliminary extraction is not necessary.

Ten grms. of the material are placed in a small porcelain dish and well mixed with 2–3 grms. of freshly calcined magnesia. This mixture is very carefully triturated with 14 c.c. of water until every particle is wetted. The dish containing the damp mixture is then placed on the water-bath for half an hour to dry it partially. The mass should be mixed at intervals to prevent any part becoming perfectly dry. At the end of the half-hour the mixture is well triturated and transferred to a flask of about 250 c.c. capacity; 150 c.c. of tetrachlorethane are added, and the whole is boiled under a reflux air condenser for half an hour. It is then filtered, *while almost boiling*, into a second flask. The residue is transferred, together with the filter paper, to the first flask, 120 c.c. of tetrachlorethane added, and the mixture boiled for twenty to thirty minutes. The liquid is then filtered into the second flask, and the residue washed twice more as before. The united washings are distilled through an air condenser until reduced to 3 or 5 c.c. The residue is cooled, and 60–70 c.c. of ether (methylated, of sp. gr. 0.720) are added, the whole well mixed, and allowed to stand overnight. The precipitate is then collected on a tared filter paper, and washed with ether, dried at 100° C., and weighed. To this weight is added 0.004 grm., as representing the amount of theobromine dissolved in the 70 c.c. of ether used in the precipitation and in the washings.

Details and Precautions.—The material should only be extracted with petroleum spirit in those cases where it is impossible, owing to the fat present, to wet the material with water—*i.e.*, cocoa beans or mass. When extracted, even with petroleum

spirit, there is always a very slight loss of the alkaloid. The author has proved during his work that theobromine is slightly soluble in cacao butter, which accounts for the loss, also found by Süß, during extraction. Süß suggested that the loss was due to its being carried over mechanically, for he found theobromine absolutely insoluble in cacao butter. The author has found that the cacao butter of commerce is already saturated with both caffeine and theobromine at about 80° C. Süß's failure therefore to detect the solubility was possibly due to his using commercial fat. The amount of water necessary for the proper wetting of the material varies between 9 and 20 c.c. according to the substance used. Cocoa powder generally requires 14 c.c., but for cocoa shell 20 c.c. are required. The water should be added a few c.c. at a time, and the mixture well triturated after each addition. When the mixing, which should be very thorough, is finished, the whole mass should be easily compressible into a firm cake. The most convenient mixer for this process is a glass rod, with one end flattened. During the half-hour's drying on the water-bath the material should soon become easily broken up, and granular in appearance. When this happens the stirring should become a little more frequent. At the end of the half-hour the whole should be very thoroughly mixed again, so as to assure that every particle is damp, as this is very necessary for the extraction. When transferring the material to the flask a little of the powder will adhere to the basin owing to its dampness; this can be wiped off with a piece of moist filter paper, and the paper dropped into the flask. The filtered solvent from the extraction should, when hot, be perfectly clear and almost colourless, and, when a high percentage of alkaloid is expected, a fourth washing should be given. The final distillation of the solvent should be taken as low as possible, the alkaloid commencing to precipitate out some minutes before the end of the distillation. In practice it is found best to distil the majority of the solvent from each filtration whilst the next boiling is taking place. This enables a smaller flask to be used, and facilitates the final distillation, which should be carried as far as possible. After precipitating the alkaloids with ether, it will be found in most cases that one hour's standing is sufficient to ensure a complete precipitation of the theobromine. However, if the supernatant liquid be even slightly turbid, it should be allowed to stand overnight, and, where time is no object, this is the best method. The resulting alkaloid should be white and clean. It sometimes contains a small amount of insoluble matter and ash, amounting together to about 0.004 grm. on the average. For greater accuracy, therefore, it is advisable, after weighing, to wash the filter with boiling water to dissolve the theobromine, and to weigh the residue, care being taken that the washing is very thorough, as the alkaloid is not very easily dissolved. The filtrate is evaporated to dryness, ignited, and the ash weighed. Both are deducted from the first weighing.

The table on p. 37 shows a few results of the amounts of insoluble matter and ash obtained in experiments on different materials.

The magnesia treatment is too short to allow of any decomposition of the theobromine; it decomposes any compound of the alkaloid which may be present, and it precipitates or converts into an insoluble form all the bodies which would otherwise dissolve in the solvent. The presence of the water allows of a rapid and complete

solution of the alkaloid by swelling up the material, and thus allowing the egress of the theobromine from the otherwise almost impermeable cells through the medium of the water. The method thus combines the advantages of groups B and D described above, while overcoming their drawbacks. The time taken for a complete estimation on a cocoa powder may be as short as five or six hours, and two estimations can be performed as easily as one in the same time. The process estimates only the theobromine, which is the principal alkaloid of cocoa; the caffeine is left in solution when the theobromine is precipitated with the ether.

	Weight of Precipitate.	Weight of Insoluble Substances.	Weight of Ash.
	Grm.	Grm.	Grm.
A.	0.226	0.002	0.002
B.	0.238	0.002	0.001
C.	0.225	0.003	0.002
D.	0.199	Nil.	0.001
E.	0.220	Nil.	0.001

PURITY OF THE THEOBROMINE OBTAINED.—With concentrated sulphuric acid at 100° C. the product gives only a very light-brown coloration. The ash and the amount insoluble in water are practically nil. The nitrogen content estimated by the "absolute" method is 31.07 per cent., which is equivalent to 99.9 per cent. theobromine, the theoretical result being 31.11 per cent. The nitrogen in theobromine cannot be estimated accurately by the Kjeldahl method owing to the alkaloid volatilising without decomposition. By this method the nitrogen in pure theobromine varies between 27 per cent. and 29.5 per cent. according to the speed of heating and other details of manipulation. Thus the estimation of nitrogen in cocoa by the Kjeldahl method never includes the whole of that present in the theobromine. The percentage of silver in the salt obtained from the alkaloid is found to be 37.4, which is equal to 99.5 per cent. theobromine, the theoretical yield being 37.59 per cent. By the precipitation of the theobromine as the silver salt caffeine has been shown to be absent.

Thus the alkaloid obtained by the above method has a purity of 99.5 to 99.9 per cent.

The work was carried out in Messrs. Cadbury Bros'. Research Laboratory, and I wish to thank the firm for their permission to publish the results.

