period during the fast. This finding was interpreted as indicating that the high water intake had caused a stimulation of protein catabolism. The total nitrogen was also increased. Knowing the percentage of purine-nitrogen in flesh and the percentage of total nitrogen in flesh a relationship was established between the output of purine nitrogen and the total nitrogen excretion. By this means it was learned that, provided sufficient flesh was catabolized to yield the increased output of total nitrogen eliminated during the water period, 46 per cent. of the purine-nitrogen of this quantity of flesh was unaccounted for. This quota of purine-nitrogen might have been oxidized to stages below allantoin and thus escaped determination.

We submit the data showing an increased output of total purine-nitrogen (allantoin-nitrogen + purine-nitrogen) under the influence of a high water ingestion as evidence in favor of the hypothesis that the water stimulated protein catabolism.

URBANA, ILL.

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY OF HARVARD MEDICAL SCHOOL.]

THE DETERMINATION OF BENZOIC ACID.

BY OTTO FOLIN AND FRED F. FLANDERS.

Received August 4, 1911.

A recent investigation on cranberries undertaken in this laboratory involved a great many determinations of benzoic acid. Although methods for benzoic acid have undergone much improvement recently, it was soon evident that a great deal of time would be consumed in carrying out the large number of determinations which the work demanded.

The method first used was the second modification of the LaWall and Bradshaw method, described in Bulletin 132 of the Bureau of Chemistry.[1] Considerable experience had previously been had by one of us[2] with this method, and its weak points were well known. The filtration of the alkaline solution is usually very tedious and often nearly impossible. The extraction of the acidified filtrate is slow and difficult, as the tendency to form an emulsion is very great. After the extraction the chloroform must be allowed to evaporate and the residue dried in a desiccator to remove acetic acid, which requires much time. Finally the titration in alcoholic solution with phenolphthalein as indicator is open to criticism. This latter objection has been in some degree overcome by Clark[3] by adding water and titrating directly in the chloroform with aqueous alkali.

It occurred to us that this titration might be better made in the chloroform directly, according to the principle recently advanced by Folin and

[1] U. S. Dept. Agr., Bur. of Chem., *Bull.* **132**, p. 140 (method 2).

[2] Flanders.

[3] U. S. Dept. Agr., Bur. of Chem., *Bull.* **132**, p. 147.

Wentworth.[1] It would only be necessary to make the extraction with chloroform, remove all acids except benzoic from the extract and titrate at once with sodium alcoholate solution.[2] In the work on cranberries, this proved a rather simple matter and the results were satisfactory.

In order to make the method of more general use, its application to the determination of benzoic acid in catchup was attempted. Here the problem was more difficult. A number of acids are extracted from catchup by chloroform. This has already been noticed by Poppe, Jackson[3] and others in connection with the high results generally obtained, but has been attributed in most cases to mineral acids carried through by the emulsion. A very few experiments by our method indicated that oxalic, malic, tartaric, citric, and aminoacetic acids were not extracted from acid salt solution by chloroform, but that acetic, lactic, glycolic, and cinnamic acids were extracted in varying amounts. Acetic and lactic acids, being normal constituents of catchup, would certainly interfere, so that the problem was to free the chloroform from these and possibly other acids without loss of benzoic acid, so that it would be fit for titration without the necessity of evaporating and drying the residue. It was found that a saturated solution of sodium chloride containing a small amount of hydrochloric acid would remove the acetic acid and still not remove appreciable quantities of benzoic acid. When the principle was applied to catchup it was found that some acid or acids were present which were not so readily removed as acetic acid, and that more washings would be required than would be needed to remove acetic acid alone. Even with two washings, using 200 cc. of salt solution for each, the results were always too high by a nearly constant amount. This and other facts indicate that a higher fatty acid is present in catchup in small amount. Numerous tests on different catchups, by our method, show that the amount of this acid is equivalent to about 0.017 per cent. calculated as benzoic acid. Blank tests by the regular method yielded on Snider's catchup 0.017 per cent. and on Heinz's catchup 0.012 per cent. acid calculated as benzoic, when none had been added. Obviously the cause of the high results is to be sought in this non-volatil acid.

In order to simplify the process, the attempt was made to extract the catchup, without filtering. At first the tendency to emulsify made extraction impossible; also much coloring matter and fatty constituents were extracted. Then it was discovered that a small amount of concentrated nitric acid would lessen the trouble with emulsion. Later by adding sodium nitrite, the extractions were made without difficulty, and the coloring matter was almost completely destroyed so that the

[1] *J. Biol. Chem.*, **7**, 423.
[2] Previous citation.
[3] U. S. Dept. Agr., Bur. of Chem., *Bull.* **132**, p. 142.

chloroform extract was nearly colorless. Also the amount of fatty material extracted was apparently much diminished. We have also been able to overcome the tendency to emulsify by boiling gently for $1^1/_2$ hours with dilute acid under an air tube condenser, filtering, and extracting the filtrate, after saturating with salt. This was not found practicable with catchups which contained more than 0.1 per cent. of benzoic acid as some of the acid separates out and is lost in filtering. It has given good results, however, in the case of jellies and products which do not need filtering, but in such cases the heating should be brief, only 10 or 15 minutes, else much caramel is formed, which discolors the chloroform.

After numerous experiments the following procedure was adopted:

Twenty-five grams of catchup are weighed out in a 50 cc. beaker, 2 cc. concentrated nitric acid added and the mixture well stirred. Several small portions, about 0.2–0.3 gram in all, of sodium nitrite are added, stirring well after each addition. Without filtering, the bleached mass is rinsed into a 500 cc. separatory funnel by means of 200 cc. of saturated ammonium sulfate solution. Five extractions are made with chloroform using 50, 35, 25, 25, and 25 cc. respectively. The shaking is done by a gentle rotatory motion and the separatory funnel is not to be shaken violently. The separate portions are drawn down as closely as possible, into a second separatory funnel and shaken with 200 cc. of a saturated solution of chemically pure sodium chloride, to each liter of which has been added 3 cc. of 10 per cent. hydrochloric acid. After clearing, the chloroform is drawn into a third separatory funnel and again shaken with a fresh 200 cc. portion of the acidified salt solution.

It is then drawn into a 500 cc. Erlenmeyer flask, 4 or 5 drops of alcoholic phenolphthalein added and titrated with standard sodium alcoholate solution. The first persistent pink color is taken as the end point, although the color may fade on standing. With some samples the chloroform has a light straw color, which may deepen slightly as the titration nears the end, but this causes no difficulty, the end-point being generally sharp and definit.

The standard sodium alcoholate is prepared by dissolving 2.3 grams of cleaned metallic sodium in one liter of absolute alcohol.[1] It is best standardized against pure benzoic acid in the same volume of chloroform as used for the titration, namely 165 cc. It must here be noted that some samples of chloroform develop hydrochloric acid on standing and, particularly when used for standardization, should be shaken with distilled water or dilute sodium hydroxide just before use.

The sodium alcoholate may also be standardized against sodium benzoate by extracting a weighed portion from a saturated salt solution made

[1] *J. Biol. Chem.*, **7**, 423.

distinctly acid with hydrochloric acid, passing the chloroform through a small filter and titrating as before.

It has been stated that the sodium alcoholate may be standardized against aqueous acid.[1] Our recent experience does not quite bear out this statement. The standard in aqueous solution appears to be slightly too high. Numerous experiments indicate that the results on catchup are too high by an amount equivalent to about 0.35 cc. of 0.1 *N* sodium alcoholate when the weight of sample taken is 25 grams.

A number of titrations of known amounts of sodium benzoate are given. If this amount, 0.35 cc., is deducted, the results are in fair agreement with the amounts added.

Sodium benzoate added. Per cent.	Titration. Cc.	Found by deducting 0.35 cc. Per cent.	Brand of catchup.
0.064	1.55	0.0696	Snider's
0.160	3.1	0.1595	"
0.320	5.7	0.3103	"
0.064	1.6	0.0725	Heinz's
0.160	3.1	0.1595	"
0.320	5.85	0.319	"

That at least two acids besides acetic are extracted by the chloroform is shown by the following experiment: An amount of sodium benzoate solution equivalent to 1.1 cc. of the sodium alcoholate was added to 25 grams of catchup and the extraction made as directed. The chloroform was then shaken with successive 100 cc. portions of the acidified salt solution, titrating after each shaking. One acid appears to be removed by the first few treatments with the salt solution, but the other remains constant. It appears also that no benzoic acid is lost by the treatment for the titration remained constant, even after several additional shakings.

	Titration.	Theory.
2nd shaking.....	2.4 cc. sodium alcoholate	1.1 cc.
3rd shaking.....	1.7 cc. "	"
4th shaking.....	1.5 cc. "	"
5th shaking.....	1.4 cc. "	"
6th shaking.....	1.4 cc. "	"
7th shaking.....	1.4 cc. "	"

As before stated, glycolic, lactic, acetic and cinnamic acids were found to interfere. This acid which remains with the benzoic might be cinnamic, but could not be one of the three first mentioned as they are all readily removed by washing with the salt solution. Cinnamic acid acts exactly like benzoic acid and will, when present, be estimated with it.

The apparent advantages of the method are the short time required for analysis, usually only $1^1/_2$ hours, ease of manipulation, time and

[1] *J. Biol. Chem.*, 7, 424.

trouble of filtering and aliquoting saved, and recovery of the chloroform. Also, the method of titration is itself capable of great exactness. The method would appear to be well adapted to the needs of the manufacturer, for after the extent of the blank had been well established on a given stock, the results should be quite satisfactory.

As a qualitative test, the method is both quick and reliable. It is only necessary to shake the chloroform, after titration, with a small amount of water, separate and filter the aqueous solution to remove emulsion, and test with ferric chloride or apply Mohler's test. The used chloroform may be recovered by shaking several times with water made alkaline with sodium or potassium hydroxide. It should be tested by shaking 100 cc. with dilute hydrochloric acid, separating and titrating with the 0.1 *N* sodium alcoholate. Not more than 0.02 cc. should be required to give the end point with phenolphthalein.

THE CRYSTALLIN ALKALOID OF CALYCANTHUS GLAUCUS.

[FOURTH PAPER.]

SOME SALTS OF A NEW QUATERNARY BASE OBTAINED BY METHYLATING ISOCALYCANTHINE.

BY H. M. GORDIN.

Received August 1, 1911.

It was shown in the last paper on this subject[1] that anhydrous isocalycanthine has the formula $C_{11}H_{14}N_2$, and that when recrystallized from a mixture of acetone and water, it contains some water of crystallization, the exact amount of which is difficult to determin, owing to the extreme slowness with which this water is given off. When the alkaloid is kept *in vacuo* over a drying agent, the loss of the water of crystallization is at first fast, but very soon slackens down to such an extent that it can be observed only when working upon considerable quantities and weighing every few weeks. I have been keeping 1.9862 grams of the alkaloid in a vacuum desiccator over sulfuric acid for nearly twenty months. So far the loss amounts to 0.0894 gram, and the weight has not changed within the last two months. Supposing there will be no further loss, the amount of water of crystallization found would be 4.5 per cent., corresponding to half a molecule of water. Calculated for $C_{11}H_{14}N_2$. $^1/_2H_2O$, 4.92 per cent. H_2O.

It was also shown in that paper that the alkaloid contains an NH group, since when treated with nitrous acid, it gives an insoluble nitrosoamine. It was therefore expected that it would react with one molecule of methyl iodide to form a tertiary methyl isocalycanthine of the formula $C_{11}H_{13}(CH_3)N_2$, and with two molecules of methyl iodide to form a neutral

[1] THIS JOURNAL, 31, 1305.