On shaking Fraction 5 with an equal volume of concentrated sulfuric acid a slight odor of hydrogen sulfide was noted. On treating with bromine a slight amount was absorbed, but no crystals separated on standing. Testing for limonene by means of the nitrosochloride gave a blue coloration at first, but this color rapidly disappeared. Evidently there is little, if any, limonene in the oil.

Fractions 6 and 7, which consist of 9.6% of the original oil, are levorotatory and of relatively high specific gravity. Their composition was not established.

Summary.

The sample of volatile oil of *Euthamia caroliniana* (*L*). *Greene* examined, consists mainly of dipentene with a trace of pinene and possibly a small amount of limonene. No free acids were present, although the oil had been distilled and stored for about 15 months. A small percentage of combined acids, probably formic and acetic were present. Esters were present to the extent of 2.10% calculated as $CH_3COOC_{10}H_{17}$. The presence of aldehydes was established by means of Schiff's reagent. The total amount of alcohols present was 7.01%, of which 5.35% are free, and 1.66% combined. In addition to the compounds identified, a compound or compounds having levorotatory properties and a comparatively high density, are present to the extent of approximately 10%. That portion of the sample of oil that boiled above 180° at 45 mm. pressure with decomposition constitutes approximately 10% of the volume of the original oil.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

THE CHEMICAL COMPOSITION OF OSCILLARIA PROLIFICA.

BY B. B. TURNER.[1]

Received April 4, 1916.

This plant, one of the *Cyanophyceae*, was first brought to the attention of the previous authors in connection with the systematic examination of the public water supplies of Massachusetts under the direction of the State Board of Health. This study was undertaken and carried on for a number of years prior to Mrs. Richards' death, because the plant is a type of blue-grass alga that has caused much trouble at water works and to consumers. It was felt that by a study of the plant some plan could be devised for successfully dealing with the class.

The life history and chemical composition of the alga have already been the subject of three papers.[2] It is there described how the alga

[1] This work was made possible through a fund established as a memorial to the late Ellen H. Richards.

[2] Isabel F. Hyams and Ellen H. Richards, *Technology Quarterly*, 14, 302 (1901); 15, 308 (1902); 17, 270 (1904).

grew in great quantities in the water of Jamaica Pond near Boston and contaminated the water supply, till it was destroyed by treatment with copper sulfate. The present paper is a continuation of the work on the chemical constitution of the organism begun by the former authors, and many of the lines followed were suggested by them.

The material available at this time consisted of some kilograms of the dead alga, partly dried and partly preserved in various liquids, particularly in alcohol and in glycerol. Some of the samples labelled "fermented" had evidently been put up after undergoing partial decay on exposure to air. As the samples were all several years old, it was not possible to be sure than some of the smell and fermentative processes which had gone on in those samples free from preservatives, were not partly due to moulds and other organisms which had grown somewhat upon the dead material. It is not believed, however, that the changes from this source, in the tightly-closed bottles, had been great.

Composition in General.—Using the ordinary methods of analysis of foodstuffs, it was found that the air-dried material contained 9.7% of moisture and 6.4% of ash, in close agreement with earlier work. A small part of the latter is due to fine sand unavoidably collected with the alga.

The proportion of nitrogen was found by the Kjeldahl method to be 7.4%, equivalent to 46.25% of protein by the usual factor of 6.25. The dried alga was next extracted with ether in a Soxhlet's apparatus, whereby 2.2% was removed. The extract contained some chlorophyll and a very small amount of fat.

The remaining 35.5% consisted principally of carbohydrates, as will be shown later, along with which would be included the more or less insoluble and indigestible substances usually classed as "fiber."

Extractions with Solvents.—Since ether extracts comparatively little, in order to study the nature of the material extracted it was necessary to submit a large quantity of the alga to the action of the solvent.

For this purpose an apparatus was devised by which any ordinary flask could be used and an intermittent flow obtained as in the Soxhlet extractor. As it is thought that this may prove useful to other investigators who have to work with large quantities of material, it is here described in detail.

New Form of Extraction Apparatus.—In order to obtain the same action as in a Soxhlet's extractor without using a flask having an outlet or side tube below the level of the neck, it is only necessary to use a siphon with equal limbs and upturned ends, so as never to run dry, at the height of the upper level to which the flask is to be allowed to fill, enclosed in another siphon, the inner limb of which reaches to the bottom of the flask, the outer limb being still lower. The air in the outer siphon, above the level of the ends of the inner siphon, is always at the same pressure in

both limbs, since they communicate freely, and this pressure varies with the action of the outer siphon, becoming lower by the expansion of the enclosed air as the liquid in the flask runs down when the siphon is in action, just as the pressure of a bubble of air trapped in the bend of an ordinary siphon would do. The principle and manner of action of the siphon is shown diagrammatically in the accompanying sketch. The inner siphon, once filled, will remain full of liquid since the open ends A and A′ are on the same level *1* and higher than the bends down which the liquid would have to flow to empty the siphon. As the liquid accumulates in the flask, the outer siphon being empty and the outer limb open to the air at C, it will rise in the limb AB level with the surface of the liquid in the flask, till it reaches level *2*, a little above that of the end A when the inner siphon will come into action and carry the liquid over to A′, and so fill the outer limb A′C. This will reduce the pressure of air in the upper part of the outer siphon, carrying over the extra volume due to expansion and the liquid will now be drawn out of the flask as by an ordinary siphon, flowing up to A even when the level in the flask has sunk below level *1*, by atmospheric pressure, passing over to A′ by the inner siphon and then descending to C. This will, obviously, continue till the flask is emptied down to level *3* when air will enter at B, and passing by the upturned end of the inner siphon A, restore the atmospheric pressure in the outer siphon causing the outer limb A′C to empty itself, when the action of the siphon will be broken, allowing the liquid again to accumulate gradually in the flask till it once more reaches level *1*. This double siphon will therefore act just like the intermittent siphon in a Soxhlet apparatus, though there is no communication through the walls of the flask below the level of the neck.

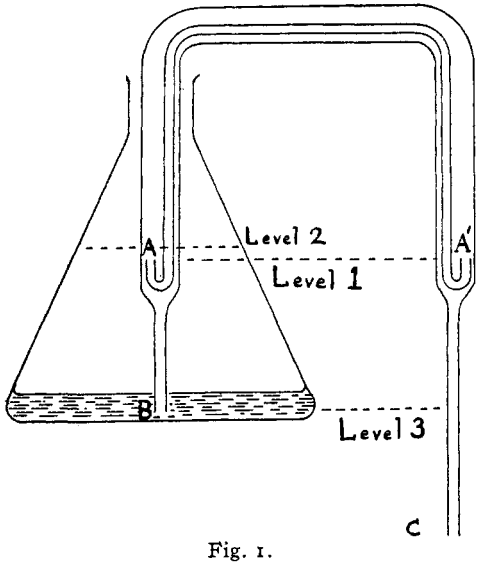

Fig. 1.

In practice, the siphon is made as in the accompanying sketch, the upturned ends of the inner siphon being replaced by cups at A and A′, which rest by means of small knobs of glass fused to them, on the constricted part of the outer tubes without closing the same. At D and D′, the inner tubes are fused into the wider tubes, which are connected at E and F by a cross tube sealed into both. It was also found advan-

tageous to have at G, where the narrower is joined to the wider part of the inner limb, a small jet, projecting inwards, and turned a little to one side so that when the siphon empties, the inrush of air from B may be divided into small bubbles and directed past the cup at A without risk of forcing its way into the inner siphon, as may happen if the free passage of the bubbles is obstructed. The outer tubes must not be too wide, nor the inner ones too narrow, as a large volume of air in the outer siphon delays, by its expansion, the filling of the outer limb A'C, while, if the inner tubes are not wide enough to allow a fairly rapid flow of liquid from one cup to another, the outer limb may not fill at all, but the liquid may continue gradually trickling over, in which case the level of liquid in the flask cannot be drawn down below that of the cups. When properly proportioned the apparatus works well for long periods. The stopcock K is introduced for convenience in filling. If the siphon is fitted up so that V is beneath the level of liquid in the outer flask or reservoir, then as soon as the inner flask fills up to the level of B, a slight suction through K will fill the inner siphon, when K is closed and the subsequent action is automatic.

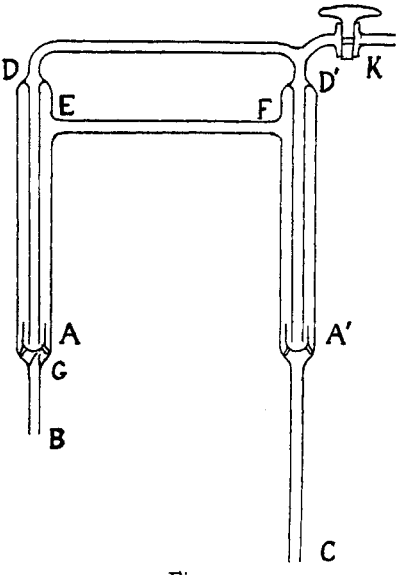

Fig. 2.

A form of the same apparatus which is easier to construct, may be made by using corks at D and D' in place of the sealed joints, and making the outer siphon in two parts connected by a rubber joint on the tube EF.

Extraction with Alcohol.—Extraction of the air-dried material with petroleum spirit (boiling at 40–60°) gave only about 0.1% of extracted matter, and ethyl ether extracted, as stated above, only about 2.2%.

Extraction with alcohol yields larger quantities of material.

The samples of oscillaria which had been standing several years in "strong" alcohol still contained a large amount of substance which could be extracted by that solvent. A portion of this partially extracted alga, after draining off the liquid, which weighed 25 g. when air-dry, was triturated in a mortar with successive quantities of about 30 cc. of absolute alcohol as long as the green color remained. After about twenty such operations, the combined extracts were mixed and an aliquot part evaporated to dryness. In this manner 1.66 g. was found to have been ex-

tracted. Allowing for moisture in the air-dried sample, and loss in the process of decanting and filtering, about 7 to 8% of the substance is seen to be soluble in cold absolute alcohol.

If the alga is treated with hot alcohol containing some water, the amount extracted is not so great. In four treatments of 100 g. of dried alga with 150 to 200 cc. of 80% alcohol, the total filtrate contained only 2.9%. If this treatment is now followed by absolute alcohol, very little more is extracted, about 0.1%. The larger amount extracted in the cold appears to be due to some coagulation, or other change, caused by the heat, which renders some constituents insoluble.

Cold water in repeated treatments extracted about 12.5%. If the aqueous extraction was preceded by alcohol, the amount withdrawn by the water was a little less. In four extractions of a lot of 100 g., following on the treatment with 80% alcohol and absolute alcohol described above, a total of 12.18% was obtained.

Boiling water acting directly on the original oscillaria extracted very little, but a characteristic swelling of the mass occurs to a voluminous jelly, about ten times the volume originally occupied by the solid in the loose, moist state. At the same time, the filtrate which first comes through soon gelatinizes. The carbohydrate of the alga seems to consist largely of a pectin-like substance.

The cold water extract is of a pale straw color, and rotates the plane of polarization of light to the right. It gives no precipitate on boiling the slightly acidified solution, but gives a slight yellow coloration with nitric acid. Saturation with ammonium sulfate causes a precipitate to form, as does phosphotungstic acid. Proteoses appear, therefore, to be present. The chief constituent present is apparently a carbohydrate which is dextrorotary but has little action on Fehling's solution until hydrolyzed by boiling with acids, when it causes copious reduction. The same action reduces the positive rotation, in case of prolonged treatment with acid, to zero or somewhat below.

The residue from aqueous extraction was then treated with acids. Dilute hydrochloric or sulfuric acid (1 to 2%) acts on the carbohydrate present very slowly. Accordingly, recourse was had to 5% sulfuric acid and digestion on the water bath (at about 96°) for from 6 to 10 hours at a time. This process not only hydrolyzed and dissolved carbohydrates but also decomposed proteins and the resulting liquid therefore gave a copious precipitate with phosphotungstic acid.

Carbohydrates.—The following experiments indicate the nature of the carbohydrates present. One hundred grams of air-dried oscillaria were extracted with 600 cc. of distilled water, protecting the extract from bacteria by a little toluene. The filtered extract, after precipitation with basic lead acetate and removal of excess of lead by Na_2CO_3(H_2S leads to solu-

tions which are almost impossible to free from colloidally suspended lead sulfide) and neutralizing contained by Fehling test, reducing sugar equivalent to 0.171 g. dextrose. The rotation was +0.40° for 1 decimeter.

Other similar aqueous extractions showed an average rotation of about +0.4° and a cupric reducing power of about 0.001–0.002 g. per cc.

The residue from the aqueous extraction was then treated with successive quantities of about 800 cc. 5% H_2SO_4 on the water bath for periods of about 10 hours. The first acid extract neutralized with $CaCO_3$ and filtered measured about 500 cc. and showed the high rotation +2.35°. After removal of sulfates by $BaCl_2$ addition of basic lead acetate gave a copious, flocculent white precipitate. After removing excess of lead with Na_2CO_3 and neutralizing, the solution, made up to 500 cc. in a measuring flask, showed a rotation of only +0.965°. This solution contained 0.0476 g. per 1 cc. total solids, and had a reducing power equivalent to 0.005 g. dextose per 1 cc., or a total of 2.5 g. By further heating for 6 hours with 5% H_2SO_4, its rotation was reduced to +0.475° (for the same volume) and its reducing power rose to 0.0075 g. per 1 cc. of the original volume.

By heating with phenylhydrazine hydrochloride and sodium acetate on the water bath for several hours, a small quantity of a crystalline precipitate was obtained from the above solution which showed a rotation of +2.35°. This, when crystallized from 50% alcohol with a little pyridine, melted, when rapidly heated, at 217–218° (corr.). Recrystallized from the same solvent, it formed beautiful clusters of very minute yellow needles, and melted sharply at 217°. The quantity obtained was too small for complete analysis, or further investigation, but a microkjeldahl analysis with 0.0056 g. of the once crystallized substance gave 0.00062 g. nitrogen, equivalent to 11.7%. This agrees with the formula of a hydrazone of a hexose ($C_6H_{12}O_5.N_2HC_6H_5$ N = 10.37%), or the osazone of a disaccharide ($C_{12}H_{20}O_9.(N_2HC_6H_5)_2$ N = 10.77%), rather than an osazone of a hexose ($C_6H_{10}O_4$ $(N_2HC_6H_5)_2$ N = 15.64%), but no known compound could be found with this melting point, containing less than two phenyl hydrazine groups to one (monosaccharide) sugar radical. Inactive α-acrosazone, with the last formula, melts at this temperature, while a galaheptose-bisphenylhydrazone (N = 14.43%) melts at about 218°. The high positive rotation of the above solution seems therefore to be due to a disaccharide of unknown composition which, on further hydrolysis, is slowly split up into monosaccharides with a much smaller rotation. The slowness of hydrolysis negatives the existence of any disaccharide such as maltose or galactose, and as solutions with a negative rotation of any considerable amount were not obtained (see below); this still further excludes the presence of saccharose.

Qualitative tests for pentoses with phloroglucin and orcin gave nega-

tive results, as also did the resorcin test for levulose (and indirectly, for saccharose).

Volatile Matter.—As the unpleasant odor acquired by the water in which the oscillaria was found was one of the reasons on account of which its investigation was first begun, a method was sought by which the volatile matters might be isolated. The smell of the unfermented oscillaria, in the dry state, is pungent but not unpleasant, and seems to be due to the aromatic substance soluble in petroleum spirit.

The fermented oscillaria, both that which had been put up in glycerol and that bottle without preservative, had a smell of decaying proteins and bad cheese. A quantity of the former was placed in a large flask, and the volatile substance present driven over with steam. The first portions of the distillate had the odor of feces, but later on, the smell changed to that of Limburger and Roquefort cheese, while it finally became more spicy, something like dried herring. The presence of indole and skatole were suspected in the first runnings, so these were shaken with ether. On filtering and evaporating the ether, only a small residue remained, from which the fecal odor had nearly disappeared, and no positive test for skatole could be obtained. The residue was chiefly of a fatty nature and showed microscopic needles. Indole and skatole, if present, as was strongly indicated by the smell, were plainly present in only very small quantities, and on account of their evanescent nature, the attempt to isolate them was not pursued further.

The bulk of the volatile substance present, which formed the chief cause of the offensive odor, consisted of fatty acids. By systematic extraction with ether of large quantities of steam distillate, two or three grams of these were obtained. An analysis of the barium salt showed that the acid was chiefly butyric.

Saponin.—The presence of a saponin-like substance in oscillaria prolifica is mentioned in an earlier paper,[1] "which is set free" (from the alga in its natural habitat) "to such an extent that the water froths whenever stirred by the wind, as if it contained a liberal supply of soap bark." A similar tendency to froth is easily noticed in the aqueous extract of the dried or preserved alga. But the suggestion that this tendency is due to a true saponin has not been confirmed by subsequent work. Various substances of the nature of dextrins, etc., might produce a similar result.

Since methyl alcohol constitutes the best solvent for saponin, 200 cc. were allowed to stand eleven days on 75 g. of dried, powdered oscillaria which had been previously extracted with ether. After filtering the deep green extract, ether was added to precipitate the saponin. A small quantity of a white gelatinous precipitate came down on adding about one volume of ether and this was increased a little by adding another volume

[1] *Tech. Quart.*, 15, 309–310 (1902).

of ether. A similar precipitate in about the same quantity was obtained by the action of methyl alcohol on the original dried oscillaria. This was tested for saponin in the following ways: (1) Basic lead acetate added to a solution of the above white substance gave no precipitate; (2) concentrated barium hydroxide solution gave a slight turbidity to a fairly strong solution; (3) ferric chloride and a ferricyanide was slowly reduced in the cold, somewhat faster on heating. This would agree with the presence of some saponin, as would the following two tests: (4) Mercuric chloride on heating and cooling gave with the substance in question a white turbidity; it was, however, *not* blackened by ammonia; (5) similarly, Nessler's solution was turned yellow, and, finally, gray, by this substance on heating; (6) tannic acid gave a considerable white precipitate; (7) no blue or green color, or fluorescence, however, was obtained on adding concentrated H_2SO_4 with a little alcohol, likewise (8) no green color appeared on exposure to air of a solution of the substance in concentrated H_2SO_4; (9) lastly, the hemolysis test proved negative in three to four hours; a slight hemolysis after sixteen hours was no more than would be observed with water alone. On the whole, the indications for the presence of an appreciable quantity of saponin in the oscillaria seem very doubtful. The substance isolated by methyl alcohol, which may also be the cause of the frothing, appears to be either a complex polysaccharide or more probably a glucoside. It does not reduce Fehling's solution directly, but does so strongly after it has been hydrolyzed by boiling for one-half to one hour with about 5% HCl.

A similar substance was found as a considerable constituent in all the alcoholic solutions in which the oscillaria had been preserved. These, though originally "strong" alcohol, had been diluted by the water in the fresh alga, so that they were not much more than 80%, in which strength saponin and similar substances are readily soluble.

Crystals.—A large part of the research was devoted to the search for definite substances which might be separated from the alga by crystallization. The various procedures need not be repeated in detail. With the exception of a few crystals of ammonium magnesium phosphate, only one crystalline substance was found and that in very minute proportion. This was obtained from the aqueous extract of the fermented oscillaria after it had been distilled with steam. The extract was treated with excess of basic lead acetate, a large quantity of gummy, brown precipitate was filtered off, the excess of lead removed with H_2S and the latter boiled off, and the filtrate then treated with about 2.5 volumes absolute alcohol, which brought down another amorphous precipitate. The filtrate from this was treated with ether till a slight turbidity was formed and then allowed to stand. Among the gummy drops which settled out in one or two days were found minute white spherules and star-shaped aggregates of

almost microscopic needles. By resolution of these in a very little water and reprecipitation with alcohol, and ether, they were obtained nearly free from gum, and the final separation was effected by rapid washing with water and decantation. By the microkjeldahl process described below, they were found to be free from nitrogen. When ignited in a little platinum boat, the ash was found to be 17.4%, consisting almost entirely of magnesia. The equivalent weight of the acid, assumed to be monobasic, is about 118, which agrees with that of caproic acid, $C_6H_{14}O_2$.

Coloring Matter.—Extraction with alcohol or ether of the air-dried alga, as well as that which had been preserved in alcohol, gave a brownish green solution characterized by a spectrum with five absorption bands. That this is caused by a mixture of two or more substances is shown by the separation of the bands, as indicated below. The original spectrum is as follows, the first four bands being given in the order of their intensity:

I. A very dark band with sharp edges in the red, extending in solutions of a medium depth of color, from about wave length 680 to 645 $\mu\mu$.

II. A broad, fairly dark band in the bluish green, shading off gradually from about 513 to 493.

III. A fainter band in the orange, shading off, 625 to 600.

IV. A faint band in the green, 540 to 530.

V. A strong end absorption beginning fairly sharply at about 440.

Of these, I, III, IV and V agree with Bands I, II, IV and VI, as given for crystallized chlorophyll by Willstätter.[1] Like other samples of "chlorophyll," this substance can be separated into a green coloring matter (the purified chlorophyll) and one or more yellow substances, such as have been described as xanthophylls.

Traces of yellow and red colors were observed at various times when the oscillaria was treated with solvents, but in too small amounts to allow identification.

The most interesting color obtained, however, was seen to a slight extent (perhaps as a colloidal suspension) in the aqueous extract of the air-dried material, which in bulk showed the violet color described in a former paper,[2] reproduced as No. 3 on Plate 1, but was present in notable quantity only in one bottle in which the oscillaria had been preserved under glycerol. Attempts to reproduce this by making glycerol extracts of the dried material all failed. Probably it is necessary to extract the fresh oscillaria with glycerol at a certain state of its growth, or exposure to air, or it may be that some ferment is concerned in the liberation of the color, which has lost its activity in the years since the alga was collected. Adding a little of the colored solution to a fresh glycerol extraction did not affect the result.

[1] *Handbuch d. Bioch. Arbeitsmethoden* II, p. 683.

[2] *Tech. Quart.*, 17, 3, 271 (1904).

The glycerol solution already mentioned was a deep bluish green by transmitted light, but was characterized by an intense red fluorescence, so that when looked at in bulk, or even in thin layers when illuminated from the side or behind the observer, it looked like blood. This fluorescence was examined with monochromatic illumination and showed a narrow band in the red at 670$\mu\mu$ which appeared unchanged in position when the wave length of the illumination varied, provided, as is required by theory, that the latter was shorter than that of the band; for longer wave lengths of illuminating light, the fluorescence disappeared.

The absorption spectrum of the blue-green solution was as follows:

I. Very dark band in red with a sharp edge on the red side at about 658 (shading at most to 660 or 662), and shading very gradually on the yellow side from about 643 almost to the D lines (*i. e.*, to about 600).

II. A very faint band in the yellowish green at about 577 to 565.

III. End absorption commencing about 450 and strong from 440 onwards.

Of these the third seems to be due to admixture with a green "chlorophyll," like that already described, as it disappears on purification, as appears in the following; the second agrees in position with Band III of Tswett's[1] Chlorophyllin α.

Both ether and alcohol precipitate the color, but it is changed in the process. The precipitate has a purple hue but is much less intensely colored than the original solution. Acids and alkalies also destroy the color. Heating to about 70° causes a highly colored dull blue flocculent precipitate to collect, as also does the addition of lead acetate, but in neither case can the coloring matter be redissolved unchanged.

When the glycerol solution is mixed with about two-thirds of its volume of a saturated solution of ammonium sulfate, the coloring matter is precipitated, which precipitate may be readily redissolved, after filtration, on adding a little distilled water. It then shows a pure blue color and the same intense red fluorescence. Repetition of the process requires a greater concentration of ammonium sulfate, as the glycerol has been removed, but the substance comes down readily on saturating its aqueous solution with the salt. In this way, it may be obtained in solution free from glycerol.

The color may be purified by precipitation with ammonium sulfate, and the spectrum of this purified solution, which is a pure and very intense blue in color, strongly resembling in appearance the color of cuprammonium hydroxide, shows Band I as described, very strongly without the end absorption III.

This blue coloring matter with the strong red fluorescence, for which the name "algocyan" is proposed, is of a very labile character. It keeps fairly well in aqueous solution (in presence of some ammonium sulfate)

[1] *Handbuch Bioch. Arb. Meth.*, II, p. 691.

if covered with toluene, but slowly fades on exposure to light for some days. Strong mineral acids or alkalies in solution of 1%, or more, decolorize it rapidly, producing a slight white precipitate. The same effect is produced, but more slowly by alcohol. If this be added gradually, there is no perceptible change at 10 or 15%, but when 50% is reached the solution, though still blue, has lost its fluorescence and appears very faintly turbid. The disappearance of the fluorescence seems to coincide with the change of state of the substance from solution to colloidal suspension. Addition of water at this stage does *not* redissolve the substance, and the fluorescence does not reappear. The algocyan is "denatured." Further addition of alcohol causes coagulation of the precipitate; if this be filtered off at once, it may be redissolved in *very* dilute ammonia (about 1 in 1000) but is bleached almost instantly. The neutral solution of unaltered algocyan in water is similarly bleached by ammonia, though a few drops per 1 cc. of 1 in 100 NH_4OH may be added without affecting the color or fluorescence. The coloring matter is not carried down by ammonium magnesium phosphate in the solution. The fluorescence is destroyed by dilute acetic acid, indicating that the solution has been changed to a colloidal suspension, as when alcohol is added, but there is a difference in this case, as the fluorescence reappears on adding dilute ammonia till the substance redissolves.

Dilute ferric chloride added cautiously (1 or 2 drops of 1 in 300 solution to each 1 cc. of very deep blue solution of algocyan) precipitates a substance apparently as blue as the original, which redissolves in very dilute ammonia (1 in 1000) to a violet solution of a paler color, from which saturation with ammonium sulfate precipitates a dull blue substance with apparent loss of color.

Microkjeldahl Method.

The well known Kjeldahl method for the estimation of nitrogen, as ordinarily carried out, requires the sacrifice of one or two decigrams, or more, of material for each determination. Where only very small quantities of substance are available, satisfactory results can still be obtained by a modification of the details of manipulation. After some experimentation, a method was found which would yield useful information as to the nature of a substance by the analysis of from 5 to 10 mg., and with care should give results accurate to about 1%, or even less, with such minute quantities. While the modifications introduced are only of the nature of details, the great usefulness of a method of obtaining such information, when obliged to work with almost "microchemical" quantities, seems to make it advisable to publish those details here.

For such minute quantities of material, the amount of sulfuric acid taken must be correspondingly reduced, not only to avoid an appreciable error in the correction for the nitrogen in the "blank," but chiefly to re-

duce the volume of the total liquids in the distillation. Half a cubic centimeter of concentrated sulfuric acid was taken each time, measured in a capillary pipet graduated to hundredths, about 0.5 g. K_2SO_4, and, when necessary to accelerate digestion, one milligram of mercuric oxide. The digestion was performed in a test tube of about one centimeter diameter in the end of which a bulb about 1 inch in diameter had been blown, which gave a space of about 10 cc. for the bursting of the bubbles should the liquid begin to "bump." The tube was used in an inclined position so that the drops should be thrown against the sides, but in spite of this precaution, bumping was the commonest source of trouble in the method and great difficulty was experienced in avoiding errors from this cause. While a slight bumping might occur without introducing serious error, it only needed the loss of a very small drop, where such minute quantities were used, to make the results entirely misleading. However, by the use of a specially small "microburner" in heating, the liquid could usually be made to boil steadily with minute bubbles, or to digest at a temperature a little below its boiling point. For this purpose, a pinhole flame may be used, by burning ordinary gas from a glass capillary about one millimeter in diameter, but besides covering the bulb with soot, the yellow flame was found inferior in another way to that about to be described. To obtain a concentrated heat at a very small point, which is the least likely to cause bumping, and will not boil the acid too rapidly, a blue flame on the principle of the Bunsen burner, not exceeding 6 to 8 millimeters high and 3 or 4 in diameter, was obtained by using a burner, easily made from glass tubing, of the shape and size shown in the sketch. The glass jet A is sealed at B into another piece of tubing BC, in which a slight expansion has been blown just below the point to which the end of the jet A will reach, and in the opposite sides of this expansion, two thin-walled bulbs at D,D. These are then crushed by a smart blow and the edges of the holes rounded in the blow pipe. The opening at C is drawn out to about three millimeters diameter. The size of the air holes at D,D is not very material. They cannot, of course, be regulated, according to the size of the flame, as in an ordinary burner, but for small flames, the inflow of air is nearly self-regulating as the flow of gas alters and there is no danger of the flow "striking back." It was found, how-

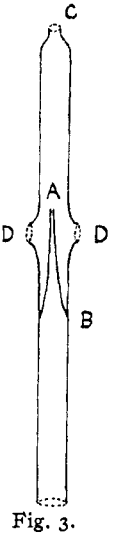

Fig. 3.

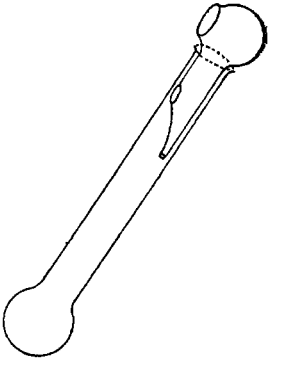
Fig. 4.

ever, that the flames were often insufficiently oxygenated, however wide the air holes were made, owing to the jet A being too wide, so that the gas did not issue at a high enough velocity to draw in the air required. Care must be taken, therefore, to make this hole small enough, while not so small that the maximum flame, when the gas stopcock is turned full on, is less than that mentioned above. The easiest way to ensure this is to test the jet with full gas pressure before sealing it in: if the yellow flame so obtained is about a centimeter long, the hole is of the right size; if larger, it may be readily contracted by cautious heating of the edges in the blow pipe. A properly adjusted burner gives, with the gas full on, a bright blue flame like that of an ordinary burner which has a slight over-supply of air, about 10 to 12 mm. high; and this can be reduced to half the height without danger of extinction or turning yellowish. In use, the flame is placed close up to the bulb to be heated so that its point just touches the glass, adjusting it carefully beneath the part covered by the acid. If the glass towards the edge of the liquid be directly heated, there is a steadier evolution of bubbles and less tendency to bump, but loss of experiments by cracking of the bulbs is very frequent.

To obviate further the danger of loss by spurting out of minute drops, it was found advisable to place a funnel in the mouth of the tube. An ordinary funnel soon becomes sealed by drops of condensed liquid, which are then spurted out by each sudden evolution of vapor, so a form was designed in which a free passage to the outer air would always remain open, while no drops could possibly be spurted straight through. This consisted in a piece of glass tube just narrow enough to slip easily into the mouth of the test tube with a spherical bulb about 5 mm. wider in diameter blown on the upper end, in which a large round hole, like the opening of a thistle-funnel, has been blown, facing at right angles to the axis of the tube. On the same side, which in use becomes the upper side, a smaller hole not less than 6 or 7 mm. diameter, is blown in the tube at a point half an inch from the bulb and below this the tube is drawn out to a narrower "tail" about half an inch long, bent a little so as to rest against the lower side of the test tube. Any liquid collecting in this funnel runs down into the tail and through the opening in this, back into the test tubes, and although this hole at once becomes stopped by a drop of liquid, gases have always free exit by the opening on the upper side. The larger outer opening being at right angles insures that even if a small drop were accidentally shot through the stem of the funnel, it would strike the side of the bulb and run back. The shape of the funnel allows of its being rinsed out easily, with a few drops of water, outside and in.

Digestion having been effected in the test tube and the acid diluted with not more than 6 or 8 cc. of water, including that used to rinse the funnel, the whole is poured into a distillation flask of special design, and of such

a size that the whole of the ammonia vapor can be driven over with 10 cc. of water or less. This consisted of a small flask with a bulb holding about 25 cc. and a neck about eight inches long, with an inner up-turned tube as a trap, sealed in, where the side tube was attached to the neck. The latter tube is bent down at right angles and has a small oval bulb just below the bend, from which it tapers down to a long, narrow point, which was inserted in a cork fitted into the mouth of a $6 \times {}^{3}/_{4}$ in. test tube. This cork was also fitted with an exit tube bent at right angles; a small funnel with a narrow capillary stem reaching down to the bottom of the tube passed through the cork closing the neck of the flask.

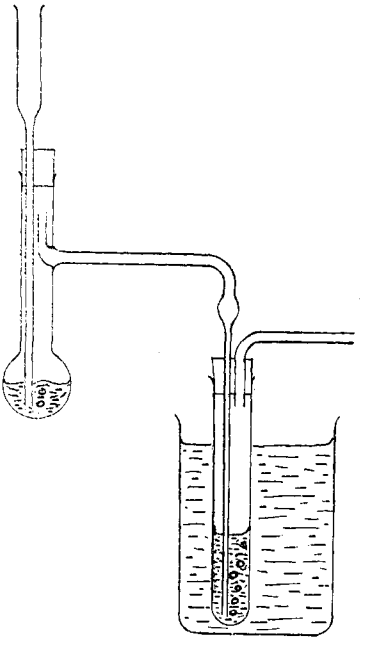

Fig. 5.

Having rinsed out the digestion tube into the flask with not more than 5 cc. of water in two small quantities, a measured quantity of strong caustic soda (2.5 cc. of a nearly saturated solution) which had been found to be slightly more than enough to neutralize the acid, was added and the liquid, which did not measure over 20 cc., distilled into an accurately measured quantity of 0.02 N hydrochloric acid in the test tube, a rather rapid current of air being drawn through the apparatus during the operation, by connecting the exit tube to an air pump. In five minutes after boiling the ammonia has all passed over, except for negligible traces, so that this stage of the operation is very quick and easy. Even where mercury has been used, in which case sodium sulfide is also added before distilling, the rapid air current prevents bumping, provided the funnel dips to the bottom of the flask. Frothing rarely causes trouble and can be instantly controlled by removing the source of heat, when the cooling effect of the air current is rapidly felt. The effectiveness of this rapid distillation, and of the trap in preventing the carrying over of alkaline spray, was demonstrated by experiments with known quantities of 0.02 N ammonium chloride.

The following results, with known substances, will show the degree of accuracy attained when bumping was avoided:

Antipyrine, taken 0.0075 g. Nitrogen found 0.0011 g. equivalent to 15%. Theory requires 14.9%.

α-Naphthylamine, taken 0.0092 g. Nitrogen found 0.00087 g. equivalent to 9.4%. Theory requires 9.8%.

Caffeine, taken 0.0066 g. Nitrogen found 0.00185 g. equivalent to 28.0%. Theory requires 28.9%.

While a certain percentage of results vary 1% or more from the theoretical requirements, indicating that the method of digestion is still capable of improvement, the close agreement of most results, when the very small quantities used are considered, indicates the usefulness of the method as a guide to the composition of an unknown substance, where the quantities available are limited.

Conclusions.

1. The proportion of protein, fat, moisture and ash has been determined in air-dried Oscillaria prolifica.

2. A systematic extraction of the alga has been carried on with various solvents and the approximate proportions extracted have been determined.

3. A new form of extraction apparatus suitable for continuous extraction with volatile solvents of large quantities of material, is here described. This consists of a double siphon, so constructed that an intermittent flow is obtained, as in a Soxhlet's apparatus, allowing of the periodic filling and emptying automatically, of an ordinary flask, between two fixed levels, without a tubulure or aperture in the side of the flask.

4. Crystalline and easily identified characteristic substances were not found in any considerable quantity in the alga. A small quantity of a crystalline magnesium salt of an organic acid (possibly caproic) has been obtained.

5. Saponin of characteristic properties was not obtained in appreciable quantities; a glucoside or polysaccharide having physical properties similar to saponin exists in the plant.

6. The bad smell and taste of the decaying alga appears to be due largely to higher acids of the fatty (butyric) series, some of which were separated: indole or skatole from decomposition of the proteins seems also to be present in traces. The fresh alga contains an aromatic compound soluble in petroleum spirit which causes a characteristic odor.

7. The spectra have been determined of various coloring matters from the alga, a "chlorophyll" similar to that of the higher plants and a blue substance soluble in water and in glycerol, with an intense red fluorescence, having properties which indicate that it is either associated with and carried down by the proteins in solution, or itself has similar precipitation properties. This substance, which is believed to be new, and may be allied chemically to the chlorophyll of the alga, has been named "algocyan."

8. The chief carbohydrate in the plant is a pectin-like substance insoluble in water with a great power of forming jelly on heating. It is hydrolyzed with remarkable slowness by boiling with 5% sulfuric acid; the examination of the products indicated the presence of a nonreducing

substance with a high positive rotation, and a reducing sugar with smaller rotation (eventually becoming zero or slightly negative) formed from this by further hydrolysis. A small quantity of a phenyl hydrazine compound was obtained which in the pure state melted at 217° (corrected) and contained about 11% of nitrogen: this can not be identified with any known compound.

9. A modification of the details of manipulation of the Kjeldahl method of nitrogen determination has been worked out, allowing of approximate analyses of as little as five to ten milligrams of material, containing about one milligram or less of nitrogen.

BOSTON, MASS.

THE POISONOUS PRINCIPLE OF POISON OAK

(*Rhus diversiloba*, T. and G.).

BY JAMES B. MCNAIR.

Received December 13, 1915.

Botanical Similarity between Poison Oak (*Rhus diversiloba*) **and Poison Ivy** (*Rhus toxicodendron*). — The difference between *Rhus diversiloba* and *Rhus toxicodendron* is so small that their proper classification forms a bone of contention between botanists. Those botanists that believe in innumerable species are in favor of their separation, while the more conservative are opposed to it. Greene,[1] considers *Rhus diversiloba* "a peculiar type of *toxicodendron* belonging exclusively to the Pacific coast." Engler[2] believes *diversiloba* a sub-species of *toxicodendron*. The only botanical ground for the separation of the two into different species is a slight difference in the shape of their leaflets.[3]

Is the Poison a Glucoside of Rhamnose, Gallic Acid and Fisetin?— As far as is known to the writer no work has been published on the chemistry of the poisonous principle of poison oak (*Rhus diversiloba* T. and G.). Because of the very close botanical relationship existing between poison oak and poison ivy (*Rhus toxicodendron*, L.) and because of their identical pharmacological action it has always been held among the medical profession, as well as among botanists, that the poisons were identical. Not only are they similar in pharmacological action, but they are also similar in solubility, rapid oxidation, etc.

In seeking to find out the constitution of the poisonous compound of poison oak the work done on its very closely related plant has been investigated. Perhaps the most recent work is that of Syme, 1906,[4]

[1] Ed. L. Greene, "Leaflets of Botanical Observation and Criticism" (1903–1906), Vol. 1, p. 119.

[2] Candolle, "Monographic Phanerogamarum," Vol. 3, p. 395; Engler und Prantl, "Die Natürlichen Pflanzen-familien," 111–5, p. 168 (1897).

[3] Gray, "Synoptical Flora" (1895–7), Vol. 1, Pt. 1, Fasc. 1–2, p. 382–383.

[4] W. A. Syme, "Some Constituents of Poison Ivy Plant," Johns Hopkins University Dissertation, 1906; Acree and Syme, *Am. Chem. J.*, 36, 303, 316 (1906).