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THE DETECTION OF PENTOSE, FORMALDEHYDE AND METHYL ALCOHOL

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I have recently attempted to improve the well-known Bial's reagent[1] for pentoses so that it will keep, and have found that when the 6 g. of orcinol and 40 drops of 10% ferric chloride solution are dissolved together in 200 cc. of ethyl alcohol without the addition of hydrochloric acid, the solution is stable. Fifteen drops of this alcoholic solution, 5 cc. of the sugar solution and an equal volume of fuming hydrochloric acid are mixed and heated in boiling water. A clear blue color always develops when 1 mg. of arabinose or xylose is present. When more pentose is present a precipitate quickly forms. With less than 1 mg. the color is greenish. When considerable quantities of hexoses obscure the test the precipitate can be filtered off, washed with water and dissolved in alcohol, when some green color will be observed if the proportion of hexose to pentose is not too great. The absorption band is between the C and D lines.

While testing the specificity of this reagent I have found that it gives interesting results with formaldehyde. With 2 mg. of formaldehyde in 2 cc. of water, 10 drops of the orcinol solution and 2 cc. of concd. hydrochloric acid cause the immediate formation of a white precipitate, consisting of very small spheroids. With only 0.2 mg. of formaldehyde the precipitate does not appear for several minutes. Heating hastens its appearance. With still lower concentrations of formaldehyde no precipitate is formed and the solution becomes yellow upon heating. Acetaldehyde gives a similar precipitate with orcinol, but when a sufficient amount of water is present and the material is heated at once, no precipitate is formed unless a very large amount of acetaldehyde is present and the inside of the test-tube is covered with scratches.

When the material in the test with formaldehyde is heated for 15 minutes in boiling water, the precipitate turns brown and the addition of an excess of alkali causes it to dissolve to a pink solution, or if a large amount of precipitate was present, pink flocks will be formed. The precipitate formed by acetaldehyde does not turn brown upon heating and the addition of an excess of alkali causes it to dissolve to form a yellow solution.

With quantities of formaldehyde smaller than 0.1 mg., where there is no precipitate upon heating for 15 or 20 minutes, the addition of an excess of sodium hydroxide produces a pink or salmon color with an intense green fluorescence. So intense is this fluorescence that the test will readily

[1] Hawk, "Physiological Chemistry," Blakiston, 1923, 8th edition, 644.

show one part of formaldehyde in ten million parts of water. Acetaldehyde treated in the same manner gives a solution that shows no fluorescence at all.

Instead of heating with hydrochloric acid, the test can be heated directly with sodium hydroxide, but carried out in this manner is somewhat less delicate.

Another way of testing for the presence of formaldehyde is to add a few drops of an alcoholic solution of orcinol, omitting the ferric chloride, and 2 drops of 20% sodium hydroxide solution. The solution soon becomes pink when formaldehyde is present, owing to oxidation by atmospheric oxygen, the formaldehyde probably acting as an oxydase. As little as one part of formaldehyde in one million of water can be detected in this manner, but only if interfering substances are absent. Traces of copper also produce a pink color, and acetaldehyde causes the appearance of a greenish-yellow color. Orcinol alone in the presence of alkali slowly develops a pink color.

While the production of the green fluorescence appeared to be too delicate to be put to use for the detection of methyl alcohol (after its oxidation to formaldehyde), the production of the white precipitate seemed suitable. I have found that this reaction can readily be used, and that its use is limited chiefly by the process employed to oxidize the alcohol. The application of a red hot copper spiral produces a very considerable amount of formaldehyde from pure ethyl alcohol. Using this procedure it is difficult to distinguish between pure ethyl alcohol and ethyl alcohol containing 1% of methyl alcohol.

Gettler[2] in his excellent review of 58 tests for the detection of methyl alcohol has recommended potassium dichromate and sulfuric acid as an oxidizing agent that forms very little formaldehyde from ethyl alcohol. I have tried this means of oxidizing the alcohols and have found it to be extremely satisfactory. As applied to the new method, removal of the chromium and sulfuric acid is not necessary, nor is it necessary to separate the acetaldehyde from the formaldehyde by fractional distillation. However, it is advisable to expel some of the acetaldehyde by heating in a large test-tube in boiling water, as acetaldehyde in large amounts sometimes forms a precipitate with orcinol. This seems to be due to the use of scratched test-tubes or test-tubes that have been attacked by hot phosphoric acid. The test-tubes must not be too narrow or the acetaldehyde will have difficulty in escaping. The amount of alcohol used in the test is 9 times greater than the quantity theoretically necessary to reduce all of the dichromate. An aqueous 0.5% solution of orcinol is used, and the addition of ferric chloride omitted.

The procedure is as follows.

[2] Gettler, *J. Biol. Chem.*, **42**, 311 (1920).

Place in a large test-tube which is 22 mm. in diameter and free from scratches, 1 cc. of the suspected alcohol, 2 cc. of 6.7% potassium dichromate solution and 2 cc. of 1:2 (62%) sulfuric acid. Mix at once after adding the sulfuric acid and allow to stand at room temperature for about 10 minutes. The reduction of the chromic acid to blue chromic sulfate should take about 40 seconds, and if it takes much longer than this the alcohol used contains too much water. Add 15 cc. of distilled water, mix thoroughly and heat in boiling water for 10 minutes. Now add 5 mg. of orcinol in 1 cc. of water, mix very thoroughly and heat in boiling water for 30 minutes. If the alcohol contained 5% or more of methyl alcohol, a precipitate will be formed after about 5 minutes heating. With 1% of methyl alcohol a precipitate will form after 15 minutes' heating. The test will show methyl alcohol down to 0.5%, although in this case it may be necessary to heat for 30 minutes and then allow the solution to cool before a precipitate forms. If the precipitate is filtered off it will be seen to be distinctly brown or yellow.

Quantities of methyl alcohol smaller than 0.5% can be detected by precipitating the chromium by adding a slight excess of sodium hydroxide and heating. When this is filtered, the clear filtrate possesses a green fluorescence if even traces of methyl alcohol were originally present. This last procedure is of doubtful value, as it is to be expected that traces of methyl alcohol derived from pectinous substances may possibly be present in beverages from fruits.

The alcohol used in the test is obtained by distilling the suspected solution or beverage, using a Vigreux column to obtain as complete a separation from the water as possible. The temperature of the upper portion of the column should not be allowed to exceed 80°.

Formic acid, amyl alcohol, acetone and furfurol do not interfere with the test. Glycerol does not interfere because it is eliminated in the process of distillation; but if added to the alcoholic distillate glycerol gives a positive test if as much as 5 mg. is present. I believe, therefore, that the procedure for the detection of methyl alcohol can be used also for the detection of small amounts of glycerol. It is impossible at the present time to say that there are found in alcoholic beverages no substances capable of interfering with the detection of methyl alcohol as carried out by this method, but I have as yet found none and believe the method to be more time saving and more trustworthy than other chemical tests that have been proposed in such large numbers for the identification of methyl alcohol in alcoholic beverages.

Summary

1. A new method is proposed for using Bial's test for pentoses.

2. An extremely delicate test for formaldehyde is described.

3. A new method is given for the detection of methyl alcohol in alcoholic beverages.

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