Phenyl *p*-nitrobenzyl ether, $C_6H_5OCH_2C_6H_4NO_2$, m. 91°, *o*-Cresyl *p*-nitrobenzyl ether, *o*-$CH_3C_6H_4OCH_2C_6H_4NO_2$, m. 89.7°, *m*-Cresyl *p*-nitrobenzyl ether, *m*-$CH_3C_6H_4OCH_2C_6H_4NO_2$, m. 51°, *p*-Cresyl *p*-nitrobenzyl ether, *p*-$CH_3C_6H_4OCH_2C_6H_4NO_2$, m. 88°, Thymyl *p*-nitrobenzyl ether, $CH_3(C_3H_7)C_6H_3OCH_2C_6H_4NO_2$, m. 85.5°, Eugenyl *p*-nitrobenzyl ether, $C_3H_5(OCH_3)C_6H_3OCH_2C_6H_4NO_2$, m. 53.6°, and vanillyl *p*-nitrobenzyl ether, $CH_3O(CHO)C_6H_3OCH_2C_6H_4NO_2$, m. 124.5°.

BALTIMORE, MD.

[CONTRIBUTION FROM THE FOOD INVESTIGATION LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE.]

THE VOLATILE REDUCING SUBSTANCE IN CIDER VINEGAR.

BY R. W. BALCOM.

Received November 27, 1916.

The presence of a volatile reducing substance in vinegar appears to have been first noted by Farnsteiner.[1] In following the changes which occurred when some wines were allowed to undergo acetic fermentation he observed a progressive increase in the apparent sugar content, which could not be accounted for by any increase in concentration due to evaporation during the fermentation process. Further investigation showed that the distillate obtained by distilling some of the partially fermented vinegar, as well as that obtained by distilling some of the finished vinegar, reduced Fehling's solution strongly, even at room temperature, and separated silver from an alkaline silver solution. The distillates from some wine vinegars which had been kept for four years exhibited this same reducing power. Since distillates from wines which had not undergone acetic fermentation showed no such reducing properties, Farnsteiner concluded that a volatile aldehyde-like substance was formed during the acetic fermentation of wines. The presence of this substance was found to account in large part for the apparent increase in sugar mentioned above.

Farnsteiner found that in the distillates which he prepared, furfural was either absent or present only in traces, and that formaldehyde was present in very small amounts only. In spite of the fact that this volatile reducing substance appeared to be oxidized to acetic acid by silver oxide, the assumption that it was acetaldehyde was found to be untenable, because dilute solutions of acetaldehyde did not react in the same way as the distillates, with Fehling's solution, Schiff's reagent or with sulfurous acid.

In a second paper[2] Farnsteiner records the result of further attempts

---

[1] K. Farnsteiner, *Z. Nahr.-Genussm.*, 2, 198–209 (1899).

[2] *Ibid.*, 15, 321–6 (1908).

to identify this substance. In some of its properties it resembled acetol, but on heating some of the distillate with an excess of phenylhydrazine hydrochloride and sodium acetate he obtained a crystalline osazone which melted at 243° (uncor.), while acetolphenylosazone melts at 145°. Along with this crystalline osazone there appeared to be formed an oil-like substance which led him to believe that the distillate contained more than one substance capable of reacting with phenylhydrazine. Farnsteiner did not succeed in establishing the identity of the volatile reducing substance but states that the problem did not appear to be hopeless and suggests that it be attacked from the bacteriological as well as from the chemical side.

In the meantime C. A. Browne[1] in an investigation of the effects of fermentation upon the composition of cider and vinegar, isolated an osazone melting at 240 to 242°, from a precipitate obtained by the direct addition of phenylhydrazine dissolved in acetic acid to a sample of "over-aged" cider vinegar. The elementary composition and other properties of this osazone agreed with those of diacetylphenylosazone first prepared by v. Pechmann.[2] As stated by Browne, this osazone can be formed either from diacetyl, CH_3-CO-CO-CH_3, or from the compound CH_3-CHOH-CO-CH_3, which is variously designated as acetylmethylcarbinol, methylacetol, dimethylketol and dimethylglycolose.

A little later Pastureau[3] published an article on the presence of acetylmethylcarbinol in certain commercial vinegars. The particular kind of vinegar is not stated, but these were undoubtedly wine vinegars. All of them reduced Fehling's solution in the cold, and one of them, which possessed this reducing power to a greater degree than the others, was subjected to distillation. The distillate reduced Fehling's solution and Tollens' reagent energetically in the cold. It gave no reaction for furfural with aniline acetate and had no action on fuchsin solution decolorized with sulfurous acid. On warming some of the distillate with phenylhydrazine acetate Pastureau obtained a crystalline osazone, lemon-yellow in color, insoluble in water, and difficultly soluble in other solvents tried. After recrystallization from acetic acid, this osazone melted at 243°. Pastureau made no analysis of this osazone but concluded, from certain reactions which he describes, that it was the osazone of acetylmethylcarbinol, since the reducing properties of the distillate seemed to him to eliminate the possibility of the presence of diacetyl. This conclusion was undoubtedly correct, but Pastureau states that his examination of these vinegars had shown them to be abnormal (*vinaigres de fécule*). Both Browne's and Pastureau's work appears to have been done without

---

[1] THIS JOURNAL, 25, 29–31 (1903).

[2] H. v. Pechmann, *Ber.*, 21, 2754 (1888).

[3] M. Pastureau, *J. pharm. chim.*, 21, 593–5 (1905).

knowledge of Farnsteiner's earlier publication since no reference to it is made by either of them.

Farnsteiner's work led the writer, when serving as an associate referee of the Association of Official Agricultural Chemists, to make some investigation of the occurrence of volatile reducing substances in cider vinegar, since, if these were present in normal vinegars of this kind, the method which was then being used for the determination of reducing sugars in cider vinegar would have to be modified. The results of that investigation are given in the associate referee's report[1] to the Association in 1910. Since then the method for the determination of reducing sugars in vinegar has been modified so as to eliminate the volatile reducing substances before the determination is made. At present the amount of volatile reducing substances in terms of invert sugar, obtained by difference, is usually stated in giving the results of a complete analysis of vinegar.

The boiling point of acetylmethylcarbinol is 141° to 142°.[2] This fact when considered in connection with the work of Farnsteiner, Browne, and Pastureau, would lead one to expect the reducing power of the distillate obtained from cider vinegars to be due, either wholly, or in part, to the presence of acetylmethylcarbinol. However, Browne's results were obtained on a sample of "overaged," therefore, abnormal cider vinegar, and Pastureau's results were obtained on a sample of wine vinegar which was also abnormal. Since all cider vinegars, so far examined, have been found to contain a volatile reducing substance or volatile reducing substances, further work seemed to be necessary for the purpose of determining to what extent, if any, the volatile reducing substance or substances might consist of acetylmethylcarbinol.

It is the object of this paper to present the results of the work undertaken for this purpose. Two different samples of cider vinegar were used. One was a sample of known history prepared under the writer's direct supervision from the time the apples were ground and pressed until the finished vinegar came from the generators. This was found to contain volatile reducing substances equivalent to 0.24 g. of invert sugar per 100 cc. of sample. The other was a market sample that contained volatile reducing substances equivalent to 0.40 g. of invert sugar per 100 cc., the largest amount which has as yet been found in a cider vinegar, so far as the writer knows. Distillates prepared from both of these samples by distilling under diminished pressure at a temperature of about 60° were found to have the same reducing action on Fehling's solution as the corresponding distillates obtained by distilling at ordinary pressure under the same conditions. This furnished additional evidence, if any

---

[1] U. S. Dept. Agr., Bur. Chem., *Bull.* **137**, 57–61 (1911).

[2] H. v. Pechmann, *Ber.*, **22**, 2214 (1889).

were needed, that the volatile reducing substances exist as such in the vinegar and are not formed during the distillation process.

In the experimental work described below, a distillate prepared from the commercial sample was used, since this contained relatively large amounts of the volatile reducing substance. A distillate prepared in the same way from the sample of known history behaved qualitatively in every way like that from the commercial sample. The relation between the amount of osazone obtained and the copper-reducing power was the same in each case, so that any conclusions drawn from results obtained on the one distillate are applicable to the other as well.

Experimental.

Several liters of distillate were prepared by adding one volume of water to four volumes of the commercial cider vinegar above referred to, and distilling until four volumes of distillate had been collected. This distillate was colorless and contained acetic acid to the extent of 3.5 g. per 100 cc. Its reducing power on Fehling's solution, determined by Munson and Walker's method, was equivalent to 0.334 g. of invert sugar per 100 cc. (average of several determinations), and when heated on the steam bath with phenylhydrazine it readily gave a precipitate which could be filtered off, dried, and weighed. As reagent, phenylhydrazine hydrochloride dissolved in a solution of sodium acetate was used. The sodium acetate solution contained 150 g. of sodium acetate per liter, and 10 cc. of this solution were used to dissolve each gram of phenylhydrazine hydrochloride taken. The reagent was freshly prepared each time by warming the hydrochloride with the sodium acetate solution on the steam bath until solution had taken place, and then filtering.

It was found that when sodium acetate was added to the distillate also, the precipitate obtained with the phenylhydrazine reagent was greater in amount and cleaner in appearance than when such addition was not made. To obtain complete precipitation it was found necessary to add an amount of phenylhydrazine hydrochloride equal to about five times the weight of the invert-sugar reducing-equivalent of the distillate and to heat for about two hours on the steam bath. The procedure used to obtain the maximum amount of phenylhydrazine precipitate with this particular distillate was as follows:

To each 100 cc. of distillate were added 15 g. of sodium acetate and after the sodium acetate had dissolved, 20 cc. of the phenylhydrazine reagent. The mixture was then heated on the steam bath for two hours, cooled, and filtered through a weighed Gooch crucible. After washing the precipitate with a little cold water it was dried to constant weight in a water-jacketed oven. When so treated 100 cc. of the distillate gave 0.67 g. of precipitate.

Twelve to fifteen grams of this phenylhydrazine derivative were prepared and recrystallized in small portions at a time from a mixture of chloroform and alcohol, as follows: Two grams of the precipitate were dissolved in 200 cc. of chloroform in a 300 cc. Erlenmeyer flask by warming on the steam bath. The solution while still warm was filtered into a second flask of the same size, using suction and a small Büchner funnel into which two pieces of filter paper had been fitted. The filtrate was warmed on the steam bath until the precipitate which had settled out had redissolved and the heating was continued until the volume of the solution was about 100 cc., when an equal volume of hot alcohol was added. The mixture was then cooled, and the precipitate filtered off, using suction, a glass funnel, and a small Hirsch filter plate. The precipitate was washed with about 10 cc. of a mixture of equal volumes of chloroform and alcohol, removed from the filter paper and dried. Yields of from 75 to 80% were obtained from the first recrystallization. A further recovery of about 10% could be obtained from the mother liquor.

One such recrystallization gave a clean crystalline product. A second recrystallization in which yields of from 85 to 90% were obtained did not change the appearance of the product.

Duplicate nitrogen determinations[1] made upon some of the twice recrystallized product, using a modification of the Kjeldahl method, gave 21.08% and 21.01% of nitrogen. The theoretical amount of nitrogen in diacetyl phenylosazone, $C_{16}H_{18}N_4$, calculated from 1916 atomic weights, is 21.05%. The product therefore appeared to be diacetylphenylosazone, but to prove beyond question that it was such, and to ascertain whether it came from diacetyl or from acetylmethylcarbinol, it was decided to prepare these two compounds for the purpose of comparing some of the properties of their solutions with the corresponding properties of the vinegar distillate.

About 200 g. of diacetyl, b. p., 85° to 90°, were prepared according to the method given by Diels and Stephan.[2] From a portion of this about 10 g. of acetylmethylcarbinol were obtained by reduction of the diacetyl with zinc and sulfuric acid, essentially as outlined by Diels and Stephan (*loc. cit.*), except that the ether extraction was carried out by extracting for 24 hours with the continuous liquid extraction apparatus devised by Bacon and Dunbar.[3] On redistilling the acetylmethylcarbinol the portion, approximately 5 g., which came over at about 140° (uncor.) was collected separately and used for the further work with this substance.

---

[1] Kindly made by H. W. Daudt, of the Organic Investigations Laboratory of this Bureau.

[2] Otto Diels and Erich Stephan, *Ber.*, 40, 4337–8 (1907). The methylethylketone used was obtained from the Norwich Chemical Company, manufacturers of acetone. It may not be generally known that methylethylketone of a high degree of purity can now be obtained in this country at a very reasonable price.

[3] U. S. Dept. Agr., Bur. Chem., *Cir.* No. 80 (1911); *J. Ind. Eng. Chem.*, 3, 930–1 (1911).

A solution of diacetyl in 3.5% acetic acid, containing 2 g. of diacetyl per liter, was distinctly yellow in color. This yellow color was still perceptible when the solution was diluted with water or with 3.5% acetic acid until it contained only 0.2 g. of diacetyl per liter. The solution containing 2 g. of diacetyl per liter had no appreciable reducing action upon Fehling's solution in the cold, even when allowed to stand for 18 hours. When 250 cc. of the diacetyl solution was distilled 90% or more of the diacetyl, judged by the color of the solution remaining in the distillation flask, came over in the first 50 cc. of the distillate, which was colored a deep yellow. 200 cc. of the diacetyl solution, when neutralized with barium carbonate, heated in a pressure flask with 2 g. of hydroxylamine hydrochloride and an excess of 3 g. of barium carbonate for 4 to 5 hours, cooled, filtered, the filtrate slightly acidified with hydrochloric acid and extracted three times with ether, gave a quantitative or nearly quantitative yield of diacetyldioxime. 200 cc. of the vinegar distillate when treated in the same way gave no dioxime or at most a trace. The vinegar distillate, therefore, did not contain diacetyl.

A solution of acetylmethylcarbinol in 3.5% acetic acid was prepared, of such a strength that its reducing action on Fehling's solution was approximately the same (equivalent to 0.337 g. of invert sugar per 100 cc., average of two determinations) as that of the vinegar distillate, which was equivalent to 0.334 g. of invert sugar per 100 cc. This was a colorless solution, the action of which on Fehling's solution in the cold was, so far as could be observed, exactly the same as that of the vinegar distillate. 250 cc. of this solution were subjected to fractional distillation, four portions of 50 cc. each of distillate being collected, leaving a fifth 50 cc. portion in the distilling flask. The reducing power of each of these five portions on Fehling's solution was determined, using Munson and Walker's method, and calculating the results in terms of invert sugar. In the following table these results are tabulated in such a way that they may be directly compared with the results obtained on 250 cc. of the vinegar distillate treated in the same way:

REDUCING POWER OF DISTILLATE EXPRESSED AS GRAMS INVERT SUGAR.

	1st 50 cc.	2nd 50 cc.	3rd 50 cc.	4th 50 cc.	50 cc. residue.	Total in different portions.	Total in 250 cc. taken.
Acetylmethyl carbinol solution..	0.214	0.187	0.170	0.149	0.119	0.839	0.843[1]
Vinegar distillate.	0.212	0.186	0.170	0.146	0.114	0.828	0.835[2]

100 cc. of the acetylmethylcarbinol solution, to which 15 g. of sodium acetate had been added, when heated on the steam bath with 20 cc. of the phenylhydrazine reagent for two hours, gave 0.65 g. of precipitate similar in appearance to that obtained from the vinegar distillate. 0.61 g.

---

[1] Calculated from determinations made on the acetylmethylcarbinol solution.

[2] Calculated from determinations made on the original vinegar distillate.

of this precipitate, when recrystallized from a mixture of chloroform and alcohol by the method already described, gave 0.49 g. of recrystallized product. 0.63 g. of the precipitate obtained from the vinegar distillate gave 0.48 g. of recrystallized product when similarly treated. These two recrystallized products as well as the corresponding product obtained from the diacetyl solution were identical in both macroscopic and microscopic appearance. All three preparations melted at the same temperature, separately and when mixed, namely, 243-244° (uncor.), and all three responded with equal readiness to v. Pechmann's test[1] for α-diketones.

From the results recorded above it is evident that the reduction of Fehling's solution by cider vinegar distillates is due largely, if not wholly, to acetylmethylcarbinol. On the assumption that Fehling's solution oxidizes acetylmethylcarbinol to acetic acid it was found possible, from the amount of cuprous oxide produced by a given volume of distillate or of acetylmethylcarbinol solution of varying concentrations, to calculate very closely the amount of precipitate which would be obtained with the phenylhydrazine reagent. This would be possible only if the reactions involved were at least approximately quantitative. The identification of acetic acid as the oxidation product formed in the action of Fehling's solution upon acetylmethylcarbinol has not yet been carried out, but there can be little doubt that acetic acid is the final oxidation product of this reaction. This would be in agreement with the fact that in oxidizing with silver oxide the distillate which he obtained from wine vinegar Farnsteiner found only acetic acid.

Further investigations in connection with the occurrence of acetylmethylcarbinol in vinegars and other products and of the conditions under which it is formed are in progress.

Summary.

The volatile reducing substances in cider vinegar consist largely, if not wholly, of acetylmethylcarbinol.

Acetylmethylcarbinol (methylacetol, dimethylketol, or dimethylglycolose) is a normal constituent of cider vinegar.

WASHINGTON, D. C.

ON THE DIGESTIBILITY OF BREAD.

II. SALIVARY DIGESTION OF ERYTHRODEXTRIN IN VITRO.

By J. C. Blake.

Received December 18, 1916.

One conclusion of the first article of this series, namely,[2] that the activity of the amylases worked with did not seem to vary appreciably with moderate variations in their concentrations, seemed to press for further ex-

---

[1] *Ber.*, 21, 2752–3 (1888).

[2] This Journal, 38, 1259 (1916).