Table II. Degradation of Acetate-Carbon-14

Compound	Reaction	Products of Reaction: Per Cent Recovery CO_2	Per Cent Recovery CH_3NH_2[b]	Specific Activity[a] CO_2	Specific Activity[a] CH_3NH_2[c]
Acetate-1-carbon-14	$K_2S_2O_8$ oxidation	96	..	22.7	...
	Azide reaction	92	90	46.0	0
Acetate-2-carbon-14	$K_2S_2O_8$ oxidation	96	..	18.2	...
	Azide reaction	90	91	0	35.9
Acetate-carbon-14[d]	$K_2S_2O_8$ oxidation	90	..	54.7	...
	Azide reaction	95	93	34.7	79.9

[a] Counts/min./mg. $BaCO_3$ assayed with an end-window Geiger-Müller tube.
[b] Determined by titration of volatile base.
[c] Oxidized to CO_2 with $KMnO_4$.
[d] Biologically prepared (*4*).

Methylamine can also be combusted directly after the Schmidt reaction, without distillation. The contents of the vial are transferred, with water, to the main compartment of a reaction vessel, and the combustion is conducted as previously described (*6*). This direct combustion greatly simplifies the procedure. However, if any unreacted acetate is present, it also will be oxidized.

Table II shows that the recoveries of both carbon dioxide and methylamine were about 90%. The specific activities of the labeled carbons of either acetate-1-carbon-14 or acetate-2-carbon-14 were twice the average values obtained by persulfate combustion. It appears that there is no significant cross-contamination by this method.

The advantages of this procedure are especially apparent when many samples are degraded simultaneously. The method was also found suitable for the degradation of propionate and butyrate. It is likely that a variety of degradations can be carried out by means of the simple apparatus described here, with a considerable saving of time. In this laboratory the complete degradation of serine by the periodate method of Sakami (*11*) was performed in the combustion-diffusion vessel.

LITERATURE CITED

(1) Aronoff, S., Barker, H. A., and Calvin, M., *J. Biol. Chem.*, **169**, 459 (1947).
(2) Baker, N., Feinberg, H., and Hill, R., ANAL. CHEM., **26**, 1504 (1954).
(3) Barker, H. A., and Kamen, M. D., *Proc. Natl. Acad. Sci., U. S.*, **31**, 219 (1945).
(4) Dauben, W. G., Abraham, S., Hotta, S., Chaikoff, I. L., Bradlow, H. L., and Soloway, A. H., *J. Am. Chem. Soc.*, **75**, 3038 (1953).
(5) Daus, L., Meinke, M., and Calvin, M., *J. Biol. Chem.*, **196**, 77 (1952).
(6) Katz, J., Abraham, S., and Baker, N., ANAL. CHEM., **26**, 1503 (1954).
(7) Markham, R., *Biochem. J.*, **36**, 790 (1942).
(8) Phares, E. F., *Arch. Biochem.*, **33**, 173 (1951).
(9) Phares, E. F., personal communication.
(10) Roseman, S., *J. Am. Chem. Soc.*, **75**, 3854 (1953).
(11) Sakami, W., *J. Biol. Chem.*, **187**, 369 (1950).
(12) Wood, H. G., Lifson, N., and Lorber, V., *Ibid.*, **159**, 475 (1945).

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Argentimetric Procedure for Borohydride Determination

HERBERT C. BROWN and ALFRED C. BOYD, JR.
Department of Chemistry, Purdue University, Lafayette, Ind.

In order to avoid errors arising from loss of borohydride under acidic conditions, an analytical procedure was developed which permits the determination of borohydride under alkaline conditions. The analysis is based upon the reaction: $8Ag^+ + BH_4^- + 8OH^- \xrightarrow{\text{ethylene-diamine}} 8Ag \downarrow + H_2BO_3^- + 5H_2O$. The precipitated silver is removed by filtration and the excess silver ion in the solution is determined by standard volumetric procedures. The procedure allows the determination of borohydride in the presence of iodate and shows that the reaction of iodate with borohydride is very slow. No interference occurs with potassium chlorate, sodium formate, ethyl alcohol, acetone, and cyclohexanone. Benzaldehyde interferes, resulting in high borohydride values. An ammoniacal solution of silver nitrate provides a convenient sensitive spot test for borohydride solutions.

IN THE original investigations of the chemistry of the borohydrides, the determination of active hydrogen utilized hydrolytic decomposition of the borohydride ion under acidic conditions, followed by measurement of the hydrogen evolved (*4*, *5*, *9*). Volumetric methods based upon the oxidation of borohydride by iodine (*7*), hypochlorite (*3*, *8*), and iodate (*6*) have been proposed.

The iodate method is a simple procedure which appears to have many advantages for the rapid analysis of borohydride solutions. An excess of standard potassium iodate solution is added to the aqueous borohydride sample, stabilized by alkali. A large excess of solid potassium iodide is added, followed by 4*N* sulfuric acid. After 2 to 3 minutes in the dark, the liberated iodine is titrated with standard sodium thiosulfate solution.

An attempt was made to simplify the iodate procedure by eliminating the need for two standard solutions. Lyttle *et al.* had stated that the borohydride reduction of iodate appears to be an instantaneous reaction (*6*). It therefore appeared that the analysis could be carried out by the addition of a large unknown excess of iodate to the borohydride solution, conversion of the iodide (presumably formed in the rapid reduction) to free iodine by acidification, followed by titration of the free iodine by standard arsenious oxide under controlled pH.

The results obtained were erratic. In the course of investigating the cause of the difficulties, it became evident that the reaction of iodate with borohydride is not so fast as it had been postulated to be. The iodate procedure must depend upon the formation of iodine or other intermediate oxidation products, upon the acidification of the iodate-iodide solution, followed by the reaction of these products with the borohydride.

In the course of this study an argentimetric procedure for borohydride determination was developed which permits the analysis of borohydride solutions under strongly alkaline conditions and the determination of borohydride in the presence of iodate. Application of the method definitely established the slowness with which iodate and borohydride react under alkaline conditions.

The new analytical procedure depends upon the reduction of silver ion by borohydride ion under alkaline conditions. In order

$$8Ag^+ + BH_4^- + 8OH^- \rightarrow 8Ag \downarrow + H_2BO_3^- + 5H_2O$$

to maintain the silver ion in solution under alkaline conditions,

a complexing agent must be used. Both ammonia and ethylenediamine were investigated. The latter proved advantageous in a number of respects. Consequently, all analytical data reported in the present paper are derived from the procedure utilizing this complexing agent.

PROCEDURE

Aqueous sodium borohydride solutions, 2*M* in sodium hydroxide, were employed for the analyses. In the presence of the strong base such solutions were observed to be relatively stable for long periods of time. The ethylenediamine solution contained 40 grams of ethylenediamine per liter of solution (approximately 0.7*M*). Standard solutions of silver nitrate (0.2000*N*) and ammonium thiocyanate (0.1000*N*) were prepared and standardized.

The reagent is prepared by mixing 25.00 ml. of the standard silver nitrate solution with 25 ml. of the ethylenediamine solution. As this mixture is swirled in an Erlenmeyer flask, a 2.00-ml. sample of the borohydride solution is added. The solution immediately darkens as metallic silver precipitates. The mixture is immediately poured into a sintered-glass funnel of fine porosity. The filtrate is collected under slight vacuum. The flask in which the reduction had occurred is washed with 10 to 20 ml. of water, and this wash water is used to rinse the precipitated metal in the funnel. The wash water is drawn into the flask containing the original filtrate. At least three washings are made similarly to ensure quantitative removal of the silver ion. The precipitate is not permitted to become dry until the final washing has been made.

The filtrate is now made acid with 5 ml. of concentrated nitric acid, 2 ml. of standard ferric ammonium sulfate indicator are added, and the solution is titrated in the usual manner with standard ammonium thiosulfate solution (*10*). Addition of 20 ml. of nitrobenzene shortly before the stoichiometric point sharpens the end point considerably (*2*).

RESULTS AND DISCUSSION

Typical results obtained by the argentimetric procedure as compared with the iodate procedure are summarized in Table I.

In order to ascertain whether the analysis was sensitive to interference by substances that might be present in solutions being analyzed for borohydride, a number of tests were made by adding several millimoles of the substances examined to the silver nitrate–ethylenediamine solution immediately before addition of the borohydride sample and carrying out the analysis as described. The results are summarized in Table II.

The precision of these results is somewhat lower than those reported in Table I, presumably because of the necessity for rapid addition and mixing of the reagents. The results, however, clearly establish that the reaction of borohydride with the silver ion reagent is much faster than the reaction of the reducing agent with the aldehyde or ketones used, or with the other added materials investigated.

Table I. Determination of Borohydride

Dilution[a]	Borohydride Found, Meq./Ml. Iodate method	Silver method
None	1.574	1.576
	1.581	1.576
	1.581	1.592
	1.597	1.588
	1.605	1.590
	Av. 1.588 ± 0.011	1.584 ± 0.007
3:1	1.196	1.186
	1.204	1.195
	1.196	1.197
	1.194	1.187
	1.206	1.194
	Av. 1.199 ± 0.005	1.192 ± 0.004
1:1	0.806	0.792
	0.815	0.799
	0.817	0.800
	0.805	0.795
	0.803	0.801
	Av. 0.809 ± 0.005	0.798 ± 0.003

[a] A standard solution of sodium borohydride in 2*M* sodium hydroxide was diluted with 2*M* sodium hydroxide in ratio indicated.

Table II. Borohydride Determination in the Presence of Added Substances

Substance Added	Borohydride Found, Meq./Ml. Detn. 1	Detn. 2	Av.
None	1.611	1.593	1.60
2.5 mmole cyclohexanone	1.630	1.593	1.61
2.5 mmole benzaldehyde	1.859	1.717	1.79
2.5 mmole acetone	1.622	1.609	1.62
None	1.592	1.566	1.58
2.5 mmole ethyl alcohol	1.599	1.618	1.61
2.5 mmole benzaldehyde	1.755	1.748	1.75
2.5 mmole sodium formate	1.590	1.557	1.57
5.0 meq. potassium chlorate	1.622	1.633	1.63

Of the materials tested, only benzaldehyde interfered. In the absence of borohydride, the addition of freshly distilled benzaldehyde to the silver nitrate–ethylenediamine reagent in the presence of alkali results in the reduction of silver and the consequent loss of silver ion from solution. The high figures for borohydride in the presence of benzaldehyde (Table II) presumably are due to this side reaction.

The reaction was applied to an examination of the postulated rapid rate of reaction of borohydride with iodate. Two solutions were prepared which contained identical concentrations of sodium borohydride in the presence of 0.1*M* sodium hydroxide. One solution was made 0.1*N* with respect to potassium iodate. The solutions were maintained at room temperature. At appropriate times, aliquots were removed from each solution and analyzed for borohydride content (Table III). It is apparent that in 3 hours the decrease in borohydride concentration is approximately 1% and after 102 hours more than half of the original borohydride is still present.

Table III. Decrease of Borohydride Concentration with Time in Presence and Absence of Potassium Iodate[a]

Time, Hr.	Borohydride Found, Meq./Ml. Blank[b]	Iodate soln.[b]
0.3	2.58	2.63
3.5	2.57	2.61
8.0	2.51	2.44
24	2.48	2.23
102	2.36	1.59

[a] Room temperature.
[b] Solutions 0.1*N* in sodium hydroxide.

Reduction of iodate would presumably form iodide ion. This should remove silver ion from solution. Consequently, the analyses in Table III should be considered to represent only approximately the amount of borohydride remaining. It is probable that the amount left is somewhat less than that given by the analytical figures. The data nevertheless establish that iodate does not interfere with the proposed method of borohydride analysis and that the reaction of potassium iodate with sodium borohydride is very slow.

The analytical procedure could be considerably simplified if the excess silver ion could be titrated in the presence of the precipitated silver. However, the black color of the precipitate makes a colorimetric determination of the end point impractical. Thus an attempt to apply the titration method of Bloom and McNabb (*1*) failed for this reason. No attempt was made to apply conductometric or potentiometric methods to such a titration.

An ammoniacal silver nitrate solution provides a sensitive qualitative test for borohydride ion. The test is run by placing 1 drop of a 1*M* silver nitrate solution in concentrated aqueous ammonium into a depression of a white, glazed porcelain spot test plate and allowing a drop of the borohydride test solution to fall into the silver nitrate drop. A black precipitate forms immediately. The limit of detection by this method was determined to be $1 \times 10^{-4}M$ with respect to hydride ion, or $2.5 \times 10^{-5}M$ with

respect to borohydride ion. (The usual precautions with ammoniacal silver nitrate solutions should be observed.)

The iodate method has consistently yielded excellent results in this laboratory. This method has yielded low results in other laboratories. In view of the present study, it is probable that these low results arise from failure to acidify the borohydride-iodate solution promptly and carefully, thus resulting in hydrolytic loss of active hydrogen. Where acidification of the reaction solution can be tolerated, the iodate method appears the most satisfactory of those now available. The argentimetric procedure of the present paper provides a satisfactory alternative procedure for borohydride determination, which should be especially useful in cases where acidification is undesirable.

LITERATURE CITED

(1) Bloom, A., and McNabb, W. M., IND. ENG. CHEM., ANAL. ED., 8, 167 (1936).
(2) Caldwell, J. R., and Moyer, H. V., *Ibid.*, 7, 38 (1935).
(3) Chaikin, S. W., ANAL. CHEM., 25, 831 (1953).
(4) Chaikin, S. W., and Brown, W. G., *J. Am. Chem. Soc.*, 71, 122 (1949).
(5) Davis, W. D., Mason, L. S., and Stegeman, G., *Ibid.*, 71, 2775 (1949).
(6) Lyttle, D. A., Jensen, E. H., and Struck, W. A., ANAL. CHEM., 24, 1843 (1952).
(7) Mathews, M. B., *J. Biol. Chem.*, 176, 229 (1948).
(8) Pecsok, R. L., *J. Am. Chem. Soc.*, 75, 2862 (1953).
(9) Schlesinger, H. I., *et al.*, *Ibid.*, 75, 215 (1953).
(10) Volhard, J., *J. prakt. Chem.*, 117, 217 (1874).

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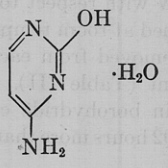
CRYSTALLOGRAPHIC DATA

90. Cytosine Hydrate (4-Amino-2-hydroxypyrimidine Hydrate)

Contributed by HARRY A. ROSE, Lilly Research Laboratories, Indianapolis, Ind.



Structural Formula for Cytosine Hydrate

CYTOSINE may be easily recrystallized from water, giving flat blades. The hydrate may lose water upon standing at room conditions for some time. The dehydration process can be speeded by heating the hydrate to 110° C. for 15 minutes.

The optical properties of the hydrate have been briefly described (*2*, *3*). X-ray powder diffraction data have also been given for the anhydrous crystals (*1*), but the fact that the data were for the anhydrous material was not made clear in the paper.

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Blades lying on {100} elongated parallel to *b*, showing the prism {110}, clinodome {011}, and basal pinacoid {001}.

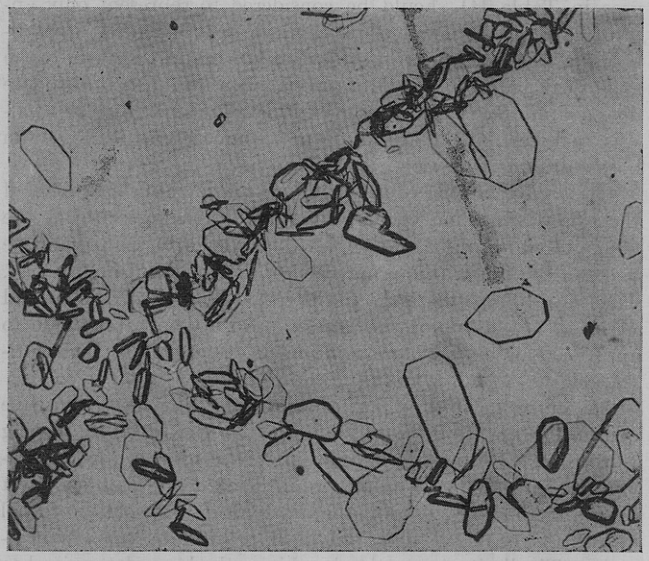


Figure 1. Crystals of Cytosine Hydrate from Hot Water on Microscope Slide

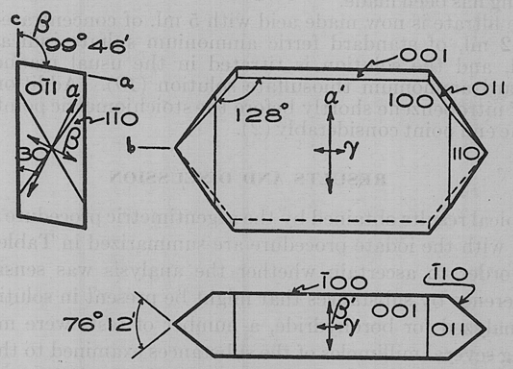


Figure 2. Orthographic Projection of Typical Crystal of Cytosine Hydrate

Cytosine Hydrate Powder Data

d	*I*/*I*₁	*hkl*	*d*(Calcd. from *a*, *b*, and *c*)
7.70	1.00	100	7.70
6.05	0.33	110	6.06
5.06	0.20	11$\bar{1}$	5.07
4.92	0.20	020	4.91
4.12	0.33	120	4.14
3.85	0.07	200	3.85
3.78	0.53	002	3.78
3.53	0.66	121, 012	3.50, 3.53
3.19	1.00	102, 201	3.19, 3.19
3.03	0.07	220, 112, 211	3.03, 3.03, 3.03
3.01	0.07	130	3.01
2.939	0.07	22$\bar{1}$, 20$\bar{2}$	2.931, 2.939
2.865	0.07	13$\bar{1}$	2.865
2.828	0.13	21$\bar{2}$	2.816
2.740	0.07	131	2.737
2.566	0.07	300, 30$\bar{1}$	2.567, 2.567
2.451	0.27	040	2.455
2.435	0.20	13$\bar{2}$	2.436
2.237	0.13	12$\bar{3}$	2.245

Anhydrous Cytosine Powder Data

d	*I*/*I*₁	*d*	*I*/*I*₁
6.46	1.00	2.45	0.03
5.33	0.66	2.39	0.27
4.43	0.66	2.27	0.07
3.65	0.27	2.22	0.07
3.53	0.66	1.849	0.07
3.40	0.66	1.820	0.03
3.28	1.00	1.716	0.13
3.09	0.20	1.640	0.07
3.07	0.20		
2.97	0.13		
2.89	0.13		
2.85	0.53		
2.73	0.13		
2.70	0.07		
2.55	0.27		