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A New Spectrophotometric Micro Determination of Vicinal Diols[1]

HANSJÖRG EIBL AND WILLIAM E. M. LANDS

The Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104

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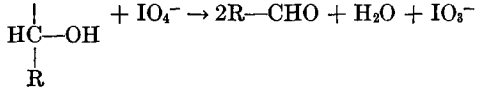
The quantitative determination of vicinal diols by periodate was introduced by Malaprade (1). One mole of a vicinal diol reacts with one mole of periodate and forms two moles of aldehyde and one mole each of iodate and water:



Generally an excess of periodate is used to ensure complete reaction of the glycol (2). Thus, periodate and iodate are always present in the reaction mixtures along with other reaction products, depending on the kind of diol or polyol which is analyzed. Some of the products may be quantitatively measured, particularly formic acid, formaldehyde, and micro analyses have been developed for certain types of compounds (3).

A general method for the quantitative determination of various diols should, however, be concerned with measuring either the disappearance of periodate or the formation of iodate. Potassium iodide reacts quantitatively with periodate in solutions buffered with bicarbonate at pH 8 to form iodine, but the corresponding reaction with iodate does not occur. Thus, periodate is easily determined in the presence of iodate but not vice versa. It is not surprising then, that the disappearance of periodate has been used in the quantitation of vicinal diols rather than the appearance of iodate (1–5).

The iodometric method of determining periodate with sodium arsenite at pH 8 is much less sensitive than that at acid pH values where iodate

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also forms iodine. Since we wished to analyze the very small amounts (10–200 nmoles) of vicinal diols produced in enzymic reaction mixtures, we examined the possibility that iodate could be measured directly after the selective removal of periodate. Rather than attempt a quantitative removal of periodate as the insoluble potassium salt, we examined the recent report that quantitative coprecipitation of periodate with aluminum hydroxide is possible in solutions containing small amounts of periodate and an excess of iodate (6). If relatively small amounts of iodate could be quantitatively retained in solutions having an excess amount of periodate, a direct determination of iodate formation should be possible in the reaction mixtures after removing the periodate by precipitation and centrifugation. The present report indcates that a sensitive iodometric assay for glycols can be developed using such a procedure.

REAGENTS

Sodium periodate solution (37 m*M*): 40 mg $NaIO_4$ (Mallinckrodt analytical reagent) was dissolved in 5 ml 0.1 *M* boric acid. The solution was always prepared immediately before use.

Potassium iodate standard solution (9.5 m*M*): 203 mg KIO_3 (Mallinckrodt analytical reagent) was dissolved in 100 ml distilled water.

Aluminum potassium sulfate solution (21 m*M*): 10 gm $K_2Al_2(SO_4)_4 \cdot 24H_2O$ (Mallinckrodt analytical reagent) was dissolved in 500 ml distilled water.

Potassium iodide solution (0.2 *M*): 3.3 gm KI (Baker analyzed reagent) was dissolved in 100 ml distilled water.

D-Mannitol was purchased from Nutritional Biochemicals Corporation.

1,2-Propanediol was a product of Matheson, Coleman & Bell.

1-Stearoylglycerol (90%) was purchased from California Corporation for Biochemical Research.

APPARATUS

A Cary model II recording spectrophotometer and a Beckman DU spectrophotometer were used for the measurements.

RECOMMENDED PROCEDURES

(*A*) The diol (0.1 to 1 μmole) was dissolved in absolute alcohol (0.2 ml) and 0.2 ml of the periodate solution was added. The reaction mixture was shaken and kept in the dark. Aliquots of 0.1 ml were taken after 20, 40, and 60 min and transferred to 6.3 ml of distilled water in a test tube. 2 ml of the aluminum potassium sulfate solution (42 μmoles) was added and then 0.5 ml of ammonia (0.72 *M* solution in distilled

water). After each addition, the contents of the tubes were mixed thoroughly. Centrifugation in a small centrifuge for 2 min resulted in a tight pellet. The supernatant was decanted into a tube containing 1 ml of 1 *N* sulfuric acid; then 0.1 ml of the potassium iodide solution was added and the contents were mixed again. The tubes were flushed with nitrogen and the absorbance at 350 nm of the solution was read after 10 min of standing in the dark. A blank without added diol was always included in the determinations. The final volume was 10 ml. The diol cleavage was finished normally after 40 min, and the determination after 60 min was only necessary to confirm that the reaction was, in fact, complete.

(*B*) The diol (50 to 600 nmoles) was treated as described in *A*. After 20, 40, and 60 min, 0.1 ml of the reaction mixture was removed and added to 5.4 ml of distilled water and treated with aluminum hydroxide as described above. Finally 1.0 ml of the potassium iodide solution was added and the absorbance read at 350 nm.

RESULTS

The reaction of periodate with diol was always performed in the dark as recommended in earlier review articles (2, 5). Blanks without added diol were always used to correct for the small reaction of periodate with alcohol. Alcohol was used in the reaction medium to get full solubility of lipoidal substrates, and was necessary especially for the analysis of monoacylglycerols.

The absorbance of iodine produced by adding potassium iodide to acidic solutions of iodate was linear with respect to concentration, and the absorption maximum at 350 nm was chosen for further determinations.

Table 1 shows that the removal of periodate by coprecipitation with aluminum hydroxide was essentially complete. The periodate in these determinations was dissolved in solvent mixtures which are commonly used in periodate determinations of vicinal diols (2, 5). For reasons, which will be discussed later, we have chosen solvent system *B*—0.1 *M* boric acid/95% alcohol (1/1)—for the quantitative determination of diols.

Table 2 shows the simulation of the products of a diol reaction mixture with periodate. No interference in the determination of iodate was observed, whether we analyzed iodate alone, iodate after treatment with aluminum hydroxide, or iodate after precipitation of the periodate with aluminum hydroxide. Although the molar ratio of periodate to iodate in part C ranged from 35 to 11, the determination of iodate in the super-

TABLE 1

Iodine Produced after Removal of Periodate with Aluminum Hydroxide

The solutions were kept in the dark for the indicated time periods and aliquots were analyzed according to the recommended procedure. The amount of oxidant remaining in the supernatant was determined as iodine and is expressed in parentheses as per cent of the total amount added.

	Periodate added, μmoles				
	0	0.58	1.15	2.30	4.60
Maximum possible iodine	0	(2.32)	(4.60)	(9.20)	(18.40)
Experiment	Iodine observed in supernatant, nmoles				
A (0.1 *M* boric acid)					
20 min	8	23 (1.0)	60 (1.3)	92 (1.0)	166 (0.9)
B (0.1 *M* boric acid/95% alcohol (1/1))					
20 min	8	32 (1.4)	60 (1.3)	120 (1.3)	239 (1.3)
40 min	8	32 (1.4)	68 (1.5)	123 (1.3)	272 (1.5)
60 min	8	34 (1.5)	68 (1.5)	126 (1.4)	284 (1.5)
C (0.1 *M* boric acid/dioxane (1/1))					
20 min	8	77 (3.3)	148 (3.2)	292 (3.2)	515 (2.8)

natant was not influenced by the large excess of periodate, indicating that the precipitation of periodate was quantitative.

A standard curve for the transformation of absorbances in nmole of iodate is presented in Figure 1. The molar absorbance calculated from this graph for the absorbance of iodine is 1.2×10^4 (A) or 2.3×10^4 (B), depending upon the iodide content of the solution.

TABLE 2

Absorbances Produced in a Periodate Reaction Assay System[a]

Exptl. condition	Added KIO_3, nmoles			
	0	66.5	133	199.5
A	(0.030)	0.239	0.489	0.721
B	(0.040)	0.235	0.485	0.734
C	(0.150)	0.236	0.483	0.729

[a] Three different amounts of iodate were checked in three experimental conditions: A, Iodate was analyzed after adding the indicated amounts to 8.4 ml of distilled water; 0.5 ml of ammonia (0.72 *M*) was then added and continued as described in the recommended procedure. B, Iodate was analyzed exactly as described in the standard procedure. C, Iodate in the presence of 2.3 μmole of added periodate was determined by the standard procedure. Absorbances were corrected for the blank values (shown in parentheses) obtained when iodate was omitted.

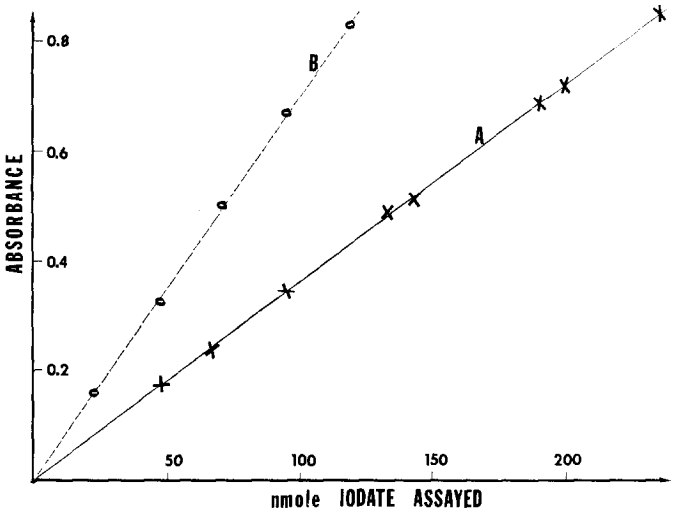

FIG. 1. Standardization curve measuring absorbance at 350 nm of iodine produced. The amounts of iodate in nmole per tube were as indicated: 8.4 ml (A) or 7.5 ml (B) of distilled water, 0.5 ml of ammonia (0.72 *M*), 1 ml of 1 *N* sulfuric acid, and 0.1 ml (A) or 1.0 ml (B) of 0.2 *M* potassium iodide were added and mixed with a final volume of 10 ml.

As a final test of the method we checked the assay system with some vicinal diols of known structure. In all cases, the estimated content of vicinal diol which was determined by measuring the formation of iodate was close to the theory—one mole of iodate formed per mole of diol (shown in Table 3). The final readings after 45 min were always taken

TABLE 3

Assay of Vicinal Diols of Known Structure

The amount of iodate formed in relation to the equivalents of added diol is described also as a percentage value.

Compound	Amt., nmoles	Diol-equivalent, nmoles	Iodate formed, nmoles	%
D-Mannitol	50	250	244	98
	100	500	478	96
	150	750	750	100
1,2-Propanediol	130	130	127	98
	260	260	262	101
	390	390	380	97
1-Stearoylglycerol	312	281	280	100
(90%)	625	562	550	98
	937	843	840	100

for the calculations, although the increase in product during the last 15 min was almost negligible as shown in Table 4 and Figure 2.

Figure 2 presents the reaction of 1-acylglycerol and 2-acylglycerol with periodate. Even after 45 min the formation of iodate in the case of 2-acylglycerol was negligible—in contrast to 1-acylglycerol, which had almost completely reacted as expected within 15 min.

DISCUSSION

The method described in this paper for the quantitative determination of vicinal diols is simple and allows the accurate measurement of diols in 60 min. In contrast to many methods of measuring the disappearance of periodate, we were able to directly analyze the iodate produced in the reaction mixtures. Excess amounts of periodate used in fairly high concentrations allowed a complete reaction in relatively short time periods. The molar ratio of periodate/diol was usually 10 to 100.

The results in Table 1 indicate that the coprecipitation of periodate with aluminum hydroxide was quantitative. The iodine produced in the supernatant was a constant percentage value (e.g., approximately 1.4% for solvent B) of the added periodate. This suggests that these amounts were produced by impurities in the periodate rather than by unprecipitated periodate since the solubility of aluminum periodate in the supernatant would be expected to be constant. A constant solubility would lead to decreasing percentage values of iodine found in the supernatant with increasing amount of periodate, such as: 1.4, 0.7, 0.35, and 0.175. A possible contaminant in the periodate reagent could simply be iodate. The absorbances were always corrected for these control values obtained without added diol. In addition, the almost constant control readings over a time period of 60 min indicate that unspecific side reactions of periodate with alcohol are negligible.

TABLE 4

Time Dependence of Iodate Formation

Absorbance at 350 nm was measured after 15, 30, and 45 min, and corrected for a blank without added diol (shown in parentheses). The 50, 100, and 150 nmole of D-mannitol analyzed corresponded to 250, 500, and 750 nmole of diol. One fourth of the total sample was used at each time point.

D-Mannitol, nmoles	15 min	30 min	45 min
0	(0.150)	(0.153)	(0.157)
50	0.210	0.220	0.223
100	0.416	0.430	0.436
150	0.641	0.661	0.668

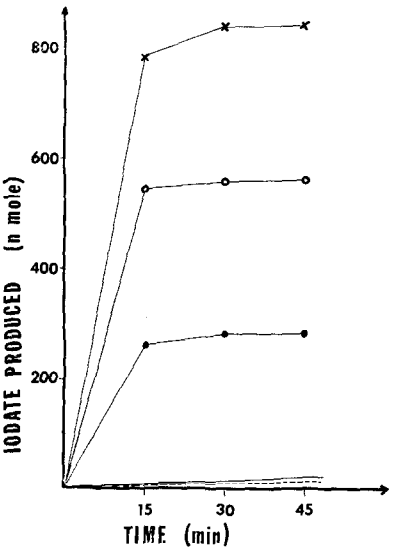

FIG. 2. Reaction of 1- and 2-acylglycerols in periodate assay system: 280 (●), 560 (○), and 840 (×) nmoles of 1-stearoylglycerol were compared with 370 (---) and 650 (—) nmoles of 2-acylglycerol.

Our interest in positional isomers of acylated glycerols led us to regard a mixture of 0.1 *M* boric acid/alcohol (1/1) as the most desirable solvent system. As reviewed recently by Serdarevich (7), boric acid stabilizes the monoacyl glycerols and prevents migration of the fatty acids. We were able to show that a borate system which hinders acyl migration did not interfere with glycol determinations.

When analyzing monoglycerides, the periodate reagent in mixtures of 0.1 *M* boric acid/alcohol (1/1) should not be added to the dry solid. Rather, the monoglycerides should first be dissolved in absolute alcohol and then the periodate in boric acid added. Otherwise there is an underestimation of 1-acylglycerols, which are not completely dissolved, especially with monoglycerides containing saturated fatty acids.

Negligible amounts of iodate were formed in the presence of 2-acylglycerols even after reaction times of 45 min. The amount of vicinal diol in the reaction mixture after 45 min was calculated to be about 2%. Extrapolation of the small absorbances measured after 30 and 45 min to time 0, however, would indicate that there was almost no contamination initially of the 2-acyl isomer by 1-acylglycerol, but that some slight acyl migration as noted by Mattson and Volpenhein (8) had occurred. The 2-acylglycerol studied had been freshly prepared from 2-acylglycerophosphorylcholine by treatment with phospholipase C. Thus monoglycerides with fatty acids in the 1- or 3-position react quantita-

tively with periodate to produce iodate whereas 2-acylglycerols do not react. This finding is now being successfully applied to the analysis of isomeric mixtures of monoglycerides derived from monoacylphosphoglyceride precursors (9).

Table 5 presents a comparison of our new method for the quantitative determination of diols with other procedures described in the literature, where generally the disappearance of periodate was measured. This method of determining directly the iodate produced is more sensitive than most reported procedures by a factor of 100. The method described by Frommhagen (15) allowed the determination of diols in amounts of 100 nmole. This method, however, as well as other procedures analyzing the disappearance of periodate spectrophotometrically at 220 nm, has some limitations (2). For instance, incubation times of 3 to 23 hours were needed because of low periodate concentrations and many inorganic materials also absorb in this region (15). The method of spectrophoto-

TABLE 5
Comparison of Different Methods for Determination of Diols

Authors	Method	Amounts used, μmoles
Van Lohuizen and Verkade (10)	Titration	800
Smith and Willeford (11)	Titration	400–600
Martin (12)	Titration	0.9–27
Karnovsky and Brumm (13)	Chromotropic (CH_2O)	2–10
Belcher *et al.* (14)	Chromotropic (CH_2O)	0.08–0.80
Frommhagen (15)	Spectrophotometric (220 nm)	0.100–1.0
New method	Spectrophotometric (350 nm)	0.01–0.250

metrically measuring formaldehyde has been developed to handle 80 to 800 nmoles (3), but this is not always applicable to all vicinal glycerols.

The present method has a wide range of applications. Most buffers and many organic compounds do not absorb at 350 nm, and the iodine is produced by a product found in all diol oxidations with periodate. An added flexibility comes from the fact that the absorbance of iodine is very much dependent on solvents or ionic concentrations. For instance, increase in the concentration of iodide by a factor of 10 (procedure B in Fig. 2) resulted in an almost twofold increase in the observed absorbance from 1.2×10^4 to 2.3×10^4. This change is a reminder of the fact that the absorbance of iodine is markedly influenced by both the equilibrium between iodine and triiodide and the dielectric constant of its environment.

In developing this method we were influenced by the sensitivity of the iodine measurements obtained in the method for alkenyl ethers as

described by Gottfried and Rapport (16). That method provides iodine absorbancies of 2.75×10^4 in 76% ethanol solutions. Our method A (Fig. 2) gave a value of 1.2×10^4 and with increased iodide (20 m*M*) we observed 2.3×10^4, which agrees more closely with that reported by others (16, 17).

The chance of selecting other solvent systems containing less polar solvents for the iodine produced offers a still wider range of sensitivities. The sensitivity of the described method, however, is sufficient to allow the accurate determination of 10 to 500 nmoles of diol. Even amounts as small as 1 nmole can be determined by reducing the final total volume in our procedure from 10 ml to 1 ml.

While this manuscript was being prepared for publication, we found a recent article by Nisli and Townshend (18), who regarded the same considerations described in this paper and devised a procedure for determining iodate in the presence of excess periodate by using the molybdate masking phenomenon described by Burnel (19). That phenomenon is sensitive to pH and to the presence of other ions, and requires small absorbance corrections for the molybdate and periodate, but does not require a centrifugation, since the periodate remains in the system. The precipitation procedure is not particularly difficult, and it allows greater flexibility by removing the major interference, periodate. Thus, two universal techniques are now available to investigators for the determination of nmole amounts of vicinal glycols whereas none were available a year ago.

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