Research Council; Standard Brands Inc. of New York, who have supported the investigation generously for many years; and the Rockefeller Foundation, who have made possible the large scale work by a substantial grant.

## Summary

The concentration and purification of pantothenic acid from liver is described. Because of the character of the material this has proved an unusually difficult task. The final preparation (amorphous) has a potency of 11,100 as compared with a standard rice bran extract which was chosen as unity. Further fractionation of this material resulted in no increased purity. For this and other reasons it is thought to be substantially pure. Its presence can be determined quantitatively when only 5 parts is present in 10 billion parts of culture medium (0.0005 $\gamma$ per ml.).

CORVALLIS, ORE. RECEIVED JUNE 8, 1938

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF OREGON STATE COLLEGE]

# A Study of Reduction with Hydriodic Acid: Use in Micro Determinations of Hydroxyl Groups

BY HERSCHEL K. MITCHELL AND ROGER J. WILLIAMS

The work described in this paper was undertaken primarily for the purpose of establishment of a new method for the micro determination of hydroxyl groups in amorphous, hydrophilic compounds such as pantothenic acid.[1] It was also desirable to develop a method for the quantitative estimation of a given type of hydroxyl as well as the total number per molecule.

A semi-micro method depending on acetylation with acetic anhydride in pyridine, has been described by Freed and Wynne.[2] Recently the description of a similar micro method has been published by Stodola.[3] A more or less standard procedure for the micro determination of hydroxyl groups in conjunction with other groups containing active hydrogens is that given by H. Roth.[4] This is based upon the Zerewitinoff (Grignard) reaction. A gravimetric method for accomplishing the same purpose, based upon the replacement of hydrogen with deuterium, has been proposed by Williams.[5]

None of the above methods proved suitable for the determination of hydroxyls in highly hygroscopic amorphous materials. In any case the adhering water interferes seriously and in the case of the Zerewitinoff method no solvent (lacking active hydrogen) could be found to dissolve the hygroscopic material.

(1) Williams, Weinstock, Rohrmann, Lyman, Truesdail and McBurney, THIS JOURNAL, **60**, 2719 (1938).

(2) Freed and Wynne, *Ind. Eng. Chem., Anal. Ed.*, **8**, 278 (1936).

(3) Stodola, *Mikrochemie*, **21**, 180 (1937).

(4) F. Pregl, "Quantitative Organic Micro Analysis," Third English Edition, P. Blakiston, Philadelphia, Penna., 1937, pp. 156–166.

(5) Williams, THIS JOURNAL, **58**, 1819 (1936).

It was believed probable that the following normal reactions of hydriodic acid with alcohols would be analytically useful

$$(1)\quad ROH + HI \longrightarrow RI + H_2O$$
$$(2)\quad RI + HI \longrightarrow RH + I_2$$

Hydroxyl groups, if completely reduced, must pass through both of the steps above. Complete reduction is difficult, however, and the second step may take place only partially. It was assumed that conditions might be found in which at least the first reaction would take place quantitatively.

If the first reaction only, takes place

Milliequiv. of OH groups = Milliequiv. of HI used up

If an additional amount of hydriodic acid is used to complete the second reaction, this amount (which is equivalent to the millimoles of iodine liberated) is subtracted from the total and

Milliequiv. of OH = Milliequiv. HI used up − Millimoles of $I_2$ liberated

The milliequivalents of OH groups reduced are equal to the millimoles of $I_2$ liberated and the milliequivalents of OH groups merely replaced are equal to the total hydroxyl groups minus those reduced.

It seemed probable that the stage of completion of reaction (2) above would give information concerning the type of grouping being reacted upon. This was based on the statement[6] given that the reactivity of alkyl halides toward hydrogen iodide is in the decreasing order; tertiary, secondary and primary.

**Other Reactive Groups.**—There are several types of functional groups that are readily at-

(6) Ogg, *ibid.*, **56**, 526 (1934).

tacked by concentrated hydriodic acid. It is well known that HI adds quantitatively to olefin double bonds and various studies of the conditions required have been made.[7] Reduction of halogen compounds also would take place. A quantitative study of a reaction of this type has been made by Shoesmith.[8] Nitroso groups,[9] phenylhydrazine,[10] thiophene or furan derivatives[11] and ethers are reacted upon readily by hydriodic acid. In many of these cases the reaction may be quantitative and hence need not prevent the simultaneous determination of hydroxyl groups.

## Experimental Procedure

Samples for analysis (0.5–10 mg. depending on hydroxyl content) are weighed into Pyrex capillary tubes on the micro balance. Forty λ (0.04 cc.) of hydriodic acid (sp. gr. 1.96) is introduced into the sample tubes with a capillary pipet. Extreme care should be taken to prevent droplets of the reagent being deposited near the top of the tubes. Following the introduction of the reagent into a capillary, the pipet is rinsed into a 50-ml. Erlenmeyer flask that has been labeled to correspond to the sample. Each tube is sealed within a few minutes by use of a fine pointed oxygen flame. The sealed tubes are then placed in a bath of constant boiling liquid for five hours (or in some cases for a different period). After the reaction has thus been carried out, each tube is placed in its correspondingly labeled flask and crushed with the end of a glass rod. The solutions are titrated to the usual starch–iodine end-point with sodium thiosulfate (0.015 *N*), followed by titrations of the same solutions to the phenolphthalein end-point with standard sodium hydroxide (0.05–0.15 *N*). Blank tubes containing 40 λ of hydriodic acid alone must accompany each set of determinations. This determines the $H^+$ and $I_2$ in the reagent itself.

In carrying out the reactions on esters, the $H^+$ from the acid produced on hydrolysis introduces a correction in the calculation. A peptide requires no correction since both acidic and basic groups are formed simultaneously on hydrolysis.

The technique in use and the description of the micro pipets used in the procedure given have been published by Kirk.[12] The Pyrex capillary chambers are thin walled, about 4 mm. in diameter and 4 cm. long.

**Materials.**—The chemicals used were, for the most part, Eastman highest purity products, and were used without further purification. The hydroxybutyric acids used were obtained through the kindness of Professor J. W. E. Glattfeld, of the University of Chicago. Kahlbaum hydriodic acid (sp. gr. 1.96) was used throughout as the reducing agent.

## Experimental Data

Table I illustrates the reproducibility of analytical results on a single compound. These were not consecutive analyses but were made over a period of several months. The effect of sample size on the amount of reduction also is shown.

TABLE I

DETERMINATION OF HYDROXYL GROUPS IN MANNITOL AT 100° IN FIVE HOURS

| | Mannitol, mmol. | Total OH groups found/mol. | Reduced OH groups found/mol. |
|---|---|---|---|
| 1 | 0.00541 | 6.0 | 4.41 |
| 2 | .00607 | 5.88 | 4.56 |
| 3 | .00616 | 6.0 | 4.63 |
| 4 | .00618 | 6.2 | 4.68 |
| 5 | .00716 | 6.0 | 4.68 |
| 6 | .00723 | 6.0 | 4.74 |
| 7 | .00766 | 5.64 | 4.98 |
| 8 | .00842 | 5.88 | 4.86 |

Table II is a condensation of analytical results obtained on several hydroxy compounds at three temperatures.

## Discussion of Results

A careful study of the data leads to the following conclusions of analytical significance.

1. Satisfactory analytical results can be obtained on primary alcohols, poly alcohols, hydroxy acids and negative substituted compounds, but simple secondary and tertiary alcohols, phenols, inositol and threonine fail to give useful results. A variation of from 0 to 4% low is noted on the more reactive types of compounds. These results compare favorably with existing methods for hydroxyl determination.

2. The reactions should be carried out in the range 100–134°.

3. From data not included here it was found that the reaction time of from five to twenty hours was satisfactory. Over the longer periods the thermal decomposition of hydrogen iodide produced a high blank and low results on compounds giving high reduction values. It was observed also that the presence of non-reducing organic compounds decreases thermal decomposition of the reagent.

It becomes evident on a careful consideration of the data that the ease of reduction is not dependent on the type of hydroxyl group as originally assumed, but does depend on the adjacent groupings about the hydroxyl. It may be stated that hydroxyls with what the organic chemists have called "negative groups" on adjacent carbon atoms, are reduced easily by hydriodic acid. The presence of adjacent methyl groups decreases the ease of the reaction. These generalizations

(7) Kharasch and Hannum, THIS JOURNAL, **56**, 1782–84 (1934).
(8) Shoesmith, *J. Chem. Soc.*, **123**, 2828–30 (1923).
(9) Earl and Kenner, *ibid.*, 2139–45 (1927).
(10) Brewster, *Trans. Kansas Acad. Sci.*, **36**, 111–12 (1933).
(11) Nellensteyer, *Chem. Weekblad*, **24**, 102–5 (1927).
(12) Kirk, *Mikrochemie*, **14**, 1 (1933).

TABLE II

| Compound | Detns. at °C. 100 | 134 | 163 | OH present | OH at 100° Red. | Subs. | OH at 134° Red. | Subs. | OH at 163° Red. | Subs. |
|---|---|---|---|---|---|---|---|---|---|---|
| Mannitol | 10 | 8 | 2 | 6 | 4.70 | 1.25 | 4.82 | 0.69 | 4.47 | 0.81 |
| Dulcitol | 4 | 1 | 1 | 6 | 4.72 | 0.79 | 4.96 | .77 | 3.22 | 0.40 |
| Erythritol | 2 | 1 | 1 | 4 | 2.88 | 1.06 | 2.86 | .86 | 2.14 | 1.18 |
| Benzyl alcohol | 1 | 1 | 1 | 1 | 0.71 | 0.24 | 0.97[a] | .63[a] | 0.69 | 0 |
| Glucose | | 1 | | 5 | | | 4.74 | .21 | | |
| Mannose | 2 | 1 | 1 | 5 | 4.07 | 0.78 | 4.35 | .70 | 3.05 | 0.90 |
| Levulose | 2 | 1 | 1 | 5 | 3.60 | 1.05 | 3.16 | 1.49 | 3.90 | .55 |
| Mandelic acid | 2 | 1 | 1 | 1 | 0.95 | 0 | 0.94 | 0.04 | 0.72 | .02 |
| Threonine | 2 | 1 | 1 | 1 | 0 | 0 | 0.725 | .055 | 1.91[a] | 0 |
| Inositol | 2 | 1 | 1 | 6 | 0 | 0 | 1.3 | .2 | 2.51 | .01 |
| 2-OH-1,4-Me-benzene | 1 | 1 | 1 | 1 | .10 | 0.09 | 0.40 | .04 | 0.54 | 0 |
| α,β-Di-OH-isobutyric acid | 8 | 3 | 1 | 2 | 1.0 | .86 | 1.81 | .23 | 1.28 | 0 |
| α,β-Di-OH-butyric acid | 2 | 1 | | 2 | 0.83 | .83 | 1.25 | .75 | | |
| β-OH-butyrolactone | 2 | 1 | 1 | 2 | 0.91 | .82 | 0.44 | 1.38 | 0.54 | .60 |
| Erythronic lactone | 8 | 5 | 1 | 3 | 1.93 | .86 | 1.95 | 1.02 | 1.13 | .82 |
| α-OH-butyrolactone | 2 | 5 | 1 | 2 | 0.05 | .05 | 0.87 | 1.09 | 1.04 | .88 |
| Tartaric acid | 1 | 1 | 2 | 2 | .1 | .2 | 0 | 0 | 1.15[a] | 1.31[a] |
| Pentaerythritol | 2 | 2 | 1 | 4 | .13 | .67 | .04 | 3.92 | 0 | 2.6 |
| Hexyl alcohol | 2 | 1 | 1 | 1 | 0 | .98 | 0 | 1.00 | 0 | 0.81 |
| Isoamyl alcohol | 2 | 1 | 1 | 1 | 0 | .96 | 0 | 0.82 | 0 | .64 |
| s-Butyl alcohol | 2 | 1 | 1 | 1 | .02 | .90 | 0 | .87 | 0 | .52 |
| t-Butyl alcohol | 1 | 1 | 1 | 1 | 0 | .25 | .01 | .46 | 0 | .44 |
| t-Amyl alcohol | 2 | 1 | 1 | 1 | 0 | .70 | 0 | .61 | 0 | .53 |
| Et acetoacetate | 2 | 2 | 1 | 1 | .09 | .84 | .14 | .81 | 0.34 | .80 |
| Et benzoate | 2 | 1 | 2 | 1 | 0 | 1.3[a] | 0 | 2.06[a] | 0 | 1.31[a] |
| m-OH-benzoic acid | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

| | 23 | 100 | 134 | HI used, calcd. | HI at 23° | subs. or add. | HI at 100° | subs. or add. | HI at 134° | subs. or add. |
|---|---|---|---|---|---|---|---|---|---|---|
| Crotonic acid | 1 | | 1 | 1 | 0 | 1.0 | | | 0 | 1.0 |
| n-Butyl ether | 1 | 1 | 1 | 2 | 0 | 0.48 | 0.1[a] | 3.7[a] | 0 | 3.7[a] |
| β-Alanine | | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] High results are due to reactions with groups other than hydroxyls.

are supported by an observation of the number of reduced hydroxyl groups as listed in Table II. The following comparisons illustrate these facts:

1. Primary alcohols such as hexyl, isoamyl or pentaerythritol substitute completely and do not reduce, while secondary butyl, tertiary butyl and tertiary amyl alcohols show only partial substitution.

2. Poly alcohols such as mannitol or erythritol show high reduction.

3. Benzyl alcohol shows a high reduction value, while an additional negative group, as in mandelic acid, promotes complete reduction.

4. Erythronic lactone and α,β-dihydroxybutyric acid have a tendency to be both substituted and reduced, while in the case of β-hydroxy-γ-butyrolactone and α-hydroxy-γ-butyric lactone less reduction follows the substitution. In the first two compounds the functional groups are all on adjacent carbon atoms, while such is not the case with the latter two substances.

5. α-Hydroxy-γ-butyrolactone reacts with hydriodic acid readily at 134° but not at 100°. Compounds with the hydroxyls adjacent react readily at 100°.

The last three compounds listed in Table II indicate application of the method described to other groups than hydroxyls. These, and others previously mentioned, must be taken into account if present in a compound along with hydroxyl groups. The reaction of butyl ether with hydriodic acid is shown to be of a more complex nature than expected from the textbook reaction

$$ROR + HI \longrightarrow ROH + RI$$

A similar observation has been made by Earl and Kenner.[9]

## Summary

1. A new method for the quantitative determination of hydroxyl groups is described and shown to be useful down to quantities of about 1 mg. Satisfactory results were obtained on primary mono alcohols, poly alcohols, hydroxy acids and various negatively substituted hydroxy compounds. The hydroxyl groups in phenols, simple

secondary and tertiary alcohols, inositol, threonine and tartaric acid could not be quantitatively determined by this method.

2. On the basis of experimental data, a generalization is offered for the variation in reactivity of aliphatic hydroxy compounds toward hydriodic acid. The presence of adjacent negative groups increases the reactivity of a hydroxyl while an adjacent methyl group decreases its reactivity.

3. The analytical data give some indication of structural relationships of the functional groups in a compound.

4. It is suggested that the analytical method described might be useful in the determination of any group readily reacted upon by hydriodic acid.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

# The Kinetics of the Periodate Oxidation of 1,2-Glycols

BY CHARLES C. PRICE AND HARRY KROLL

The cleavage by periodic acid oxidation of the carbon to carbon bond in compounds in which the two carbon atoms each bear an oxygen atom either as a hydroxyl or carbonyl group was first observed by Malaprade.[1]

$$RCHOHCHOHR + HIO_4 \longrightarrow 2RCHO + H_2O + HIO_3$$
$$RCHOHCOR + HIO_4 \longrightarrow RCHO + RCOOH + H_2O + HIO_3$$

The original investigator, as well as Fleury and Lange,[2] were concerned chiefly with the analytical applications of the reaction. Fleury, Hérissey and Joly[3] made use of the reaction in studying the problem of the ring structure of the sugars, an application which was extensively and successfully investigated by Karrer and Pfaehler and by Jackson and Hudson.[4]

Although Malaprade made the observation that the reaction was much more rapid in acid than in neutral or basic solution, no measurements of the kinetics of the reaction have been made. The purpose of the present investigation was to study the kinetics under various conditions with the object of elucidating the mechanism of this oxidative cleavage in the particular case of 1,2-glycols.

Ethylene glycol and 2,3-butylene glycol consumed the theoretical amount of periodate almost instantaneously in acid solution. Pinacol, however, was found to oxidize at a conveniently measurable rate and was therefore the material used for the kinetic investigation.

(1) Malaprade, *Bull. soc. chim.*, **43**, 683 (1928); *Compt. rend.*, **186**, 382 (1928).

(2) Fleury and Lange, *J. pharm. chim.*, **17**, 196, 313, 409 (1933).

(3) Fleury, Hérissey and Joly, *ibid.*, **20**, 149 (1934).

(4) Karrer and Pfaehler, *Helv. Chim. Acta*, **17**, 766 (1934); Jackson and Hudson, THIS JOURNAL, **59**, 994 (1937).

## Experimental

Standard aqueous solutions of periodic acid or sodium periodate and pinacol were mixed at 25.0° after adjusting the *p*H with 0.5 *N* solutions of sulfuric acid or sodium hydroxide. The course of the reaction was followed by withdrawing samples which were added to acidified potassium iodide solution, the liberated iodine then being titrated with sodium thiosulfate to the disappearance of the iodine color.

The hydrogen ion concentration of the reaction mixture was determined with a Beckmann *p*H meter. It did not vary appreciably during the course of the oxidation.

**The Kinetics of the Reaction at Constant *p*H.**—The dependence of the rate of the oxidation on the concentrations of the periodate and pinacol was first determined by measurements at a particular hydrogen ion concentration. The reaction was found to follow simple second order kinetics, the rate being directly proportional to the glycol and periodate concentrations as is illustrated by the results of these experiments summarized in Table I. The average deviation for the constants in any of the experiments was less than 5%.

TABLE I

THE RATE CONSTANTS FOR EXPERIMENTS WITH VARIOUS PINACOL AND PERIODATE CONCENTRATIONS AT A CONSTANT *p*H

*p*H, 5.5–5.6; temp., 25.0°

| [Periodate][a] | [Pinacol][a] | $k$[b] |
|---|---|---|
| 0.00550 | 0.02327 | 0.205 |
| .00611 | .03116 | .207 |
| .00916 | .01871 | .199 |
| .01221 | .01558 | .202 |
| .01221 | .03513 | .197 |
| .01486 | .02180 | .197 |
| .01960 | .05270 | .196 |

[a] Concentrations in moles per liter. [b] $dx/dt = k \times$ [Periodate][Pinacol]; the [Periodate] being the total periodate as determined by titration.

**The Effect of Changing *p*H.**—It was found that the value of $k$ for the simple second order reaction varied greatly with the *p*H of the reaction mixture. Since this