and 6-sulfonic acids.[12] The barium salts were fractionally crystallized, converted to the sodium salts and the position of the sulfonic group proved by conversion to the acid chloride and to the amide. The products were bioassayed as the sodium salts.

1-Nitro-2-methylnaphthalene.—This compound was prepared by nitration of 2-methylnaphthalene by the method published by Fierz-David and Mannhart.[13] Recrystallized from methanol; m. p. 81–82°.

1-Amino-2-methylnaphthalene-4-sulfonic Acid.—The 1-nitro-2-methylnaphthalene was reduced, and the amine sulfate converted to the 1-amino-4-sulfonic acid as directed by Fierz-David and Mannhart.[13] The product was a fine, white light powder. *Anal.* Calcd. for $C_{11}H_{11}O_3NS$: N, 5.90. Found: N, 5.93.

The author wishes to express thanks to Mr. E. F. Shelberg for the microanalyses here reported, and to Mrs. Flemintine Peirce Dann for the bioassays.

(12) Dziewónski, Schoenówna and Waldmann, *Ber.*, **58**, 1211–1218 (1925).

(13) Fierz-David and Mannhart, *Helv. Chim. Acta*, **20**, 1024–1040 (1937).

Summary

Compounds formed by the reaction of 2-methyl-1,4-naphthoquinone with various metallic or amine bisulfites have been found to be highly water-soluble and to possess a degree of Vitamin K activity equivalent to that of the 2-methyl-1,4-naphthoquinone contained therein.

Other sulfonated 2-methylnaphthalenes were prepared and tested for antihemorrhagic activity. The sulfonic acid group was found to be relatively inert from the antihemorrhagic viewpoint. In the 1-position only slight activity is conferred. This is likewise true of 1-amino-2-methylnaphthalene-4-sulfonic acid. In the 3-position in such an active molecule as 2-methyl-1,4-naphthoquinone the sulfonic group reduces the activity. 2-Methylnaphthalene sulfonated at the 6- or 8-position showed no antihemorrhagic activity at high levels.

NORTH CHICAGO, ILLINOIS RECEIVED APRIL 18, 1941

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, No. 246]

The Action of Hydrogen Peroxide in *t*-Butanol upon *d*-Glucal and Triacetyl-*d*-glucal in the Presence of Osmium Tetroxide

BY ROBERT C. HOCKETT, ALVA C. SAPP[1] AND SARAH R. MILLMAN[2]

Among the several methods available for conversion of an aldose sugar into its epimer, none is so simple and efficient as could be wished.[3] The procedure of Bergmann[4] in which, by the action of benzoperacid and subsequent hydrolysis, the elements of hydrogen peroxide are added to the double bond of a glycal, results in formation of a mixture of epimeric aldoses in which one isomer generally predominates to a high degree. If, therefore, the glycal be prepared originally from that epimer which is less abundant among the products of hydroxylation the series of transformation may represent an epimerization on a practical scale in this direction. In the reverse direction, the extremely small yield will usually render the procedure useless for preparative purposes. In several instances, the fortunate preponderance of a less easily available epimer over its abundant relative in such products, has made the method valuable in practice. Thus ribose has been prepared from arabinose,[5] talose from galactose[6] and various glycosyl-mannoses[7] from disaccharides containing glucose as the reducing part.

In all these cases the conversion was from a sugar possessing the *trans* configuration of the hydroxyl groups on carbons two and three into the epimer with the *cis* configuration in these positions[8]

(1) A preliminary study of this problem was submitted by Mr. Alva Charles Sapp as a thesis in partial fulfillment of the requirements for the degree of Master of Science in October, 1937.

(2) The study was extended by Miss Sarah Ruth Millman as part of a thesis which was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in June, 1940. Miss Millman was Ellen H. Richards Fellow in Chemistry from 1935 to 1938. A paper including the present report was presented at the Boston Meeting of the American Chemical Society in September, 1939.

(3) Tollens–Elsner, "Kurzes Handbuch der Kohlenhydrate," Leipzig, 1935, p. 16.

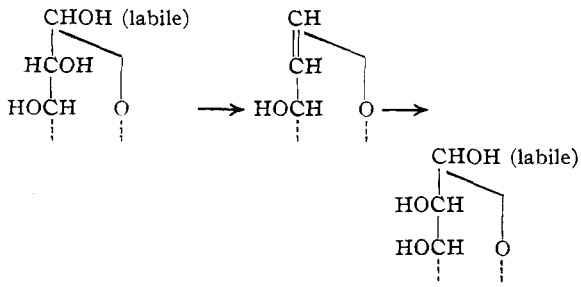
(4) Bergmann and Schotte, *Ber.*, **54**, 440, 1564 (1921).

(5) Gehrke and Aichner, *Ber.*, **60**, 918 (1927); Austin, THIS JOURNAL, **56**, 1152 (1934); Karrer, *et al.*, *Helv. Chim. Acta*, **18**, 1435 (1935).

(6) Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931); Komada, *Bull. Chem. Soc. Japan*, **7**, 211 (1932).

(7) Watters and Hudson, THIS JOURNAL, **52**, 3473 (1930); Evans and Dauben, *ibid.*, **60**, 886 (1938); Haworth, *et al.*, *J. Chem. Soc.*, 2336, 2644 (1930).

(8) Since the configuration of carbon one is labile on account of prototropic changes, no direct evidence has been obtained to show whether additions to the glycal double bond are of the "*cis*" or "*trans*" type.



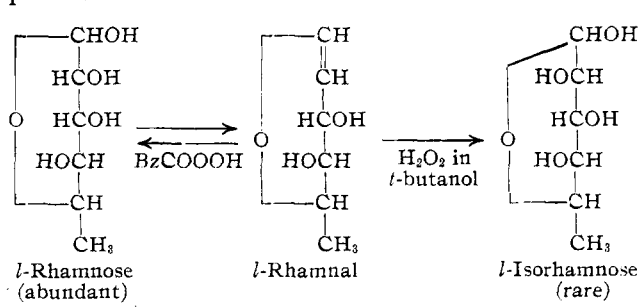
On the other hand, sugar derivatives with a substituent replacing the hydroxyl hydrogen of carbon three generally are converted by Bergmann's method preponderantly to sugar derivatives with the hydroxyl groups of carbons two and three in a *trans* position.[9]

Both the glycals and the benzoperacid necessary for this reaction are relatively unstable and need to be prepared immediately before use. The substitution of a more stable hydroxylating agent which could be kept in stock ready for immediate use, would be very advantageous. Professor N. A. Milas of this Laboratory with his collaborators has observed the activity of hydrogen peroxide in *t*-butanol solution in adding hydroxyl groups at the olefinic linkages of many compounds when osmium tetroxide acts as a catalyst.[10] He has kindly given us permission to study the action of this agent upon unsaturated sugar derivatives. Our objectives were to learn whether glucal and its acetate would add the elements of hydrogen peroxide without much decomposition, to discover which of the possible stereoisomers would predominate among the products, and to learn whether the presence of acetyl groups would affect the course of the reaction.[6]

It has been found that both 3,4,6-triacetyl-*d*-glucal and *d*-glucal itself will add hydroxyl groups at the unsaturated link. The acetylated compound was incompletely transformed at 20° when treated with an equimolecular quantity of hydrogen peroxide in *t*-butanol at a concentration of about 0.46 mole per liter. When the proportion of peroxide was doubled, the reaction was complete in six days according to polarimetric observation and no unchanged triacetylglucal was recovered. Glucose was isolated in a yield of 55 to 60% as β-pentaacetyl-*d*-glucose. Mannose was detected in only a faint trace as mannose phenylhydrazone. A three to one molar concentration of peroxide gave very nearly the same results.

Glucal itself was treated with equimolecular proportions of peroxide, with 10% excess over theory and with double molecular proportions. Reactions could not always be followed polarimetrically because of color, but usually the disappearance of color permitted observing the endpoint which occurred in about six days. Mannose was obtained in about 2% yield and glucose in yields ranging from 8 to 18%. The glucose was isolated both as osazone, after removal of mannose phenylhydrazone, and as β-glucose pentaacetate. In both cases, some acid was found as a by-product, the quantities being greater from the free glucal.

These results show an interesting difference between the action of benzoperacid and the present reagent upon these unsaturated sugar derivatives. Both glucal and its triacetate give mainly a glucose derivative on hydroxylation with hydrogen peroxide in tertiary butanol in the presence of osmium tetroxide instead of behaving differently as is the case when benzoperacid is used. The relatively good yield of glucose derivative obtained when the acetylated glucal is so treated[9] suggests that the present method may become a valuable supplement to the Bergmann method of epimerization. In a case such as that of *l*-rhamnose which has the mannose type of configuration and was recovered in high yield when *l*-rhamnal was treated with benzoperacid,[4] the present method when applied either to *l*-rhamnal or rhamnal diacetate might be expected to give mainly the more rare *l*-isorhamnose (6-desoxy-*l*-glucose) or its derivative.



Our studies will be extended to answer these questions more fully.

We wish to express our gratitude to Professor Werner Freudenberg, formerly of Fordham University, for seed crystals of triacetylglucal.

(9) (a) Levene and Raymond, *J. Biol. Chem.*, **88**, 513 (1930); (b) Tanaka, *Bull. Chem. Soc., Japan*, **5**, 214 (1930); (c) Hirst and Woolvin, *J. Chem. Soc.*, 1131 (1931).

(10) Milas and Sussman, THIS JOURNAL, **58**, 1302 (1936); **59**, 2345 (1937); Milas, Sussman and Mason, *ibid.*, **61**, 1844 (1939).

Experimental

The Peroxide Reagent.—To 100 cc. of 27% hydrogen peroxide (du Pont Albanal) was added 400 cc. of *t*-butanol. Anhydrous sodium sulfate was added until layers formed. The alcoholic layer, which contained most of the peroxide, was separated and kept over anhydrous sulfate. A certain amount of water remains in a solution so prepared and this has an advantageous effect so that stronger desiccating agents are not recommended. The peroxide content as determined by adding an aliquot part to potassium iodide in glacial acetic acid and titrating with standard sodium thiosulfate solution, was 5.63%. The solution could be kept indefinitely.

Osmium Tetroxide Solution.—A vial of this oxide was broken under tertiary butanol and the solution was diluted to a strength of approximately 0.5% with the same alcohol. Care must be taken that the vapors do not come into contact with the eyes, on which this oxide has a strong effect, causing blindness.

Hydroxylation of Triacetylglucal.—In a typical experiment, 2 g. (0.00732 mole) of triacetyl-*d*-glucal prepared according to Bergmann[4] and showing m. p. 51–53° was mixed with 8.87 cc. of 5.63% hydrogen peroxide in *t*-butanol (0.0116 mole) and one cc. of 0.5% osmium tetroxide solution and diluted to 25.0 cc. The mixture became yellow but could be read in a polarimeter. Readings in a one-dm. tube changed from −0.20° to +7.16° in six days whereupon they remained constant. The color meanwhile disappeared and unconsumed peroxide could be detected. After removal of solvent, peroxide and catalyst by concentrating under reduced pressure at 60°, traces of these substances were removed by adding butanol and reconcentrating. The residue was deactylated by treating it in 20 cc. of dry methanol with 4 cc. of approximately 1 *N* barium methylate overnight. After removal of barium ion quantitatively with standard sulfuric acid and of methanol by evaporation, the residue was made up to 25.0 cc. with water. A test with phenylhydrazine showed a mere trace of mannose. The polarimetric reading of this solution in a 1-dm. tube was +1.56; if calculated as due entirely to glucose this reading would indicate a recovery of 73.6% of the theoretical amount. Titration with hypoiodite[11] would indicate only 61.2% recovery of glucose if other sugars are considered absent. The discrepancy between these determinations indicates that by-products are present.

In a separate experiment where a trimolecular proportion of peroxide was used, the entire product was concentrated dry and acetylated using 11.44 cc. of acetic anhydride and 0.72 g. of anhydrous sodium acetate. By pouring into water and eventually crystallizing the precipitated oil from alcohol, there was isolated 1.672 g. of β-glucose pentaacetate melting 127–128° and rotating +4.05° (*C*, 0.60; $CHCl_3$). This corresponds to a yield of 58% of the theoretical amount of glucose and indicates that *at least* this much glucose was formed in the reaction.

Titration of aliquot parts of the sugar solution by standard alkali shows the presence of non-volatile acids to the extent of 0.053 equivalent per mole of triacetylglucal taken.

Hydroxylation of *d*-Glucal.—Free glucal was prepared by deacetylating weighed portions of pure glucal acetate with barium hydroxide as described by Meisenheimer and Jung.[12] Excess base was precipitated with carbon dioxide and after filtration and evaporation, the glucal was extracted from residues of barium salts by means of absolute alcohol. The ethanol was then removed by evaporation under reduced pressure and replaced by *t*-butanol. Reactions were run using an equimolar proportion of peroxide, a 10% excess and a double molar proportion. The conditions used and the yields of products are indicated in the table.

Glucal, g.	Molar proportion of peroxide,	Total volume, cc.	Vol. of 0.5% O_8O_4, cc.	Yield of glucose, %	Yield of mannose, %
1.04	2	25	1	8.10[a]	Trace
2.55	1	25	0.5	16.7[b]	2.2
2.55	1.10	25	.5	18.5[b]	1.6

[a] Isolated as β-glucose pentaacetate. [b] Isolated as glucosazone.

The mannose phenylhydrazone melted at 188° and glucosazone at 204°.

Alkali titration of an aliquot portion of the sugar solution from the first of the runs listed, showed that non-volatile acids were present to the extent of 0.078 equivalent per mole of glucal taken.

Summary

1. The action of hydrogen peroxide in *t*-butanol upon triacetyl-*d*-glucal and *d*-glucal in the presence of osmium tetroxide has been investigated.

2. An equimolar proportion of peroxide gives a very incomplete conversion of triacetyl glucal to sugar.

3. Bi- or tri-molecular proportions of hydrogen peroxide in *t*-butanol with osmium tetroxide present convert triacetylglucal in six days at room temperature into a glucose derivative to the extent of at least 55–60% as shown by isolation of β-pentaacetyl-*d*-glucose on further acetylation. Only a trace of mannose derivative is formed and non-volatile acids to the extent of five mole per cent.

4. Equi- or bi-molecular proportions of hydrogen peroxide in *t*-butanol with osmium tetroxide present, in six days at 20° will convert *d*-glucal into *d*-glucose to the extent of 8 to 18% and into *d*-mannose to the extent of 2%. Non-volatile acids are formed to the extent of nearly 8 mole per cent.

5. The conversion of glucal acetate in good yield to glucose derivatives provides a new method of reversing the epimerization reaction sequence of Bergmann.

CAMBRIDGE, MASSACHUSETTS RECEIVED MAY 14, 1941

(11) Cajori, *J. Biol. Chem.*, **54**, 622 (1922).

(12) Meisenheimer and Jung, *Ber.*, **60**, 1462 (1927).