THE MECHANISM OF CARBOHYDRATE OXIDATION. XIV.[1] THE ALKALINE DEGRADATION OF CELLOBIOSE, LACTOSE, MELIBIOSE AND GENTIOBIOSE BY POTASSIUM HYDROXIDE[2]

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In a recent study by Marjorie P. Benoy and one of us[4] concerning the action of potassium hydroxide on maltose, an effort was made to establish the molecular mechanism through which this disaccharide passes in the course of its decomposition by an alkali. The following three possibilities were pointed out. (a) The maltose molecule might be hydrolyzed into two molecules of glucose,[5] in which case the solution of the disaccharide should react like an equivalent solution of the hexose. (b) By reason of its being a reducing sugar, maltose might also react as though a series of maltose enediols were formed. (c) Obviously, both of these reactions could take place simultaneously, the relative rates of the two processes being determined by the experimental conditions which had been chosen.

The quantitative data which were obtained in the alkaline degradation of maltose were best understood on the assumption that both reactions were taking place simultaneously and also that the hydrolytic process was much less rapid than the formation and fission of the possibly formed enediols. Furthermore, the interpretation set forth for the behavior of maltose necessitated the assumption that the disaccharide molecule would not form a 3,4-enediol by reason of the hexosido attachment on carbon atom 4. This hindering action[6] was conceived as exerting a decided influence on the course of the maltose decomposition, which resulted in a marked difference between the quantitative data obtained from it and those obtained from an equivalent solution of glucose. To test further the validity of these assumptions with other known disaccharides was the first objective of this study.

Since the hexosido sections of the cellobiose and gentiobiose molecules are glucose in each case and those of lactose and melibiose are galactose, it is clear that the quantitative data obtained from these two kinds of disac-

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[1] Contribution XIII of this series, THIS JOURNAL, 52, 4065 (1930).

[2] This paper is an abstract of a thesis presented by Robert Casad Hockett in December, 1929, to the Graduate School of The Ohio State University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[3] E. I. du Pont de Nemours and Co. Fellow, 1928–1929.

[4] Evans and Benoy, THIS JOURNAL, 52, 294 (1930).

[5] Cf. (a) Lewis, Am. Chem. J., 42, 301 (1909); (b) Nef, Ann., 357, 232 (1907); (c) Nef, ibid., 403, 299 (1914).

[6] (a) Ref. 5 c; (b) Lewis and Buckborough, THIS JOURNAL, 36, 2385 (1914).

charides should be different by reason of the fact that it has been shown previously that the action of potassium hydroxide solutions on the two substituent hexoses, glucose and galactose, is not quantitatively the same in each case.   To ascertain the facts with reference to the part played by the hexosido section of these disaccharides, was our second objective in this work.

Not only by reason of the glucosido and galactosido character of the hexosido section of the disaccharides, but also on account of the difference in the point of hexosido coupling within these four disaccharides, the enediol theory of sugar behavior would make it possible to predict a marked difference in the yields of a given reaction product, such as lactic acid, which might be obtained by the action of potassium hydroxide on these sugars. Lactose[7] is a 4-galactosido-glucose, while melibiose is a 6-galactosido-glucose.[8]   On the other hand, cellobiose,[9] like maltose, is a 4-glucosido-glucose, while gentiobiose is a 6-glucosido-glucose.[10]

To ascertain whether the experimental data in each of these cases are in harmony both with this general point of view and the more detailed outline given in the theoretical part of this report was the third objective of this work.

We wish to express our thanks to E. I. du Pont de Nemours and Company for their valuable assistance which made this work possible.

Experimental Part

The general procedures employed in these experiments were almost identical with those described in previous reports[11] from this Laboratory on the action of potassium hydroxide on certain carbohydrates.

Materials.—(a) Carbohydrates.—α-Lactose monohydrate was purchased on the market and by determinations of ash, moisture and optical rotation, found to be of sufficient purity for use in this work.   β-Melibiose dihydrate was similarly purchased and examined.   β-Cellobiose octacetate was presented by Dr. C. A. Browne of the United States Bureau of Chemistry and Soils and the acetyl groups removed by the methods of

[7] (a) W. N. Haworth and C. W. Long, *J. Chem. Soc.*, **131**, 544 (1927); (b) G. Zemplén, *Ber.*, **59**, 2402 (1926); (c) P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **71**, 471 (1926–1927).

[8] (a) W. N. Haworth, J. V. Loach and C. W. Long, *J. Chem. Soc.*, 3146 (1927); (b) B. Helferich and H. Bredereck, *Ann.*, **465**, 166 (1928); (c) P. A. Levene and E. Jorpes, *J. Biol. Chem.*, **86**, 403 (1930); (d) K. Josephson, *Ann.*, **472**, 230 (1929).

[9] (a) W. N. Haworth, C. W. Long and J. H. G. Plant, *J. Chem. Soc.*, **131**, 2809 (1927); (b) G. Zemplén, *Ber.*, **59**, 1254 (1926); (c) P. A. Levene and M. L. Wolfrom, *J. Biol. Chem.*, **77**, 671 (1928).

[10] (a) W. N. Haworth and B. Wylam, *J. Chem. Soc.*, **123**, 3120 (1923); (b) B. Helferich and co-workers, *Ann.*, **440**, 1 (1924); **447**, 27 (1926); **450**, 225 (1926).

[11] (a) Evans, Edgar and Hoff, THIS JOURNAL, **48**, 2665 (1926); (b) Evans and Hass, *ibid.*, **48**, 2703 (1926); (c) Evans and Cornthwaite, *ibid.*, **50**, 486 (1928); (d) Evans and Hutchman, *ibid.*, **50**, 1496 (1928);   (e) Evans and O'Donnell, *ibid.*, **50**, 2543 (1928); (f) Evans and Conaway, *ibid.*, **52**, 3680 (1930); (g) Ref. 4; (h) Evans, *Chem. Reviews*, **6**, 281 (1929).

Maquenne and Goodwin[12] and of Zemplén.[13]   The anhydrous β-form of the sugar was obtained, purified and standardized.   β-Gentiobiose octaacetate was presented by Dr. Joseph F. Leete, now of The Applied Sugar Laboratories, Inc., New York City, by Dr. A. F. O. Germann of The Laboratory Products Company, Cleveland, Ohio, and by Dr. Burckhardt Helferich, then of the University of Greifswald, Germany.   We are deeply indebted to these well-known workers for this valuable material.   Deacetylation by the method of Zemplén modified, gave the anhydrous β-sugar, which was purified and standardized as usual.   (b) Reagents.—All reagents were either prepared for use or tested by the usual methods to determine their suitability for the work.

Manipulations.—(a) The sample quantities used were these: lactose monohydrate 2.25 g.; melibiose dihydrate, 1.1819 g.; cellobiose, 1.0687 g.; gentiobiose, 1.0687 g. When the lactose samples were dissolved in 25 cc. of potassium hydroxide solution of the appropriate normality and the samples of the other sugars in 12.5 cc., the resulting solutions were all 0.25 molar with respect to sugar and of the same carbon content per cubic centimeter as the solutions of maltose, of the trioses and of the hexoses used in previous studies.[11]   Consequently, all the results obtained in this and in previous studies are strictly comparable with one another.

The solutions were agitated for forty-eight hours in a thermostat maintained at 25 or 50°.   The containing flasks were closed with well-fitting rubber stoppers during this process.   At the end of the reaction period, the solutions were neutralized with phosphoric acid and examined quantitatively for formic, acetic and lactic acids.   (b) Analytical methods.—These were very nearly the same as those described in previous papers.[11]   (c) Pyruvic Aldehyde.—The procedure for the determination of this substance as its osazone was carried out as described by Evans and Hass.[11b]

Experimental Results.—The quantitative data obtained by these procedures are expressed in graphical form in Figs. 1 to 7.

Theoretical Part

A.   4-Hexosido-glucoses—Cellobiose and Lactose.—If the alkaline decomposition of all 4-hexosido-hexoses follows the general reactions which have been previously outlined for maltose,[4] then the changes taking place in the cellobiose molecule may be represented by the following equations.[14]

(a)

Cellobiose
Gl = Glucosido
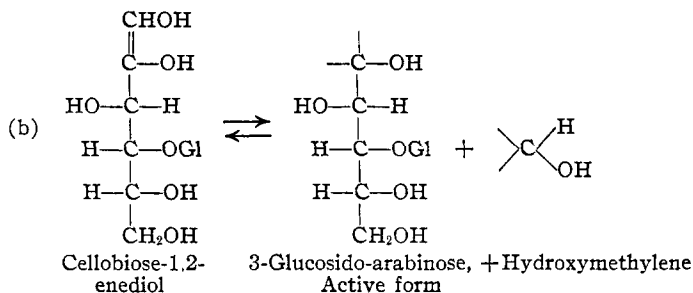
Cellobiose
Aldehydo form[15]

Cellobiose-1,2-
enediol
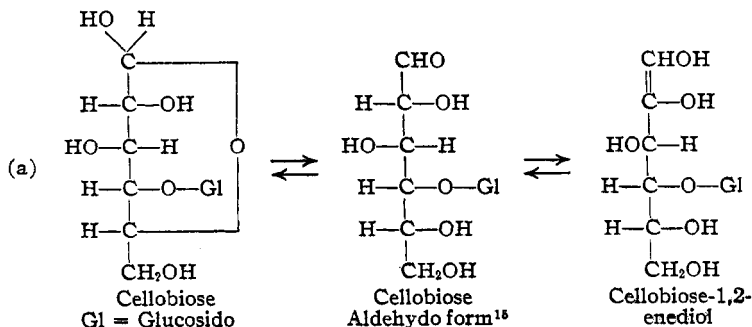
[12] Maquenne and Goodwin, Bull. soc. chim., 31, 854 (1904).

[13] Zemplén, Ber., 59B, 1258 (1926).

[14] (a) Refs. 4 and 7; (b) cf. K. Bernhauer, Biochem. Z., 210, 175 (1929).

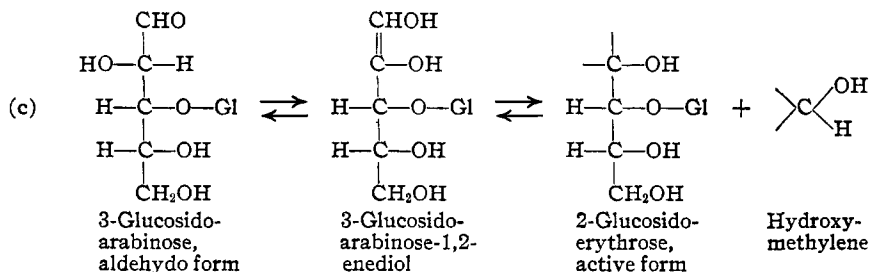[15] Wolfrom, THIS JOURNAL, 51, 2188 (1929); ibid., 52, 2464 (1930); Wolfrom and Newlin, ibid., 52, 3619 (1930).

(1) **Cellobiose 1,2-Enediol (4-β-Glucosido-glucose-1,2-enediol).**—The rupture of the cellobiose-1,2-enediol would result in the formation of 3-glucosido-arabinose and hydroxylmethylene as is shown in reaction (b).

(b)

$$
\begin{array}{ccc}
\text{CHOH} & & \\
\text{C—OH} & & \text{—C—OH} \\
\text{HO—C—H} & \rightleftharpoons & \text{HO—C—H} \\
\text{H—C—OGl} & & \text{H—C—OGl} \quad + \quad \text{C}\!\!<^{\text{H}}_{\text{OH}} \\
\text{H—C—OH} & & \text{H—C—OH} \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$

Cellobiose-1,2-   3-Glucosido-arabinose, +Hydroxymethylene
enediol   Active form

In the presence of an oxidizing agent of the appropriate potential, these decomposition products would yield 3-glucosido-arabonic and formic acids. If lactose were the sugar undergoing this series of changes then it is obvious that under appropriate oxidizing conditions 3-galactosido-arabonic and formic acids would be those obtained.
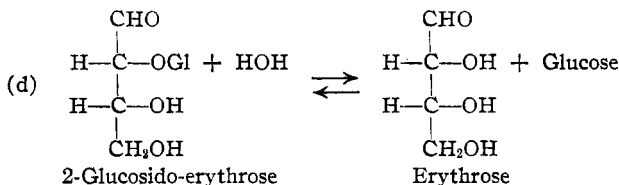
(2) **3-Glucosido-arabinose-1,2-enediol.**—The 3-glucosido-arabinose formed in the cleavage represented in reaction (b) may, in the absence of oxidizing agents, undergo the series of changes which result in enediol formation and subsequent splitting. Such reactions would result in the formation of 2-glucosido-erythrose in alkaline cellobiose solutions and of 2-galactosido-erythrose in similar solutions of lactose. This step in the case of cellobiose is shown in reaction (c).

(c)

$$
\begin{array}{cccc}
\text{CHO} & \text{CHOH} & & \\
\text{HO—C—H} & \text{C—OH} & \text{—C—OH} & \\
\text{H—C—O—Gl} \rightleftharpoons & \text{H—C—O—Gl} \rightleftharpoons & \text{H—C—O—Gl} \quad + \quad \text{C}\!\!<^{\text{OH}}_{\text{H}} \\
\text{H—C—OH} & \text{H—C—OH} & \text{H—C—OH} & \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} &
\end{array}
$$

3-Glucosido-   3-Glucosido-   2-Glucosido-   Hydroxy-
arabinose,   arabinose-1,2-   erythrose,   methylene
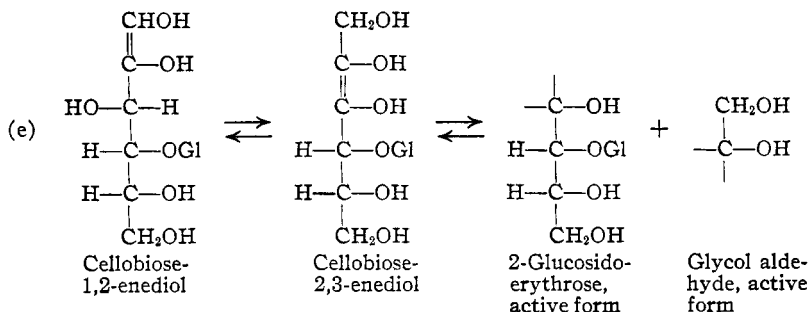aldehydo form   enediol   active form

Again it is clear that these cleavage products would yield 2-glucosido-erythronic and formic acids in the presence of an appropriate oxidizing agent. In lactose solutions under similar experimental conditions, 2-galactosido-erythronic and formic acids would be the reaction products.

(3) **Hydrolysis of 2-Glucosido-erythrose.**—At this point in the alkaline degradation of the 4-hexosido-hexoses, enolization has reached an effective[6] end, for although a new 1,2-enediol may be written, such a compound could not form any stable products on splitting. The reason for this lies in the fact that a non-labile glucosido group occupies the position in this

molecule where in the case of a monosaccharide there would be a labile hydrogen atom. Under these circumstances, it seems unlikely that such an enol would ever undergo fission even if it existed. Therefore, we deem it probable that the 2-glucosido-erythrose would remain unchanged by the alkali until the relatively slow process of hydrolysis should have broken it into glucose and erythrose in the case of cellobiose and into galactose and erythrose in the case of lactose.[16] Hence, it follows that cellobiose and maltose solutions should yield glucose and erythrose at this point in their alkaline degradation, while lactose solutions should contain galactose and erythrose. This change for cellobiose may be shown by reaction (d).

$$
\begin{array}{l}
\text{CHO} \\
|\\
\text{(d)}\quad \text{H—C—OGl + HOH} \\
|\\
\text{H—C—OH} \\
|\\
\text{CH}_2\text{OH} \\
\text{2-Glucosido-erythrose}
\end{array}
\quad\rightleftharpoons\quad
\begin{array}{l}
\text{CHO} \\
|\\
\text{H—C—OH + Glucose} \\
|\\
\text{H—C—OH} \\
|\\
\text{CH}_2\text{OH} \\
\text{Erythrose}
\end{array}
$$

(4) Cellobiose-2,3-enediol (4-β-Glucosido-glucose-2,3-enediol.—In solutions either of higher alkali normality or at higher temperatures it is conceivable that the cellobiose-1,2-enediol could undergo a transformation into cellobiose-2,3-enediol, which in turn would rupture with the formation of 2-glucosido-erythrose and glycol aldehyde as shown in reaction (e).

$$
\text{(e)}\quad
\begin{array}{l}
\text{CHOH} \\
\|\\
\text{C—OH} \\
|\\
\text{HO—C—H} \\
|\\
\text{H—C—OGl} \\
|\\
\text{H—C—OH} \\
|\\
\text{CH}_2\text{OH} \\
\text{Cellobiose-}\\
\text{1,2-enediol}
\end{array}
\rightleftharpoons
\begin{array}{l}
\text{CH}_2\text{OH} \\
|\\
\text{C—OH} \\
\|\\
\text{C—OH} \\
|\\
\text{H—C—OGl} \\
|\\
\text{H—C—OH} \\
|\\
\text{CH}_2\text{OH} \\
\text{Cellobiose-}\\
\text{2,3-enediol}
\end{array}
\rightleftharpoons
\begin{array}{l}
\text{—C—OH} \\
|\\
\text{H—C—OGl} \\
|\\
\text{H—C—OH} \\
|\\
\text{CH}_2\text{OH} \\
\text{2-Glucosido-}\\
\text{erythrose,}\\
\text{active form}
\end{array}
+
\begin{array}{l}
\text{CH}_2\text{OH} \\
|\\
\text{—C—OH} \\
|\\
\\
\text{Glycol alde-}\\
\text{hyde, active}\\
\text{form}
\end{array}
$$

Likewise, when lactose undergoes these series of changes, 2-galactosido-erythrose and glycol aldehyde would be formed, which in the presence of an appropriate oxidizing agent would be converted into 2-galactosido-ery-thronic and glycolic acids while the fission products of cellobiose-2,3-enediol would yield 2-glucosido-erythronic acid and the acid which is common to both reactions, namely, glycolic. It is clear that the 2-glucosido-erythrose formed in reaction (e) should undergo the hydrolysis shown in reaction (d).

Summarizing the changes (reactions a, b, c, d) involved in the alkaline degradation of the 4-hexosido-hexoses, it is found that one molecule of the disaccharide should yield one molecule of a hexose (from the hexosido sec-

[16] Zemplén, *Ber.*, **59**, 1254, 2402 (1926).

tion), one molecule of a tetrose (from the hexose section) and two molecules of hydroxymethylene (from the hexose section), or one molecule of a hexose, one molecule of a tetrose and one of glycol aldehyde (reaction e).

The above points of view with reference to the behavior of disaccharides in aqueous solutions of alkalies find support in the classical researches of de Bruyn and van Ekenstein[17] on the action of hot aqueous solutions of potassium hydroxide toward maltose and lactose, in which it was found that maltose yielded glucose and one other substance that gave a further amount of glucose through *acid* hydrolysis. From this latter fact they concluded that this unknown substance was an anhydride of glucose. Their experiences with lactose were even more notable in this connection. In the alkaline solution of milk sugar they obtained galactose (the hexosido portion of the molecule) *but they found that lactose did not produce glucose simultaneously*. These well-known investigators were of the opinion that the unknown substance obtained in the lactose solutions was similar to that obtained in the maltose ones. In the case of maltose it is evident that their supposed anhydride of glucose was probably either 3-glucosido-arabinose, 2-glucosido-erythrose or a mixture of these, while in the lactose solution 3-galactosido-arabinose, 2-galactosido-erythrose or both must have been present. Since, in the latter case, glucose did not form simultaneously with galactose, one is forced to the conclusion that the arabinose and erythrose galactosides were undergoing hydrolysis under the existing experimental conditions.

Still more striking confirmation of the above points of view is found in the data obtained by Glattfeld and Hanke[18] in the alkaline oxidation of maltose with hydrogen peroxide and with air. They obtained two classes of acids: (a) glucosido ones, and (b) simple (non-glucosido) ones. In the former group they obtained glucosido-erythronic and glucosido-arabonic acids, a result which is in complete harmony with reactions (b), (c) and (e) above. The simple acids were formic, glycolic, oxalic (peroxide oxidation) and glyceric, the presence of the first two being easily accounted for in part through equations (b), (c) and (e), while the oxalic may arise from the oxidation of glycol aldehyde.[19]

On the now known structure of maltose the presence of glucosido-glyceric acid in the reaction products obtained by Glattfeld and Hanke is excluded. Therefore, it follows that glyceric acid must have been formed as a result of the action of the alkali on glucose itself, thus producing glucose-3,4-enediol, which on cleavage gave two molecules of glyceric aldehyde. The data obtained both by Lewis[5a] in the oxidation of maltose with Fehling's solution and by Lewis and Buckborough[6] are in harmony with behavior

[17] De Bruyn and van Ekenstein, *Rec. trav. chim.*, **18**, 147 (1899).

[18] Glattfeld and Hanke, THIS JOURNAL, **40**, 973 (1918).

[19] Heimrod and Levene, *Biochem. Z.*, **29**, 49 (1910).

of the 4-hexosido-glucoses outlined above.   It is of great interest in this connection to note that Lewis found large amounts of glucosido-mannonic acid in his reaction mixture, a result which points to the disaccharide having undergone the de Bruyn and van Ekenstein rearrangement.

   B.   6-Hexosido-glucoses.—Since gentiobiose and melibiose are 6-hexosido-glucoses, then it follows that the de Bruyn and van Ekenstein equilibria of these two sugars must be entirely different from those arising from the action of aqueous solutions of alkalies on the 4-hexosido-glucoses. Furthermore, it is evident that the enediols of these sugars should be structurally different from those in the 4-hexosido-glucose series.   In the 6-hexosido-glucoses there is no hindering effect[6] to 6-hexosido-glucose-enediol formation due to the point of glucosidic attachment such as is the case in the 4-hexosido-glucoses.   Hence it follows that the chemical behavior of the 6-hexosido-glucoses should be qualitatively and quantitatively different in many respects from that of the 4-hexosido-glucoses when both series of sugars are subjected to the action of the same reagents under identical experimental conditions.   The truth of this assertion becomes evident from a consideration of the following reactions which on the enediol theory of sugar behavior[20] should take place when the 6-hexosido-glucoses are acted upon by aqueous solutions of potassium hydroxide.

6-Hexosido-glucose-enediols: Gentiobiose and Melibiose
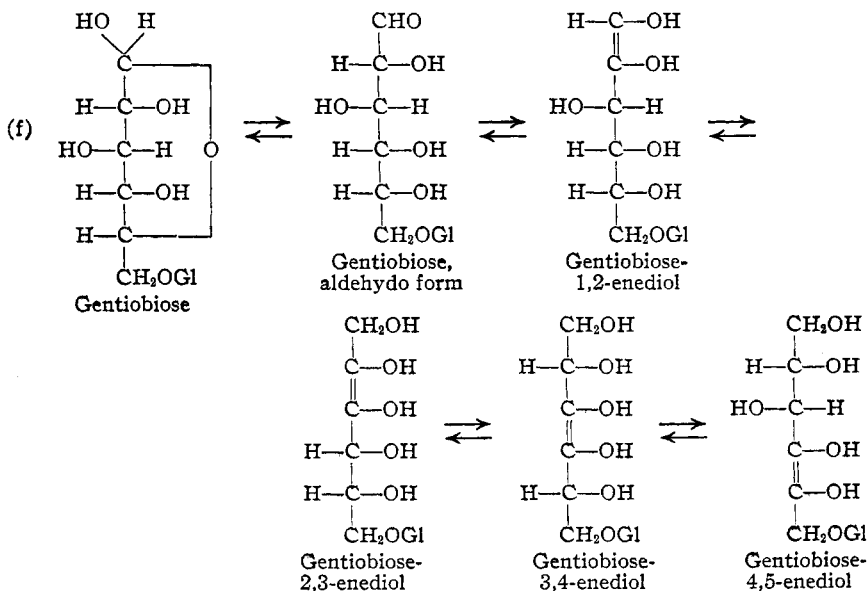
These same general reactions should take place with melibiose.   There

[20] Gross and Lewis, THIS JOURNAL, **53**, 2772 (1931).

is no *a priori* reason why one should not postulate the existence of 6-hexosido-glucose-4,5-enediol. The corresponding galactosido derivatives should form with melibiose. So far as we know the literature in this field does not contain any data with reference to the chemical oxidation of gentiobiose and melibiose, yet if the enediols indicated above should undergo a splitting in the presence of appropriate oxidizing agents it may safely be predicted that the oxidation products indicated in the following reactions (g, h, i, j) will be found in the reaction mixture, and that the amounts of them will be dependent in part on such factors as the alkali concentration,[11] the concentration of the sugar[21] and the temperature[11] employed.

(g) Gentiobiose-1,2-enediol ⟶ 5-glucosido-arabonic acid + formic acid
(h) Gentiobiose-2,3-enediol ⟶ 4-glucosido-erythronic acid + glycolic acid
(i) Gentiobiose-3,4-enediol ⟶ 3-glucosido-glyceric acid + glyceric acid
(j) Gentiobiose-4,5-enediol ⟶ 2-glucosido-glycolic acid + erythronic acid

De Bruyn and van Ekenstein[17] found that melibiose comports itself like lactose either toward potassium hydroxide or lead hydroxide. They reported the presence of galactose in such solutions but they could not prove the presence of glucose. On the basis of the behavior outlined above it is evident that the absence of glucose was to have been expected.

Discussion of Experimental Data

Lactic Acid from 4-Hexosido-glucoses and 6-Hexosido-glucoses.—On the basis of the above suggested behavior of cellobiose and lactose toward aqueous solutions of potassium hydroxide, Benoy[4] and one of us were able to account for a series of experimental observations which showed that maltose yielded approximately but one-half as much lactic acid as did an equivalent solution of glucose under exactly the same conditions. Had the fission of enediols been preceded by the hydrolysis of the disaccharide sugar, then it is evident that the yields of the acid would have been approximately the same as those obtained from the glucose solutions. It was concluded, therefore, that the lactic acid which formed in the alkaline degradation of maltose was derived from the glucose (hexosido section) obtained through the hydrolysis of the glucosido-erythrose (reaction d) formed by the successive splitting of maltose-1,2-enediol, 3-glucosido-arabinose-1,2-enediol and maltose-2,3-enediol (reactions b, c and e). If the hexosido section of the maltose molecule were the only source of lactic acid, then it is clear that the amount of this acid which may be obtained by the alkaline degradation of this well-known disaccharide should be approximately one-half of that to be expected from an equivalent solution of glucose. It has been shown previously that the tetrose sugars do not give lactic acid under these conditions.[22]

[21] Shaffer and Friedemann, *J. Biol. Chem.*, **86**, 345 (1930).
[22] Nef, *Ann.*, **376**, 40 (1910).

An application of these suggestions concerning the source of lactic acid from maltose permitted us to make some general predictions with reference to the yields of this acid which one should expect through an alkaline degradation of the following well-known disaccharides.   (a) *Cellobiose.*— It is now commonly accepted that maltose is the α-glucoside of β-glucose while cellobiose[9] is the β-glucoside of β-glucose.   Since the hexose and hexosido sections of these two disaccharides are glucose, then it is clear that the yields of lactic acid from cellobiose and maltose should be of the same general quantitative character with respect to the lactic acid that would be obtained from equivalent solutions of glucose under the same experimental conditions.   That this is so at 50° is shown in Fig. 1.   It is an interesting fact that the two curves tend to become more nearly coincident
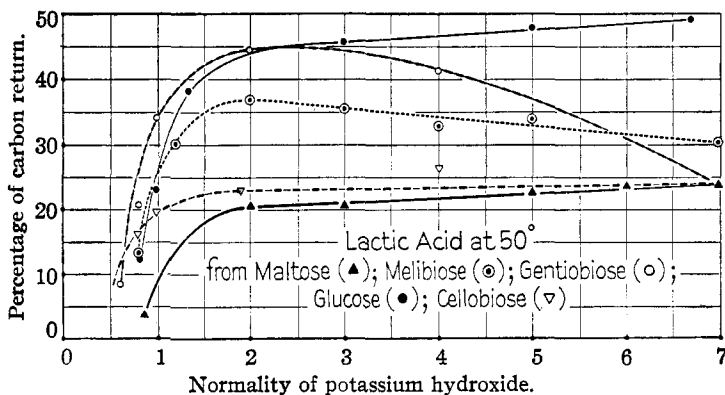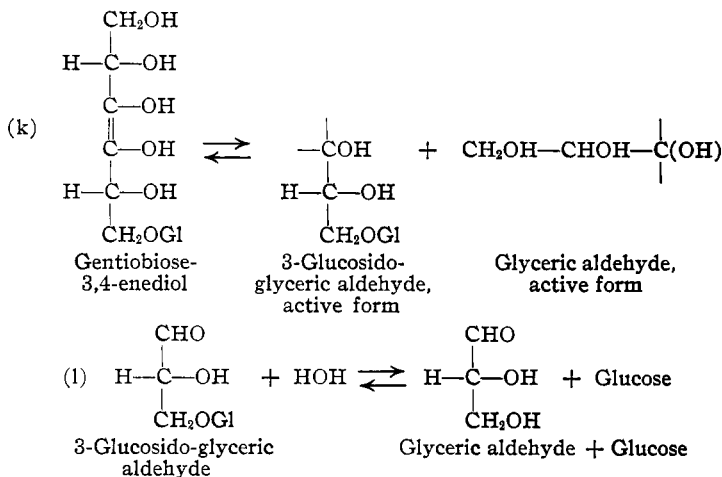

Fig. 1.

at higher alkalinities.   At the lower alkalinities one would expect those wider differences in the quantitative composition of the reaction mixtures in both maltose and cellobiose which may arise out of the differences in the complex equilibria present in each solution and also those due to the degree and rate of hydrolytic splitting of the various glucosido compounds present.[23]   This general similarity in behavior of cellobiose to that of maltose fulfils our expectation with reference to the first objective of these experiments.   (b) *Lactose.*—Since lactose is 4-galactosido-glucose,[7] then it becomes evident that the behavior of galactose itself in aqueous solutions of potassium hydroxide will be an important factor to consider in forecasting the general character of the quantity of lactic acid which is to be expected from alkaline solutions of lactose.   Hoff[11a] found that a galactose (0.5 M) solution held at 25 to 50° for forty-eight hours and over a range of alkalinity from 1.5–9.0 N, returned less than 2% of its carbon content as lactic acid.   Hence, since the hexosido sections of these sugars are

[23] Nef, *Ann.*, **403**, 204 (1914).

the sources of lactic acid, it is clear that lactose should not be expected to yield much of this acid under our experimental conditions.[24]

As a matter of fact we were unable to find lactic acid in any measurable amounts in our alkaline solutions of lactose.   (c) *Gentiobiose.*—An examination of the above suggested behavior of the 6-hexosido-hexoses toward alkalies shows that these sugars should form one molecule of 3-hexosido-glyceric aldehyde and one of glyceric aldehyde as some of the possible degradation intermediates.   Furthermore, it is evident that the 3-hexosido-glyceric aldehyde might undergo hydrolysis, thus releasing another molecule of glyceric aldehyde and one molecule of a hexose, which in turn could be acted upon by the alkali present.   These steps are given in the following reactions (k and l), in which the degradation of gentiobiose is shown.

(k)

$$
\begin{array}{c}
CH_2OH \\
| \\
H-C-OH \\
| \\
C-OH \\
\| \\
C-OH \\
| \\
H-C-OH \\
| \\
CH_2OGl
\end{array}
\;\rightleftharpoons\;
\begin{array}{c}
| \\
-COH \\
| \\
H-C-OH \\
| \\
CH_2OGl
\end{array}
\;+\;
CH_2OH-CHOH-\overset{|}{\underset{|}{C}}(OH)
$$

Gentiobiose-   3-Glucosido-   Glyceric aldehyde,
3,4-enediol   glyceric aldehyde,   active form
                    active form

(l)

$$
\begin{array}{c}
CHO \\
| \\
H-C-OH \\
| \\
CH_2OGl
\end{array}
\;+\; HOH \;\rightleftharpoons\;
\begin{array}{c}
CHO \\
| \\
H-C-OH \\
| \\
CH_2OH
\end{array}
\;+\; Glucose
$$

3-Glucosido-glyceric   Glyceric aldehyde + Glucose
aldehyde

If gentiobiose should decompose to give two molecules of glyceric aldehyde and one molecule of glucose, a hexose sugar which would undergo the same general reactions to yield two more molecules of glyceric aldehyde, then it could be predicted with a fair degree of certainty that there must be some conditions of temperature and alkali concentration under which gentiobiose more nearly resembles glucose than it does either maltose, lactose or cellobiose.   That these expectations were justified is shown in Fig. 1 (50°).   The same general relationship at 25° is shown for gentiobiose, maltose and glucose in Fig. 2.   It is seen that both glucose and gentiobiose give the same amounts of lactic acid at approximately 1.6 N potassium hydroxide at 25° and 2.37 N potassium hydroxide at 50.°   Furthermore, it should be noted that the yields of lactic acid from alkaline solutions of gentiobiose below these values are greater than those obtained from the

[24] (a) Nef, *Ann.*, **376**, 53 (1914); (b) *cf.* Hoppe-Seyler, *Ber.*, **4**, 346 (1871); Nencki and Sieber, *J. prakt. Chem.*, [N. F.] **24**, 502 (1881); (d) Duclaux, *Chem. Zentr.*, 169, I (1894).

equivalent solutions of glucose, a fact which is probably due to the difference in the character of the equilibria present in the two solutions, $i.$ $e.$, gentiobiose seems to be more sensitive to alkalies than is glucose.

Another point concerning the lactic acid yields from gentiobiose which is worthy of much note is the fact that there is a maximum (2.37 N potassium hydroxide) in the curve of this reaction. This surprising and unexpected result might be explained from several standpoints, but the enediol theory of sugar behavior seems to offer an explanation which is in harmony with the general interpretation of the studies in this series of papers. The gentiobiose may form a 4,5-enediol (reaction f) which on cleavage would give glucosido-glycol aldehyde and erythrose.[25] Should the glucosido-glycol aldehyde undergo hydrolysis under these experimental conditions

Lactic Acid at 25°
from Maltose (▽); Melibiose (○);
Gentiobiose (●); Glucose (⊙)

Percentage of carbon return.
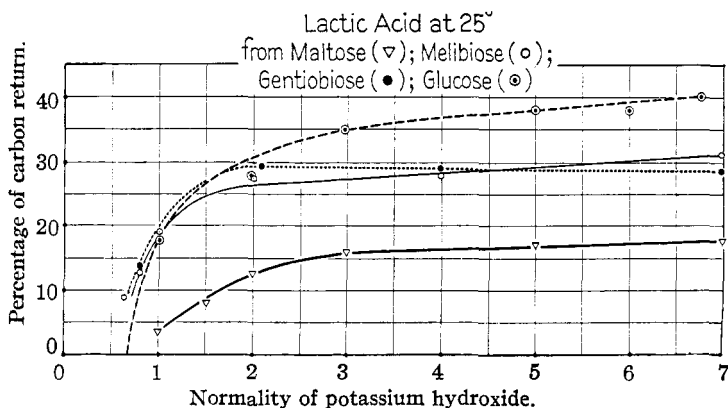
Normality of potassium hydroxide.

Fig. 2.

in the same manner as postulated for 2-hexosido-erythrose (reaction d), then the 4,5-gentiobiose-enediol molecules would have yielded by these changes one molecule of glucose, one of erythrose and one of glycol aldehyde, three compounds of which only glucose would yield lactic acid. Thus, it becomes clear that the yields of lactic acid from gentiobiose at alkali normalities higher than that of the maximum yield should approach approximately one-half that of an equivalent solution of glucose, $i.$ $e.$, the amount of lactic acid should be a decreasing function of the alkali normality.

The existence of a 6-hexosido-glucose-4,5-enediol was clearly implied first by Lewis and Buckborough[6] in their explanation of the possible presence of glucosido-glycolic acid as an oxidation product of maltose, if one were to accept the structure of that disaccharide as then understood. The possibility of hexose-4,5-enediol formation seems to find further support in the fact that the recent studies made in this Laboratory on the behavior

[25] H. O. L. Fischer, $Ber.$, **62**, 861 (1929).

of glucose, fructose and mannose[8] in alkaline solutions show that the yields of lactic acid from these well-known sugars are less at 75° than at 50°. Should the temperature effect be in the direction of a hexose-4,5-enediol, then in the glucose series such a molecule would form erythrose and glycol aldehyde, neither of which would give lactic acid. The lactic acid yields from mannose at 50° show this same tendency after about 2.5 N potassium hydroxide.[11e] (d) *Melibiose.*—Since the general structure of melibiose is like that of gentiobiose except that galactose is the hexosido portion, then the principal differences in the behavior of these two sugars must be found in the difference in the behavior of galactose and glucose in alkalies. Since galactose is a sugar that gives much smaller yields of lactic acid than glucose, we were of the opinion that the melibiose would give smaller yields of lactic acid than gentiobiose and larger ones than maltose, cellobiose and lactose (Figs. 1 and 2). Much to our satisfaction these expectations were realized. At 50°, the melibiose yields of lactic acid reached a maximum at approximately 2 N, after which there was a diminution in its production. Like gentiobiose, this disaccharide could also form a 6-galactosido-glucose-4,5-enediol, which in turn would split into erythrose and galactosido-glycol aldehyde, the latter giving galactose and glycol aldehyde on hydrolysis. Such a change would tend to lower the yield of lactic acid by reason of the small yields of lactic acid that may be obtained from galactose. From our data (Figs. 1 and 2) it is seen that the qualitative character of the hexosido portion of the disaccharides does affect the yields of lactic acid obtained from these sugars. The absence of lactose data should not be overlooked. These facts bear out the expectations of our second objective in these experiments. The comparative relationship between the yields of lactic acid obtained from cellobiose (and maltose) and gentiobiose fulfil the expectation of our third objective in these experiments, namely, that the point of glucosidic attachment (4 in cellobiose and maltose and 6 in gentiobiose) must be an important factor in the alkaline degradation of these disaccharides. The same was found to be equally true for the point of galactosidic attachment in lactose and melibiose.

Pyruvic Aldehyde Osazone from 4-Hexosido-glucoses and 6-Hexosido-glucoses.—It has been pointed out repeatedly in the literature that pyruvic aldehyde is to be regarded as the precursor of lactic acid formation in alkaline solutions of certain disaccharides, hexoses, pentoses and trioses.[26] In all the experiments carried on in this Laboratory it has been found that at a given temperature the pyruvic aldehyde osazone production reaches a maximum with respect to the alkali normality, after which it recedes fairly rapidly. In all cases the pyruvic aldehyde is regarded as a condensation

[26] (a) Ref. 11; (b) Fischler, *Z. physiol. Chem.*, **177**, 264 (1928); (c) Strain and Spoehr, *J. Biol. Chem.*, **89**, 503, 527 (1930).

product of glyceric aldehyde which in turn arises from such compounds as glucose-3,4-enediol. This latter point and the theory outlined above permit one to make a general forecast with reference to the pyruvic aldehyde osazone yields from alkaline solutions of the disaccharides under discussion.
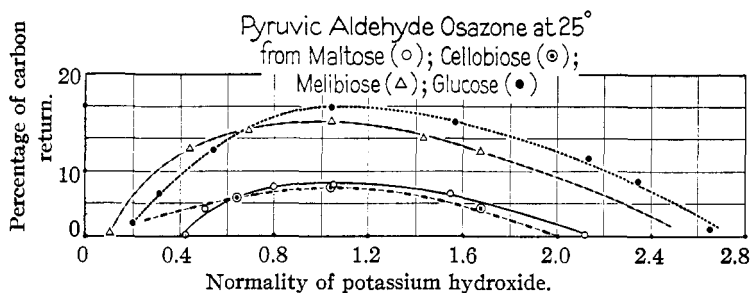

Pyruvic Aldehyde Osazone at 25°
from Maltose (○); Cellobiose (◉);
Melibiose (△); Glucose (●)

Percentage of carbon return.

Normality of potassium hydroxide.

Fig. 3.

(a) **From 4-Hexosido-glucoses.**—Since the hexosido sections of cellobiose and lactose are regarded as the portion of these disaccharides yielding lactic acid, then it is evident that cellobiose should resemble maltose in its pyruvic aldehyde production and lactose should not give any. These predictions were found to be true as shown in Figs. 3 and 4. Although
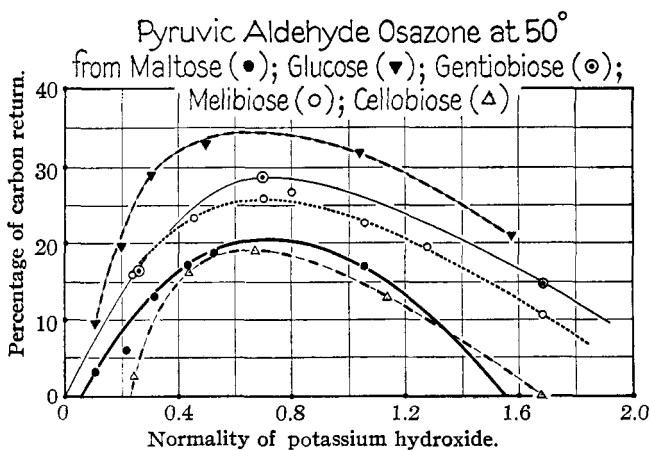

Pyruvic Aldehyde Osazone at 50°
from Maltose (●); Glucose (▼); Gentiobiose (◉);
Melibiose (○); Cellobiose (△)

Percentage of carbon return.

Normality of potassium hydroxide.

Fig. 4.

galactose is known to yield pyruvic aldehyde osazone, yet by reason of the strong resistance of lactose to alkalies, we could not obtain any of this derivative with the milk sugar.

(b) **From 6-Hexosido-glucoses.**—From the above discussion with reference to lactic acid formation from gentiobiose and melibiose, it becomes evident that gentiobiose should behave more nearly like glucose than melibiose with respect to pyruvic aldehyde osazone formation. That this is

so may be seen in Fig. 4.   By reason of the limited amount of gentiobiose in our possession, we omitted the reaction at 25°.   It is further evident on the theory set forth above that the 6-hexosido-glucoses should have been expected to yield more of the pyruvic aldehyde derivative than the 4-hexosido-glucoses.   This is found to be true.

Acetic Acid from 4-Hexosido-glucoses and 6-Hexosido-glucoses.—It has been pointed out that the acetic acid obtained in the reaction of alkalies on sugars may come from two sources: (a) that obtained through the action of alkalies on glyceric aldehyde, which in turn yields pyruvic aldehyde;[11a]   (b) that arising through the rearrangement of glycol aldehyde
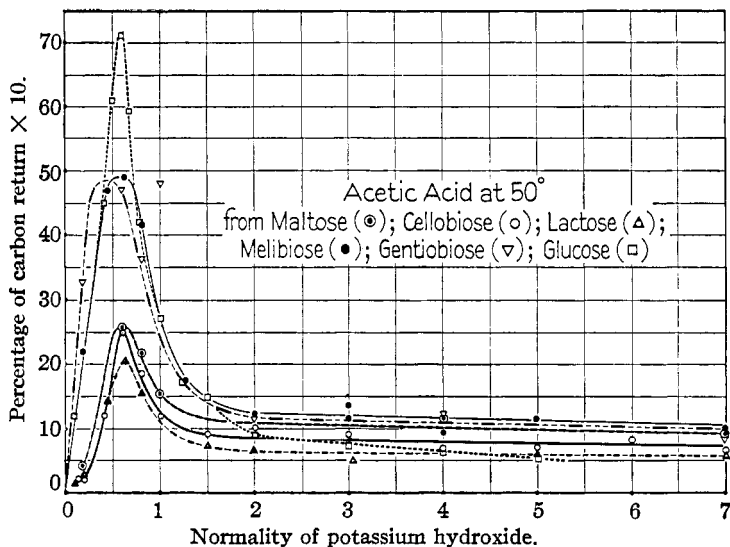

Fig. 5.

to its corresponding saccharinic acid.[27]   From the above theoretical considerations, glyceric aldehyde should be expected to form in greater amount from the 6-hexosido-glucoses than from the 4-hexosido-glucoses. Hence, gentiobiose and melibiose should more nearly approach glucose in the production of acetic acid than do maltose and cellobiose.   In the 4-hexosido-glucoses only one half of the molecule (hexosido section) will give glyceric aldehyde and the other half one molecule of a tetrose and two of formaldehyde (or one of glycol aldehyde), while in the 6-hexosido-glucose both the hexose and hexosido sections retain the possibility of yielding glyceric aldehyde.   That these grouping possibilities are borne out by the facts is seen in Fig. 5.   The occurrence of a maximum point in each case harmonizes with the point of view that as the alkali normality increases, the velocity of lactic acid formation from pyruvic aldehyde soon exceeds

[27] Evans and Hockett, THIS JOURNAL, **52**, 4068 (1930).

that of acetic acid from the same source.   The presence of acetic acid and the absence of lactic acid in the lactose degradation points to such a reaction as (e) where the milk sugar may yield glycol aldehyde, a source of acetic acid,[26] and 2-galactosido-erythrose.   That acetic acid was to have been expected from this source had been shown previously by J. E. Hutchman,[28] of this Laboratory, who found that alkaline solutions of glycol aldehyde (1.72 M), when subjected to the same procedures as were used in these experiments yielded both acetic and formic acids (Fig. 6).   Careful examination of such solutions failed to disclose the presence of either pyruvic aldehyde or lactic acid,[22] even though the reaction mixture was shown to contain the C_4 saccharinic acids, 1,3-dihydroxybutyric[29] found by Nef, and 1,2-dihydroxyisobutyric,[30] both of which were identified through the kindness of Dr. J. W. E. Glattfeld, of the University of Chicago, who furnished specimens of the phenylhydrazides of these two acids for melting point determinations.
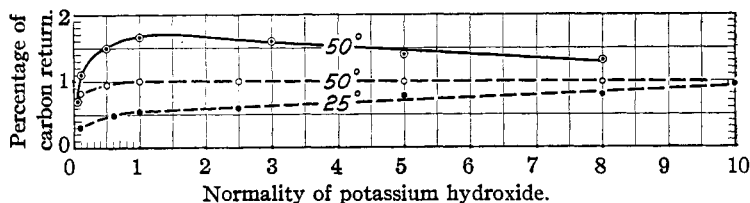

Fig. 6.—Formic acid from glycolaldehyde at 50° (⊙) and acetic acid from glycolaldehyde at 25° (●) and 50° (○).

Formic Acid from 4-Hexosido-glucoses and 6-Hexosido-glucoses.—It has been shown above that the 4-hexosido-glucoses tend to decompose into one molecule of glucose, one of erythrose and two of hydroxymethylene (or into one of glycol aldehyde).   According to Nef the tetroses form the 2,3-enediol (CH_2OH—$C(OH)$=$C(OH)$—CH_2OH), a derivative which will yield two molecules of glycol aldehyde.   Thus it is possible for the hexose section of this class of disaccharides to yield either two molecules of glycol aldehyde and two of hydroxymethylene or three molecules of glycol aldehyde, a compound which Hutchman has shown will yield more formic acid with alkalies than it does acetic acid (Fig. 6).   In the 6-hexosido-glucoses the hindering action of the hexosidic point of attachment found in the 4-hexosido-glucoses is absent and hence there is much greater freedom for 3,4-enediol formation, also a forerunner of acetic acid.   Therefore, the dominant tendency in the 4-hexosido-glucoses should be toward formic acid, a condition that is not necessarily true for the 6-hexosido-glucoses. Hence, at 50° (Fig. 7) it is seen that there is this tendency toward a group-

[28] J. E. Hutchman, Thesis for the Ph.D. degree, The Ohio State University, 1927.

[29] Glattfeld and Sander, THIS JOURNAL, **43**, 2675 (1921).

[30] Glattfeld and Sherman, *ibid.*, **47**, 1742 (1925).

ing of these disaccharides, maltose, cellobiose and lactose giving more formic acid than gentiobiose and melibiose. This grouping would probably be much more pronounced if the small quantities of formic acid could be determined more accurately.
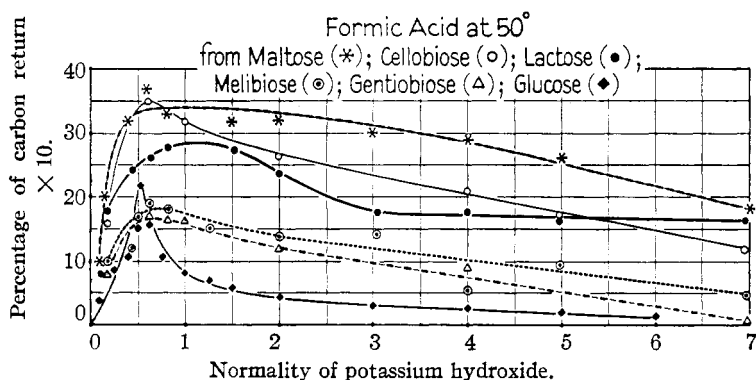

Fig. 7.

Summary

1. The three principal objectives in these experiments were as follows: (a) to verify the prediction based on maltose chemistry that cellobiose would react toward aqueous solutions of potassium hydroxide to give practically the same yields of lactic, acetic and formic acids, and pyruvic aldehyde osazone as maltose itself; (b) to verify the prediction that the glucosido sections of cellobiose and gentiobiose, and the galactosido sections of lactose and melibiose, would greatly influence the yields of lactic acid in each case, which yields would be relatively greater in the glucosido-glucoses; (c) to verify the prediction that due to the hindering action of the hexosido section of the molecules in the 4-hexosido-glucoses (cellobiose, lactose and maltose), the yields of lactic acid would not be so great in these cases as from gentiobiose and melibiose, compounds in which the hexosido attachment is on carbon atom 6. The reactions were found to be in harmony with these general points of view.

2. Since pyruvic aldehyde is regarded as the precursor of lactic acid, the same general relationships were also found in this case.

3. The yields of acetic acid from the 6-hexosido-glucoses were greater than those obtained from the 4-hexosido-glucoses, while those of formic acid show the opposite tendency. The disaccharide sugars show a maximum yield of acetic acid quite similar to that of the hexose sugars. A mechanism of acetic and formic acid production from the disaccharides is given.

4. On the enediol theory of chemical action in the disaccharides, the probable oxidation products of gentiobiose and melibiose are given.

5.   The interpretation of the maximum point in the curve of lactic acid production from gentiobiose and melibiose is based on the assumption that a 6-hexosido-glucose-4,5-enediol must have formed in each case in the regions of higher alkalinity.   A mechanism is given to explain the decreasing yield of this acid after the point of maximum production has been passed.

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[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF CHEMISTRY AND SOILS]

ROTENONE. XVI.
INTERPRETATION OF SOME CHARACTERISTIC REACTIONS OF ROTENONE

BY F. B. LAFORGE, H. L. HALLER AND L. E. SMITH

During the past four or five years, research by several groups of investigators in different parts of the world has produced a large number of important facts bearing on the structure of the rotenone molecule.

Many of the observed reactions are apparently without analogy, and it has not been possible to coördinate any group of facts with a satisfactory structural formula.

From the observations of Butenandt[1] and others it has been established that the rotenone molecule ($C_{23}H_{22}O_6$) consists of two components each containing eleven carbon atoms joined through a carbonyl group.   One of these components contains a benzene ring to which two methoxyl groups are attached;   the other is related to tubaic acid.   Cleavage takes place at the carbonyl group when rotenone and its derivatives are subjected to oxidation or hydrolysis.

$$(C_{11}H_{11}O_3)CO(C_{11}H_{11}O_2)$$
Derric acid component   Tubaic acid component

Rotenone and most of its derivatives contain a lactone group which is peculiar in that it is opened by hydrogenation, with the formation of desoxy acids.[2]   The desoxy acids are formed with loss of an asymmetric center.[3] As will be shown later, the lactone ring is located in that part of the molecule which yields tubaic acid.   The conversion of the lactones into acids in order to render them soluble in alkali, is taken advantage of in the peroxide oxidation process described in previous articles.[4]

[1] Butenandt, *Ann.*, **464**, 253 (1928).

[2] LaForge and Smith, THIS JOURNAL, **51**, 2574 (1929); **52**, 1088 (1930); **52**, 1091 (1930); **52**, 3603 (1930).

[3] Haller and LaForge, *ibid.*, **53**, 3426 (1931).

[4] LaForge and Smith, *ibid.*, (a) **52**, 1091 (1930); (b) **52**, 3603 (1930); (c) Smith and LaForge, *ibid.*, **52**, 4595 (1930).