TABLE II

IGNITION OF NICOTINE SILICOTUNGSTATE AT 650°

Time of ignition, min.	Nicotine silicotungstate mg.	Residue, mg. Found	Residue, mg. Calcd.
15	2.737	2.366	2.363
15	3.172	2.741	2.738
45	2.107	1.818	1.819
45	2.146	1.869	1.852

cal balance the rider of which was checked against a 10-mg. weight calibrated by the National Bureau of Standards. The tares were adjusted so as to avoid the use of fractional weights. Since weighings were by difference and were sometimes made several days apart, it was necessary to use similar platinum crucibles for tares to eliminate errors due to change in pressure, temperature and humidity. The use of the usual glass lead-shot tares was found to introduce an appreciable error due to these causes. Throughout the experiments the crucibles were handled with platinum-tipped forceps.

The Distilled Water.—Stock distilled water was carefully redistilled into a leached 12-liter flask, with the use of a block-tin condenser. When about half the determinations had been made, blanks were run on the water alone and with 0.1 normal solutions of the hydrochloric acid used. The procedure and apparatus were the same as were used in the preparation of the saturated solutions. On the basis of three runs each, the values of the blanks were 0.80 ± 0.14 mg. per liter for the water and 1.70 ± 0.14 mg. per liter for the acid. The amount of the correction for dissolved glass was applied in proportion to the acid concentration of the solvent.

The author wishes to acknowledge the interest of Nathan L. Drake in this work.

Summary

The solubility of nicotine silicotungstate in water and in 0.001 to 0.1 *N* hydrochloric acid solutions has been determined. The maximum solubility, 38.5 mg. per liter, is found in water and a minimum of 5.30 mg. per liter in 0.005 *N* hydrochloric acid. The solubility increases from 6.96 mg. per liter in 0.015 *N* acid to 13.4 mg. per liter in 0.1 *N* hydrochloric acid.

COLLEGE PARK, MD. RECEIVED SEPTEMBER 5, 1936

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Mechanism of Carbohydrate Oxidation. XXIII.[1] A Contribution to the Alkaline Hydrolysis of Oligosaccharides[1a]

BY HARRY GEHMAN, LEONARD C. KREIDER AND WM. LLOYD EVANS

After studying the action of aqueous potassium hydroxide on maltose (4-α-glucosidoglucose) to form lactic acid, Benoy[2] and one of us came to the conclusion that the hexose (reducing) portion of the molecule was first degraded by the alkali to form the following fragments: formaldehyde, glycolaldehyde and 2-glucosidoerythrose. Hydrolysis of the latter was then assumed to occur to produce glucose and erythrose. As it was quite certain from previous work[3] that formaldehyde, glycolaldehyde and erythrose would produce no lactic acid in alkaline solution, it was probable that when maltose was degraded by alkali the entire reducing half of the molecule was lost to lactic acid production. The hexosido-(non-reducing) portion, being released by hydrolysis as glucose, should then produce lactic acid as glucose was known to do. The fact that a solution of maltose (0.25 *M*) yielded approximately half as much lactic acid as was obtained under identical conditions from an equivalent amount of glucose (0.50 *M*) lent considerable weight to this argument.

Later in a similar study of the alkaline degradation of cellobiose (4-β-glucosidoglucose) and lactose (4-galactosidoglucose) Hockett[4] and one of us showed that these disaccharides also yielded only such amounts of lactic acid as would have been expected from their hexosido (non-reducing) portions alone.

The 6-hexosidohexoses, like gentiobiose (6-glucosido-glucose) and melibiose (6-galactosidoglucose), present a different type of behavior, for here the amount of lactic acid produced is considerably greater than that expected from the hexosido portion of the molecule alone. This was explained by pointing out that the reducing portion of these molecules contributed a part of the lactic acid. Simultaneous with the degradation

(1) Number XXII of this Series, H. W. Arnold and W. L. Evans, THIS JOURNAL, **58**, 1950 (1936).

(1a) Presented before the Organic Section of the American Chemical Society, Pittsburgh Meeting, 1936.

(2) W. L. Evans and M. P. Benoy, *ibid.*, **52**, 294 (1930).

(3) J. U. Nef, *Ann.*, **376**, 40 (1910); J. E. Hutchman, Ph.D. Dissertation, O. S. U., 1927.

(4) W. L. Evans and R. C. Hockett, THIS JOURNAL, **53**, 4384 (1931).

of the reducing half of the molecule, or following it, hydrolysis would liberate the non-reducing half and make it subject to alkaline degradation so it too could make its normal contribution of lactic acid to the total.

This paper offers a mechanism for the alkaline hydrolysis postulated above and presents additional evidence to support and extend the previous theories[2,4] of the action of alkali on oligosaccharides. The use of the recently synthesized glucosidodihydroxyacetone pentaacetate,[5] cellobiosidodihydroxyacetone octaacetate,[5] and gentiobiosidodihydroxyacetone octaacetate[5] was invaluable in the latter phase of this work.

Experimental Part

Carbohydrate Materials.—Glucosidodihydroxyacetone pentaacetate, gentiobiosidodihydroxyacetone octaacetate, and cellobiosidodihydroxyacetone octaacetate were prepared according to directions already published.[5] Dihydroxyacetone monoacetate,[6] cellobiose octaacetate, lactose octaacetate,[7] and glucose pentaacetate, were specimens prepared in the conventional ways and recrystallized until pure. The gentiobiose octaacetate was obtained from The Laboratory Products Co., Cleveland, Ohio.

Analytical Procedure.—The general procedure outlined by Nadeau, Newlin and Evans[8] was followed for the isolation of the lactic acid. The use of acetylated sugars necessitated a normality correction due to the neutralizing action of the acetyl groups and also to the greater expansion of the reaction mixture in these cases. The normalities of alkali reported are corrected values. A nitrogen atmosphere was also used in the reaction flasks instead of an air atmosphere as had formerly been customary. All the work was carried out at a temperature of 50°. The amount of carbohydrate material and volume of alkali used to degrade it are as follows: glucosidodihydroxyacetone pentaacetate, 1.4443 g. (0.25 *M*) in 12.5 cc. KOH; glucose tetraacetate, 1.0878 g. (0.25 *M*) plus dihydroxyacetone monoacetate, 0.4125 g. (0.25 *M*) in 12.5 cc. KOH; glucose pentaacetate, 2.4375 g. (0.50 *M*) in 12.5 cc. KOH; gentiobiose, 1.0687 g. (0.25 *M*) in 25.0 cc. KOH;[4] β-gentiobiose octaacetate, 1.6957 g. (0.20 *M*) in 12.5 cc. KOH; β-gentiobiosidodihydroxyacetone octaacetate 1.5002 g. (0.20 *M*) in 10.0 cc. KOH; β-cellobiose octaacetate, 1.6957 g. (0.20 *M*) in 12.5 cc. KOH; β-cellobiosidodihydroxyacetone octaacetate, 1.8760 g. (0.20 *M*) in 12.5 cc. KOH; gentiobiose octaacetate 1.6957 g. (0.20 *M*) plus dihydroxyacetone monoacetate, 0.3302 g. (0.20 *M*) in 12.5 cc. KOH; β-cellobiose octaacetate, 1.6957 g. (0.20 *M*) plus dihydroxyacetone monoacetate, 0.3302 g. (0.20 *M*) in 12.5 cc. KOH; glucose pentaacetate, 1.56 g. (0.40 *M*) plus dihydroxyacetone monoacetate, 0.3302 g. (0.20 *M*) in 12.5 cc. KOH; glucosidodihydroxyacetone pentaacetate, 0.924 g. (0.20 *M*) plus glucose pentaacetate, 0.78 g. (0.20 *M*) in 10.0 cc. KOH; β-lactose octaacetate 2.197 g. (0.25 *M*) in 12.5 cc. KOH.

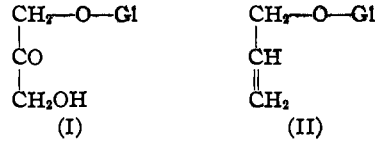
The isolation of non-volatile saccharinic acids other than lactic acid was carried out according to the procedure outlined by Plunkett.[9]

Experimental Results.—The quantitative data obtained in this work are shown in graphical form in Figs. 1 to 6. The lactic acid yields in moles per mole of sugar used are plotted as functions of the corrected alkali normalities.

Theoretical Part

The hydrolysis of glucosides and oligosaccharides usually is achieved with dilute acid and by the aid of enzymes in a neutral solution. Hydrolysis in an alkaline medium has been reported in relatively fewer instances, and hence it is not so well known. The evidence obtained from the action of aqueous alkali on certain disaccharides was of such a nature as to compel Benoy,[2] Hockett[4] and one of us to postulate alkaline hydrolysis during the alkaline degradation of the carbohydrates studied, and results reported here are in complete harmony with this view.

The following comparison of certain of our experimental data is of much interest in connection with the main purposes of this paper. Since glucosidodihydroxyacetone (I) yields lactic acid in alkaline solution, while allyl glucoside (II) does not produce this acid under the same conditions, an explanation for this difference in chemical behavior of these two compounds was sought in some possible change in molecular structure which one of them might undergo in alkaline solutions. If (I) is written in the customary *keto*-form it is evident that the structure of both compounds is the same at the glucosidic link



and hence alkaline hydrolysis would be expected in both cases if it were known to occur in either one. From our experimental results it was concluded that glucosidodihydroxyacetone in alkaline solution must possess a different structure at the glucosidic link than does allyl glucoside. If glucosidodihydroxyacetone should undergo enolization in alkaline solution[10] a possible enolic iso-

(5) Number XX of this Series, L. C. Kreider and W. L. Evans, THIS JOURNAL, **58**, 1661 (1936).

(6) H. O. L. Fischer, E. Baer and L. Feldman, *Ber.*, **63**, 1732 (1930); A. Wohl and C. Neuberg, *ibid.*, **33**, 3095 (1900).

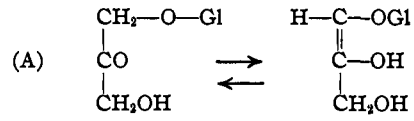
(7) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 1270 (1915).

(8) G. F. Nadeau, M. R. Newlin and W. L. Evans, *ibid.*, **55**, 4957 (1933).

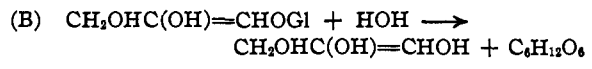
(9) R. J. Plunkett, Ph.D. Dissertation, The Ohio State University, 1936.

(10) *Cf.* A. Kusin, *Ber.*, **69B**, 1041–1049 (1936).

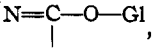
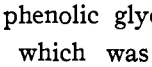
mer would be 1-triosenediol glucoside, a change which may be shown as follows



It may then be assumed that 1-triosenediol glucoside undergoes hydrolysis as shown by the equation



Both the triosenediol and glucose thus formed are sources of lactic acid. Since allyl glucoside does not yield lactic acid under our experimental conditions, it is to be concluded that hydrolysis did not take place, *i. e.*, there was no structural opportunity for isomerization to yield an enolic form.

For these reasons the authors examined the literature to ascertain whether certain given types of compounds existed which would undergo alkaline hydrolysis, and on the other hand whether certain other definite types would resist such hydrolysis. The reduction of heated Fehling's solution was accepted as a criterion of hydrolysis in the absence of direct statements, or other experimental evidence that was decisive, such as the production of lactic acid. This statement would of necessity assume that the aglycone was inactive toward the oxidizing solution. Our examination makes it fairly certain that the literature of alkaline hydrolysis is not yet sufficiently understood to justify a conclusive statement concerning the existence of sharply defined classes of compounds that on the one hand undergo alkaline hydrolysis, while another group resists this action. The following facts verify this point of view. (a) Methyl,[11] ethyl,[11] glycol,[11] propyl[12] and benzyl[13] glucosides and trehalose[14] are representatives chosen from a large number of similar compounds that undergo no hydrolysis in aqueous alkali. In our experiments we found that methyl, glycol and allyl glucosides, and trehalose, when treated with aqueous solutions of potassium hydroxide (4.0 *N* and above) for forty-eight hours at 50°, yielded no lactic acid. These results confirm the absence of hydrolysis in these cases. It should be noted that all of these compounds cannot isomerize to an enolic form as given above for glucosidodihydroxyacetone. (b) There are other glycosides that contain a still different type of an aglycone, some of which undergo alkaline hydrolysis while others do not. Helferich and Kühlewein[15] found that the glycosides of theophylline (galactose, glucose and arabinose), chlorotheophylline *d*-glucoside, tetraacetyl-trichloropurine *d*-glucoside, dichloroadenine *d*-glucoside did not reduce Fehling's solution, while the glycosides of theobromine (galactose and glucose), and tetraacetylhydroxycaffeine *d*-glucoside did reduce the same reagent. In those cases where reduction took place, the compound could be isomerized to contain the group  , while in the cases where no reduction occurred the glycosidic portion reacted with the purine compound to form the link N–C, thus excluding the possibility of isomerization after the manner indicated above. Fischer and Helferich[16] found that theobromine glucoside is decomposed by water into its components at ordinary temperature in the course of a few hours. Levene and Sobotka[17] report that tetraacetylhydroxycaffeine was found so unstable that it could not be deacetylated at all without opening the purine linkage. (c) The phenolic glycosides present much interest in this connection. Helferich and Schmitz-Hillebrecht[18] found that the acetylated glucosides of phenol, α- and β-naphthol, methyl arbutin, guaiacol and α- and β-galactosides of phenol and certain other similar glycosides reduce Fehling's solution. On the other hand Helferich and Winkler[19] report that tetraacetyl-phenol-β-*d*-mannoside would not reduce Fehling's solution without previous acid hydrolysis. In the phenolic glycosides we find the group  , which was postulated to form in alkaline solutions of compounds possessing the structure which would yield the enolic isomer, and hence were expected to undergo hydrolysis.

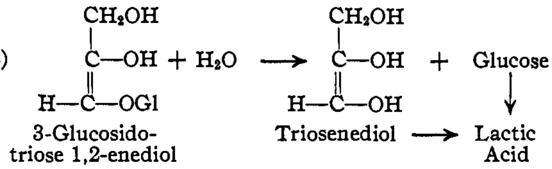
More recently Gardner and his collaborators[20] have made a study of hydrolysis of α-hydroxyanthraquinone β,*d*-glucoside and β,*d*-arabinoside,

(11) E. Fischer, *Ber.*, **26**, 2400 (1893).
(12) E. Fischer and L. Beensch, *ibid.*, **27**, 2478 (1894).
(13) E. Fischer and B. Helferich, *Ann.*, **383**, 68 (1911).
(14) C. S. Hudson, THIS JOURNAL, **38**, 1571 (1916).
(15) B. Helferich and M. von Kühlewein, *Ber.*, **53**, 17 (1920).
(16) E. Fischer and B. Helferich, *ibid.*, **47**, 210 (1914).
(17) P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **65**, 463 (1925).
(18) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).
(19) B. Helferich and S. Winkler, *ibid.*, **66**, 1556 (1933).
(20) (a) J. H. Gardner, T. F. McDonnell and C. J. W. Weigand, THIS JOURNAL, **57**, 1074 (1935); (b) H. Foster with J. H. Gardner, *ibid.*, **58**, 597 (1936); (c) J. H. Gardner and W. H. Demaree, *ibid.*, **58**, 757 (1936).

1,5- and 1,8-dihydroxyanthraquinone glucoside, and β-hydroxyanthraquinone β,*d*-glucoside in aqueous solutions of potassium hydroxide, borax and hydrochloric acid. These compounds were found to undergo hydrolysis in the three reagents chosen. (d) In striking contrast to the resistance toward alkaline hydrolysis of certain compounds noted in (b) that contain the group N–C at the purine–glycosidic union is the behavior observed by Maurer[21] and Scheidt[21] of certain glycosides of amino acid esters which were found to undergo hydrolysis in alkaline solutions.

It is evident from this brief survey that it is possible for alkaline hydrolysis of *oligosaccharides* to occur in molecules where the biosidic link exists either in the form Gl–O–C= (with a bond below the C), or where the structure can be transformed by the alkaline medium to assume that arrangement.

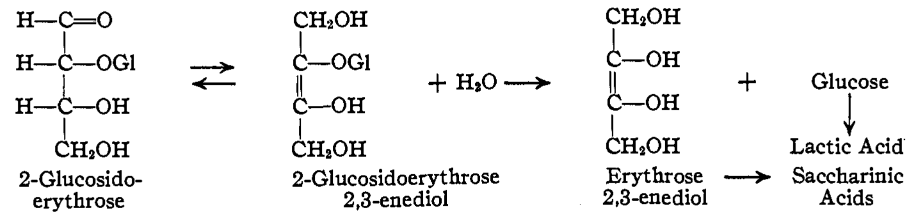
Such a structure could be induced in the fragment 2-glucosidoerythrose, which was postulated as an intermediate in the alkaline degradation of maltose[2] and of cellobiose.[4] The reactions that probably take place are shown in the following

(E) 

The actual isolation of the monosaccharides set free in such an hydrolysis is not possible when working with the stronger alkaline solutions. However, Lobry du Bruyn and Alberda van Ekenstein,[22] using much weaker alkalies, isolated galactose from lactose (4-galactosidoglucose) solutions, but obtained no glucose. This result was a reasonable one because the latter undoubtedly was partially degraded before the alkaline hydrolysis occurred.

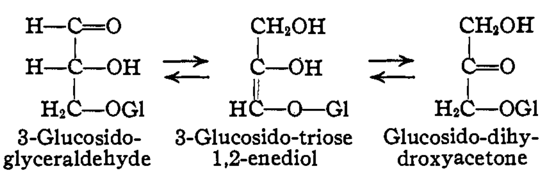
Discussion of Experimental Data

Glucosidodihydroxyacetone and Gentiobiose.—From previous studies of the action of alkali on gentiobiose,[4] it was assumed that the reducing half of the molecule was the first to suffer degradation. This action produced fragments of two types: (1) those that form monosaccharides, and

(C) 

(2) those that result in the formation of disaccharides. By the action of alkalies on the monosaccharides so formed, glycol aldehyde yields no lactic acid, the triose gives much lactic acid, and the tetrose no lactic acid. Of the possible disaccharides, 5-glucosidopentose, 3-glucosidotriose and glucosidoglycol aldehyde, the 3-glucosidotriose yields much lactic acid. The newly formed disaccharides could then suffer a similar degradation in the reducing portion of their molecules, or they could undergo alkaline hydrolysis according to the mechanism outlined above. Reactions D and E may be considered the steps in a typical example of such an hydrolysis. The glucose thus liberated could then undergo fragmentation in the presence of alkali with the resulting production of lactic acid.

In like manner the various fragments postulated in the degradation of disaccharides of the gentiobiose type[4] could undergo enediolization to form the grouping Gl–O–C= (with a bond above the C) which will then permit hydrolysis in the alkaline medium to liberate the non-reducing portion to the attack of the alkali. For example, if gentiobiose should fragment as postulated[4] to yield glyceraldehyde and 3-glucosidoglyceraldehyde, the latter could undergo the following reactions culminating in an alkaline hydrolysis to form glyceraldehyde and glucose as shown in following reactions

(D) 

An experimental study of the amounts of lactic acid produced by the alkaline degradation of the

(21) (a) Kurt Maurer, *Ber.*, **59B**, 827–829 (1926); (b) K. Maurer and B. Scheidt, *Z. physiol. Chem.*, **206**, 125 (1932); *C. A.*, **26**, 3482 (1932).

(22) Lobry du Bruyn and Alberda van Ekenstein, *Rec. trav. chim.*, **18**, 147 (1899).

individual disaccharide fragments should yield important data regarding the mechanism of the alkaline degradation of gentiobiose itself. One of these compounds, a glucosidotriose, is now available for study in the recently prepared glucosidodihydroxyacetone pentaacetate.[4] In discussing Fig. 1 three cases will be noted.

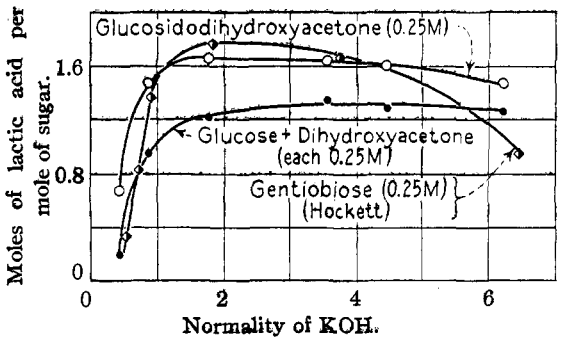


Fig. 1.—A comparison of the lactic acid yields obtained from 0.25 *M* solutions of glucosidodihydroxyacetone with those of a mixture of glucose and dihydroxyacetone, and with those of gentiobiose in the presence of potassium hydroxide at 50°.

(a) This keto sugar should react in alkaline solution in accordance with Reaction D to produce the corresponding aldo sugar, 3-glucosidoglyceric aldehyde,[23] one of the postulated intermediates formed in the alkaline degradation of gentiobiose. If the remaining fragments obtained from the gentiobiose molecule consisted chiefly of formaldehyde and glycol aldehyde, the yields of lactic acid from glucosidodihydroxyacetone at different alkalinities should be approximately the same as those obtained from gentiobiose itself provided the concentration of this glucosidotriosenediol in this very complex mixture were the same in both cases. From Fig. 1 it may be fairly concluded that at alkalinities below 1 *N* gentiobiose molecules have not undergone a sufficient degree of fragmentation to yield a concentration of the glucosidotriosenediol molecules at a given alkalinity equal to that produced by the glucosidodihydroxyacetone used. (b) At favorable alkalinities, it is conceivable for the gentiobiose molecules to undergo fragmentation in the direction of yielding one molecule each of 3-glucosidoglyceric aldehyde and glyceric aldehyde. Since glyceric aldehyde itself is also a source of lactic acid, the total yield of this acid under these conditions may be greater than in the previous case, provided the degree of fragmentation of the gentiobiose molecules is sufficiently great to furnish the required concentration of the enediolic isomers of glyceric aldehyde and of the glucosidoglyceric aldehyde necessary for this purpose. This explanation would account for the greater yields of lactic acid from gentiobiose between 1.2 and 4 *N* (Fig. 1) than those from glucosidodihydroxyacetone in the same alkaline range. (c) If at the highest alkalinities the fragmentation of gentiobiose molecule yields a tetrose and glucosidoglycol aldehyde the yields of lactic acid should tend toward that obtained from the glucose molecule (a hydrolytic product) under the same conditions. It will be shown later that in addition to the effect of such a postulated fission on the yields of lactic acid produced in these cases, the formation of saccharinic acids exercises a marked influence in this connection.

The assumption that glucosidoglyceric aldehyde is a possible intermediate in the alkaline degradation of gentiobiose seems justified by the discussion set forth above concerning the experimental facts presented in Fig. 1. Unfortunately, the other disaccharide fragments have not yet been synthesized in a pure form, so that they too could be tested for lactic acid production. However, by making use of the accumulated experience of previous workers,[2,4,24] we can predict with a fair degree of assurance that the glucosidobioses will yield about the same amount of lactic acid as their constituent glucose portions alone would produce and that glucosidotetroses and glucosidopentoses would probably yield somewhat more lactic acid than their constituent glucose portions.

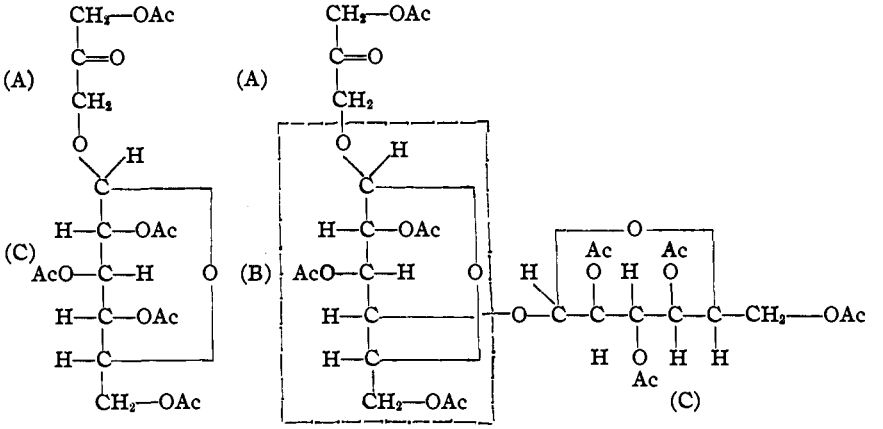
Glucosidodihydroxyacetone and Cellobiosidodihydroxyacetone.—Benoy,[2] Hockett,[4] and Evans postulated that in the alkaline degradation of 4-glucosidoglucoses, such as maltose and cellobiose, the reducing section of the molecule would decompose into fragments which were not sources of lactic acid, while the hexosido portion of the disaccharide molecule was the only available source of this acid. The recent syntheses[5] of glucosidodihydroxyacetone pentaacetate and cellobiosidodihydroxyacetone octaacetate has made it possible to test this assumption in a very simple manner.

If glucosidodihydroxyacetone pentaacetate undergoes alkaline hydrolysis in the manner indicated in Reactions D and E, two lactic acid producing compounds, namely, dihydroxyacetone

(23) L. C. Kreider and W. L. Evans, THIS JOURNAL, 57, 229 (1935).

(24) W. L. Evans, *Chem. Rev.*, 6, 281 (1929).

and glucose, would be formed in the reaction mixture. If cellobiosidodihydroxyacetone octaacetate were subjected to the same treatment under the same experimental conditions, dihydroxyacetone and glucose derived from the hexosido section of the molecule would be present in the reaction mixture in addition to the non-lactic acid producing fragments into which the hexose section of the molecule was decomposed. Thus it is seen that both systems would contain the same common lactic acid sources. A comparison of the structural formulas of these two carbohydrates will make this point more clear. The monosaccharide sections designated A and C produce lactic acid when degraded by alkali, while section B produces no lactic acid under the same conditions.[4]



Under these circumstances the yield of lactic acid in each case should be the same within the limits of experimental error. This is shown to be the case in Fig. 2.

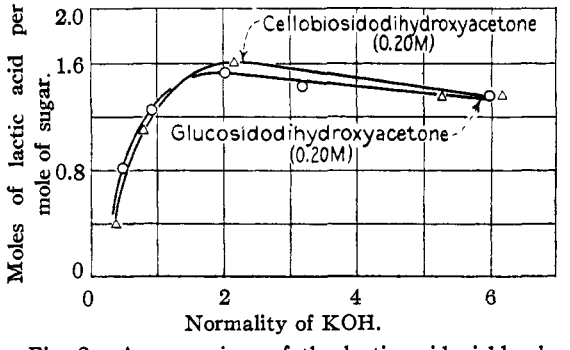


Fig. 2.—A comparison of the lactic acid yields obtained from 0.20 *M* solutions of glucosidodihydroxyacetone with those of cellobiosidodihydroxyacetone in the presence of potassium hydroxide at 50°.

Cellobiosidodihydroxyacetone and Gentiobiosidodihydroxyacetone.—In Fig. 3 we note that gentiobiose (0.20 *M*) produces between 0.5 and 0.6 mole more lactic acid at the maximum than does cellobiose (0.20 *M*). Since a comparable difference exists between the gentiobiosidodihydroxyacetone and the cellobiosidodihydroxyacetone it is evident that whatever may be the mechanism of the fragmentation of these molecules its effects on the parent disaccharides are analogous. The degradation proceeds so that the cellobiosidic portion reacts as cellobiose and the gentiobiosidic portion reacts as gentiobiose. This is evidence that the mechanism of break at the oxygen link connecting the dihydroxyacetone is such that the non-reducing portions of the residual disaccharides are not disturbed until after their hydrolysis has taken place, and that all alkaline degradations originate at the reducing regions only.

Relative Yields of Lactic Acid from Oligosaccharides and their Hydrolytic Fractions.—The lactic acid yields from these oligosaccharides can be shown to differ markedly from those of their possible hydrolytic fractions. The yield of lactic acid from glucosidodihydroxyacetone is much higher than the yield obtained from an equivalent mixture of glucose plus dihydroxyacetone (Fig. 1). The lactic acid produced by the

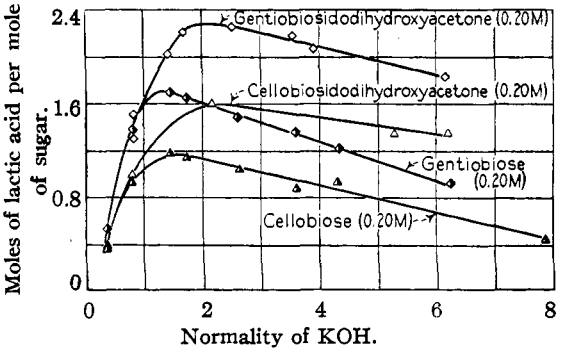


Fig. 3.—A comparison of the lactic acid yields obtained from 0.20 *M* solutions of gentiobiosidodihydroxyacetone with those of gentiobiose, and cellobiosidodihydroxyacetone with those of cellobiose in the presence of potassium hydroxide at 50°.

alkaline degradation of cellobiosidodihydroxyacetone differs greatly from the possible hydrolytic products that might be formed (Fig. 4). In

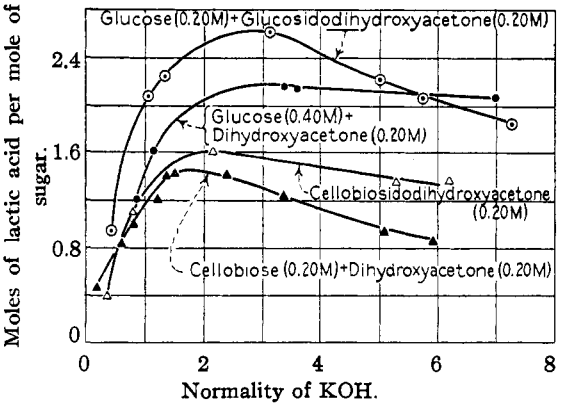


Fig. 4.—A comparison of the lactic acid yields obtained from 0.20 *M* solutions of cellobiosidodihydroxyacetone in the presence of potassium hydroxide at 50° with mixtures of its theoretically possible saccharidic components.

the same way there is an absence of any correlation between the lactic acid yields obtained from gentiobiosidodihydroxyacetone and from any of its possible hydrolytic products is shown in Fig. 5. These facts can best be interpreted

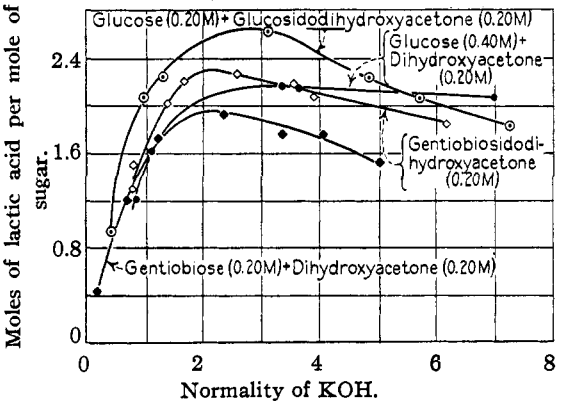


Fig. 5.—A comparison of the lactic acid yields obtained from 0.20 *M* solutions of gentiobiosidodihydroxyacetone in the presence of potassium hydroxide at 50° with mixtures of its theoretically possible saccharidic components.

as evidence that, in general, oligosaccharides do not hydrolyze immediately and entirely when they are placed in alkali, but rather that hydrolysis is a relatively slow process and is probably preceded by a partial alkaline degradation of the reducing end of the molecule. It should also be pointed out that the possible hydrolytic fractions and their degradation products tend to undergo rearrangements in alkaline solutions to form their respective saccharinic acids, thus proportionally reducing the yields of lactic acid, which itself may be considered the saccharinic acid of the trioses.

The yields of lactic acid obtained from glucosido-dihydroxyacetone, cellobiosidodihydroxyacetone, and gentiobiosidodihydroxyacetone are in general greater than those obtained from mixtures simulating the products obtained in the first step in the alkaline hydrolysis of these oligosaccharides, *i. e.*, glucose and dihydroxyacetone, cellobiose and dihydroxyacetone, and gentiobiose and dihydroxyacetone, respectively (Figs. 1, 4 and 5). In the case of mixtures representing other possible hydrolytic reactions, the yields of lactic acid tend to be higher than those obtained from the oligosaccharide itself.

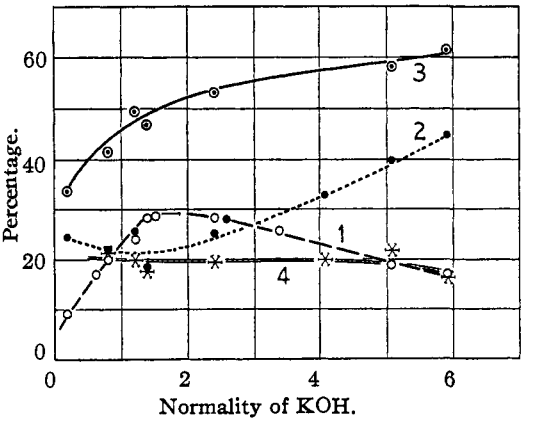


Fig. 6.—A comparison of the lactic acid and saccharinic yields obtained from a mixture of cellobiose octaacetate (0.20 *M*) and dihydroxyacetone monoacetate (0.20 *M*) in the presence of potassium hydroxide at 50°: 1, percentage of the sugars returned as lactic acid; 2, percentage of the sugars returned as saccharinic acids other than lactic acid; 3, total saccharinic acids recovered; 4, zinc oxide content of the salts obtained in 2.

The much slower release of the carbohydrate material of the parent oligosaccharide into the alkali sensitive hydrolytic portions makes possible a more efficient formation of lactic acid. This result is in agreement with an earlier observation of Shaffer and Friedemann[25] concerning the effect of the concentration of sugar molecules on the yields of lactic acid.

Lactose (4-Galactosidoglucose).—The use of an improved technique enabled us to obtain well crystallized zinc lactate in small amounts from

(25) P. A. Shaffer and T. E. Friedemann, *J. Biol. Chem.*, **86**, 345 (1930).

the alkaline degradation of this sugar. Lactose being a disaccharide linked in the same fashion as cellobiose, it was expected that its lactic acid yields plotted on a molar basis should closely approximate those of galactose just as those of cellobiose[4] and maltose[2] approximate those of glucose. This was found to be true.

Non-volatile Saccharinic Acids Other than Lactic Acid.—The materials extracted at this stage were found to be distinctly salt forming in nature with the zinc oxide content of these salts varying widely according to the carbohydrate source. As stated above it is reasonable to assume that the formation of these other saccharinic acids occurs at the expense of lactic acid formation so that a reciprocal relationship with the latter would be established. This condition was not always realized experimentally, but Fig. 6 shows the results from the cellobiose octaacetate and dihydroxyacetone monoacetate mixture. The acids involved here are undoubtedly of varying carbon content.

The zinc oxide content of the acids obtained in the case mentioned above remains constant at about 20% (approximately that required for a 6-carbon saccharinic acid). However, in some cases the cellobiosidodihydroxyacetone, for example, these salts showed a regular rise from about 10% zinc oxide content at the lower alkalinities to that required for the C_6 acids. Undoubtedly the lower zinc oxide indicates the formation of glycosidic saccharinic acids, the lactic acid yields always being low in these cases. The method used here for their isolation was not such as to permit their identification, a feature of the work that should be investigated further. It was not possible to account for 100% of the sugar because of its removal by decolorizing agents of tars which probably consist of polymeric acetol, formaldehyde, and condensed forms of other carbonyl compounds. The formation of acetic and formic acids in the alkali reactions is well known.

Summary

1. Following the interpretation of Benoy,[2] Hockett and Evans[4] for the alkaline degradation of reducing disaccharides, a mechanism has been proposed for the alkaline hydrolysis of the theoretically possible intermediates that may form. These intermediates are assumed to form glucosidic enediols that in turn are hydrolyzed to glucose and an enediol which in the case of trioseneediol is converted to lactic acid, and in that of tetrosenediol is converted to the corresponding saccharinic acids. The glucose released in the hydrolysis is also a source of lactic acid.

2. A comparative study of the lactic acid yields obtained from gentiobiose and glucosidodihydroxyacetone justifies the assumption that the corresponding glucosidoaldotriose, 3-glucosidoglyceric aldehyde, is a fragmentation product of gentiobiose in alkaline solution.

3. A comparative study of the lactic acid yields obtained from alkaline solutions of glucosidodihydroxyacetone and cellobiosidodihydroxyacetone confirms[2,4] the view that the hexosido section of the 4-hexosidohexoses is the source of the lactic acid and not the hexose section.

4. A comparative study of the yields of lactic acid obtained from gentiobiosidodihydroxyacetone and gentiobiose, and also those from cellobiosidodihydroxyacetone and cellobiose confirms the general view expressed in 3.

5. The yields of lactic acid obtained from mixtures of the possible hydrolytic products of the oligosaccharides investigated are greater than those from the parent carbohydrate in each case except in those instances involving the release of dihydroxyacetone as the first step in these alkaline hydrolyses. This may be ascribed either to a slow degradation of the oligosaccharides, or to the concurrent rearrangements taking place with the resulting formation of saccharinic acids, or both.

6. A study of alkaline solutions of varying normalities which contained a mixture of cellobiose octaacetate and dihydroxyacetone monoacetate shows that there is a definite relation between the yields of lactic acid obtained and those of the saccharinic acids.

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