NOTE.

Natural Indicators.—Since the publication of the article on "Some Natural Indicators" in the September number of THIS JOURNAL, Mr. G. A. Fraps has called my attention to an article entitled "The Wide Occurrence of Indicators in Nature," by himself, published in the *American Chemical Journal* for September, 1900, in which he has recorded some similar observations. H. W. BRUBAKER.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY.]

THE STRUCTURE OF MALTOSE AND ITS OXIDATION PRODUCTS WITH ALKALINE PEROXIDE OF HYDROGEN.

BY W. LEE LEWIS AND SIEGEL A. BUCKBOROUGH.

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Nef[1] and his students have established the methods for oxidizing the sugars with various agents and for separating and identifying the resulting products. Nef[2] has recently submitted a complete system of dissociation of the sugar molecule in explanation of these oxidations and in explanation of the reciprocal conversion of certain sugars under the influence of dilute alkalies.

One[3] of us investigated the products formed when maltose is oxidized with alkaline cupric sulfate. This work brought out that maltose is oxidized largely as an unhydrolyzed disaccharose, forming glucosido-acids, whose subsequent hydrolysis gives dextrose and simpler acids. There were thus obtained from 100 g. of anhydrous maltose, 34.72 g. of hydrolyzed dextrose, 29.78 g. of hexonic acids, 2.86 g. of glycollic, 0.25 g. of oxalic, 3.46 g. of formic acids, and 7.74 g. of carbon dioxide. Of unidentified material, believed to contain glycerinic and trioxybutyric acids, there remained 27.29 g., with 2.15 g. lost during the various manipulations. The ratio of the various products found was quite different from that observed by Nef[4] in a study of the oxidation products of the simple hexoses, dextrose, levulose, and mannose, especially in respect to the larger amount of mannonic lactone (21.00 g.) formed from the disaccharose. This investigation of maltose, however, failed to throw any light on the constitution of that sugar, largely because the amount of oxygen taken up by each molecule was insufficient.

The present study was therefore undertaken in the hope that the more complete destruction of the maltose molecule, under the influence of alkaline hydrogen peroxide, might permit a better quantitative separation of the products, thus reflecting the point of the glucosido union between

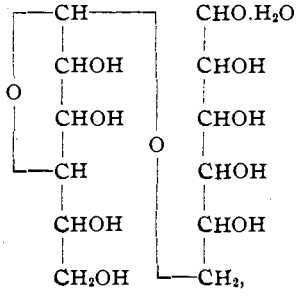
[1] *Ann.*, **357**, 214–312; **376**, 1–119; **403**, 204–383.

[2] *Ibid.*, **403**, 204–242.

[3] Lewis, *Am. Chem. J.*, **42**, 301–319.

[4] *Ann.*, **357**, 259.

the two constituent dextrose groups in malt sugar. The results confirmed the previous findings that maltose oxidizes largely without hydrolysis and that saccharinic acid formation does not take place under the conditions. A larger amount of oxygen is taken up with alkaline peroxide as evidenced in the larger yield of acids containing few carbon atoms. One hundred grams of anhydrous maltose gave, by this method, 22.97 g. of hydrolyzed dextrose (corr. 24.97 g.) 0.16 g. of mannonic lactone, 16.04 g. of glycollic, 0.11 g. of oxalic, 55.37 g. of formic acid and 4.44 g. of carbon dioxide. Of unidentified material there remained 1.18 g., believed to contain erythronic and *l*-threonic acids. One gram of material was used up in titrations and otherwise lost in manipulation. Especially noteworthy are the larger amounts of formic and glycollic acid found in comparison with the previous work using alkaline copper sulfate. Herein it is believed are to be found the proofs indicated by Nef[1] which establish the structure of maltose as originally assumed by Fischer,[2] as a γ-*d*-glucosido-*d*-glucose hydrate,



in which the primary alcohol hydroxyl functions in the glucosido union.

Fischer[3] established the structure of maltose[3] as a disaccharose composed of two molecules of *d*-glucose and containing a γ-lactone ring similar to his synthetic alkyl glucosides. According to Armstrong[4] maltose is an α-glucoside, as established by selective enzyme action. The resulting formula $C_6H_{10}O_5.\overset{5}{C}H_2OH(\overset{4,3,2,1,}{C}HOH)_4.CHO.H_2O$ does not, however, determine which of the carbon atoms 1, 2, 3, 4, and 5, holds the hydroxyl group taking part in the glucosido union. Carbon atoms 1, 2 and 3 may be at once eliminated as possibilities from the following consideration: *d*-Maltose with $1^1/_2$ molecules of calcium hydroxide at ordinary temperature gives a very large quantity of glucosido α and β-*d*-isosaccharinic acids,[5]

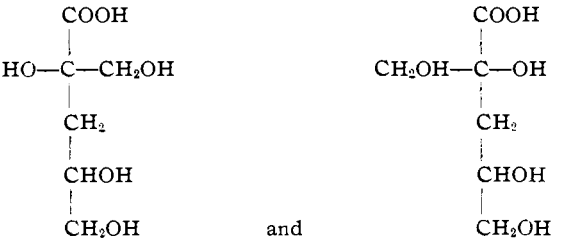
[1] *Ann.*, **403**, 299–303.

[2] *Ber.*, **27**, 2988.

[3] *Ibid.*, **35**, 3141; **28**, 1145; Nef, *Ann.*, **403**, 299.

[4] *Trans. Chem. Soc.*, **85**, 1305.

[5] Kiliana, *Ber.*, **18**, 631, 2514; **38**, 2668; Nef, *Ann.*, **357**, 306; **376**, 54–56.



This product can only come about through enolization of malt sugar with subsequent addition and splitting off of water forming intermediately

γ-*d*-glucosido-*d*-fructose, $C_6H_{10}O_5.CH_2OH(CHOH)_3—\overset{\overset{O}{\|}}{C}—CH_2OH$, after the analogy of the interconversion of simple hexoses under the influence of alkalies.[1] This product similarly goes over into γ-*d*-glucosido ortho-hexoson, $C_6H_{10}O_5.CH_2OHCHOHCH_2—\overset{\overset{O}{\|}}{C}—\overset{\overset{O}{\|}}{C}—CH_2OH$, which, by the benzilic acid rearrangement, can give the final product γ-*d*-glucosido α- and β-isosaccharinic acids. Hydrolysis of the latter yields *d*-glucose and α- and β-isosaccharin. It may be seen that these transformations involve the three hydroxyl groups attached to carbon atoms 1, 2 and 3, which therefore must be present as such in the original maltose molecule. The participation of any one of these in the glucosido union is therefore precluded.

The selection of the correct hydroxyl group, as between the two remaining, is fixed upon that attached to carbon atom 6 by the following considerations:

Nef[2] and Glattfeld[3] have shown that, when glucose is treated with alkali of a certain concentration, there results six sugars; *i. e.*, *d*-glucose, *d*-mannose, *d*-fructose, *d*-pseudofructose and α- and β-*d*-glutose. The intermediate 1,2-hexose dienols of this transformation undergo dissociation into hydroxy methylene and methyleneols of the pentoses. There result, finally, through further dissociation, various sugars containing one, two, three, four and five carbon atoms $(CH_2O)_x$, the oxidation of which, accompanied in some instances by the benzilic acid rearrangement, produces the ultimate products found in sugar oxidation.

It is altogether probable that maltose under the influence of alkalies enters into a similar equilibrium[4] of the six glucosido hexoses of the glucose series. The intermediate glucosido hexosedienols undergoing

[1] *Ber.*, **28**, 3078; *Rec. trav. chem. Pays-Bas.*, **19**, 1 (1900).

[2] *Ann.*, **403**, 362.

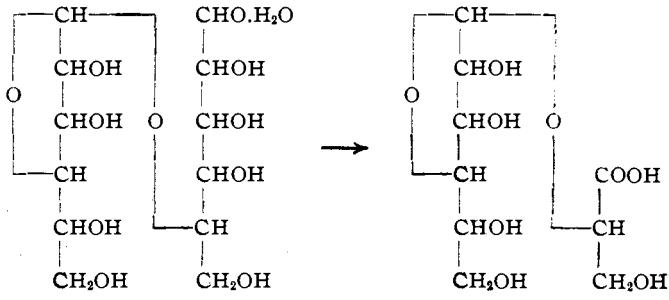
[3] *Am. Chem. J.*, **50**, 137.

[4] Nef, *Ann.*, **403**, 300, 381–382.

dissociation, oxidation, etc., would produce the glucosido acids whose hydrolysis would give the final products found in this study.

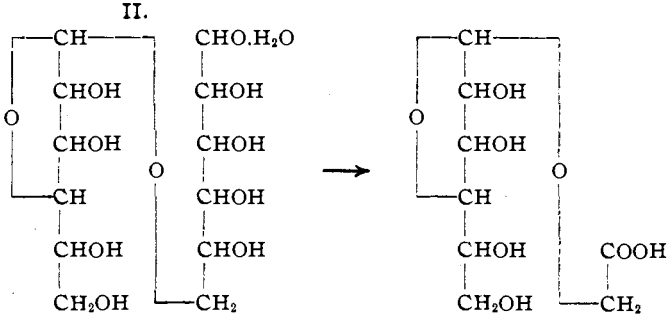
The step-by-step splitting off of oxymethylene with the formation of formic and carbonic acids, as the main course of the reaction, could not go beyond the carbon atom whose hydroxyl enters into the glucosido union, otherwise glucosido acids would not be the principal product of the oxidation. In the following equation it may be seen that if Formula I were correct for maltose the principal product of the oxidation would be glucosido glycerinic:

I.



If Formula II were correct, the principal product would be glucosido glycollic acid.

II.



One hundred grams of maltose with alkaline peroxide gave finally 16.04 g. of pure crystalline glycollic acid while glycerinic acid was not found present.

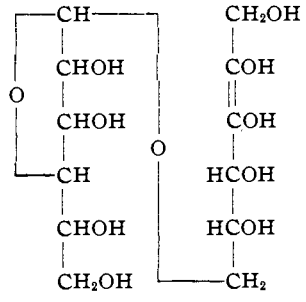
The ratio and nature of the oxidation products of *d*-glucose with alkaline hydrogen peroxide are quite different from those of maltose, especially in respect to the small amount of glycollic acid[1] (4.3 from 100 g.) and the presence of *d*-arabonic lactone[2] in the former. These differences must be due to the effect of the above glucosido bond.

Regarding the source of the other products found, the large quantities

[1] Spoehr, *Am. Chem. J.*, **43**, 238.

[2] Glattfeld, *Ibid.*, **50**, 135–157.

of carbonic and formic acids undoubtedly result from the oxidation of dissociated hydroxymethylene, $>CHOH$. Oxalic acid could result from a more complete oxidation of diose methyleneol, $HOCH_2COH<$.[1] Erythronic and *l*-threonic acid were indicated in the results but not established because of the small amounts. Their formation is most probable from the dissociation of glucosido 2,3-*d*-glucose dienol,



into the methylenols of diose, $HOCH_2C(OH)<$, and of glucosido-*d*-erythrose. The osone of the latter, formed by oxidation, could undergo the benzilic acid rearrangement (asymmetric in part or entire) to give C_4 acids.

Mannonic lactone, on the other hand, has been proved to arise from the hydrolysis of glucosido mannonic acid through the action of dilute alkalies on maltosone.[2] That the benzilic acid rearrangement often takes place asymmetrically has been pointed out by Nef[3] in explanation of the preponderating gluconic acid in oxidation of the simple hexoses and of mannonic acid when maltose is acted upon by Fehling's solution.[4] In the oxidation of maltose with alkaline peroxide there is formed intermediately therefore some maltosone.

Experimental Part.

A solution of 3.22 g. of maltose in 80 cc. of 3% hydrogen peroxide (6.5 molecules) was prepared and added with vigorous shaking through a period of ten minutes to a solution of 5.62 g. of 85.7% potassium hydroxide (equivalent to 4.82 g. of potassium hydroxide net, being 7.7 molecules) in 100 cc. of water. The total volume was then increased to 200 cc., making the concentration of the alkali approximately half normal. Under similar conditions 3.22 g. of maltose were dissolved in 160 cc. hydrogen peroxide and the mixture poured with vigorous shaking through a period of ten minutes into a solution of 5.62 g. of potassium hydroxide in 40 cc. water.

[1] Cf. Anderson, *Am. Chem. J.*, **42**, 406.

[2] Lewis, *Ibid.*, **42**, 315–319.

[3] *Ann.*, **357**, 231–2, 284.

[4] In unpublished notes one of us (Lewis) has obtained mannonic lactone in quantity from the oxidation of lactose with Fehling's solution.

Three solutions of each concentration were prepared and kept at room temperature, being protected from the carbon dioxide of the air by means of soda lime tubes.

In none of the above mixtures was any change in temperature or appearance of the solution noted. The final solutions were in all cases colorless.

By testing with Fehling's solution the oxidation was found in the first three trials (80 cc. hydrogen peroxide) to be complete after seven to ten days and in the last three (160 cc. hydrogen peroxide) after fourteen to seventeen days, as evidenced by the absence of reduction. While the solutions were standing, as well as at the conclusion of the oxidation, the continued presence of an excess of hydrogen peroxide was proved by frequent tests with starch potassium iodide paper. The excess of hydrogen peroxide was finally removed by the addition of a little platinum black and vigorous stirring.

(1) *Quantitative Determination of the Amounts of Carbon Dioxide Formed in the Oxidation.*—To determine the amount of carbon dioxide in each case, an apparatus was set up in which a wash bottle of concentrated potassium hydroxide was connected with a large U-tube filled with soda lime, and this in turn with the flask containing a sugar solution. To the other side of the flask was attached a reflux condenser in series with six towers containing a saturated solution of barium hydroxide. The calculated amount of hydrochloric acid was then added to the alkaline reaction mixture by means of a dropping funnel, and air free from carbon dioxide was slowly and continuously drawn through the apparatus. The flask was finally heated in an oil bath at 110° to 120° for one hour. The barium carbonate precipitate was then thoroughly washed, dried at 100° and weighed.

Blank experiments were also made to determine the amount of carbon dioxide in 5.62 g. of potassium hydroxide. Three determinations gave respectively, 0.2430 g., 0.2439 g. and 0.2435 g. of barium carbonate.

The three solutions of maltose with 80 cc. of hydrogen peroxide, after the correction was made for the potassium hydroxide, gave respectively, 0.8584 g., 0.8556 g. and 0.8540 g. of barium carbonate. Two of the solutions prepared with 160 cc. hydrogen peroxide, after the correction, gave 0.9851 g. and 0.9629 g. of barium carbonate.

TABLE I.—SUMMARY OF RESULTS FOR CARBON DIOXIDE.

Amount of maltose. Grams.	Amount of hydrogen peroxide. Cc.	Time for oxidation. Days.	Grams of carbon dioxide.	Per cent. of theoretical yield.	Per cent. of total.
3.22	80	7–10	0.137	2.91	4.52
3.22	80	7–10	0.134	2.84	4.41
3.22	80	7–10	0.136	2.85	4.47
3.22	160	14–17	0.220	4.67	7.21
3.22	160	14–17	0.215	4.56	7.05

(2) *Quantitative Determination of the Amounts of Volatile Acids.*—As before, three lots of 3.22 g. of maltose with 80 cc. hydrogen peroxide were set aside under like conditions, also three lots with 160 cc. hydrogen peroxide. The time periods for complete oxidation were the same as in the first series. After adding the platinum black to the solutions and heating the flasks to remove the excess of hydrogen peroxide, theoretical amounts of hydrogen chloride were added. Each solution was then separately distilled from a flask provided with a Kjeldahl bulb to prevent the volatilization of possible glycollic acid. A pressure of 10–25 mm. was maintained and the flask finally heated for some time in a boiling water bath. The residues were several times dissolved in 100 cc. of water and the distillation repeated. The distillate, which proved to be free from hydrogen chloride, was then made up to a definite volume and the formic acid determined by titrating an aliquot part with 0.1 *N* sodium hydroxide. The Jones[1] method was also used, in which the formic acid was oxidized to carbon dioxide with 0.1 *N* permanganate. The two methods agreed perfectly, thus proving formic the only volatile acid present.

TABLE II.—SUMMARY OF RESULTS FOR FORMIC ACID.

Amount of maltose. Grams.	Amount of hydrogen peroxide. Cc.	Time for oxidation. Days.	Formic acid by permanganate. Grams.	Formic acid by sodium hydroxide. Grams.	Per cent. of theoretical yield.	Per cent. of total weight.
3.22	80	7–10	1.377	.....	27.84	44.90
3.22	80	7–10	1.336	1.334	27.14	43.80
3.22	160	14–18	2.070	2.065	42.00	67.85
3.22	160	14–18	2.007	2.012	40.60	65.55
3.22	160	14–18	2.047	.....	41.40	66.85

(3) *The Nonvolatile Acids.*—In the determination of the nonvolatile acids left behind with the salt residue after the distillation of formic acid, it was decided to use larger quantities of the materials in the same proportion as in the preliminary experiments, in which 80 cc. hydrogen peroxide were used. Eight 25.76 g. lots of maltose (equivalent to 195.77 g. of anhydrous sugar) were thus set aside. In each case the strength of the hydrogen peroxide was again determined just before using and correction made so as to keep the concentration uniform. No change in the temperature or color of the solution was ever noticed on addition of the hydrogen peroxide solution to the sugar. There were only slight differences in the time required for complete oxidation, the average being eleven days. After no reduction was shown with Fehling's solution, the contents of each flask were heated for a half hour and shaken repeatedly with platinum black to remove the excess of hydrogen peroxide. Then 6% in excess of the theoretical amount of hydrochloric acid was added, and the solution distilled at a temperature of 45° to 50° under a pressure

[1] *Am. Chem. J.*, **17**, 539.

of 15–25 mm. The residue in the flask was dried at 80° for half an hour, redissolved in 150 cc. water and again distilled. This process of redistillation was continued until the nonvolatile acids were entirely free from hydrogen chloride.

The amount of formic acid in the filtrate from each lot was determined by the Jones method, the yields being as follows: 13.20 g., 13.48 g., 13.70 g., 14.19 g., 13.50 g., 13.45 g., 13.28 g., and 13.67 g., respectively. These results are nearly 25% higher than those obtained with small quantities of sugar.

The salty residues of acid gum from each lot of sugar were taken up in 95% alcohol, thus separating most of the potassium chloride. The 95% alcohol residues from each lot were then combined and refluxed with absolute alcohol, thus eliminating more of the salt. After filtering and concentrating somewhat, the filtrate was left at a low temperature for twenty-four hours. A little more of the salt separated out, together with a small amount of material which reduced Fehling's solution and which apparently was hydrolyzed sugar.

The final product, dried at 75° and 20 mm., weighed 105 g., which is 53.6% of the weight of the sugar used.

The gums, which were slightly darkened, were dissolved in five parts of 5% sulfuric acid and heated on the boiling water bath for ten hours under the reflux. Then the theoretical amount of barium hydroxide, necessary to remove the acid, was dissolved in 300 cc. of hot water and slowly added. After heating on the boiling water bath for another half hour the mixture was filtered. On concentrating the solution to two liters, the amount of split off sugar was determined by the Munson and Walker[1] method and also by the Fehling solution method. The results by the former in two determinations were 0.3808 g. and 0.3812 g. of cuprous oxide. This weight of cuprous oxide from 8 cc. of the solution corresponds to 178.4 mg. of dextrose, equivalent to 44.6 g. of this sugar in the 2000 cc. By the latter method 20 cc. of the sugar solution were diluted to 100 cc. and 11.20 cc. of this were required for 10 cc. of Fehling's solution, corresponding to a total of 45.0 g. of dextrose.

In order to determine to what extent dextrose was destroyed during the ten hours hydrolysis with five parts of 5% sulfuric acid, an independent experiment was conducted in which 100 g. of C. P. dextrose hydrate (90.90 anhydrous) was refluxed on the boiling water bath ten hours with 500 cc. of 5% sulfuric acid. The solution darkened and showed a final content of 80.12 g. of anhydrous dextrose (91.43 g. hydrated) or a loss of 8.56%.

The solution was now adjusted so that a few drops gave the slightest precipitate with a 2% solution of sulfuric acid, filtered and concentrated

[1] THIS JOURNAL, **28**, 663; **29**, 541.

to about 1500 cc. To remove the dextrose, the solution was heated in a boiling water bath with 60 g. of calcium carbonate for ten hours and filtered. The filtrate was of a golden red color and syrupy odor. Thirty-eight grams of calcium carbonate were filtered off and digested with 5% acetic acid. An insoluble residue was left which was dissolved in hydrochloric acid and reprecipitated with ammonia several times until perfectly white. This gave 0.3047 g. of calcium oxalate. When dried to a constant weight at 100° and analyzed the following result was obtained: 0.3047 g. of the salts gave on ignition 0.1168 g. CaO.

Calculated for $CaC_2O_4.H_2O$: CaO, 33.89; found, 38.33.

The aqueous solution of lime salts and dextrose was then concentrated in two hemispherical evaporating dishes on steam baths. Cold dilute alcohol was added with much stirring and decanted several times. The darkened lime salts thus obtained were taken up in water and decolorized with animal charcoal. On repetition of the above process the lime salts became granular in appearance and so free from sugar that 0.5 g. showed no reduction with Fehling's solution. The air-dried lime salts weighed 60.7 g. and on ignition 0.3978 g. of calcium salts gave 0.0718 g. or 18.05% calcium oxide.

The calcium was split off by treating the lime salts in a hot dilute solution with a slight excess of oxalic acid. After filtering, the aqueous solution was distilled under reduced pressure, as usual, and the residue dried. The thin syrupy acids which weighed 40.5 g. dissolved, with the exception of 0.3 g., in 500 cc. hot absolute alcohol. This solution was concentrated several times and set aside in the ice box, but no crystals formed. Finally it was concentrated to a weight of 80 g., representing 40 g. of gums and 40 g. of alcohol. There were now added slowly 250 cc. of absolute ether with much shaking and the whole mixture placed in the ice box over night. On decanting the ether solution and distilling, 30.9 g. mobile light brown residue, Fraction A, was obtained. The portion insoluble in absolute ether was darker and thicker and weighed 9.1 g. To the latter was added acetic ether in repeated portions of 300 cc. each, and the mixture refluxed till no more of the gum went into solution. The acetic ether solution was concentrated and set away but no crystals formed. Finally the ether was removed by distillation and 8 g. of gum, Fraction B, obtained. The remaining gum, Fraction C, soluble in absolute alcohol weighed 0.9716 g.

Fraction A.—On standing for some time after careful drying, the entire ether residue weighing 30.9 g., solidified to a homogeneous mass of leafy crystals characteristic of glycollic acid. Its identity with this acid was established in five different ways.

The melting point of the crystals was found to be 80°.[1]

[1] Nef, *Ann.*, **357**, 223.

A portion was carefully dried in a vacuum desiccator, weighed and dissolved in water. A part of this solution, equivalent to 0.2678 g. of crystals, on titration required 34.72 cc. of 0.1 *N* sodium hydroxide, or 64.8 cc. for 0.5 g. The theoretical amount required for 0.5 g. of glycollic acid is 65.8 cc.

To 4.1 g. of A was added 4 cc. 50% alcohol and 7.09 g. of phenyl hydrazine. After standing three or four days at room temperature the mixture suddenly became a mass of fine crystals. These were filtered off and recrystallized twice from 30% alcohol. Three and one-tenth grams of shiny hexagonal plates with a melting point of 100°[1] were obtained.

Four and six-tenths grams of the crystals from the residue A were digested eight hours on a boiling water bath with 5 g. of quicklime. An excess of 5 g. of calcium hydroxide was filtered off. On concentration of the filtrate, 3.4 g. of crystals of calcium glycollate were obtained and recrystallized. 1.0100 g. of air-dried salt lost on drying to constant weight at 100° to 120° 0.2838 g. of water.

Calculated for $Ca(C_2H_3O_3).4H_2O$: H_2O, 27.48; found, 28.09.

The remaining 0.7266 g. of anhydrous calcium salt gave on heating 0.2126 g. of CaO.

Calculated for $Ca(C_2H_3O_3)$: CaO, 29.47; found, 29.27.

Four and five-tenths grams of the residue A on being treated with an excess of strychnine in the usual manner gave on crystallization 8.0 g. strychnine glycollate melting at 185° to 190°.[2]

On heating the strychnine glycollate with an excess of quicklime for ten hours there was obtained 3.4 g. of calcium salt. 1.2125 g. of air-dried salt lost on being dried in the air bath to constant weight as above 0.3420 g. of water.

Calculated for $Ca(C_2H_3O_3)4.H_2O$: H_2O, 27.48; found, 28.20.

The remaining salt, 0.8705 g., gave on further heating 0.2550 g. of CaO.

Calculated for $Ca(C_2H_3O_3)$: CaO, 29.47; found, 29.29.

Fraction B.—This residue of 8.0 g. was diluted with water to 250 cc. Ten cc. of this solution containing 0.32 g. of the original gum was diluted to 100 cc. and treated with 49.8 cc. 0.1 *N* sodium hydroxide. This was heated for ten minutes on the boiling water bath and the excess of sodium hydroxide titrated with 0.1 *N* hydrochloric acid. A total of 23.19 cc. 0.1 *N* sodium hydroxide was thus used to neutralize 0.32 g. of the acid. On the basis of this titration, the calculated amount of brucine, 25.5 g., was added to the acid solution, together with a small amount of alcohol.

[1] *Ann.*, **357**, 233.

[2] Nef, *Ibid.*, **357**, 238.

This was digested on the boiling water bath one hour after the complete solution of the brucine. On concentrating the solution under reduced pressure a white precipitate of 5.1 g. formed which was filtered off and proved to be brucine.[1] The water was removed by distillation and the residue taken up in half its weight of water and five times its weight of absolute alcohol, after which seven crops of crystals, totalling 20 g., were obtained as follows: 5 g., 1.9 g., 1.5 g. and 1 g. of transparent plates, melting respectively at 198–202°, 195–202°, 202–205° and 190°; 8 g., 1.6 g. and 1 g. of small cubes all melting at 175°.

Fraction C.—This alcohol-soluble residue of 0.9716 g. was diluted with water to 100 cc. and titrated as above. Ten cc. of the solution required 7.25 cc. of 0.1 *N* sodium hydroxide. To the remaining solution was added the calculated amount of brucine, 2.85 g. On taking up the salts in alcohol 0.2 g. melting at 184° and 0.2 g. melting at 185° crystallized out. After concentration a residue of 1.5 g. was left which, combined with a corresponding residue from Gum B, made 9.5 g. In order to convert this combined residue into free brucine and acid, sodium hydroxide was added on the basis of one and one-half molecules of sodium hydroxide to one molecule of the brucine salt of an assumed four-carbon atom acid. Eight grams of brucine were filtered off. To neutralize the sodium hydroxide a slight excess of hydrochloric acid was added and the solution distilled to dryness. The salty residue was dissolved in water and redistilled to remove all traces of hydrogen chloride. The acid was then taken up in absolute alcohol and, after the removal of the alcohol, 2.052 g. of gum were obtained. This residue was titrated as before in a 2% solution and 0.5 g. was found to require 38.49 cc. 0.1 *N* sodium hydroxide.

The remaining portion of the solution was digested eight hours on the boiling water bath with 3.3 g. of quicklime. The filtrate on standing gave 0.7869 g. of crystals. On heating these to a constant weight 0.7245 g. was obtained. After ignition 0.1102 g., or 10.85%, of calcium oxide was left.

The high melting salts, including the first four crops from B and the two small crops from C, were combined, making 9.8 g. in all. Likewise the remaining low melting salts from B were combined, making 10.6 g. On treatment with sodium hydroxide in the usual way the high melting salts gave 2.4 g. of acid gum and the low melting salts 3.2 g. On standing, after having been freed from absolute alcohol by distillation, the low melting salts gave 0.52 g. of leafy crystals resembling in appearance glycollic acid and melting at 79°. This was boiled with strychnine, and after filtering off the excess gave, on concentration, 0.2 g. of strychnine glycollate crystals melting at 185°. The remainder of the solution of this residue was added to a similar residue from the high melting salts.

[1] Anderson, *Am. Chem. J.*, 42, 410 (foot-note) (1909).

The 2.4 g. of gum from the low melting salts, on being taken up in alcohol and allowed to stand for some time, gave 0.32 g. of the characteristic crystals of mannonic lactone melting at 150°. Some of this was mixed with pure mannonic lactone with no change in melting point.

After various futile attempts at identification, the remaining portion from the low melting salts was combined with that from the high melting salts, giving 2.32 g. An optical determination of the latter gave: d = 1.054; p = 2; *i. e.*, 0.5223 g. substance and 25.5927 g. water; $[\alpha]$ in a 1 dcm. tube equals —0.70°; whence $[\alpha]_D^{20}$ = —33.2°.[1] Of the residual gum 0.5 g. was found to require 37.55 cc. 0.1 N sodium hydroxide. With quicklime the final portion, gave 0.8364 g. air-dried, calcium salt. This, on drying to constant weight, lost 0.0886 g. or 10.5% water. On ignition 0.1271 g., or 17% of CaO was obtained.

Summary.

1. Saccharinic acid formation does not take place at room temperature when maltose is treated with an alkaline solution of hydrogen peroxide with an alkalinity of 0.43 N.

2. The ratio and nature of the oxidation products from maltose with alkaline peroxide are quite different from that of glucose with the same reagent, a fact which must be attributed to the effect of the glucosido bond.

3. Approximately half of the maltose in the reaction mixture used, oxidizes as such. The remainder is apparently hydrolyzed before oxidation.

4. The formation of glucosido acids in the oxidation of maltose explains why a molecule of dextrose requires 2.48 atoms of oxygen by Fehling's solution while the larger maltose molecule requires but 2.86 atoms with the same reagent.

5. The formation of α- and β-d-isosaccharinic acids from maltose under the influence of mild alkalies involves free hydroxyl groups on the first, second and third carbon atoms from the free aldehyde group. These carbon atoms are therefore eliminated as having taken part in the glucosido bond.

6. The formation of relatively large amounts of γ-d-glucosidoglycollic acid in the oxidation of maltose rather than γ-d-glucosidoglycerinic acid indicates that the terminal or primary alcohol carbon atom functions in the glucosido union of the two d-glucose molecules which go to make up maltose.

7. The formula of maltose is that of a γ-d-glucosido-d-glucose with the glucosido union on the primary alcohol carbon.

8. It is probable that maltose under the influence of alkalies enters into an equilibrium of the six glucosido-hexoses of the glucose series, the

[1] Glattfeld, *Am. Chem. J.*, **50**, 150.

dissociation and oxidation of whose intermediate hexose-dienols result in the various oxidation products found.

This work was originally undertaken under the mistaken impression that it was a part of certain problems on sugar oxidations granted by Dr. Nef to one of us (Lewis) in 1909. The investigation was well under way before the error, fully acknowledged here, was discovered. The results are now published, however, with the full consent of Dr. Nef, grateful recognition of whose generosity in the matter is herewith freely accorded.

The oxidation products of Fehling's solution on maltose and on lactose are now being studied in this laboratory and, with their completion, the authors will discontinue, as requested by Dr. Nef, all research on the oxidation products of the sugars with inorganic reagents.

BRAIN CEPHALIN: I. DISTRIBUTION OF THE NITROGENEOUS HYDROLYSIS PRODUCTS OF CEPHALIN.

By C. G. MacArthur.

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The constitution of cephalin is uncertain. Though the nature of the glycerophosphoric acid produced on hydrolysis is fairly well established,[1] the nitrogenous substances[2] and the fatty acids[3] present are not definitely known either as to identity or quantity. This series of investigations was started three years ago in an attempt to clear up these two uncertainties in the cephalin molecule.

This paper considers the preparation of cephalin, the methods used in determining quantitatively its various nitrogenous products and the data obtained by these methods.

Preparation and Purification.—Fresh sheep brains were cleaned carefully, ground in a meat grinder with a small amount of thymol, spread in very thin layers on glass plates, and placed in an air drier. By frequent turning the tissue dried in a day. The dry material was scraped off and placed in a vacuum desiccator. In some cases, instead of the above air-drying method, the dehydration was accomplished by adding to the tissue twice its weight of alcohol or acetone and filtering after a day's standing.

After complete desiccation the cholesterol was extracted by continuously shaking the tissue with twice its weight of acetone. Two such treatments of about four hours each removed practically all the cholesterol.

[1] Dimitz, *Biochem. Z.*, **21**, 337.

[2] Thudicum, "Die chemische Konstitution des Gehirns," p. 142; Koch, *Z. physiol. Chem.*, **36**, 134; Neubauer and Frankel, *Biochem. Z.*, **21**, 321.

[3] Cousin, *J. pharm. chim.*, **24**, 101 and **25**, 177; Dimitz and Frankel, *Biochem. Z.*, **21**, 337; Parnas, *Biochem. Z.*, **22**, 411.