THE CONDENSATION OF FORMALDEHYDE

AND

THE REACTION OF FORMALDEHYDE WITH ASCORBIC ACID

by

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THE CONDENSATION OF FORMALDEHYDE AND THE CATALYSIS OF THE REACTION

Introduction

The subject of the production of sugars and sugar-like substances from formaldehyde has long been of interest to the chemical world, not alone as a chemical problem, but also because of its bearing on the photosynthetic production of carbohydrates by plants. Whether or not formaldehyde is an actual intermediate in this process is still a debatable question, but as a matter of fact most of the theories of the mechanism of photosynthesis, with the notable exceptions of those of Liebig (1), Ballo (2), and Tscholinzoff (3), assume formaldehyde to be the final stage of the reduction of carbon dioxide by the plant.

It is the purpose of this dissertation to outline briefly the 80 years of investigation which has led up to our present knowledge and concepts regarding the condensation of formaldehyde to sugar-like products and the recent studies of catalysis of the reaction. Experimental work will be presented which is concerned with two phases of this problem; (1) the catalysis of the condensation by various substances and, (2) the reaction between formaldehyde and ascorbic acid. The results obtained in this work will be discussed in the light of their possible relation to photosynthetic processes, but it must be borne in mind that reactions which may be brought about in vitro represent at best only possible analogies to the reactions taking place in life processes.

Before the discovery of formaldehyde, the Russian chemist Butlerow (4) had obtained, by the action of methyl iodide on silver oxalate, a white solid substance which he called dioxymethylene and to which he ascribed the formula $C_2H_4O_2$. By treating this substance with alkalies he obtained a sugar-like substance which he called methylenoitan, with the formula $C_6H_{12}O_6$. He considered this product a sugar, "the first example of the synthetic production of a substance having the properties of a sugar by means of the simples compounds of organic chemistry".

In 1869, some ten years later, Hofmann discovered formaldehyde and showed that Butlerow's dioxymethylene was a polymerized form of his formaldeigde, which from vapor density determinations had the formula CH_2O.

A few years later Baeyer (5) applied the work of Butlerow and Hofmann to a discussion of the mechanism of photosynthetic production of sugar from formaldehyde. His theoretical deductions on the subject of photosynthesis have been widely quoted and have, in fact, been taken as the starting point of a great deal of the later work on photosynthesis. One of the important assumptions of Baeyer's theory was that formaldehyde is condensed to sugars under conditions existing in the plant. Numerous later workers have directed their attention to the study of this condensation under various experimental conditions.

Many modifications of the original Butlerow condensation have been devised, but in all of these the principle is much the same, the modifications consisting mainly in the use of weaker alkalies and lower temperatures than those used by Butlerow. Loew (6) introduced the use of calcium hydroxide at temperatures of from 15 to 20° C. and called the product obtained formose. Lobry de Bruyn and van Ekenstein (7) found lead hydroxide to be an excellent condensing agent. Many other substances bring about the condensation: the hydroxides of sodium, potassium, magnesium and barium, finely divided zinc and lead, basic lead salts and sodium acetate. With weakly alkaline solutions, higher temperatures favor the condensation, although above about 70° the alkalies bring about decomposition of the products.

Formose prepared by any of these methods is a complex mixture of substances, the nature of which has for the most part not been definitely established. Fischer and Passmore (8) discovered that formose contains d-acrose, a synthetic sugar mixture obtained by the condensation of the trioses, glyceric aldehyde and dioxyacetone. This discovery is of considerable significance in relation to the origin of sugars in nature, since Fischer and Tafel (9) have shown that α-acrose contains some of the naturally occurring sugars, i. e. the inactive forms of glucose and fructose. Nef's studies on the products obtained by the action of alkalies on sugars (10) led him to the conclusion that formose consists of a mixture of half pentoses and half hexoses. Of the hexoses, Nef considered 24 members to be present in the formose mixture, viz. 8 aldohexoses, 8 2-ketohexoses, and 8 3-ketohexoses, the aldoses constituting about one-half of the mixture and being primarily dl-glucose and dl-galactose. Nef's statements, however, require further substantiation. Indeed, there is a great field of sugar chemistry yet to be worked out in the study of the formose sugars.

The Mechanism of Condensation

The mechanism by which formaldehyde condenses to hexose and pentose sugars has not been definitely determined. There are at least three suggested possibilities, and theories have been built around each. These proposed theories include:

1. The formation of glycolaldehyde as an intermediate product, and the condensation of this to tetroses

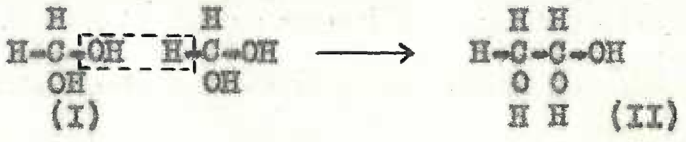
and hexoses. H. and A. Euler (11) have reported the formation of glycolaldehyde from formaldehyde by the action of calcium carbonate, although Nef (10) maintains that in the experiments of the former authors the calcium carbonate must have contained some calcium hydroxide, for Nef could not obtain any sugar formation with either calcium carbonate or barium carbonate, even after heating the mixture for days. Pribram and Franke (12) obtained glycolaldehyde by illuminating solutions of formaldehyde in quartz vessels with ultraviolet light. It is quite generally established that glycolaldehyde readily condenses to tetroses and hexoses under the influence of alkalies. Neuberg (13) claims that pentoses are also formed in this reaction, which implies a splitting of some sort. This does not seem at all unlikely in the light of Nef's studies of the action of alkalies on sugars (10).

2. The intermediate formation of the trioses, glyceric aldehyde and dioxyacetone, and the condensation of these to hexose sugars. As mentioned above, α-acrose is obtained by the condensation of the trioses, and because of the fact that he found α-acrose in the formose mixture (8), Fischer (14) considered it probable that the trioses are intermediate products in the condensation of formaldehyde. H. and A. Euler (11) have reported finding dioxyacetone in a mixture obtained by treating formaldehyde with calcium carbonate.

3. The successive formation of diose, triose, tetrose, pentose and hexose sugars, by the stepwise addition of one molecule of formaldehyde at a time. This is essentially the mechanism by which Baeyer (5) conceived the formation of hexose sugars from formaldehyde. Although no direct evidence has been brought to show that this stepwise condensation occurs, it is plausible, and readily accounts for the presence of odd-carbon molecules in the resulting mixture, even under conditions such that the splitting of larger molecules might be doubtful.

Considering the mechanism more in detail, that is in regard to the manner in which one molecule of formaldehyde is combined with another, regardless of the products formed, whether they be diose, triose or higher sugars, we are still at a loss for definite evidence. Several possibilities have been put forward, including:

1. The addition of water to the formaldehyde molecule to form the compound $CH_2(OH)_2$. Baeyer (5) based his theory in part on the assumption of such a mechanism, pointing out the instability of the double hydroxyl grouping. Two or more such molecules then combine by eliminating water between them, thus:



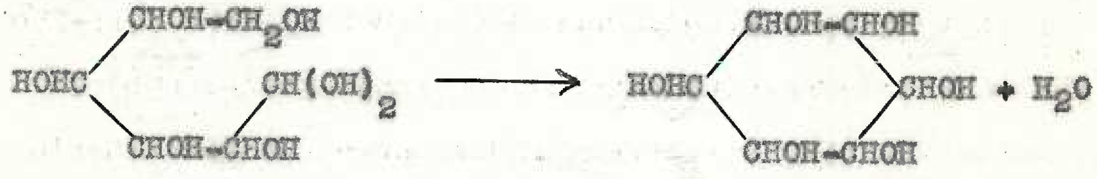
$$\mathrm{H{-}\overset{H}{\underset{OH}{C}}{-}\boxed{OH\ \ H}{-}\overset{H}{\underset{OH}{C}}{-}OH} \longrightarrow \mathrm{H{-}\overset{H}{\underset{\overset{O}{H}}{C}}{-}\overset{H}{\underset{\overset{O}{H}}{C}}{-}OH}$$

(I) (II)

This process is repeated until six carbon atoms are linked together (III), when the elimination of another molecule of water may result in the formation either of an aldohexose (IV), or of a cyclic hexahydric alcohol (V):

$$\underset{\text{(III)}}{CH_2OH\text{-}(CHOH)_4\text{-}CH(OH)_2} \longrightarrow \underset{\text{(IV)}}{CH_2OH\text{-}(CHOH)_4\text{-}CHO} + H_2O$$

or



(III) (V)

It is readily seen that elimination of water from (II) could give rise to glycolaldehyde (VII):

$$\underset{\text{(II)}}{CH_2OH\text{-}CH(OH)_2} \longrightarrow \underset{\text{(VII)}}{CH_2OH\text{-}CHO} + H_2O$$

Similarly, glyceric aldehyde (IX) and dioxyacetone (X) might arise from the compound $CH_2OH\text{-}CHOH\text{-}CH(OH)_2$, thus:
(VIII)

$$CH_2OH\text{-}CHOH\text{-}CH\begin{matrix}OH\\OH\end{matrix} \longrightarrow CH_2OH\text{-}CHOH\text{-}CHO + H_2O$$

(VIII) (IX)

$$CH_2OH\text{-}CHOH\text{-}CH\begin{matrix}OH\\OH\end{matrix} \longrightarrow CH_2OH\text{-}CO\text{-}CH_2OH + H_2O$$

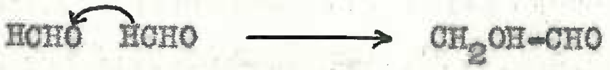
(VIII) (X)

2. The reaction of formaldehyde in an "active" or "aldehyde-enol" form. Nef (10) was an early proponent of this theory, and believed that in alkaline solution aldehydes exist in the enolic form:  $\overset{R}{\underset{HO}{C}}=$. Two such active molecules might add together directly and, by subsequent tautomeric shift, form glycolaldehyde (VII):

$$HOHC= \;= CHOH \longrightarrow \underset{(VII)}{CH_2OH{-}CHO}$$

The enolic form of (VII),  $CH_2OH{-}\overset{OH}{C}=$, Could then add another molecule of formaldehyde, or another like itself, forming a triose or tetrose as the case might be.

3. A third possibility, though it seems less probable in alkaline solutions, is the shifting of a hydrogen atom from one molecule of formaldehyde to the oxygen atom of another, with consequent linking of the two carbons:



$$HCHO\ \ HCHO \longrightarrow \underset{(VII)}{CH_2OH{-}CHO}$$

This is the mechanism proposed originally for the so-called "aldol" type of condensation.

Whether the reaction takes place essentially according to one of these systems, or in some other manner, is an unsettled problem. At least we seem safe in referring to the reaction as a condensation, inasmuch as it appears to result in the linking of carbon to carbon.

The great amount of chemical work which has been done on the condensation of formaldehyde in the presence of various alkalies offers little evidence of validity for the theories of photosynthetic production of sugars, beyond the fact of demonstrating that it is chemically possible to obtain sugars which occur in the plant. To sum up the work outlined above, in Spoehr's words (15): "Baeyer's theory is a good suggestion of a possible mechanism of photosynthesis; the chemical evidence which has been advanced in support of it may serve as a good clue for further investigations with very much more refined methods". Some of the more recent additions to this volume of chemical evidence will be brought out in subsequent parts of this dissertation.

THE CATALYSIS OF THE CONDENSATION OF FORMALDEHYDE

Schmalfuss (16) investigated the synthesis of sugars from formaldehyde in the presence of magnesium oxide at 120°, and observed that the reaction was markedly accelerated by the addition of glucose or fructose. He suggested that possibly simple sugars play a similar catalytic role in the photosynthetic production of sugars by the plant. More recently, Kusin (17,18) has made a further study of the activity of these and other compounds in catalyzing the condensation in the presence of calcium hydroxide and lead hydroxide at more moderate temperatures. Of the series of compounds which he investigated, those capable of forming an enediol group in alkaline solution possessed catalytic properties, and Kusin was led to conclude that the enediol structure was itself the active catalytic agent.

The importance of this work in relation to proposed theories of photosynthesis led the writer to undertake a more extended study of the condensation of formaldehyde in the presence of calcium hydroxide, using improved methods of analysis. The work was begun with the intention of determining the effect of ascorbic acid (vitamine C) on the condensation, its use being suggested by the fact that, according to the formula proposed by Hirst and coworkers (19,20), this compound contains the enediol structure and should, if Kusin's theory be correct, catalyze the reaction. The widespread occurrence of ascorbic acid in green plants lends significance to the discovery that this compound is a very active catalyst, and led the writer to a further study of the compound in relation to a possible photosynthetic function.

When the study was extended to a number of other compounds, results were obtained which are not entirely in accord with the theory of Kusin. Some of the experimental data presented here show that other types of structure appear to be as active in catalyzing the condensation as is the enediol group, indicating that Kusin's theory may not be entirely correct in this regard.

Experimental

When experiments were begun on the catalysis of the formaldehyde condensation, it was found that the methods used by Kusin were more or less unsatisfactory. His data were obtained by formaldehyde determinations by the ammonia method. Inpreliminary experiments, the writer found that accurate results could not be obtained by this method. Vorländer (21) and others (22,23) have described the use of dimethyldihydroresorcinol (referred to hereinafter as dimedon) for the quantitative precipitation of aldehydes. A satisfactory procedure for the present experimental work, based on the use of this reagent, was developed.

Kusin showed only qualitatively the presence of reducing compounds in the products of the reaction. The accurate estimation of the reducing substances formed in the reaction required a method of removing the unchanged formaldehyde from the mixture, and correction for the reducing power of the catalyst as determined in control experiments. The formaldehyde was removed by treating with dimedon, the excess of the latter being effectively removed by means of a mercuric sulphate-ferric sulphate reagent. In the case of ascorbic acid and reductone, these catalysts were also destroyed by this treatment.

In the experiments in which he used calcium hydroxide, Kusin carried out the reaction in a solution of 4 per cent formaldehyde in 40 per cent methyl alcohol, in order to minimize the loss of formaldehyde by the Cannizaro reaction. The catalysis by ascorbic acid was studied in both aqueous and 40 per cent methyl alcoholic solution. The results showed a considerable delay in the catalytic activity in the presence of the methyl alcohol, for which reason the subsequent experiments were carried out in aqueous solution. Although there was considerable loss of formaldehyde, due to intermolecular oxidation and reduction, the course of the condensation could be followed very well by means of determinations of reducing substances formed.

Controls in which water was used in place of the formaldehyde solution were run on each substance used as a catalyst, in order to correct for the reducing power of the catalyst itself, thus making possible the calculation of net reducing substances actually formed in the reaction.

The catalytic activity of approximately equimolecular quantities of the various compounds was determined. 1 gram of glucose or fructose and equivalent amounts of the other catalysts in most cases were used. Data are given for catalyses by the following compounds:

Glucose. Bureau of Standards dextrose.

Fructose. Pfahnstiehl purified fructose.

Kojic acid. Supplied by Dr. Smits of Kansas State Agricultural College.

Ascorbic acid. Merck and Co., Cebione.

Glucosamine. Recrystallied glucosamine hydrochloride.

Reductone. Prepared according to Euler and Martius (24).

Syrup obtained from formaldehyde and ascorbic acid in the presence of calcium carbonate as described below.

Calcium Lactate. Prepared from lactic acid and calcium carbonate and recrystallized.

Calcium propionate and tetramethylglucose had no catalytic activity. The series of catalysis experiments is at present being extended to include a number of other compounds, in an effort to arrive at more definite conclusions as to the structural basis of the catalytic activity.

The condensations were carried out in a 100 ml. Kjeldahl flask fitted with a mechanical stirrer and having a side tube in such a position that samples could be pipetted directly from the reaction mixture. The flask was immersed in a constant temperature water bath at 40° C. ($\pm 0.2^{\circ}$). The catalyst (when used) was dissolved in 100 ml. of a 4 per cent solution of formaldehyde, either aqueous or 40 per cent methyl alcoholic. 2 grams of high grade calcium hydroxide were added through the side tube. Vigorous stirring was continued throughout the experiment, and 2 ml. samples withdrawn for sugar and formaldehyde analyses at appropriate intervals. In the experiments in which ascorbic acid was used, the reaction was carried out in an atmosphere of nitrogen. Later results indicated that this precaution was probably not necessary, since the formaldehyde effectively prevents the oxidation of the ascorbic acid by air. Control experiments were run on formaldehyde with calcium hydroxide, in the absence of any catalyst, in both aqueous and methyl alcoholic solution.

Determination of formaldehyde. 2 ml. of the reaction mixture were carefully pipetted into a 250 ml. Erlenmeyer flask and made just acid to phenol red indicator with 0.5 N HCl. 200 ml. or less (depending on the disappearance of the formaldehyde from the reaction mixture) of a saturated aqueous solution of dimedon were added, and the mixture heated in a boiling water bath for ten minutes. A white flocculent precipitate formed when formaldehyde was present. After cooling to room temperature and allowing the mixture to stand overnight, the precipitate was collected by suction in a weighed Gooch crucible, rinsed with water, dried in the oven at 100-110°, and weighed. The weight of the precipitate multiplied by the factor 0.10274 gives the weight of formaldehyde in the 2 ml. sample. The concentration of the stock 4 per cent solution was determined similarly.

Determination of reducing substances. 2 ml. of the reaction mixture were pipetted carefully into a large test tube, made just acid to phenol red with 0.5 N HCl, and diluted to about 20 ml. 1 gram (or less when the formaldehyde was diminished) of solid dimedon was added, and the tube and contents heated in a boiling water bath for ten minutes. After cooling thoroughly, the contents of the tube were filtered with suction through a fritted glass filter into a side-arm test tube, graduated at 50 ml. The precipitate was rinsed thoroughly with small portions of water, care being taken to keep the total volume of the filtrate below about 45 ml. The filtrate was treated with 3 ml. of the mercuric sulphate-ferric sulphate reagent (containing 9 per cent $HgSO_4$ and 30 per cent $Fe_2(SO_4)_3$ in 0.25 N H_2SO_4) and allowed to stand ten minutes. The mixture was diluted to the 50 ml. mark, transferred without rinsing to an Erlenmeyer flask, and neutralized to litmus with barium carbonate. The solution was filtered by gravity, acidified to Congo red with a drop or two of concentrated sulphuric acid, and treated with hydrogen sulphide to remove the mercury. The excess of hydrogen sulphide was removed by means of a current of air. After pipetting 5 ml. samples into sugar tubes, they were neutralized to phenol red with a few drops of 0.5 N NaOH. Sugar determinations were run by the Shaffer-Hartmann method, using the Shaffer-Somogyi reagent (25) with a carbonate-bicarbonate ratio of 3.

Samples taken from the reaction mixture after the disappearance of all formaldehyde (as indicated by the lack of any precipitate with dimedon in the formaldehyde analyses) were acidified to phenol red, diluted to about 40 ml. and treated with the iron-mercury reagent. The subsequent treatment was the same as given in the procedure above.

Results and Discussion

The data obtained in the series of experiments, all carried out at 40°C., are tabulated below for each catalyst used, and for control experiments. A discussion of the results accompanies each set of related data. The values for reducing substances determined are in each case total amounts of reducing substance found.

1. Control.

2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

Time-minutes	0	120	180	210	240	300
Reducing subst. mgs. per 100 ml.	0	0	178	232	149	89
Formaldehyde per cent	4.04	2.42	0.42	0	-	-

1a. Control

2 gms. of $Ca(OH)_2$ in 100 ml. 4 per cent formaldehyde in 40 per cent methyl alcohol.

Time-minutes	0	120	180	210	225	255	315
Reducing subst. mgs. per 100 ml.	0	0	244	298	238	256	190
Formaldehyde per cent	4.36	4.33	2.02	0	-	-	-

As mentioned above, Kusin used the 40 per cent methyl alcoholic solution in all cases in which calcium hydroxide was the condensing agent, in order to minimize the loss of formaldehyde by the Cannizaro reaction. As the data above shows, there is a considerable decrease in the formaldehyde concentration in the aqueous solution before the appearance of reducing substances, while in the case of the methyl alcoholic solution there is very little drop. However, the condensation apparently began to take place rapidly at about the same time in the two controls. The lower values for reducing substances determined in the samples from the aqueous control is probably due to the decreased amount of formaldehyde present to take part in the condensation. Kusin's control at 40° C. with 1 per cent calcium hydroxide showed 93 per cent of the formaldehyde present after 5 hours, whereas in the experiments performed here the reaction is well under way by the end of 3 hours, and no formaldehyde is present after 3½ hours. It may be assumed that this difference is due to the higher concentration of calcium hydroxide and to the fact that the reaction mixture was stirred continually, of which Kusin makes no mention, and which undoubtedly facilitates the reaction as well as giving uniformity of the mixture.

2. Glucose.

1 gm. of glucose and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	1089	901	1071	1273	821	723	612
Formaldehyde per cent	4.12	3.72	1.20	0	-	-	-

2a. Glucose.

1 gm. of glucose and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	987	764	679	572	507	402	333

3. Fructose.

1 gm. of fructose and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	782	427	572	450	414	334	236
Formaldehyde per cent	3.98	1.49	0	-	-	-	-

3a. Fructose.

1 gm. of fructose and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	732	314	167	104	84	56	42

Kusin found both glucose and fructose to be active catalysts. It was desired, however, to obtain data on these compounds as a basis for comparison under the new experimental conditions. The somewhat more marked catalytic action of fructose as compared to glucose is in accord with the more active chemical nature of the former. The much more rapid and greater decline in reducing power in the control using fructose affords an indication of the relative alkali-lability of the two sugars. According to Kusin's theory, these compounds owe their catalytic activity to the formation of the enolic form of the sugar group, namely -COH=COH- in the fructose molecule, and -COH=CHOH in the case of glucose.

4. Ascorbic acid.

1 gm. of ascorbic acid and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	0	19	111	328	186	170	114
Formaldehyde per cent	3.98	2.40	1.02	0	-	-	-

4a. Ascorbic acid.

1 gm. of ascorbic acid and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent formaldehyde in 40 per cent methyl alcohol.

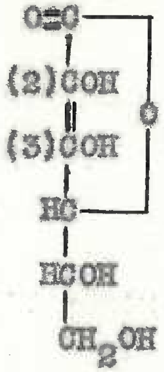
Time - minutes	0	15	45	60	120	180	240
Reducing subst. mgs. per 100 ml.	0	0	0	69	224	424	417
Formaldehyde per cent	4.08	3.95	3.42	3.30	1.17	0	-

4b. Ascorbic acid.

1 gm. of ascorbic acid and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	0	0	0	0	0	0	0

According to Hirst and coworkers (19) ascorbic acid has the structure



containing the enediol grouping on the second and third carbon atoms. It was thought at first that the catalytic activity of ascorbic acid in these experiments might lend support to the proposed structure for the compound, assuming Kusin's theory to be correct. This idea was discarded, however, when it was found that other compounds, incapable of forming the enediol structure, catalyzed the condensation.

The activity of ascorbic acid in aqueous formaldehyde is of very nearly the same order as that of glucose. The reason for the much slower action in the case of methyl alcoholic solution is not known, but may be explained on the basis of repressed ionization of some of the reactants.

5. Kojic acid.

1 gm. of kojic acid and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

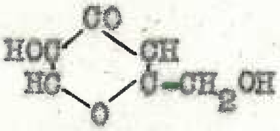
Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	24	44	101	340	280	238	179
Formaldehyde per cent	4.16	3.14	1.94	0	-	-	-

5a. Kojic acid.

1 gm. of kojic acid and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	0	0	0	0	0	0	0

Kojic acid (26) has been assigned the structure



and, although it apparently has no possibility of forming an enediol group, it catalyzes the condensation nearly as well as glucose or ascorbic acid. The available data as to the chemical behavior of kojic acid (26,27) indicate that it is quite stable toward alkali, and not likely to break down under the conditions of these experiments into products which might be the source of the catalytic activity. Further, the control experiment was repeated, the samples being neutralized with oxalic acid, diluted to volume, and filtered with analytical filter-aid. Sugar determinations on these solutions gave constant reducing values throughout the two-hour period of reaction, showing rather conclusively the stability of the compound under these conditions.

The iron-mercury treatment effectively destroyed all reducing power in the control experiment, but left small amounts in the condensation experiment, in the case of samples taken before the formation of any reducing products from the formaldehyde. This was found to be true, as shown in subsequent data, in the case of certain other catalysts, and apparently is due to the presence of the dimedon at the time of treatment with the iron-mercury reagent. The reducing power of dimedon alone was found to be quantitatively destroyed by the reagent.

6. Glucosamine.

1.2 gms. of glucosamine hydrochloride and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

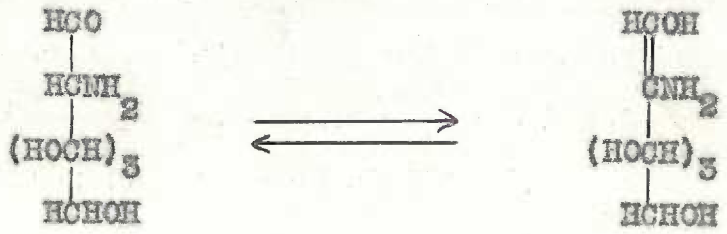
Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	785	660	773	1190	1207	1035	1023
Formaldehyde per cent	4.16	2.63	1.45	0.15	0	-	-

6a. Glucosamine.

1.2 gms. of glucosamine hydrochloride and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	271	256	269	244	248	263	340

The definite catalytic activity of glucosamine is not in accord with the theory of Kusin, as in this compound the possibility of an enediol grouping is eliminated by the presence of the NH_2 group on the second carbon atom. Enolization gives rise to an "ene-aminol" structure:



$$\begin{array}{c}\mathrm{HCO}\\ |\\ \mathrm{HCNH_2}\\ |\\ \mathrm{(HOCH)_3}\\ |\\ \mathrm{HCHOH}\end{array} \rightleftharpoons \begin{array}{c}\mathrm{HCOH}\\ \|\\ \mathrm{CNH_2}\\ |\\ \mathrm{(HOCH)_3}\\ |\\ \mathrm{HCHOH}\end{array}$$

Whether this grouping on the first and second carbon atoms is the active agent in catalyzing the condensation we cannot say, but it seems probable, since Kusin showed mannitol to be inactive as a catalyst. This would seem to eliminate the possibility of any catalytic function by the hydroxyl groups on the remaining four carbon atoms.

Glucosamine is known to be quite stable toward alkalies, and the control experiment shows quite uniform reducing values, indicating that there is no degradation of the molecule under these conditions.

It is not understood why the initial value for the reducing substances found is so much higher in the catalysis experiment than in the control. Repeated runs gave similar results. Calculation of actual amounts of reducing substances formed is thus made impracticable, but the catalytic effect is apparent from the markedly increased reducing power and the coincident disappearance of formaldehyde from the solution.

7. Reductone.

0.49 gm. of reductone and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent aqueous formaldehyde.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	100	357	699	470	405	342	268
Formaldehyde per cent	4.16	2.26	0	-	-	-	-

7a. Reductone.

0.49 gm. of reductone and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

Time - minutes	0	15	30	45	60	90	120
Reducing subst. mgs. per 100 ml.	0	0	0	0	0	0	0

Reductone (II) was isolated by Euler and Martius (24) and regarded by them as the enediol of tartronic aldehyde (I). It may also be considered as the enediol of hydroxymethyl glyoxal (III) (28). The relationships of these three compounds are shown as follows:

CHO	HCOH	CH_2OH
CHOH	COH	CO
CHO	CHO	CHO
(I)	(II)	(III)

As is to be expected from the enolic nature of the compound, reductone is one of the best catalysts among the series of compounds investigated, being comparable to fructose in its activity.

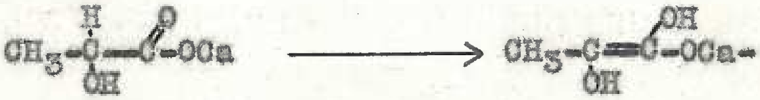
As was the case with kojic acid, the reducing power of the catalyst is completely destroyed in the control experiment but not in the condensation experiment.

8. Calcium lactate.

1.7 gms. of calcium lactate and 1.8 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent formaldehyde.

Time - minutes	0	30	60	90	105	120
Reducing subst. mgs. per 100 ml.	0	0	0	268	256	238
Formaldehyde per cent	4.16	-	2.56	0	-	-

The catalytic activity of calcium lactate was less to be expected than that of certain other compounds. However, it shows a definite action, which supposedly may be due to the following type of enolization, not usually considered as occurring:



$$\mathrm{CH_3-\underset{OH}{\overset{H}{C}}-\overset{O}{\overset{\|}{C}}-OCa} \longrightarrow \mathrm{CH_3-\underset{OH}{C}=\overset{OH}{C}-OCa-}$$

Since it was found that the calcium salt of propionic acid did not catalyze the condensation, the activity seems to center about the hydroxyl group of the α-carbon in lactic acid. Other α-oxy acids are being tried as catalysts, with the corresponding reduced analogs as controls.

Conclusion

As the data discussed above show, there are apparently several structural groupings which possess the ability to catalyze the condensation of formaldehyde to reducing sugars in the presence of calcium hydroxide. It is the hope of the writer to extend these studies to a sufficient series of compounds that certain deductions may be made.

It has become evident that the theory of Kusin, namely that the enediol configuration is the catalytic agent in this reaction, does not completely state the facts. It is our hope to determine more carefully the type or types of structural relationship to which the catalytic action is due.

Kusin suggested (17) that simple sugars, since they catalyzed the formation of sugars from formaldehyde in vitro, might play some such catalytic role in the production of carbohydrates from formaldehyde in the green plant. The evidence given here for the catalytic activity of ascorbic acid, its widespread occurrence in the plant world, and the discovery, discussed in a subsequent part of this dissertation, that ascorbic acid forms a compound or compounds with formaldehyde, suggest that this compound may have a more likely role as a photosynthetic catalyst than do the simple sugars.

THE REACTION OF ASCORBIC ACID WITH FORMALDEHYDE

Explanation

In attempting to follow the rate of disappearance of ascorbic acid in the experiments in which this compound was used as a catalyst for the condensation of formaldehyde, it was found that the formaldehyde interfered with the determination of ascorbic acid either by the indophenol titration method (30-33), using 2,6-dichlorphenolindophenol, or by iodine titration. Investigation into this phenomenon led to the discovery that ascorbic acid and formaldehyde form a labile compound in solution even in quite acid medium, thus blocking the titration of the ascorbic acid. The solution reduced indophenol very slowly, but did not permit a normal titration of the acid. The acidity of the ascorbic acid, as measured by titration with alkali, was found to remain unchanged. Formaldehyde could be recovered quantitatively from this combination by treating the solution with dimedon, the precipitate forming more slowly than was the case with solutions of formaldehyde alone.

Later it was found that if ascorbic acid is treated with an excess of formaldehyde in the presence of calcium carbonate a syrupy compound is formed. The product has sugar-like properties, and apparently corresponds to the addition of two molecules of formaldehyde to one molecule of ascorbic acid. No formaldehyde can be recovered from this compound by treating it with dimedon, and it does not reduce indophenol.

In earlier experiments, in which crystalline ascorbic acid was exposed to the vapors from 40 per cent formalin, a thick syrupy substance was obtained. As a small amount of precipitate could be obtained from this syrup by treating it with dimedon, and as it retained some reducing power toward indophenol, it now appears to have been a mixture of the compound obtained in the presence of calcium carbonate and the labile combination formed in aqueous solution.

EXPERIMENTAL

The Reaction of Formaldehyde with Ascorbic Acid in Acid Solution

The titration of ascorbic acid in solutions containing various amounts of formaldehyde was compared with that of pure ascorbic acid solutions by titration with indophenol, iodine, and sodium hydroxide.

Titrations with 2,6-dichlorphenolindophenol. 61 mg. of ascorbic acid were dissolved in 6 ml. of 4 per cent formaldehyde solution and aerated with nitrogen (omitted in later experiments). 1 ml. samples were titrated, after addition of 2 ml. of 1N acetic acid to each, at the end of 2, 10 and 20 minutes reaction time. The dye solution was standardized against a solution of a known amount of ascorbic acid in water. The results are tabulated below:

Time of Reaction minutes	Titration ml.	Ascorbic acid titrated per cent
2	25.9	49.9
10	0.9	1.7
20	0.8	1.5

Higher concentrations of formaldehyde gave more nearly quantitative results. A solution of 23.6 mg. of ascorbic acid in 25 ml. of 1N acetic acid was prepared. As the protective action of formaldehyde in preventing the oxidation of ascorbic acid had by this time been established, the use of a nitrogen atmosphere was deemed unnecessary. 2 ml. samples of the solution used 9.65 ml. of the dye solution. 2 ml. samples treated with 5 ml. of 40 per cent formaldehyde and allowed to stand fifteen minutes required but one drop of the dye to give a pink color (end-point) which lasted for two minutes, fading slowly at the end of this time. The fact that the titrations of ascorbic acid, especially in the presence of the lower concentrations of formaldehyde, showed a fading end-point indicates that the combination is more or less dissociable under these conditions.

Titrations with Iodine. Determinations of ascorbic acid with 0.01N iodine solution gave similar results. A solution of ascorbic acid containing 1 mg. per ml. in 1N acetic acid was prepared. 3 ml. samples required 3.5 ml. of the iodine for titration, starch being used at the end-point. 3 ml., after standing 10 minutes with 5 ml. of 4 per cent formaldehyde solution, required 1.5 ml. of iodine. 5 ml. of 40 per cent formaldehyde added to a3 ml. sample reduced the titration to 0.15 ml. Formaldehyde solutions alone gave no titration with either the iodine or the indophenol.

Attempts to make use of this reaction of formaldehyde with ascorbic acid in the quantitative determination of the latter are being made. The reaction may also prove to be of value in the isolation of ascorbic acid from natural sources, since formaldehyde apparently protects the ascorbic acid against oxidation.

Titrations with 0.1N NaOH. A solution of 0.4877 gm. of ascorbic acid in 50 ml. was prepared in 40% formaldehyde solution. 5 ml. samples were taken by pipette at intervals, and titrated with 0.1N NaOH to the end-point of phenolphthalein. The titrations, corrected for acidity of the formalin, are given below:

Time of Reaction	Titrations ml.	Acid Titrated per cent
5 min.	2.6	94
10 min.	2.5	90
20 min.	2.5	90
50 min.	2.5	90
44 hours	2.6	94

An aqueous solution containing 0.4940 gm. of ascorbic acid in 50 ml. was prepared and 5 ml. samples titrated after 5 and 30 minute intervals. Both samples required 2.8 ml. of 0.1N NaOH, the calculated equivalent of base.

The interpretation of these data will require further study along this line. It has been stated (19) that the lactone structure of ascorbic acid is very stable, the acidity of the compound being due to the hydrogen of the enolic hydroxyl groups. It is generally accepted that this enediol structure is also responsible for the indophenol reduction. The data given above show that the reducing power of ascorbic acid toward indophenol may be quantitiatively blocked without appreciably affecting the titrable acidity of the compound. Further investigation of this phenomenon is in progress, including the determination of pH curves with the glass electrode.

The Reaction of Formaldehyde with Ascorbic Acid in the Presence of Calcium Carbonate

5 grams of crystalline ascorbic acid were dissolved in 50 ml. of 40 per cent formalin and 5 grams of calcium carbonate added. The mixture was placed in an open crystallizing dish under the drying oven, where the temperature was about 50-55°C., and allowed to evaporate. Water was added as needed in order to redissolve the solid polymerized formaldehyde. When the last traces of formaldehyde had disappeared (usually after a week or ten days) a syrupy residue containing the excess calcium carbonate remained. This material was softened by adding a small amount of water and stirring thoroughly. The mixture was then thoroughly triturated with 250-300 ml. of absolute alcohol and filtered by suction through a fritted glass filter, followed by washing with small portions of alcohol. The alcohol was removed from the filtrate by evaporation under the oven in the manner described above. The syrup remaining after the removal of the alcohol was dried under the hyvac pump over Drierite, whereby a sweet, thick syrup, clear and nearly colorless, was obtained.

The quantitative yield was about 133-134 per cent of the weight of ascorbic acid used. Varying the amount of formaldehyde in the preparation yielded the same amount of syrup. 1 gram of ascorbic acid with 0.8 gram of formaldehyde gave 1.34 gms. of the product, showing that the compound is not formed from formaldehyde alone. No ascorbic acid residue was found in the precipitate after the trituration with alcohol. The syrups often retained traces of formaldehyde. These could be removed, however, by means of dimedon, without altering the properties of the syrupy compound. The analyses and properties of the compound are discussed below.

Molecular weight. Determinations were made by the freezing-point depression method, water being used as a solvent. A solution of 0.8885 gm. of the syrup in 35.2681 gms. of water (triple-distilled) had a freezing-point of -0.24°C. A sample of the solvent under the same conditions had a freezing-point of -0.04°C., the true depression being, therefore, 0.20°. The molecular weight of the compound, as calculated from these data, is 234. The method was applied to a sample of pure glucose with an error of less than 1 per cent in the molecular weight.

Optical activity. The specific rotation in water varied from $[\alpha]_D^{25°}$ -10.2° to $[\alpha]_D^{25°}$ -12.8°, the latter value being found for several preparations of syrup. The variations may have been due to varying small amounts of water in the different preparations. No mutarotation was observed.

The fact that the compound is laevo-rotatory is of considerable interest, since ascorbic acid itself has a specific rotation of +49° (33). A solution of ascorbic acid in 40 per cent formaldehyde had a specific rotation of + 55° (calculated for the ascorbic acid), the rotation remaining unchanged during a period of 24 hours. This increased dextrorotation of the labile ascorbic acid-formaldehyde combination is in marked contrast to the laevo-rotation of the product formed in the presence of calcium carbonate.

Acetylation of the syrup. Determination of the hydroxyl groups in the compound was carried out essentially according to the improved method of Hafner, Swinney and West (34), the acetylation being done in the cold (0°C. for 6 days). Close agreement between the results on different size samples indicates complete acetylation under these conditions. Results of the acetylation of samples of the compound and of ascorbic acid are tabulated below.

Experiment No.	Sample		Reagent	Titration mls.	Acetyl No*
1	0.0414 gm.	syrup	4 ml. of 1:7 reagent	18.98	761.3
1a	0.0407 gm.	"	"	19.00	763.7
3	0.0394 gm.	"	4 ml. pyridine only	0.02	-
2	0.1043 gm.	"	4 ml. of 1:7 reagent	16.75	763.7
2a	0.1050 gm.	"	"	16.72	764.7
3a	0.0947 gm.	"	4 ml. pyridine only	0.02	-
4	-		4 ml. of 1:7 reagent	20.45	-
5	-		"	20.44	-
6	0.0520 gm.	ascorbic acid	"	19.55	789.
6a	0.0509 gm.	"	4 ml. pyridine only	1.00	-
7	0.0886 gm.	"	4ml. 1:7 reagent	18.75	845.4
7a	0.0802 gm.	"	4 ml. 1:7 reagent	18.90	850.
8	0.1021 gm.	"	4 ml. pyridine only	2.00	-
9	-		4 ml. of 1:7 reagent	20.45	-

Reducing equivalent. The glucose reducing equivalent of the compound was determined by the Shaffer-Somogyi reagent 50 (25). The reducing equivalent is expressed here as the ratio of the reduction by the compound to that by an equal weight of glucose. The reducing equivalents were determined on a number of preparations, and these were found

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*The acetyl number is defined as the mgs. of acetyl bound per gram of substance acetylated.

to possess from 52 to 57 per cent of the reducing power of glucose. These variations may be due, as suggested in the case of the optical rotatory power, to varying water contents of the preparations. This seems especially likely since the variations in the two properties were generally in the same direction. The compound did not reduce indophenol solution and did not reduce the Shaffer-Somogyi reagent 50 in the cold. Addition of strong alkali, either before or after addition of the copper reagent, caused rapid reduction of the copper to take place in the cold. A solution of the syrup, treated with the iron sulphate-mercury sulphate reagent as described above, lost over 90 per cent of its reducing power. The reducing power of ascorbic acid is quantitatively destroyed by this treatment, whereas it has been shown that the common reducing sugars lose only 7 per cent or less of their reduction by this treatment (35). This may be taken as evidence of the presence of an ascorbic acid nucleus in the compound.

<u>Ash</u>. Samples of various preparations were burned in an electric muffle furnace, and the following amounts of ash (Ca) found: 1.7, 2.0, 1.0, 1.2, 1.08, and 1.46 per cent.

<u>Elementary analysis</u>. Carbon and hydrogen determinations were made by the micro method of Pregl. The values given here are tentative at the present time, as analyses

have been temporarily interrupted.

$C_8H_{12}O_8$ Calculated C 40.68, H 5.12
236.1 Found C 39.62 H 6.71

Benzoylation of the syrup. 10 grams of syrup were dissolved in 50 ml. of dry pyridine, 50 ml. of chloroform added, and the mixture cooled in an ice bath. 15 ml. of benzoyl chloride were added drop by drop for 2 hours, with vigorous stirring. After standing overnight at room temperature, 4 ml. of water were added. After ½ hour the mixture was diluted with ice water and extracted five times with 50 ml. portions of chloroform. The extract was washed five times with 50 ml. of cold dilute sulphuric acid, followed by three washings with 50 ml. of cold water. The solution was dried with anhydrous sodium sulphate, and the chloroform removed with a stream of air. The product obtained was a mixture of crystals and syrup. A crystalline, ether- and water insoluble compound was separated and analyzed.

The compound melted at 205°C. with browning. Preliminary determinations of molecular weight by the method of Rast (36, 37) gave a value of 630. The elementary composition was found to be: C 66.45, H 4.98.

Controls. Ascorbic acid treated with calcium carbonate in water in place of formalin, and carried through the treatment described above, gave a brownish colored hard syrup which reduced indophenol and showed a varying dextro-rotation, usually about +17.4°. It apparently consisted of a mixture of decomposition products of ascorbic acid.

40 per cent formalin with calcium carbonate gave no sugar-like product. Formic acid (calcium formate) was determined in the residue in amounts which accounted for the total loss of carbonate in this control and in the syrup preparations.

1 gram of ascorbic acid and excess of 40% formaldehyde in the absence of calcium carbonate yielded a thick syrupy material (1.3 gms.). A solution of the syrup reduced indophenol, rapidly at first but without a sharp end-point. It reduced the Shaffer-Somogyi reagent 50 in the cold. The optical rotation was $[\alpha]_D^{25} - 6.3^\circ$. The properties of the material vary and certainly it does not resemble closely the compound formed in the presence of calcium carbonate.

Catalysis of the Formaldehyde Condensation. It appeared to the writer to be somewhat significant to study the activity of this compound of formaldehyde with ascorbic acid in catalyzing the condensation of formaldehyde to sugars. The experiment was carried out in the manner described in an earlier section of this dissertation, the data being more logically included at this point:

1.3 gms. of syrup and 2 gms. of $Ca(OH)_2$ in 100 ml. of 4 per cent formaldehyde.

Time - minutes	0	15	30	45	60
Reducing subst. mgs. per 100 ml.	48	739	657	620	459
Formaldehyde per cent	4.16	0	-	-	-

1.3 gms. of syrup and 2 gms. of $Ca(OH)_2$ in 100 ml. of water.

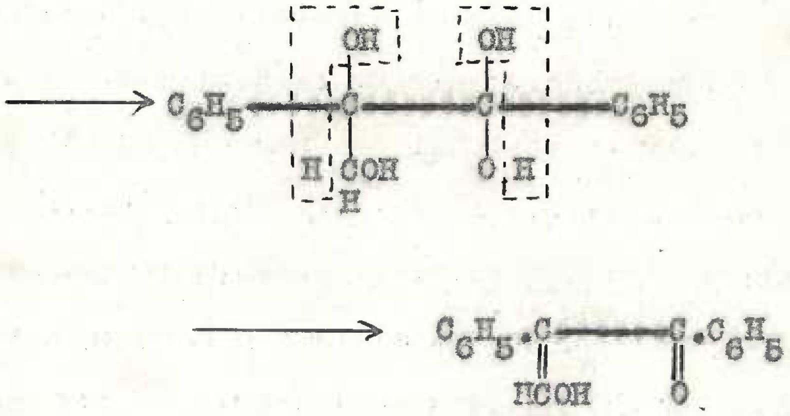
Time - minutes	0	15	30	45	60
Reducing subst. mgs. per 100 ml.	48	119	107	92	85

It is interesting to note that the catalysis by this compound of ascorbic acid and formaldehyde is much more marked than in the case of any other compound studied in the series above. The nature of this compound, and the basis for this unusually rapid catalytic effect will be taken up in the following theoretical considerations.

Theoretical Discussion

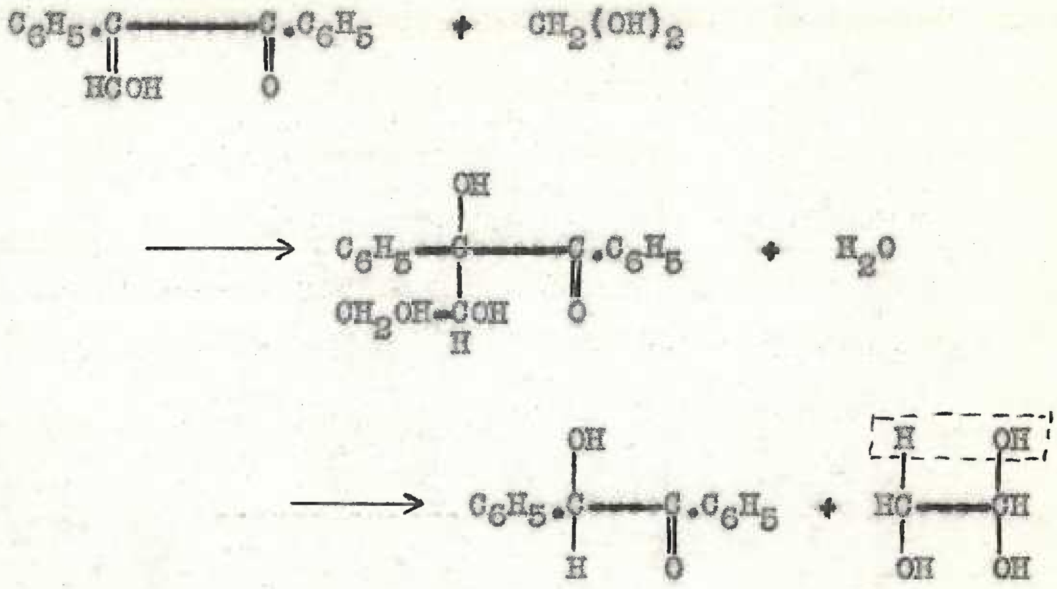
In studying the catalysis of the formaldehyde condensation by benzoin, Kusin (18, 38) postulated the formation of certain intermediate compounds of formaldehyde with benzoin, and indeed succeeded in isolating one product which he called oxymethyl benzoin. It represented the addition of one molecule of formaldehyde to one molecule of benzoin, and, according to Kusin, was formed by the reaction of benzoin with the hydrated form of formaldehyde, thus:

$$C_6H_5.C(OH):C(OH).C_6H_5 \quad + \quad CH_2(OH)_2$$



$$\longrightarrow \quad C_6H_5.\underset{\substack{\| \\ HCOH}}{C}—\underset{\substack{\| \\ O}}{C}.C_6H_5 .$$

Following the addition of a second molecule of formaldehyde to this compound, the product broke down to give rise to glycolaldehyde, regenerating benzoin:



The mechanism of formation of a ketohexose from glycolaldehyde and formaldehyde was further postulated, represented thus:

$$HC(OH){:}CH(OH) + CH_2(OH)_2 \longrightarrow H_2C(OH).CH(OH).CH(OH)_2$$

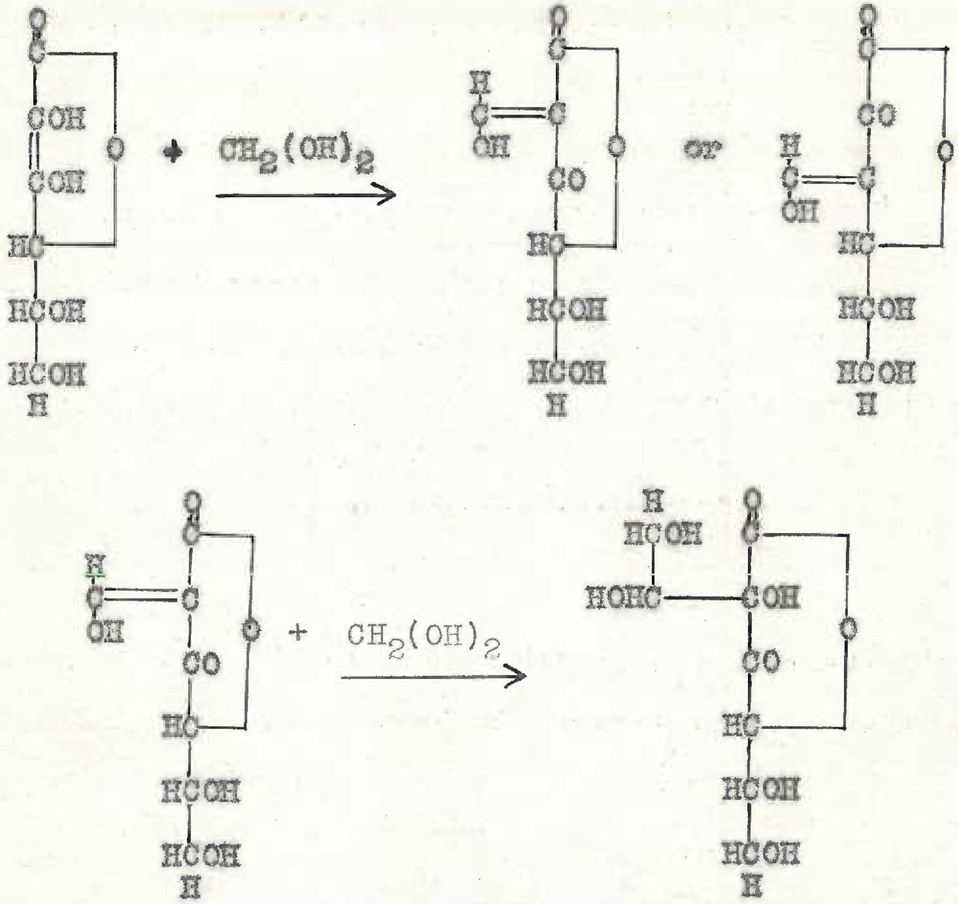
$$H_2C(OH).C(OH){:}CH(OH) \quad + \quad H_2C(OH).CH(OH).CH(OH)_2$$

$$\longrightarrow H_2C(OH).C(OH)_2.CH(OH).CH(OH).CH(OH).CH_2(OH)$$

$$\longrightarrow H_2C(OH).CO.[CH(OH)]_3.CH_2(OH) \quad + \quad H_2O$$

While this theory is not entirely demonstrated by fact, there is some evidence in support of it, and it offers a good working basis for the mechanism of catalysis by compounds containing two hydroxyls at the double bond.

Assuming that the reaction of ascorbic acid with formaldehyde follows this type, it may be illustrated graphically thus:



The first product, containing one added formaldehyde molecule, has not been isolated in this case, but we may assume that it is this combination which so effectively blocks the dye-reducing power of ascorbic acid. The syrupy compound formed in the presence of calcium carbonate may be the second product, corresponding to the addition of the second molecule of formaldehyde. Indeed, a number of the properties of the material support this view.

Thus, the increase in weight in the preparations was found to correspond to the addition of two formaldehyde molecules per molecule of ascorbic acid. The molecular weight was found to be about 234. The theory for the compound $C_8H_{12}O_8$ is 236.1.

The acetyl number obtained by acetylation of the syrup indicates the presence of four acetylated hydroxyl groups. While there are five hydroxyls in the proposed structure, one of them is tertiary in character. Tertiary hydroxyls have been shown to be very poorly acetylated. The benzoylation product should be the tetrabenzoyl derivative, since there appear to be four active hydroxyl groups. Such a compound would have the theoretical composition: $C_{36}H_{28}O_{12}$. C 66.26, H 4.29, molecular weight 652. Found C 66.45, H 4.98, molecular weight 630.

The fact that there may be ~~two~~ four isomers of this compound probably accounts for the failure of separation of a crystalline product in the reaction, and also for the fact that besides the crystalline benzoate, a thick syrupy product, yet to be analyzed, is obtained.

According to this proposed and partially verified structure for the compound, there is no opportunity for the formation of an enediol group. In the light of the information derived from the catalysis experiments, namely that other types of structure seem to possess catalytic activity, we might expect to find here a certain degree of activity, but nothing of the extreme order that was observed.

Insofar as we seem to have borne out Kusin's theory thus far, we may assume one further hypothesis to hold good, and ascribe the catalytic effect in this case to the splitting out of glycolaldehyde, or a similar compound, under the influence of the alkalinity produced by the calcium hydroxide. Some evidence, although as yet unsatisfactory, has been obtained for the presence of glycolaldehyde in a solution of the syrup treated with calcium hydroxide.

GENERAL DISCUSSION AND CONCLUSIONS

As was stated in the introduction of this dissertation, the whole problem of the condensation of formaldehyde to reducing sugars or sugar-like compounds has long been of interest because of its possible part in the processes of photosynthesis. The work which the writer has discussed above was carried out largely on the basis of this interest, although some of the facts which were brought to light are significant as chemical data alone.

In speculating on the significance which any of these experiments may have, it must be remembered that these observations are made in vitro, that the conditions under which they are made do not approach the physiologic in most respects, and that most of the forces at work in the plant are as yet unknown. With these reservations in mind, we may venture an hypothesis.

It is a fact of common knowledge, of course, that ascorbic acid is very generally distributed throughout the realm of green plants. Its functions in the animal body, as one of the essential vitamines, are becoming known. What part it plays in the life of the plant is still more or less a mystery. It may be that this problem will be answered, in part at least, by the recognition of a photosynthetic role for ascorbic acid.

It seems likely that if formaldehyde is formed in the photosynthetic reduction of carbon dioxide, it must be combined quickly with ascorbic acid to form the labile complex found to exist in solutions of the two compounds. Under conditions existing in the plant, the syrupy compound, obtained in the presence of calcium carbonate, may also be formed. The preparations in the presence of calcium carbonate were carried out at a pH slightly below 7, as measured at various stages of the reaction. This mild reaction is in contrast to the extremes of alkalinity employed by various workers thus far in condensing formaldehyde to sugar-like materials.

If, then, a two-carbon fragment may be split off from this compound of ascorbic acid and formaldehyde, glycolaldehyde may be formed and built up into hexoses, and ascorbic acid regenerated.

It is the hope of the writer to take part in further work on this problem, with the view of verifying by in vivo experiments some of the observations made in vitro, and thus piecing together the mechanisms involved in the photosynthetic production of sugars.

SUMMARY

A brief historical account has been given of the study of the condensation of formaldehyde to sugar-like products in the presence of various alkalies and catalysts; the mechanism of the condensation is discussed briefly.

Data are presented to show the effect of a number of compounds in catalyzing the formation of reducing compounds from formaldehyde in the presence of calcium hydroxide; improved procedures for the determination of formaldehyde and reducing substances, adapted to the conditions of these experiments, are presented.

Two reactions of ascorbic acid with formaldehyde are described: a reaction in acid solution whereby the ascorbic acid is protected against oxidation by iodine or indophenol, and the formation of a syrupy compound in the presence of calcium carbonate. The properties and probable constitution of this compound are discussed.

The experimental data included in this dissertation are considered from the stand point of their possible relation to the photosynthetic formation of carbohydrates. The possibility that ascorbic acid may function as an important photosynthetic catalyst is suggested.

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