1935

The preparation of erythrose and some of its derivatives

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NOTE TO USERS

THE PREPARATION OF ERYTHROSE AND SOME OF ITS DERIVATIVES

By

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Plant Chemistry

Approved

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Iowa State College
1935

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. R. M. Hixon and to Dr. Werner Freudenberg for the suggestion of this problem and for their generous advice and encouragement given during the progress of the work.

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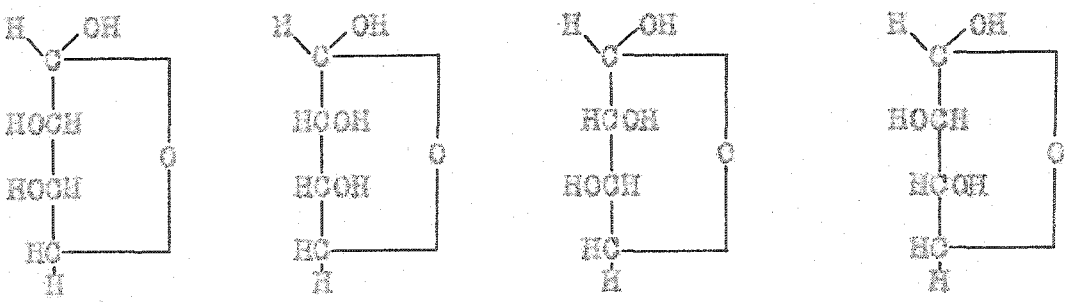
INTRODUCTION

In spite of the comprehensive work on the chemical and physiological properties of the monosaccharides and their derivatives, the four carbon sugars have been very much neglected. This disregard can in part be accounted for by the nonoccurence of the tetroses in natural products and by their difficult synthetic preparation. It is probably also due to the lack of facts which might direct the interest of chemists toward this field. The investigation reported in this thesis was undertaken with the twofold purpose of helping to fill in to some extent this gap in chemical knowledge and of directing attention to a potentially important class of compounds.

The study reported in this thesis concerns l-erythrose which is one of the four possible aldo-tetroses. The other tetroses are called d-erythrose and l- and d-threose. Their relationships are shown by the following formulae:

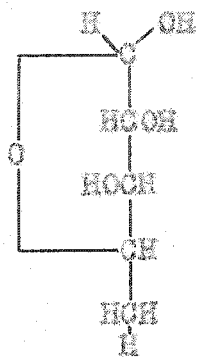


l-erythrose d-erythrose l-threose d-threose

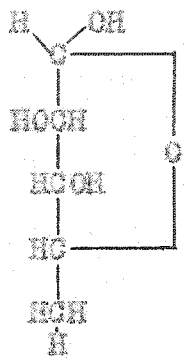
The methyltetroses are a very similar group. They differ only in having a methyl group in the place of one of the

hydrogens on carbon atom 4* of the tetrose.

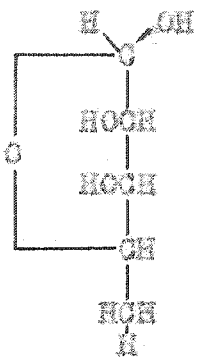
The possession of three asymmetrical centers calls for the formation of the eight following methyltetroses.



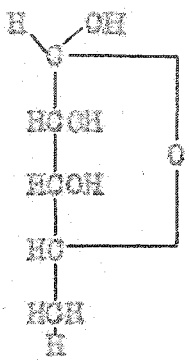
l-arabino-methylose



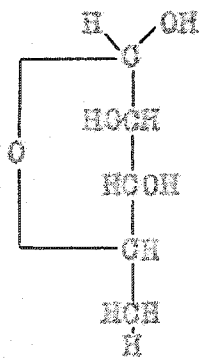
d-arabino-methylose



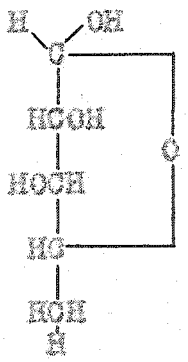
l-ribo-methylose



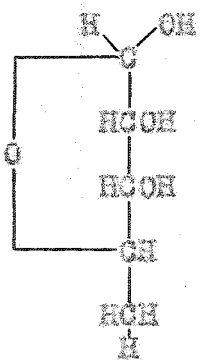
d-ribo-methylose



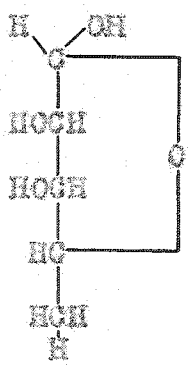
l-xylomethylose



d-xylomethylose



l-lyxomethylose



d-lyxomethylose

Since the typical sugar reactions depend upon the arrangement of the aldehyde and hydroxyl groups, any reaction reported for one of the tetroses or methyltetroses would be shown with equal facility by all of the members of this group.

*The carbon atoms in sugars are numbered consecutively starting from the aldehyde end of the chain.

Compounds similar to those reported for l-erythrose could therefore be formed for all of the other tetroses and methyl tetroses.

The tetroses were prepared for the first time during the last decade of the nineteenth century. The most important results were obtained by Fischer, Wohl, and Ruff. Fischer (1) prepared the original tetrose mixtures by oxidising the alcohol, erythritol. The sugar obtained was identified by the preparation of erythrosazone. Wohl (2,3) and Ruff (4, 5) used their famous sugar degradation reactions on the then available pentoses to obtain several of the four carbon sugars. After the completion of the investigations by the three chemists mentioned, only one noteworthy research experiment in this field was reported during the course of the next thirty years. In 1917 Weermann (6) oxidized the acid amides of several pentonic acids with sodium hypochlorite, producing thereby small quantities of the corresponding tetroses. Within the last six years the experiments by Deulofeu (7, 8) in Argentina and Hockett (9) and Werner Freudenberg (10) in the United States have again contributed to the knowledge of the tetroses.

The more recent experiments have been prompted by various reasons. Dr. Evans of Ohio State University aroused Hockett's interest in the tetroses because of his desire to use them as material for investigations on the alkaline oxidation of sugars. In these researches the products of oxidation of

hexoses are compared with the products from pentoses in order to determine if the oxidation proceeds through the removal from the hexose of carbon atom 1 with the formation thereby of the related pentose. In order to extend this comparison a supply of pure tetrose was desired.

Venancio Deulofeu was mainly interested in determining the molecular weight of a four carbon sugar. The aldo-triose, glycerose, shows in solution a double molecular weight. The five and six carbon sugars exhibit normal molecular weights. Using the purest sirups that he could obtain, Deulofeu found the tetroses to be approximately monomolecular.

The tetroses possess properties which make them of fundamental interest. Their largest possible ring structure is the five membered furanose ring. The furanose sugars are noted for their great reactivity. Those possessing an unprotected aldehyde group will reduce Fehling's solution without heating in contrast to the boiling required for a positive reaction with the pyranoses (six membered rings). The five membered ring seems to exist in solution with the ring-aldehyde equilibrium shifted much more to the aldehyde form than is the case with the six membered ring (11). The pyranoses give a negative Schiff's test whereas the furanoses exhibit a positive although slow reaction. The formation of hydrazones is also a great deal faster with the smaller ring form (11). The properties mentioned for the furanose ring have usually been discovered by a study of sugar derivatives

in which the ring can not shift. The properties due to the furanose ring in the free sugars can only be studied by the use of the tetroses as they can not assume a pyranose structure.

Are the tetroses of physiological importance? This question has been raised many times but the physiologist has never been able to answer it because the chemist has as yet failed to produce a pure easily accessible tetrose. It is known that yeast does not ferment tetroses. In this property they are like the pentoses. Do they also resemble the pentoses by being non-metabolizable? That is not now known nor will it be known until uncontaminated tetroses can be prepared and tested.

METHODS OF PREPARING ERYTHROSE

The methods for the preparation of erythrose can be divided into two groups depending on whether optically active or inactive forms are produced.

The optically inactive erythrose has been prepared by the condensation of glycol aldehyde in the presence of alkali (12, 13). This reaction was of importance in showing a possible mechanism of carbohydrate formation in nature but it yielded a mixture of products which was unsuitable for separation into the various sugars formed. The alcohol, erythritol, has been oxidized to d,l-erythrose by nitric acid (1), hydrogen peroxide with ferric acetate as a catalyst (14), alcoholic quinone (15), bromine and sodium carbonate (16), sunlight and uranium salts (17), and electrolytically (18). These preparations all had the disadvantages of poor yields and of inseparable mixtures.

Theoretically the active erythrose could be prepared either by a degradation reaction from the related pentoses, arabinose or ribose, or by a building up reaction from the triose, glycerose. The preparation from glycerose is impractical on account of the great difficulty in obtaining the active starting material which can only be prepared by a long series of tedious transformations. Ribose occurs in natural products to only a very limited extent. Arabinose, on the contrary, is found abundantly as the polysaccharide,

araban, from which the free sugar is obtained by hydrolysis with dilute sulfuric acid. The configuration of the naturally occuring form is that of the l-arabinose. Recently directions for the preparation of d-arabinose from the easily available and cheap d-glucose have been published (19). This announcement makes both the l-arabinose and the d-arabinose quite easily accessible. The experiments reported herein were started when good methods of preparation for only the l-arabinose were known. Therefore, l-arabinose was chosen as the starting material for the preparation of l-erythrose.

The first degradation on l-arabinose was reported in 1899 by Wohl (3). The preliminary steps consisted of the preparation of l-arabinose oxime by the action of hydroxylamine on arabinose and then the acetylation of the oxime by acetic anhydride and sodium acetate which yielded the tetraacetyl arabononitrile. The nitrile was treated with silver oxide and one or two drops of ammonia solution. The resulting product, triacetyl erythrose, was obtained in a crystalline form. However, on attempting to hydrolyze it the reaction proceeded so slowly and with such poor conversion that the preparation of the free l-erythrose by that method was abandoned. The nitrile, however, by means of silver oxide and a large quantity of ammonia was converted into l-erythrose diacetamid. This product was obtained in 26 per cent yield based on the original arabinose. The l-erythrose diacetamid was saponified by means of dilute sulfuric acid. The rotation change during

the reaction was followed and the final specific rotation calculated for the theoretically possible amount of erythrose was +32.7°. The osazone of erythrose was prepared in 20 per cent yields. Attempts to free the sirup of acetamid, ammonium sulfate, and ammonium acetate were not successful in producing a product that would crystallize.

In 1901 Ruff (5) reported the results of the application of his procedure to the degradation of l-arabinose. His supply of arabinose was obtained from cherry gum. The method consisted of the oxidation of arabinose to arabonic acid by means of bromine. The acid was isolated as its calcium salt. The salt was oxidized by hydrogen peroxide with ferric acetate as a catalyst to yield an erythrose containing solution from which l-erythrose benzylphenylhydrazone separated on the addition of benzylphenylhydrazine. The l-erythrose was regenerated by splitting the hydrazone with formaldehyde. This preparation showed an upward mutarotation with an equilibrium value of +21.5°. The sirup could not be crystallized even by drying over phosphorus pentoxide or cooling with liquid air.

Much later (1917) Weermann (6) applied his method of degradation to the sugars. This method was based on the action of sodium hypochlorite on the amid of the corresponding acids. l-Arabinose was among the sugars degraded. l-Arabonic acid admid was prepared from calcium arabonate after precipitating the calcium with oxalic acid. The amid was then treated with sodium hypochlorite. Benzylphenylhydrazine was added to the

solution and l-erythrose benzylphenylhydrazone precipitated. Attempts to split the hydrazone with benzaldehyde gave a highly colored impure sirup. Treatment with formaldehyde gave no better results. The sirup obtained did not crystallize on standing for one-half of a year. The rotation immediately after dissolving was zero and the equilibrium was reached at +23.6°. The l-erythrose benzylphenylhydrazone was undoubtedly pure but no satisfactory method for hydrolyzing it to yield a pure product has as yet been developed.

The most recent attempt to degrade l-arabinose was reported in 1929 by Deulofeu and Selva (7). They employed the method of Zemplen which was based upon the action of sodium methylate on the acetylated nitrile. A yield of 40 per cent of the theoretical was calculated from the reducing power of the solution obtained.

A purer product was secured by treating the arabononitrile with silver oxide and barium hydroxide. The yield was 13 per cent which was likewise calculated from the reducing power of the solution. The osazone was prepared but in only extremely small quantities. From 5 grams of tetraacetyl arabononitrile only 0.03 grams of erythrosazone was obtained, much less than had been expected from the reducing power of the solution.

Deulofeu (20) has also applied Wohl's method to the degradation of d-arabinose. He secured results similar to those reported for l-arabinose. Ruff (4) investigated the

degradation of d-arabinose as well as l-arabinose.

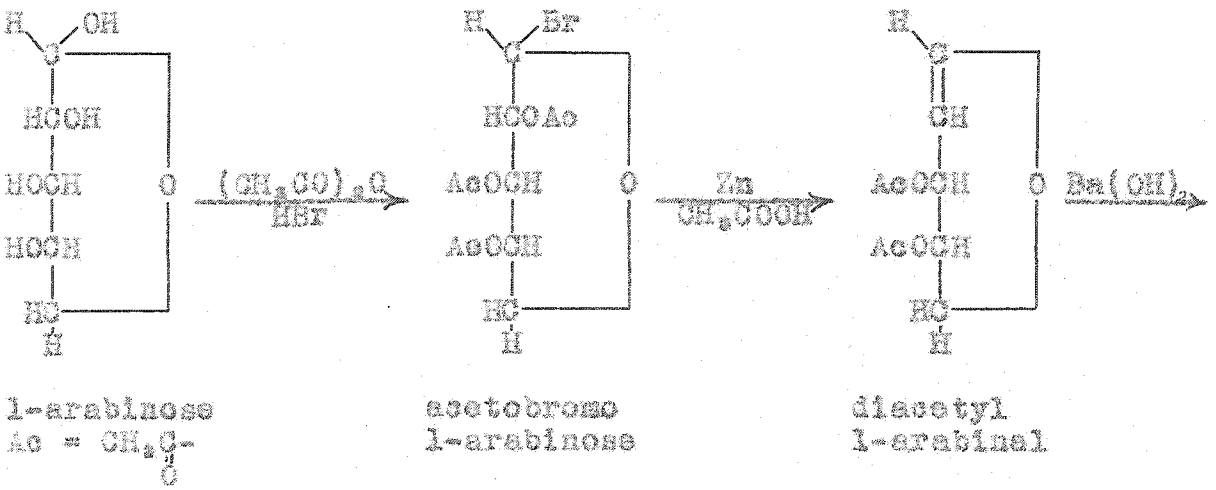
It can be seen from this review that no convenient degradation method has yet been found for the preparation of erythrose. This statement likewise applies for the preparation of the other tetrose, threose, with but one possible exception.* In view of the poor results secured by the methods previously used, it was decided to investigate a new degradation method.

*Robert C. Hockett reported to the 1934 fall meeting of the American Chemical Society that d-threose diacetamid could be prepared by Wohl's method in very excellent yields, if the silver oxide was omitted in the degradation process and only a strong solution of ammonia used.

OZONE SPLITTING OF ARABINAL

The possibility of removing carbon atom 1 from arabinose by the formation of a 1,2 unsaturated derivative and then splitting the double bond with ozone was suggested by similar work on derivatives of glucose and xylose. In 1920 Fischer, Bergmann, and Schotte (21) reported, in proving the structure of triacetyl glucal through the action of ozone, the formation from it of triacetyl d-arabinose. Later Bergmann and Freudenberg (22) used ozone to split the unsaturated sugar, diacetyl pseudo-glucal, in order to determine the position of the double bond. The possibility of using this procedure for making accessible sugars with a shorter carbon chain was first used by Freudenberg (10), who converted d-xylal into what was thought at the time to be crystalline d-threose. The success with this preparation would seem to justify the attempt to prepare erythrose by a similar procedure.

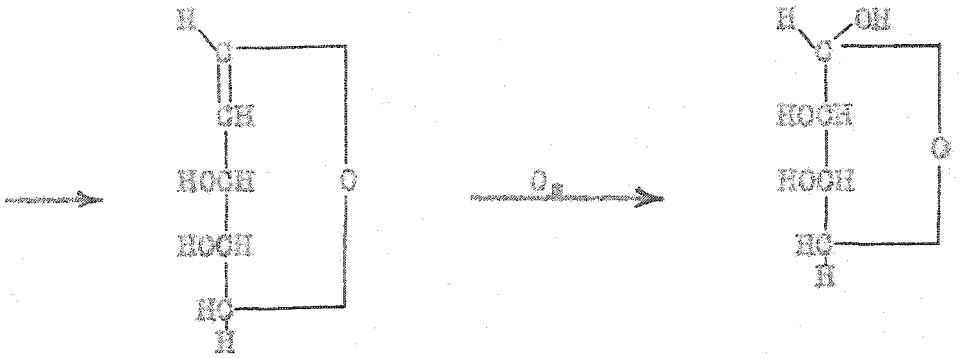
The application of this method to the degradation of l-arabinose requires the following steps.



l-arabinose
Ac = $CH_3C(=O)-$

acetobromo l-arabinose

diacetyl l-arabinal

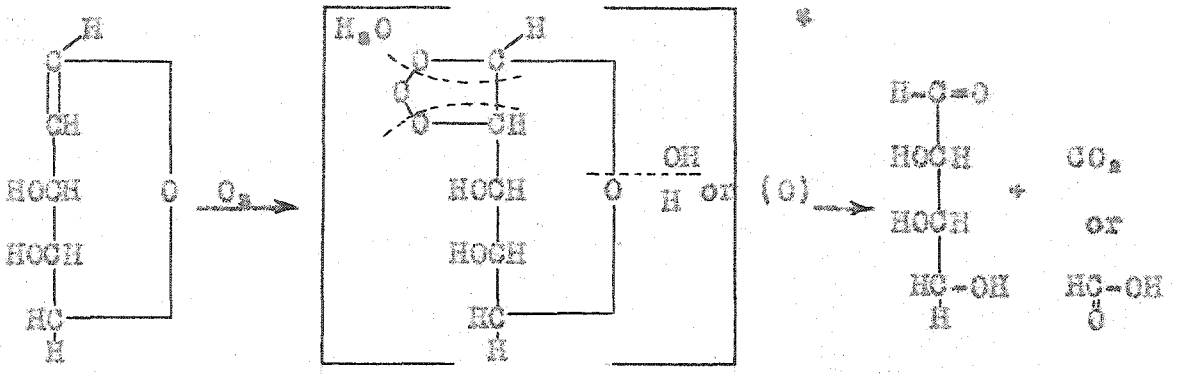


l-arabinal l-erythrose

The l-arabinose can be transformed into acetobromo arabinose by the action of hydrobromic acid in acetic anhydride. Acetobromo arabinose is reduced by means of zinc dust and acetic acid to diacetyl arabinal. The acetyl derivative can be hydrolyzed with barium hydroxide to the free arabinal which is degraded to l-erythrose by the action of ozone.

No great difficulty was encountered in the transformation from l-arabinose to l-arabinal. The yields previously reported (25) for the reduction of acetobromo arabinose into diacetyl arabinal could only be duplicated if a catalyst were used. Platinum chloride served in this capacity very well (24). The reaction of ozone on a glacial acetic acid solution of pure crystalline arabinal originally was thought to result mainly in the formation of l-erythrose since the sirups obtained yielded l-erythrosazone. However, the osazone was much more difficult to prepare than had been expected. It was also found to be formed in much smaller quantities than anticipated. The erythrose sirup reduced Fehling's solution very strongly in the cold and gave quickly a very intensive red coloration to

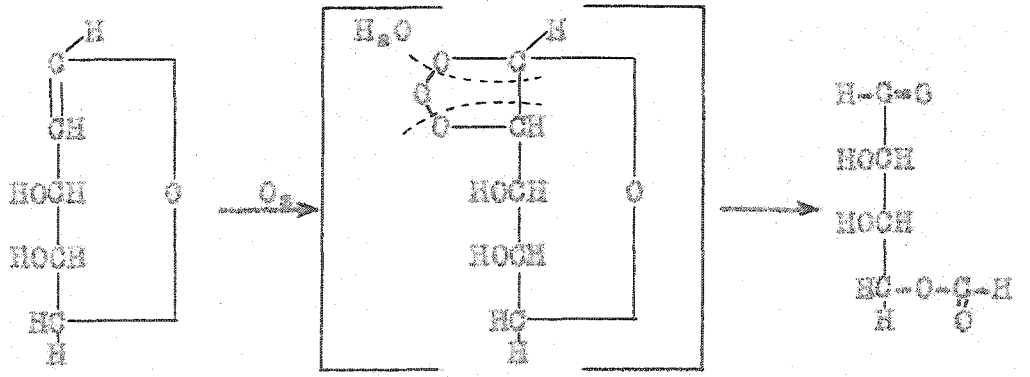
Schiff's reagent. Other tetrose sirups had been reported to give only slowly a positive Schiff's test. This quick coloration led to the suspicion that a more reactive aldehyde group was present than could be accounted for by the erythrose and brought up the question of how the action of ozone on arabinal proceeded. Two possible courses were deemed most likely. They are shown in the following equations.



l-arabinal

EQUATION I

l-erythrose



l-arabinal

EQUATION II

4-formyl l-erythrose

*No attempt was made to isolate any ozonide. The reaction was carried out in glacial acetic acid and this solvent facilitates the hydrolysis of the ozonide.

If the reaction proceeded according to equation I, the end products would have been erythrose and either carbon dioxide or formic acid. All of the previous investigators who had used this reaction seemed to be of the opinion that the reaction proceeded according to this scheme. Fischer, Bergmann, and Schotte (21) said that the ozone treatment of triacetyl glucal yielded a triacetyl arabinose. They made no attempt to isolate the triacetyl compound or to determine its acetyl value but hydrolyzed their preparation by refluxing with 0.05 N hydrochloric acid. The product of hydrolysis was identified by the preparation then of the arabinose benzylphenylhydrazone and of the arabinose *p*-bromophenylosazone. These derivatives proved that arabinose had been formed and that the double bond therefore in the triacetyl glucal was between carbon atoms 1 and 2 but they did not give any clue as to what had become of carbon atom 1 of the triacetyl glucal. The investigators made no statements as to how the ozone reaction proceeded but from their mentioning that a triacetyl arabinose had been formed it seems logical to conclude that they considered the terminal carbon atom to have been oxidized off completely.

F. Micheel (25) in proving the structure of digitoxose used as one step the ozone splitting of the unsaturated diacetyl digitoxoseen. The sirup remaining was designated as diacetyl d-ribomethylose. In this work just as in Fischer's the product was identified by preparing an osazone derivative after dilute acid hydrolysis. Thus the configuration of the

sugar and also the position of the double bond were proven but no light was cast upon the mechanism of the reaction with ozone.

Freudenberg's (23) report of the preparation from diacetyl d-xylal of a crystalline diacetyl d-threose and from d-xylal of a crystalline d-threose seemed to incontrovertibly prove that the reaction proceeded according to equation I. This evidence, however, is questionable in the face of the reports of Hockett* who prepared threose sirups by both Ruff's and Wohl's methods. These sirups gave rotations that differed from Freudenberg's preparation not only in magnitude but also in sign. Hockett was aware of the rotation published by Freudenberg and he therefore checked his rotations carefully. It then seemed evident that Freudenberg either misread the sign of the rotation of his product or else the rotation was that of a different compound.

It therefore was evident that previous use of ozone to split double bonds in sugar derivatives offered only doubtful evidence for the reaction proceeding according to equation I.

If the reaction was correctly portrayed by equation I, there would be formed in addition to erythrose either carbon dioxide or formic acid. Carbon dioxide would be formed, if the formyl group resulting from the hydrolysis of the ozonide was oxidized further by ozone or hydrogen peroxide which was

*Hockett's findings were communicated to the 1934 fall meeting of the American Chemical Society but have not yet been published.

formed when the ozonide split. Formic acid would result, if the formyl group were hydrolyzed from the sugar residue. It seemed extremely unlikely that any appreciable amount of such hydrolysis should take place in view of the fact that acetyl derivatives under the same conditions were unaffected. That the formyl group should be oxidized to carbon dioxide seemed to be possible, however, no trace of carbon dioxide was found either in the stream of gas passing through the reaction vessel during the ozonization or in the gas given off during the ozonide hydrolysis treatment.

The formation of erythrosazone by the action of phenylhydrazine on the erythrose sirup prepared from arabinal showed conclusively that some free erythrose had been formed. The osazone formation took place in dilute acid solution and without heating so that any formyl group present would not have been hydrolyzed. Furthermore the rotation of the unpurified sirup agreed fairly well with those previously reported for l-erythrose as can be seen from the following table.

TABLE I

Investigator	Substance	Initial $[\alpha]_D^{\theta}$	Equilibrium $[\alpha]_D^{\theta}$
Ruff	d-erythrose	+1.	-14.5
Wohl	l-erythrose	--	+32.7
Ruff	"	+2.4	+21.5
Weermann	"	0	+23.6
Felton	crude "	+24.6	+31.2
Felton	purified " *	+11.5	+30.5

* Prepared by hydrolysis of acetone methyl erythroside.

The osazone formation and the rotation of the crude erythrose sirup were the evidence in favor of equation I as portraying the ozone reaction.

Facts which could be explained only by accepting equation II as accounting for an appreciable amount of the degradation were gradually accumulated. As has been mentioned there was no evolution of carbon dioxide at any stage of the reaction. Quite large samples of arabinal were ozonized for these tests so that a large volume of carbon dioxide would have been formed, if the reaction proceeded with the complete oxidation of the terminal carbon atom.

The crude erythrose sirup showed a very great reactivity. It would color immediately Fuchsin sulfurous acid a very intensive red. Samples of erythrose which were known to be free of any substituting group colored the same reagent only very slowly.

Alkali would immediately turn the crude erythrose sirups an intensive yellow color. This test has been used to differentiate between derivatives that might occur either with a free aldehyde or with a ring structure (26). Those derivatives that contain free aldehydes are known to be very sensitive to alkali. This sensitivity makes it impossible to determine the acetyl value of the erythrose sirup.

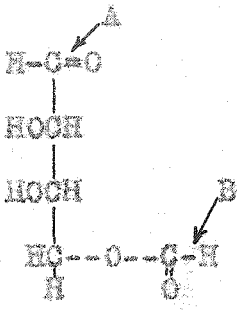
Attempts to acetylate the crude erythrose sirups also showed the great instability of the product. Even with the use of cold pyridine and acetic anhydride the reaction mixture

became immediately a deep red color. No product could be obtained after the completion of the reaction. Similar results (unpublished) were obtained by Freudenberg on attempting to acetylate the d-threose sirups prepared from d-xylal. This result can be contrasted with the successful acetylating of threose sirups prepared by Ruff's method (Hockett unpublished).

If the crude erythrose sirup were treated with phosgene in trying to prepare the carbonate, the material was completely decomposed yielding only a tarry substance.

The only reactions which would yield any quantity of compound that could be purified at all either by distillation or crystallization were those in which methyl alcohol and an acid were used. These reagents will not only react with glycosidic hydroxyls but will also protect free aldehyde groups. There were at least two products from the reaction between the crude erythrose sirup and methyl alcohol containing 0.75 per cent hydrochloric acid. After distillation in a vacuum one product crystallized. The properties and analysis showed this compound to be the methyl glycoside of a desoxypentose instead of the sought for methyl erythroside. The oily part of the distillate was very unstable. Immediately after the distillation the sirup would not reduce Fehling's solution but within a few hours the test became positive. The sirup would also change from a colorless to a yellow liquid. The methoxy content of the total mixed distillate was much higher than

that for the crystalline desoxypentoside alone and also much higher than for methyl erythroside. The high methoxy value was probably due to the full acetal of 4-formyl erythrose. It was not possible to obtain the sirupy product alone as the only separation of the crystals from the oil that was successful was the absorption of the oil by a porous clay plate. The oil could not be extracted from the plate because it was so quickly decomposed.



4-formyl erythrose

The difference in the action of the sugar aldehyde (A) and of the formic acid aldehyde (B) should be noted. The sugar aldehyde will reduce Fehling's solution whereas the formic acid aldehyde will not. On the other hand the formic acid aldehyde will reduce a hot mercuric chloride solution whereas the sugar aldehyde will not. By the use of these tests the presence or absence of a formyl group in erythrose derivatives was determined.

The crude erythrose sirups would reduce both Fehling's solution and a mercuric chloride solution. The freshly distilled methylated product would not reduce Fehling's solution until after it had been hydrolyzed by acid but it

would reduce mercuric chloride. These results showed the presence of a formic acid aldehyde and of a sugar aldehyde which was protected by a group that was sensitive to acid. After treating the methylated product with barium hydroxide the resulting sirup could be distilled. This alkali hydrolyzed methylated sirup would then no longer reduce mercuric chloride but its action against Fehling's solution was the same as it was before the barium hydroxide treatment. These findings showed that there was a formyl group present in the crude erythrose sirup and in the methylated sirup and that this group was removed by the alkali hydrolysis.

The glycosidic hydroxyls of sugars can be methylated not only by methyl alcoholic HCl but also by the use of diazomethane. These two methylating agents differ, however, with respect to their action on aldehyde groups. Methyl alcohol containing HCl will form a dimethyl acetal with an aldehyde. Diazomethane forms ketones from aldehydes (20). The crude erythrose sirup yielded with diazomethane no methylated derivative, in spite of the fact that a reaction took place between the two. This result added still more evidence to confirm the belief that the main product of the ozone reaction had the properties which would be expected to be shown by 4-formyl erythrose.

From the reactions of the erythrose sirup with the above mentioned methylating agents, it can be concluded that sugar derivatives, in which only three or four membered rings are possible, react in an aldehyde form and not in a ring form

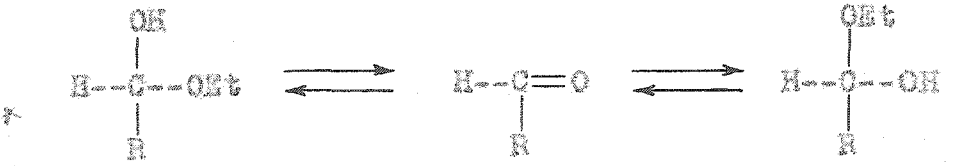
at least with methyl alcoholic HCl. It has been shown by Brigl (26) that 3,4,5,6 tetrabenzoyl aldehydo-glucose reacts with diazomethane as an aldehyde and not as a ring sugar derivative. It has been known for several years that sugars react frequently with five and six membered rings and recently Micheel (27, 28) has prepared by an ingeneous synthesis sugar derivatives with definitely proven seven membered rings. Ringless sugar derivatives have been studied by Levene (29), Brigl (30), and Wolfrom (31). Thus it is seen that five, six, and seven membered rings, and ringless derivatives are known and that a three membered ring has been shown not to be voluntarily formed. The 4-formyl erythrose is a substance which could form either a three or four membered ring. The apparent reaction of 4-formyl erythrose in an aldehyde form shows that a four membered ring is not voluntarily formed. Micheel (28) found the 1,6 rings to be stable which was in accordance with the tension theory. It was predicted that the 1,2 and 1,3 rings would be very unstable and not likely to be easily formed.

The present knowledge of the ring forms of sugar derivatives is best shown by the pentaacetates of galactose. There are seven different crystalline pentaacetyl galactoses. These are the pentaacetates of aldehydo galactose, of α- and β-galactofuranose, of α- and β-galactopyranose, and of α-β-galactoseptanose. There are yet possible four more pentaacetyl galactose derivatives which would have 1,2 and 1,3

rings. From the behavior of 3,4,5,6-tetrabenzoyl glucose and of 4-formyl erythrose, it would seem that these unknown acetates of galactose and similar derivatives for other sugars would be difficult to prepare.

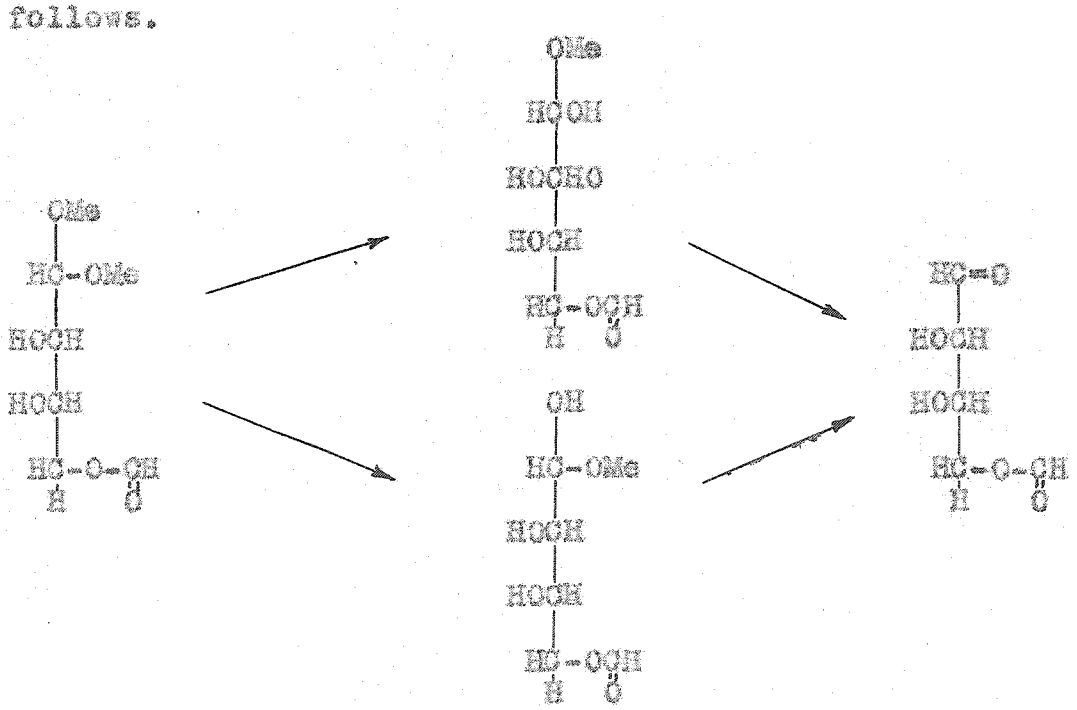
The rotation changes during dilute acid hydrolysis of the impure dimethyl acetal of 4-formyl erythrose were very unusual for sugar derivatives. The rotation increased to a maximum in two hours and then declined gradually for two days. That this unusual change in direction was not due to the known crystalline impurity was determined by running a similar hydrolysis experiment on the purified solid substance. A similar curve is given by the mutarotation of the ethyl hemi-acetal of aldehydo-galactose pentaacetate (52, 53). The two curves are compared on a graph in the experimental section.

The mutarotation of the ethyl hemi-acetal of aldehydo galactose pentaacetate in chloroform was explained by Wolfrom as proceeding according to the following equation.



The change in rotation on hydrolysis of the dimethyl acetal of 4-formyl erythrose can be explained likewise only by a two step reaction. The initial rotation was +52.8°. The final rotation was +39.8°. If the reaction proceeded in one step from the initial to the final product, then the rotation change

would have been constantly downward. The fact that the rotation became more dextro for a time and then less dextro showed that a more dextro intermediary product must have been formed. For this intermediate a methyl hemi-acetal can be postulated. There are two possible methyl hemiacetals, so that the intermediate product can be a mixture of these two. In view of the results obtained on the hydrolysis of mercaptals, it would be expected that one form of the hemi-acetal would predominate. The postulated course of the hydrolysis is as follows.



The treatment of the crude erythrose sirup with methyl alcohol, acetone, sulfuric acid, and copper sulfate followed by the subsequent neutralization with calcium hydroxide and the repetition twice of the same procedure resulted in the preparation of acetone methyl erythroside which could be

sufficiently purified by distillation to yield a product that analyzed as calculated. The alkali used in the reaction undoubtedly accounted for the removal of the formyl group. The methyl group was very stable as compared to the methyl groups of the 4-formyl erythrose dimethyl acetal. The rotation also changed in only one direction during acid hydrolysis.

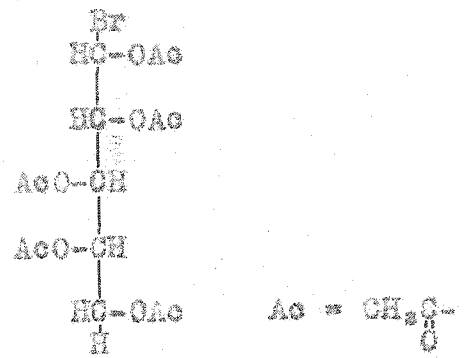
The hydrolysis of acetone methyl erythroside by dilute sulfuric acid yielded a quite pure erythrose sirup. This sirup would reduce Fehling's solution in the cold but gave only a faint Schiff's test and gave no reduction with mercuric chloride. The sirup had a mutarotation of from $+11.6^{\circ}$ to $+30.5^{\circ}$. The erythrose showed no tendency to crystallize and slowly became yellow colored on standing.

NEW SUGAR DERIVATIVES

During the course of the studies on the preparation of erythrose several new sugar derivatives were investigated. These included a second acetobromo arabinose, a tetraacetyl disaccharide derivative of a desoxypentose, and the methyl glycoside of a desoxypentose.

The second acetobromo arabinose was not investigated completely because of the lack of material. The seperation from the β-acetobromo arabinose was quite often not successful. The new compound was more soluble in ether than the ordinary acetobromo arabinose derivative so that it often crystallized as a second crop. The rotation of β-acetobromo arabinose was +288.4°. The rotation of the second acetobromo was about -130°. The closeness of this rotation to that calculated for α-acetobromo arabinose (-110°) suggested that here might be a mixture of α- and β-acetobromo arabinoses. This explanation was completely disproved by the quantitative analysis. The analysis showed a bromine content of 18.19 per cent as compared to 25.6 per cent for α-acetobromo arabinose. The acetyl value was very high. After hydrolysis the resulting sugar formed with benzylphenylhydrazine the benzylphenyl-hydrazone of arabinose. This hydrazone formation showed the bromine containing compound to be an arabinose derivative. The data agreed quite well with that calculated for a pentaacetyl bromo arabinose but the proof was not complete.

A possible structural formula and a comparison of the calculated and experimental value of the bromine analysis are as follows.



Ac = $CH_3C(=O)-$

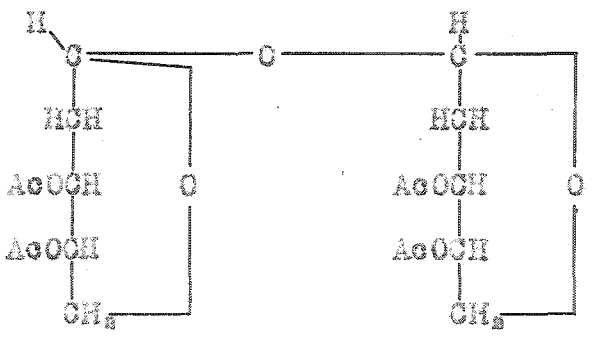
Calculated	Found
% Br	% Br
18.12	18.17

The reduction of acetobromo arabinose yielded two products besides the diacetyl arabinal. The by-product formed in greatest abundance was triacetyl arabinose, which was identified by Gehrke and Aichner (34). A second by-product could be crystallized from the residue which remained after the two volatile products mentioned had been distilled off. This solid product was formed in varying amounts in every reduction. The quantities were usually very small ranging from a few tenths to one gram for each 100 grams of acetobromo arabinose reduced. The properties of this crystalline product were so unique that it was thought worthwhile to investigate it.

The new reduction product did not reduce Fehling's solution. This failure at once suggested that perhaps the bromine had been replaced by hydrogen to form an acetylated anhydro alcohol. Emil Fischer had expected to prepare such

a product by the zinc in acetic acid reduction of acetobromo glucose but he found instead the unsaturated triacetyl glucal. However, a Fehling's test after acid hydrolysis showed considerable reduction. This result eliminated at once the anhydro alcohol possibility as it showed the presence of a reducing group protected by a glycosidic group.

An elementary analysis of the new compound showed it to be halogen free. Its molecular weight was found to be about 400. The molecular weight and properties of a glycoside proved that the compound was made up of the transformation products from two molecules of acetobromo arabinose. The acetyl determination showed the molecule to contain four acetyl groups. Therefore, for a symmetrical product two acetyls were left on each arabinose chain. The carbon-hydrogen values agreed quite well with those calculated for a dissaccharide made up of two diacetyl desoxypentose units joined through their reducing groups. These findings can be depicted by the following structural formula.

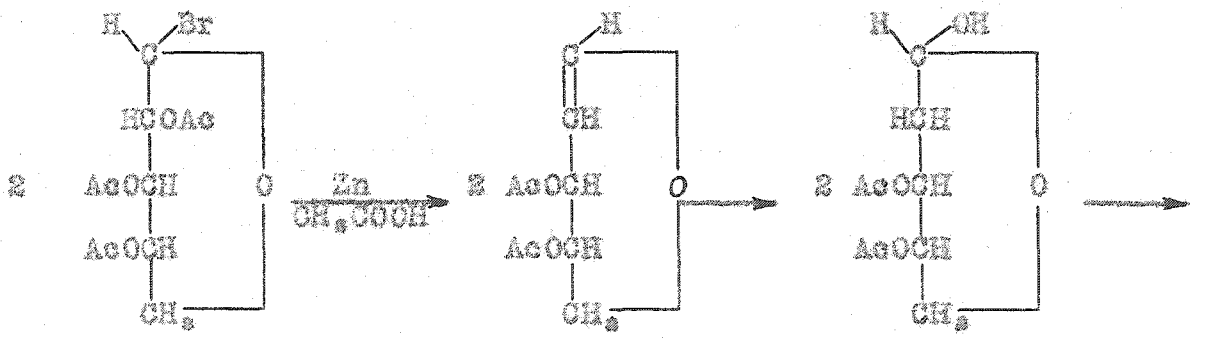


Ac = $CH_3C(=O)-$

Such a compound would be named tetraacetyl l-ribodesoside l-ribodesoside. However, the positions of the desoxy groups

have not been proven. From the mode of formation they would be expected to have the above positions.

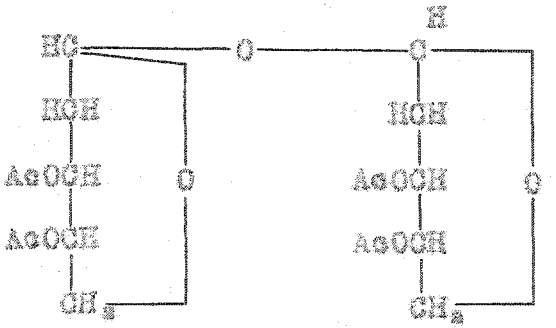
How could such a compound as the one portrayed above be formed? The simplest explanation is that first the diacetyl arabinal was formed. The unsaturated sugars in the presence of dilute mineral acid are known to be transformed into the 2-desoxy sugars. The same transformation might also have occurred to a small extent in the acetic acid solution during the reduction. Two diacetyl desoxy pentose molecules might then lose a molecule of water to form the disaccharide. It seems most likely that this loss of water took place during the distillation of the diacetyl arabinal which occurred in a vacuum at a temperature of over 100°C. These steps are shown as follows.



acetobromo arabinose

diacetyl arabinal

diacetyl 2-desoxy ribose (or arabinose)



tetraacetyl 2-desoxy riboside 2-desoxy riboside

This new disaccharide possessed two different crystalline forms. When it was crystallized from absolute alcohol it melted at 185.5° C. If it were crystallized from chloroform, it melted at 167-169°. When crystallized from 95 per cent alcohol, it came down sometimes in the one form and sometimes in the other. The difference in melting points was not due to solvent of crystallization as both forms exhibited exactly the same rotation $[\alpha]_D = +69.3°$.

By removing the acetyl groups with alkali the parent disaccharide was obtained. The analysis agreed quite well with that calculated for a desoxypentose disaccharide.

All attempts to prepare a benzylphenylhydrazone failed. The acetyls were removed and the disaccharide linkage was split by dilute hydrochloric acid. The resulting sirup was quite dark colored. This decomposition was not altogether unexpected because of the known sensitivity of desoxy sugars to acids.

As was mentioned before, in attempting to prepare methyl erythroside a crystalline compound possessing glycosidic characteristics was obtained. The methoxy and carbon and hydrogen analysis gave values corresponding to those which were calculated for a methyl desoxypentoside.

In explaining the formation of a desoxy pentose the fact that the arabinal used was a pure crystalline compound must be considered. The pure arabinal precludes any possibility of the desoxy pentose having been carried along as an impurity through several steps. The most logical explanation seemed to be that the desoxy sugar was formed under the

influence of the acetic acid used as a solvent for the ozone splitting.

Hydroarabinal was prepared in order to complete the reduction products of the unsaturated pentoses. Its diacetyl derivative was already known as also were the dihydro xylal and the diacetyl dihydro xylal.

The reduction was carried out in alcohol solution using paladium black as a catalyst. The arabinal quickly took up an equivalent of hydrogen. The product obtained boiled under a pressure of 1 mm. at 85-85° C. It had a rotation of $[\alpha]_D = 48.3°$ and a refractive index of $n_D = 1.4848$.

EXPERIMENTAL

Preparation of l-Arabinose.

The arabinose was prepared from mesquite gum by the procedure of Anderson and Sands (35). For the preparation of acetobromo arabinose a sufficiently pure product was prepared by warming with glacial acetic acid, cooling, filtering, and washing with methyl alcohol.

Preparation of Acetobromo Arabinose.

The acetobromo arabinose was prepared according to the directions of Meisenheimer and Jung (36). Much larger quantities of arabinose were used which necessitated some slight changes in technique. In brief the method employed consisted in the suspension of 100 grams of arabinose in 500 cc. of acetic anhydride, maintained at 0°C, and the introduction thereto of dry hydrobromic acid gas until saturated. The HBr was prepared by dropping bromine onto red phosphorus covered with water and then passing the gas over moist red phosphorus to free it of bromine vapor. The yields could be raised by from five to ten per cent over those previously reported for this method with corresponding quantities by allowing the reaction mixture to stand over night at room temperature. When good yields were obtained the acetobromo arabinose always began to crystallize out of the acetic anhydride solution during the over night storage.

The acetic anhydride solution was worked up by diluting

with 1500 cc. of chlorofrom and then washing three times with 500 cc. of ice cold water. The solution was dried over calcium chloride and the solvent evaporated off in a vacuum. The acetobromo arabinose crystallized during the evaporation of the solvent. The crystals were washed from the distilling flask with ether and the entire mass placed in the ice box for at least twelve hours in order to complete the separation. The acetobromo arabinose was then filtered with suction and washed with ice cold ether. The while crystals remaining melted at 139°, were quite pure, and served very well for the reduction to diacetyl arabinal. The rotation of a sample recrystallized from ether was $[\alpha]_D = +288.3^\circ$ in chloroform. The average yield was 90 grams which was about 40 per cent of the theoretical. The mother liquor was used to prepare a second crystalline acetobromo arabinose.

Preparation of a New Acetobromo Arabinose.

The ether was evaporated from the mother liquor after the crystallization of the β-acetobromo arabinose. The residual sirup was then placed in an ice box until another crop of crystals formed. This second crop was sometimes only another quantity of the ordinary acetobromo arabinose but occasionally a new acetobromo arabinose was obtained. The yields varied upward to five grams from 100 grams of arabinose. The product could be recrystallized with fair success from ether. It then had a melting point of 132°. The rotations in

chloroform of four different preparations were as follows.

TABLE OF ROTATIONS

Sample grams	Length of tube dm.	Temp. degrees	α	$[\alpha]_D$
0.1089	1.1	31	-1.55	-129.1
0.2898	1.1	38	-4.22	-132.1
0.1623	1.1	23	-2.34	-130.9
0.2416	1.1	23	-3.59	-134.6

The rotation of the possible α-acetobromo arabinose, calculated according to Hudson's values, was found to be approximately $[\alpha]_D$ = -110. The method of preparation and the somewhat similar rotations at first suggested the possibility of this second compound being α-acetobromo arabinose. In view of the fact that only one halogeno-acetyl derivative of any aldose sugar was known* the above mentioned compound seemed to be worthy of investigation. Hudson (39) in discussing the halogeno-acetyl forms said "the discovery of the so-called α-forms, isomeric with the common β-halogeno-acetyl sugars, will unquestionably open a rich field of synthetic exploration in the sugar group". It was later found that some of the then designated bromoacetyl sugars were in reality α-forms but in no case are an α- and β pair known.

The bromine analyses of the new acetobromo arabinose averaged 18.16 per cent as compared with 23.6 per cent

*Fischer and Armstrong (37) reported a description of two forms of acetobromo glucose but Fischer (38) later was unable to reproduce the preparation of the α-form.

calculated for a triacetylbromo arabinose. The value agreed quite well with that calculated for a pentaacetylbromo arabinose which would have 18.12 per cent bromine.

TABLE OF BROMINE ANALYSES

Sample grams	AgBr grams	Br %	Br calc. for pentaacetyl %	Br calc. for triacetyl %
0.2403	0.1038	18.25	18.12	23.6
0.2647	0.1155	18.22	18.12	23.6
0.1858	0.0787	18.03	18.12	23.6

The bromine analyses immediately proved that the compound was not α-acetobromo arabinose.

Only one complete carbon and hydrogen analysis was run because of the lack of material. In a second determination the carbon dioxide absorption tube was spoiled. The values agreed approximately with that calculated for pentaacetyl-bromo arabinose.

CARBON AND HYDROGEN ANALYSIS

Sample grams	CO_2 grams	H_2O grams	Found %C	Found %H	Calc. %C	Calc. %H
0.1842	0.2701	0.0743	40.01	4.52	40.81	4.79
0.1081	--	0.0445	--	4.61	--	4.79

The acetyl determination gave a very startling result. More alkali was used to neutralize the acid formed by the hydrolysis than would have been required if the sample had been made up entirely of acetic acid and hydrobromic acid no matter in what proportion they might have been mixed. This

result could only be explained by the decomposition of the sugar residue into acids during the alkali treatment.

ACETYL DETERMINATION

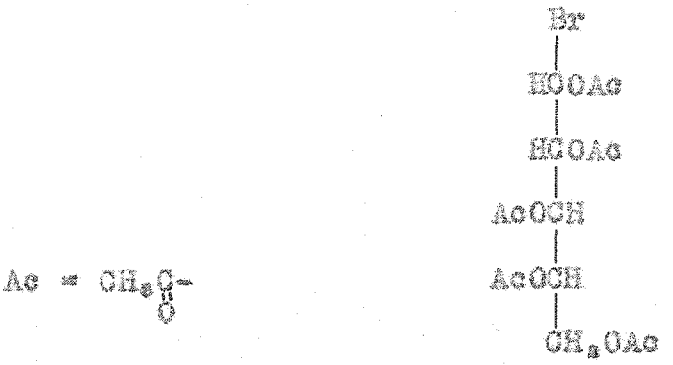
Sample	Found 0.1 N NaOH	Calc. for pentaacetyl bromo arabinose	% NaOH
grams	cc.	cc. 0.1 N NaOH	%
0.2503	42.6	32.7	70.8
0.2647	45.9	38.0	69.4
0.2986	52.9	40.6	70.86

Another noteworthy point brought out was the fact that the solution during the hydrolysis turned a very deep yellow color. Alkali did not cause β-acetobromo arabinose solutions to become yellow during hydrolysis. The yellow coloration with alkali indicated the formation of a free aldehyde during the reaction.

A sample of the second acetobromo arabinose was hydrolyzed by refluxing with 0.005 N HCl until in solution. The solvent was evaporated off in a vacuum and arabinose benzylphenyl-hydrazone was prepared from the residual sirup by treating with an equivalent amount of benzylphenylhydrazine hydrochloride and sodium acetate in 75 per cent alcohol solution. The hydrazone was identified by its melting point of 172-174°.

The arabinose benzylphenylhydrazone formation proved the compound to be an arabinose derivative. The bromine analysis and inconclusive carbon and hydrogen determinations all pointed toward a pentaacetyl-bromo arabinose. Since the high acetyl determination could only be explained by the formation of

acids from the arabinose part of the molecule, it was apparent that the new acetobromo derivative had a different structure than β-acetobromo arabinose and that the arabinose was present in a form which was very sensitive to alkali. The results of the reactions and analyses of the new acetobromo arabinose suggested the following structural formula.



Preparation of Diacetyl Arabinal.

The diacetyl arabinal was prepared according to the procedure used by Levene and Mori (40) for the corresponding xylose derivative. The yield of diacetyl arabinal from 90 grams of acetobromo arabinose averaged 34 grams, if a few drops of chloroplatinic acid were used as a catalyst (24). The diacetyl arabinal was purified by distillation under reduced pressure.

Preparation of a Tetraacetyl Desoxypentose Disaccharide.

After the distillation of the diacetyl arabinal and a second fraction consisting of triacetyl arabinose (34), using a maximum bath temperature of 150°C and a pressure of 2 mm., the residue remaining in the distilling flask would crystallize. If 90 grams of acetobromo arabinose had been used, then the

residue was taken up in 10 cc. of warm 95 per cent alcohol. The alcohol solution was allowed to remain at room temperature during the crystallization of the new disaccharide. If the solution were cooled to 0°, a sirupy impurity was precipitated along with the crystals. Seed crystals were occasionally needed to induce the separation of the desoxypentose derivative. The yield varied from 0.1 to 1 gram for each preparation from 90 grams of acetobromo arabinose. The quantities obtained were not constant but some of the compound always was secured.

The compound was easily soluble in chloroform, warm alcohol, ethyl acetate, and acetic acid, slowly soluble in benzene, insoluble in cold alcohol and ether, and very insoluble in water.

Recrystallization could be carried out very easily from 95 per cent alcohol. The solid substance was dissolved in the smallest possible amount of hot alcohol. The solution was filtered while hot through a warmed funnel. The crystallization occurred very rapidly and completely at room temperature. The crystals would come out in two different forms. One form melted at 167-169° and the other at 184.5 to 185.5°. It was impossible to predict which form would be obtained. Since the compound showed no loss in weight on drying, the different forms were not due to solvent of crystallization. Further proof that no solvent was included in the solid was given by the rotations which were practically the same for both forms.

ROTATIONS OF TETRAACETYL DESOXYPENTOSE DISACCHARIDE

M.P. °C	Sample grams	α	$[\alpha]_D$
164.5-165.5	0.1705	+1.50	+69.2
167-169	0.1774	+1.36	+69.5

The samples were dissolved in chloroform and made up to a volume of 9.93 cc. The rotations were measured in a tube 1.1 cm. long.

After the evaporation of the chloroform both samples melted at 167-169°. These results showed that in spite of the different melting points of the two forms used both went into the same form in solution and therefore gave the same rotations and the same melting points after the solvent had been removed.

A molecular weight determination was made using benzene as solvent. 0.5706 grams of compound dissolved in 80.5 grams of benzene gave a freezing point lowering of 0.094°. The molecular weight was found to be 590.

$$\text{Molecular weight} = \frac{(0.5706)(5.12)(1000)}{(0.094)(80.4)} = 390$$

The compound was tested for halogen by the usual sodium fusion method. No halogen was found. A test with bromine showed that there was no unsaturation in the tetraacetyl disaccharide. Fehling's solution was not reduced until after the compound was hydrolyzed with acid. These tests showed that the substance under investigation contained no halogen, free aldehyde group, or double bonds but did contain a

glycosidic linkage.

Acetyl determinations and carbon and hydrogen analyses gave the following results.

ACETYL DETERMINATIONS

Sample grams	0.1 N alkali cc.	$CH_3C{=}O$ %
0.1943	18.46	40.7
0.2609	25.7	40.7

CARBON AND HYDROGEN ANALYSES

Sample grams	CO_2 grams	H_2O grams	C %	H %
0.2045	0.3903	0.1118	52.08	6.12
0.1100	0.2069	0.0612	51.44	6.25

The data presented compare with that calculated for a tetraacetyl desoxypentose disaccharide as follows.

	Calc.	Found
%C	51.65	51.44, 52.08
%H	6.26	6.25, 6.12
$\%CH_3C{=}O$	41.1	40.7, 40.7
Mol. wt.	418.	390

The identification of the compound as a tetraacetyl desoxypentose disaccharide was strengthened by the removal of the acetyl groups and the subsequent analysis of the deacetylated derivative. If the compound had even only one more oxygen, as the low carbon, hydrogen and acetyl values indicated, then the relative changes in analysis would have been quite different from those which were found. It would

have required a carbon content of almost 3 per cent less than was calculated and found for the deacetylated product.

Attempts to prepare a benzylphenylhydrazone of the parent sugar were unsuccessful. A small sample was refluxed with 0.005 N hydrochloric acid until in solution. The solution was then evaporated under reduced pressure and the residue treated with the calculated equivalent amount of benzylphenylhydrazine hydrochloride and an excess of sodium acetate. The reaction was always carried out in 75 per cent alcohol solution. The acid hydrolysis seemed to cause a great deal of decomposition which may account for the lack of success in securing a benzylphenylhydrazone.

Preparation of Desoxypentose Disaccharide.

Three tenths gram of tetraacety desoxypentose disaccharide was deacetylated by dissolving in 25 cc. of water containing 1 gram of barium hydroxide. The solution was freed of excess barium hydroxide by bubbling carbon dioxide through it. The barium carbonate was filtered out and the water evaporated off under diminished pressure. The residue which still contained some inorganic salts was extracted several times with warm absolute alcohol. The alcohol was then removed in a vacuum and the residue extracted with cold water. The water was slowly evaporated in a desiccator over phosphorous pentoxide. After the water had been removed the compound solidified. The yield was 0.16 gram or 90 per cent of the theory. After

recrystallization from isopropyl alcohol and ether the compound melted at about 177-180°. The melting point was not sharp and there was also a great deal of decomposition.

The desoxypentose disaccharide would not reduce Fehling's solution until after acid hydrolysis.

The carbon and hydrogen analyses gave the following results.

Sample mgs.	CO_2 mgs.	H_2O mgs.	Found %C	Found %H	Calc. %C	Calc. %H
2.596	4.606	1.812	48.41	6.94	48.00	7.25
3.226	5.654	1.966	47.80	6.81		

Preparation of a Tetraacetyl Arabinose.

In one preparation of diacetyl arabinal in which no catalyst was used the reaction went only to a slight extent to the formation of diacetyl arabinal. From 45 grams of aceto-bromo arabinose less than 10 grams of diacetyl arabinal was obtained. A large amount of a crystalline acetylated arabinose was obtained. After recrystallization from 95 per cent alcohol it had a melting point of 95-97°.

CARBON AND HYDROGEN ANALYSES

Sample grams	CO_2 grams	H_2O grams	Found %C	Found %H	Calc. %C	Calc. %H
0.1572	0.2801	0.0820	48.59	5.84	49.03	5.70
0.1537	0.2746	0.0797	48.72	5.67	49.03	5.70

The acetyl determination showed 53.9 per cent $CH_3C{=}O$ as compared with the calculated 54.1 per cent.

The rotation of a 0.1299 gram sample made up to 9.93 cc. in chloroform gave an α = +2.103 and $[\alpha]_D^{25}$ = +146.6.

The rotation and melting point were compared with those for the known α- and β-tetraacetyl arabinoses.

MELTING POINTS AND ROTATIONS OF VARIOUS TETRAACETYL ARABINOSES

	Rotations $[\alpha]_D$	M.P. °C
α-tetraacetyl (41)	+42.9	97
β " (41)	+147.2	86
above described	+140.8	95-97

The melting point was found to agree with that given for α-tetraacetyl arabinose and the rotation was practically the same as the rotation of β-tetraacetyl arabinose. In view of the fact that the rotation was more characteristic, it seemed likely that the compound was probably β-tetraacetyl arabinose. The melting point was very carefully checked. No explanation could be offered for the different melting point but in view of the similar rotations it seemed very unlikely that this tetraacetate could be a new tetraacetyl arabinose, although that was possible.*

Ozone Splitting of Diacetyl Arabinal.

Diacetyl arabinal was treated with ozone under the conditions outlined by Freudenberg (10). For a typical

*According to Hudson and Phelps (42) β-acetobromo arabinose was transformed into α-tetraacetyl arabinose by Koenig and Knorr's synthesis which employs silver acetate and glacial acetic acid.

preparation 5 grams of diacetyl arabinal was dissolved in 50 cc. of glacial acetic acid and a stream of oxygen containing ozone was bubbled through the solution until a small test sample would no longer decolorize a carbon tetrachloride solution of bromine. Best results were obtained when the reacting products were slightly cooled. The acetic acid solution on the completion of the reaction exhibited a strong oxidizing effect on moist starch-potassium iodide paper. The solution was then diluted with 300 cc. of ether and 100 grams of zinc dust were added. The flask was cooled, if the zinc reacted too violently with the acetic acid. After the first reaction between the zinc and acetic acid had subsided, the ether was refluxed until the solution would no longer impart a coloration to moist starch-potassium iodide paper. The zinc was then removed by filtration and the solvent evaporated under reduced pressure. The acetic acid was completely removed by evaporating several times with absolute alcohol. The resulting product was an almost colorless sirup which would reduce Fehling's solution slowly even in the cold. A dilute mercuric chloride solution was reduced on warming for one to two minutes. The sirup was soluble in alcohol, chloroform, and hot water but insoluble in benzene, ether, petroleum ether, and cold water. The reaction product slowly became colored on standing and showed no tendency to crystallize. The compound could not be distilled in a vacuum.

Attempts were made to prepare hydrazones from the

ozonization product. For these tests benzylphenylhydrazine hydrochloride, phenylhydrazine hydrochloride, and 2,4-dinitrophenyl hydrazine were used. In each case an oily precipitate was very quickly formed but none of the derivatives would solidify. Semicarbazide hydrochloride likewise failed to give a crystalline derivative.

Before it was realized that the main reaction product must have a free aldehyde group and not a ring structure, the preparation of acetobromo erythrose was attempted. The product obtained was a black tarry mass.

The reaction of the ozonization product with benzyl mercaptan was investigated. Two and two-tenths cc. of benzyl mercaptan was added to 1 gram of the ozonization product dissolved in 2 cc. of benzene. A trace of dry hydrochloric acid gas was added to the reaction mixture. Immediately the solution became slightly turbid due to the separation of water. More HCl was added and the reaction allowed to continue for four hours. The solution was diluted with benzene, washed with water, and dried over calcium chloride. The solvent was then evaporated in a vacuum. The resulting sirup would solidify on cooling with an ice salt mixture but would again melt on warming to room temperature. No solvent could be found from which the reaction product would crystallize. The product would not reduce Fehling's solution. This result was in accordance with the properties shown by mercaptals. There was no doubt but that the benzyl mercaptan reacted very easily to

form a mercaptal but the purification of the mercaptal was unsuccessful.

In view of the fact that the ozonization product would decolorize Schiff's reagent, attempts to prepare a sodium bisulfite addition compound were made. If the aldehyde containing sirup were shaken with a saturated sodium bisulfite solution an evolution of heat indicated that a reaction had taken place but no crystalline product was obtained.

The reaction of the ozonization product with methyl alcoholic HCl was also studied. Five grams of the product was treated with 50 cc. of dry methyl alcohol containing 1 per cent HCl. The reaction was allowed to continue for two days at room temperature. The HCl was removed by shaking the solution with silver carbonate until it reacted neutral to litmus paper. The precipitate was filtered out and the solvent was removed under diminished pressure. The sirup remaining behind was distilled under a pressure of 4 mm. at a bath temperature of from 120 to 130°. About 1.5 grams of product distilled. The distillate was slightly decomposed even when distilled in the absence of air. The compound was quite unstable and would soon become a dark yellow color. It was investigated mainly to determine its acetyl value. The acetyl and formyl groups were only very slightly affected by 0.1 N sodium hydroxide at room temperature. When heated over night at 70 to 80°C with 0.5 N sodium hydroxide the amount of alkali neutralized was higher than was calculated for the dimethyl acetal of 4-formyl,

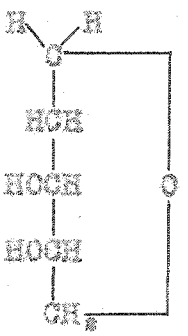
2,3-diacetyl erythrose. An attempt to measure the volatile acid given off by hydrolyzing with 15 per cent phosphoric acid yielded only a trace of acid in the distillate.

Preparation of Arabinal.

The free arabinal was prepared according to the procedure of Meisenheimer and Jung (36) which consisted of the hydrolysis of the acetyl groups from the diacetyl arabinal by a cold barium hydroxide solution. The arabinal was a white crystalline solid which melted at 81 to 82°. One preparation gave a rotation of $[\alpha]_D^{25} = \frac{(2.14)(9.98)}{(0.0975)(1.1)} = -199.5°$ which agreed quite closely with the values reported by Meisenheimer and Jung of -202.6° and by Austin and Humoller (45) of -198°.

Arabinal can not be stored in a desiccator over calcium chloride because of its great sensitivity to acids. It will turn into a dark tarry mass within a few hours.

Preparation of Dihydro Arabinal.



Dihydro arabinal

Two grams of arabinal was reduced in an alcohol solution using paladium black as a catalyst. The reduction was carried

out under only a slight pressure. In one hour most of the arabinal was reduced but the reaction was allowed to continue for seven hours more in order to hydrogenate the arabinal as completely as possible. In eight hours 2 grams of arabinal took up 485 cc. of hydrogen. The catalyst was separated by decantation and filtration. The alcohol was removed by distillation under reduced pressure. The hydroarabinal would not crystallize, however, it distilled very easily. The boiling point of redistilled hydroarabinal was 83-85° under a pressure of 1 mm. The distillate was dried at room temperature in a vacuum desiccator over P_2O_5. Dihydro arabinal was soluble in alcohol, water, and benzene and slightly soluble in ether. It had a refractive index of $n_D^{27} = 1.4848$ and a rotation of +48.2°.

ROTATIONS OF DIHYDRO ARABINAL

Sample grams	Temp. °C	α	$[\alpha]_D$
0.0954	20	+0.454	+48.1
0.1466	23	0.780	48.3

For the rotations the samples were dissolved in water and made up to a volume of 9.98 cc. The tube used was 1.1 dm. long.

CARBON AND HYDROGEN ANALYSIS OF DIHYDRO ARABINAL

Sample grams	CO_2 grams	H_2O grams	Found %C	Found %H	Calc. %C	Calc. %H
0.1273	0.2364	0.0955	50.65	8.40	50.85	8.53
0.1504	0.2744	0.1138	50.01	8.40	50.86	8.53

Ozone Splitting of Arabinal.

The ozone splitting of arabinal was at first carried out exactly as has been described for the splitting of diacetyl arabinal. One disadvantage of this procedure was the fact that the ozonized product was not completely soluble in ether. The freshly prepared sirups were also always yellow colored. An alternate procedure which appeared to be more satisfactory, consisted simply in the omitting of the zinc treatment. The glacial acetic acid solutions, after the bromine test showed no more unsaturation, were immediately subjected to a vacuum distillation. As much of the acetic acid as possible was removed by treating the residue several times with absolute alcohol and then distilling it off. The peroxide effect was destroyed just as rapidly by the distillation as it was by the zinc treatment. The freshly prepared sirups were always almost colorless. They would become yellow colored on standing. Sirups prepared in this way were used for the subsequent reactions.

Samples of the crude erythrose sirup were treated with the calculated equivalent amounts of phenylhydrazine in dilute acetic acid solutions. Erythrosazone was sometimes but not always obtained. After recrystallization from benzene it melted at 162-164°.

The crude erythrose sirups were also treated with acetic anhydride and pyridine according to the customary procedure for preparing acetates with these reagents. The solutions,

even when cooled in ice, immediately became a deep red in color. No pure product could be separated after allowing the reagents to react for the usual length of time.

The erythrose containing sirups were also treated with acetic anhydride and hydrobromic acid in an unsuccessful attempt to prepare an acetobromo derivative. The product obtained reduced silver nitrate solution only very slightly in the cold but very strongly on heating. This result indicated that a bromine containing compound had been formed.

On passing phosgene through an acetone solution of the crude erythrose sirup a rapid decomposition took place. Attempts to separate out the carbonate derivative yielded no product.

Preparation of Acetone Methyl Erythroside.

Five and one-half grams of arabinal was treated with ozone in the manner described above. The resulting sirup was shaken with a mixture of 90 cc. of dry acetone and 10 cc. of dry methyl alcohol containing 0.2 per cent sulfuric acid mixed with 10 grams of anhydrous copper sulfate. The shaking was continued for from four to six hours. Calcium hydroxide was then added and the mixture again shaken until it was no longer acid in reaction to litmus paper. The solution was then filtered from the solid. The solvent was distilled off under reduced pressure and the sirup remaining was distilled in a vacuum of 2 mm. The residue after the vacuum

distillation was treated again by exactly the same process as was described. Only half as large quantities of reagents were used in the repetition.

The distilled products from both reactions were mixed and redistilled. Under a pressure of 2 mm. the boiling point was from 45 to 50° C. The total yield was about 3 grams. The product was not all acetone methyl erythroside as different fractions of the distillate would show varying methoxy contents.

The product obtained would not reduce Fehling's solution until after it had been acid hydrolyzed. It gave no reduction with a mercuric chloride solution. The acetone methyl erythroside was stable and could be stored for long periods of time without acquiring the ability to reduce Fehling's solution.

The fraction whose analysis most closely approximated that calculated for acetone methyl erythroside showed a rotation of about +58.0°.

ROTATIONS OF ACETONE METHYL ERYTHROSIDE

Sample grams	Temp. °C	α	$[\alpha]_D$
0.1338	25	+0.965	+57.4
0.1452	27	0.918	56.0

The samples were made up to 9.98 cc. in chloroform. The rotations were measured in a tube 1.1 dm. long.

The change in rotation during the hydrolysis of the

acetone methyl erythroside by 0.05 N HCl in water was also determined. The acid removes both the methyl alcohol and the acetone groups.

HYDROLYSIS OF ACETONE METHYL ERYTHROSIDE BY HCl

Time	α	$[\alpha]_D$
0	+1.046	+67.
15 min.	1.015	65.1
30 "	1.006	64.3
18 hrs.	0.457	29.3
30 "	0.415	26.6
48 "	0.412	26.4

A 0.1557 gram sample in 9.98 cc. of solution was used for the measurements. A tube 1.1 dm. long at a temperature of 24° served for the readings of the angles of rotation.

CARBON AND HYDROGEN ANALYSIS OF ACETONE METHYL ERYTHROSIDE

Sample grams	CO_2 grams	H_2O grams	Found %C	Found %H	Calc. %C	Calc. %H
0.1775	0.3627	0.1267	55.76	8.12	55.14	8.11
0.1413	0.2843	0.1033	54.87	8.35	55.14	8.11

The methoxy content of the various fractions of acetone methyl erythroside varied from 1.6 per cent more than the theoretical value to 5 per cent less. The higher values may be due to some dimethyl acetal and the lower values to acetone protecting the reducing group instead of methyl alcohol. The fraction showing a low methoxy content also showed lower rotations than those reported above for acetone methyl erythroside. The methoxy data tabulated below was for a

sample with high rotation and of approximately the correct carbon and hydrogen content.

METHOXY ANALYSIS OF ACETONE METHYL ERYTHROSIDE

Sample grams	AgI grams	Found % OCH	Calc. % OCH
0.2167	0.3164	19.42	17.82
0.0977	0.1443	19.51	17.82

Action of Methyl Alcoholic HCl on Erythrose Sirups.

The erythrose sirup obtained by the action of ozone on 5 grams of arabinal was mixed with 40 cc. of dry methyl alcohol containing 0.75 per cent HCl. After standing at room temperature for two days the reaction mixture gave only a trace of reduction with Fehling's solution. The acid was neutralized by shaking with silver carbonate. The precipitated silver chloride was filtered. The solvent was evaporated off under reduced pressure and the sirup remaining was distilled under a pressure of 2 mm. at a bath temperature of 120-130° C. Three grams of product was obtained.

Immediately at the end of the distillation crystals began to appear in the distillate. The product was placed in the refrigerator for several days during which time it became filled throughout with crystals. No solvent was found which would separate the crystals from the oil. The separation obtained by absorbing the oil on a clay plate yielded a pure solid which could be recrystallized from Skelly Solve B or other but it resulted in a loss of the oil which was so

rapidly decomposed that it could not be extracted from the plate.

The analyses of the crystals showed that they were not methyl erythroside, although they did possess the properties of a methyl glycoside. The analysis of this crystalline product will be given later.

Since the sirupy compound could not be obtained alone, it was studied mixed with the crystals which made up only about 10 per cent of the total product. The freshly distilled mixture gave no reduction with Fehling's solution until after it had been hydrolyzed by acid. It did give a strong reduction of a dilute mercuric chloride solution.

Different preparations of the mixed methylated product would have varying methoxy contents. The methoxy values compared as follows with those calculated for methyl erythroside, methyl desoxypentoside, and dimethyl acetal of 4-formyl erythrose.

METHOXY ANALYSIS OF IMPURE DIMETHYL ACETAL OF 4-FORMYL ERYTHROSE

Sample grams	AgI grams	Found % OCH_3	Methyl erythroside % OCH_3	Methyl desoxy-pentoside % OCH_3	4-Formyl erythrose dimethyl acetal % OCH_3
0.1100	0.2811	31.35	23.13	21.0	32.0
0.1616	0.3510	28.66	23.13	21.0	32.0
0.1605	0.3419	28.14	23.13	21.0	32.0

The high methoxy values proved that the sirupy compound was not mainly methyl erythroside. They showed that for a

sugar with four carbon atoms there must be two methoxy groups for each sugar molecule. The lability of the methoxy groups also indicated that they were not present in a glycosidic form as they were hydrolyzed even more readily than methyl furanoside derivatives.

The change in rotation during acid hydrolysis was followed. A solution containing a 0.1496 gram sample was made up to 9.98 cc. in water. Its rotation was measured in a tube 1 dm. long. One drop of concentrated hydrochloric acid was then added and the changes in rotation were measured.

HYDROLYSIS OF IMPURE DIMETHYL ACETAL OF

4-FORMYL ERYTHROSE

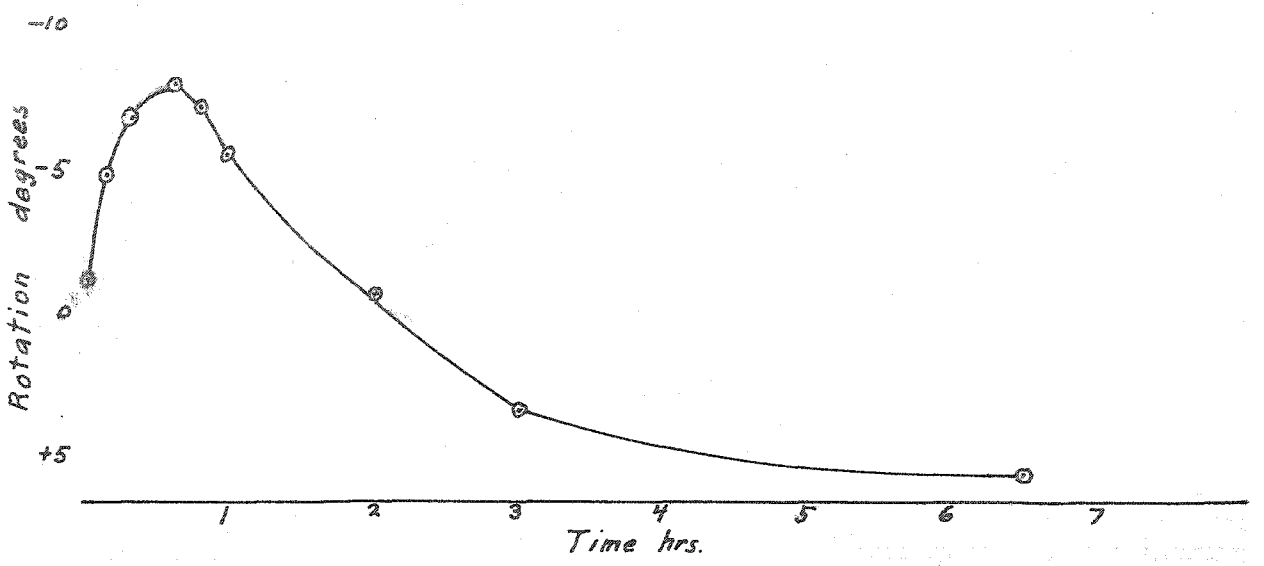
Time.	α	$[\alpha]_D$	
0	+0.88	+53.4	
15 min.	0.975	59.1	
30 "	1.024	62.1	
2 hrs.	1.101	66.7	
3 "	1.049	63.6	
4 "	1.014	61.5	
16 "	0.782	47.4	
40 "	0.684	41.5	
52 "	0.667	40.5	
70 "	0.657	39.8	(Same two days later)

These changes in rotation are compared on graph I with the mutarotation of the ethyl hemi-acetal of aldehydo galactose pentaacetate.

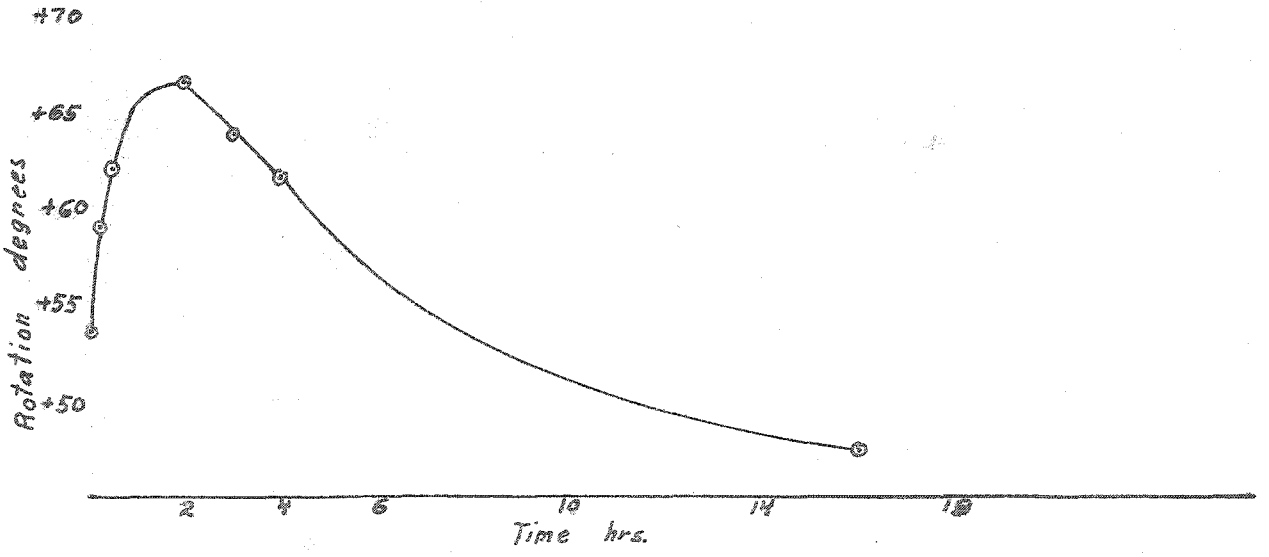
The change in rotation shown by the pure crystalline methyl desoxypentoside treated in the same manner was only very slight for the first four hours so that the quick rise

Graph I

Mutarotation of the Ethyl Hemi-acetal of Aldehydo-Galactose Pentaacetate (33)



Rotation Changes on Acid Hydrolysis of 4-Formyl Erythrose Dimethyl Acetal



and fail in rotation must have been essentially due to the sirupy component.

A small amount of the methylated mixture was treated with an excess of $Ba(OH)_2 \cdot 8H_2O$ dissolved in methyl alcohol (44). The solution at once assumed a bright yellow color, which was probably due to the fact that some of the methyl groups had been lost from the dimethyl acetal, leaving an unprotected aldehyde group which was sensitive to the strong alkali. After allowing the reaction to continue for two days the methyl alcohol solution was filtered and the solvent removed in a vacuum. The residue was extracted three times with warm acetone. The acetone was removed in a vacuum and the sirup remaining was distilled under a pressure of 2 mm. The product obtained would no longer reduce a dilute mercuric chloride solution. It also differed from the starting material in that it became red colored on decomposing rather than yellow.

The action of diazomethane on the sirups resulting from the ozonization of arabinal was tested in order to compare its action with that of methyl alcoholic HCl. The crude erythrose containing sirup was dissolved in a solution of chloroform containing diazomethane. After one day the chloroform was evaporated off. The sirup remaining behind would not distill and was not at all like the product obtained from the reaction using methyl alcohol containing HCl.

Preparation of a Methyl Desoxypentoside.

The crystals obtained by the action of methyl alcohol

containing HCl on the arabinal ozonization product had a melting point of 81-82° after being recrystallized from ether. They melted without decomposing and solidified again on cooling. Their methoxy, carbon, and hydrogen contents agreed with the theoretical for a methyl desoxypentoside.

METHOXY ANALYSIS OF METHYL DESOXYPENTOSIDE

Sample grams	AgI gram	Found % OCH	Calc. % OCH
0.1196	0.1881	20.78	21.0

CARBON AND HYDROGEN ANALYSIS OF METHYL DESOXYPENTOSIDE

Sample mgs.	CO_2 mgs.	H_2O mgs.	Found %C	Found %H	Calc. %C	Calc. %H
5.136	9.248	3.882	49.11	8.46	48.65	8.17
4.759	9.592	3.593	48.95	8.59		
6.437	11.495	4.794	48.70	8.83		

Rotation $[\alpha]_D^{25.7} = \frac{+(0.65)(1.0047)}{(0.005979)(0.5)} = +218.5$ (in H_2O)

Preparation of Erythrose from Acetone Methyl Erythroside.

One gram of acetone methyl erythroside was dissolved in 25 cc. of 0.1 N H_2SO_4. After standing for three days at room temperature 1.5 cc. of glacial acetic acid was added and the sulfate precipitated with the calculated amount of barium hydroxide solution (45). The reaction mixture was filtered through charcoal and tested for excess barium and sulfate. If necessary, small additions of barium hydroxide or sulfuric acid were made until the sulfate and barium were exactly equal.

The water was then removed under reduced pressure. The residue was dissolved in alcohol and filtered. The alcohol was distilled off in a vacuum. The sirup remaining was dried at 74° in a vacuum over P_2O_5. The sirup gave no reduction with mercuric chloride but gave a strong reduction in the cold with Fehling's solution. Fuchsin sulfurous acid was very slowly colored. The erythrose exhibited mutarotation in water.

ROTATION OF ERYTHROSE

Sample	Time	α	$[\alpha]_D$
0.8084	0	+0.365	+11.3
	24 hrs.	+1.03	+30.5 (constant)

SUMMARY

1. The ozone splitting of arabinal yielded mainly 4-formyl erythrose instead of unsubstituted erythrose.

2. 4-Formyl erythrose reacted in an aldehyde form when treated with methylalcoholic HCl, thus showing that a 1-3 ring was not voluntarily formed. This completed the tests to determine whether sugars react of their own accord in an aldehyde or ring form when a 1-2, 1-3, 1-4, 1-5, or 1-6 rings were possible. It had been previously shown that 1-2 rings were not formed and that 1-4, 1-5, and 1-6 rings were formed.

3. Acetone methyl erythroside was prepared and used as a material from which to obtain unsubstituted erythrose.

4. The reduction of acetobromo arabinose yielded, besides diacetyl arabinal and triacetyl arabinose, a tetra-acetyl desoxypentose disaccharide.

5. A desoxypentose formed during the ozone splitting of arabinal in glacial acetic acid solution was separated and identified in the form of its methyl glycoside.

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