1927

Studies with glucose derivatives: Part I, The digestion of pectin and methylated glucoses by various organisms; Part II, The structure of chloralose derivatives

Harold W. Coles
Iowa State College

Recommended Citation

Coles, Harold W., "Studies with glucose derivatives: Part I, The digestion of pectin and methylated glucoses by various organisms; Part II, The structure of chloralose derivatives " (1927). *Retrospective Theses and Dissertations*. 14232.
https://lib.dr.iastate.edu/rtd/14232

STUDIES WITH GLUCOSE DERIVATIVES

PART I- THE DIGESTION OF PECTIN AND METHYLATED GLUCOSES BY VARIOUS ORGANISMS

PART II- THE STRUCTURE OF CHLORALOSE DERIVATIVES

by

Harold W. Coles

A Thesis submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major subject- Plant Chemistry

Approved

In charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State College

1927

ACKNOWLEDGMENT

I wish to express my thanks to Dr. R. M. Hixon of the Chemistry Department and to Dr. Max Levine and Dr. J. C. Weldin of the Bacteriology Department, Iowa State College, for their cooperation and helpful suggestions given during the progress of this work.

TABLE OF CONTENTS

Part I.

Part II.

TABLE OF CONTENTS
(Continued)

PART I- THE DIGESTION OF PECTIN AND METHYLATED GLUCOSES BY VARIOUS ORGANISMS

THE DIGESTION OF PECTIN AND METHYLATED GLUCOSES BY VARIOUS ORGANISMS[1]

Introduction

This preliminary paper is the result of work started with the object of determining the effect of introduced methyl groups upon the digestion of carbohydrates by microorganisms. At the same time, it was hoped that methylated glucoses and pectin might offer a means of distinguishing between *Bact. aertrycke*, *Bact. schotmulleri*, and other closely related forms. These two organisms, up to the present time, are not distinguishable on the basis of morphological, cultural, or physiological characteristics. It was also thought that possibly a relation might exist between the digestion of pectin and the methylated glucoses.

The only paper in the literature recording the digestion of methylated hexoses by organisms is that of Irvine and Hogg (1914) who record a few experiments made with 3-monomethyl glucose. It was found by these authors that living top yeast and macerated extract, prepared from dried Munich bottom yeast, had no action upon the sugar. They also found that, of the

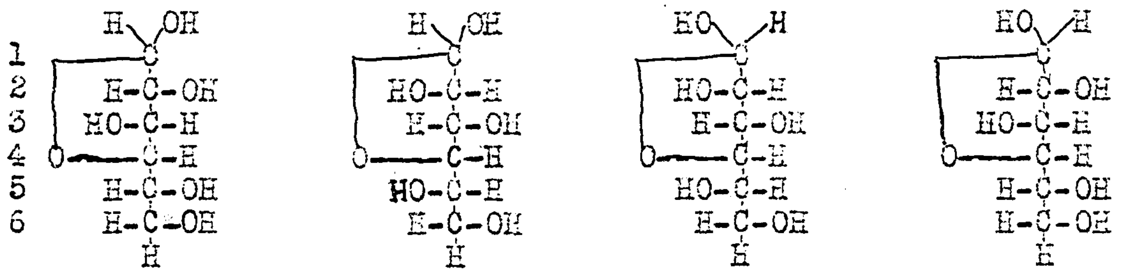
---

[1] Reported at the meeting of the Iowa Academy of Sciences, April, 1926. Published in Plant Physiology, Vol. 1, No. 4, October, 1926.

seven species of bacteria tried, *Bact. coli commune*, *Bact. lactis aerogenes*, *Bact. proteus*, *Bact. paratyphosum*, *Bact. cloacae*, *Bact. typhosum*, and *Staphylococcus pyogenes aureus*, all of which are glucose fermenters, only *Bact. cloacae* Jordan digested the methylated glucose with the production of acid and gas.

Methylated Glucoses

Glucose may have the following structures[2] depending upon the spacial configuration of the molecule:



α-d-glucose α-l-glucose β-l-glucose β-d-glucose

It is seen that we might theoretically have methoxy groups attached to carbons one to six on each of the configurations given. Furthermore, we might have all possible combinations of two to five methyl groups for each configuration, and if include the so-called γ-sugars (compounds in which the oxidic

---

[2] The recent paper of Hirst, Jour. Chem. Soc., London 129: 350. 1926, indicates that the oxide linkage of glucose should be amylene oxidic ⟨1,5⟩ instead of butylene oxidic ⟨1,4⟩ as shown.

linkage is believed to be other than butylene oxidic<1,4> the list is greatly increased.

A simple calculation will show then, that we have theoretically possible the following methylated glucoses: 20 monomethyl glucoses (including the methyl glucosides), 56 dimethyl glucoses, 76 trimethyl glucoses, 52 tetramethyl glucoses, and 20 pentamethyl glucoses, a total of 224 different methylated glucoses, not including the gamma sugars, since each glucose derivative can occur as the α- or β-modification and each of these as dextro- or levo-glucose.

A careful search of the literature revealed that only a very few of these theoretically possible sugars have been prepared. These are tabulated below, the doubtful ones being indicated by a question mark.

Table I

Summary of Methylated Glucoses described in the Literature.

Number of Methyl Groups.				
1	2	3	4	5
α-d-1	α-d-1,3	α-d-1,2,3	α-d-1,2,3,5	α-d-1,2,3,5,6
β-d-1	α-d-2,3	α-d-1,2,5(?)	β-d-1,2,3,5	β-d-1,2,3,5,6
α-d-2(?)	β-d-2,3	α-d-2,3,5	α-d-1,2,3,6	
α-d-3	α-d-2,5(?)	α-d-2,3,6	β-d-1,2,3,6	
β-d-3		α-d-3,5,6	α-d-1,4,5,6(?)	
α-d-6(?)		α-d-2,5,6(?)	β-d-2,3,5,6	
			α-d,2,3,5,6	
			α-d-3,4,5,6	

Materials and Methods

The media used in these investigations were of two kinds: One containing peptone, and the other containing no peptone, and hence no carbon except the carbon of the pectin or the carbon of the methylated glucoses. They were made up as follows:

Media with peptone.	Media without peptone.
2 grs. Bacto-peptone	2 grs. ammonium chloride
1 gr. K_2HPO_4	2 grs. K_2HPO_4
10 cc.1% Andrades Indicator	10 cc.1% Andrades Indicator
3 grs. pectin or sugar	3 grs. pectin or sugar
1000 cc.distilled water	1000 cc. distilled water

The media were adjusted to a pH of 7.0 to 7.2 and tubed.

In all of the work, twenty-four hour glucose-phosphate agar slants were used for inoculation of the culture media. Inoculations in every case were made as heavy as possible, care being especially taken with the media containing no peptone to see that the inoculations were positive. The tubes, after inoculation, were incubated 24 to 38 hours at 37° C., excepting those organisms whose optimum was 25° to 27° C., and any acid, gas or gumminess recorded each day.

The pectin, a very pure form, was obtained from the Research Laboratories of the California Fruit Growers Exchange, and was used as received. It contained, as reported by them, aluminum oxide as an impurity. It was tested for reducing sugar which was found to be absent.

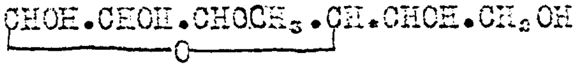
The method followed for the preparation of the 3-mono-methyl glucose was essentially that of Irvine and Hogg (1914).

The reactions involved are:

$$\alpha\text{-d-glucose} \xrightarrow[\text{1\% HCl gas}]{\text{acetone}} \text{diacetone glucose} \xrightarrow[\text{(Freudenberg and Hixon) (1923)}]{\text{sodium}} \text{Na salt}$$

$$\text{of diacetone glucose} \xrightarrow[\text{iodide}]{\text{methyl}} \text{3-monomethyl diacetone glucose}$$

$$\xrightarrow[\text{acid}]{\text{dilute}}$$



$$\text{CHOH.CHOH.CHOCH}_3\text{.CH.CHOH.CH}_2\text{OH}$$

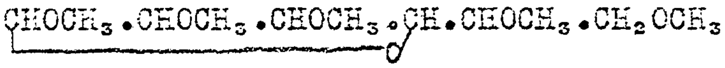
3-monomethyl glucose (I)

The 3-monomethyl glucose thus secured is readily soluble in water, less so in methyl alcohol, and is very sparingly soluble in other organic solvents. It has a melting point of 157-158° C., and a rotation in water of +55.5° (final). It exhibits mutarotation. It was purified by recrystallization from methyl alcohol.

The preparation of 1,2,3,5-tetramethyl glucose and 1,2,3,5,6-pentamethyl glucose may be described together, since they are formed in the same methylation process. The method of Haworth (1915) was used. The reaction occurs as follows:

$$\alpha\text{-d-glucose} \xrightarrow[\text{.25\% HCl}]{\text{CH}_3\text{OH}} \alpha\text{-methyl glucoside} \xrightarrow[\text{NaOH}]{\text{dimethyl sulfate}}$$

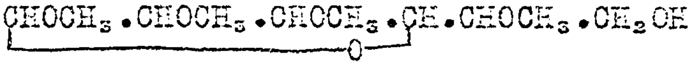
(Bourquelot 1915)



$$\text{CHOCH}_3\text{.CHOCH}_3\text{.CHOCH}_3\text{.CH.CHOCH}_3\text{.CH}_2\text{OCH}_3$$

and

pentamethyl glucose (II)



$$\text{CHOCH}_3\text{.CHOCH}_3\text{.CHOCH}_3\text{.CH.CHOCH}_3\text{.CH}_2\text{OH}$$

tetramethyl glucose (III)

The methylated glucoses thus secured are syrups. They were repeatedly fractionated for purification. They did not reduce Fehling's solution.

The tetramethyl glucose (III) is soluble in water, chloroform, methyl iodide and in common organic solvents. It has a refractive index number equal to 1.4583 and D_4^{20} equal to 1.158. It does not show mutarotation.

The pentamethyl glucose (II) is soluble in alcohol, water, acetone, ether, and methyl iodide. Index of refraction, n_d is equal to 1.4454. It has the following rotations: +147.4° in water and +153.9° in alcohol. It does not show mutarotation.

Description of Organisms

The organisms of group I belong to the colon-typhoid group and were mainly of fecal origin. Those of Group II were obtained from activated sludge from creamery wastes, and Group III are organisms from miscellaneous sources. The figures in parentheses indicate the number of strains used. All cultures which attacked pectin and the methylated glucoses were examined morphologically and culturally to determine their position in the classification of the colon-typhoid group as given by Weldin and Levine (1923).

Group I

Bacterium coli (4), *Bact. paragrunthali* (2), *Bact. communior* (1), *Bact. coscoroba* (2), *Bact. grunthali* (5), *Bact. neapolitanum* (1), *Bact. pseudocoloides* (1), *Bact. schafferi* (4), *Bact. vesiculosum* (2), *Bact. aerogenes* (9), *Bact. cloacae* (7), *Bact. levans* (3), *Bact. oxytocum* (13), *Bact. vulgaris* (6), *Bact. abortivoequinum* (2), *Bact. aertrycke* (1), *Bact. paratyphi* (5), *Bact. flexneri* (6), *Bact. schotmulleri* (4), *Bact. pullorum* (5), *Bact. morgani* (2), *Bact. enteritidis* (3), *Bact.suipestifer* (3), *Bact. ambiguum* (1), *Bact. typhi murium* (1), *Bact. dispar* (1), *Bact. typhi* (2), *Bact. alkalescens* (1), *Bact. sanguinarium* (2), *Bact. anatum* (1), *Bact. pfaffii* (1), *Bact. jeffersonii* (1), *Bact. rettgeri* (1), *Bact. shigae* (1), *Bact. rhinoscleromatis* (1), *Bact. viscosum* (1), *Bact. viscosum aerogenes* (1).

Group II

Bacillus albolactus (2), *B. cereus* (4), *B. mesentericus* (1), *B. panis*, (2), *B. megatherium* (1), *B. aterrimus* (1), *B. niger* (1), *Bseudomonas myxogenes* (1), *Pseud. fluorescens* (1), *Pseud. ovalis* (2), *Pseud. pavonacea* (3), *Flavobacterium ovalis* (2), *Flav. acetylicum* (2), *Flav. suaveolens* (3), *Flav. zettnowi* (1), *Flav. deciduosum* (1), *Serratia rubida* (2), *Ser. rubrica* (2), *Ser. amyloruber* (1), *Bact. metalkalescens* (1), *Rhodococcus coralinus* (1).

Group III

Saccharomyces ellipsoideus (2), *Sac. cereviseae* (1), *Torula rosea* (1), *B. subtilis* (2), *B. sphaericus* (1), *B. mesentericus* (1), *Staphyloccus aureus* (2), *Staph. albus* (3), *Ser. marcescens* (3), *B. fusiformis* (1), *Pasteurella cholera gallivarum* (1), *B. aceto-ethylicus* (2), *Pseud. cyanogenes* (3), *Pseud. pyocyanea* (2), *Pseud. fluorescens* (1), *Sac. pombe* (1), *Past. suiseptica* (1), *Sarcina lutea* (1), *Monilia* *variabilis* (1), *Micrococcus luteus* (1), *B. mesentericus fuscus* (1), *Bact. abortus* (1), *Micr. flavescens* (1).

Results

The cultures which attacked the pectin with the production of acid and gas are the following, the numbers in parentheses being the laboratory numbers of the cultures: Seven out of thirteen strains of *Bact. oxytocum* (305, 369, 499, 139, 261, 269, 270); four out of nine strains of *Bact. aerogenes* (117, 80, 256, 257); two out of two strains of *Bact. viscosum aerogenes* (248, 298); and two out of two strains of *Bact. acetoethylicus* (172, 173). Garden soil and manure produced acid and no gas, and garden soil produced gumminess in both media. All other cultures of Groups 1 to 3 failed to attack pectin with the production of acid, gas or gumminess. There was no difference between the two media, i. e., no culture produced acid or gas in the synthetic non-peptone medium which did not produce it in the peptone medium, and vice versa.

A larger variety of cultures produced acid and gas from the 3-monomethyl glucose. They were: Six out of seven strains of *Bact. cloacae* (233, 235, 236, 264, 136, 263); one out of three strains of *Bact. levans* (238); one out of fourteen strains of *Bact. oxytocum* (499); one strain of *Bact. pseudocoloides* (436); and one out of nine strains of *Bact. aerogenes* (268). Manure gave acid and gas, but garden soil gave acid and no gas.

The tetramethyl and pentamethyl glucoses were not digested by any of the cultures of Groups 1, 2, or 3 with the production of acid and gas. The test with manure was indefinite, a slight acidity being produced with a bubble of gas.

Discussion

From a consideration of the cultures and the cultures digesting pectin with the production of acid and gas, it is evident that only those organisms occurring in the soil are capable of attacking pectin with the production of acid and gas. None of the intestinal forms, classified in the sub-genus Escherichia of the genus Bacterium, produced acid or gas from the pectin. It was to be expected that organisms digesting pectin would be found in the soil, since the rotting of wood and plant tissue, containing pectin bodies, takes place readily in the soil.

Again, it is evident that only those organisms occurring commonly in the soil are capable of attacking 3-monomethyl-glucose with the production of acid and gas. It was expected that very few organisms, if any, would be able to touch the tetramethyl and pentamethyl glucoses. In these two sugars the hydroxyl groups are protected by methyl groups, and this parallels the cellulose molecule in which hydroxyl groups are presumably bound in ether linkages. It seems, therefore, that those organisms of the colon-typhoid group, which are vigorous fermenters of glucose, are unable to attack methylated glucoses when the number of methyl groups becomes large.

It is interesting to speculate just how the number and position of the methyl groups in the sugar molecule affect the digesting ability of the organisms. It is hoped to make these speculations the basis of a later investigation on methylated hexoses.

Conclusions

1. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by those members of the colon-typhoid group commonly found in feces.

2. The organisms attacking pectin and 3-monomethyl glucose with the production of acid and gas were those generally associated with the soil.

3. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by organisms isolated from the activated sludge of creamery wastes.

4. All of the members of the colon-typhoid group tested were incapable of digesting 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose.

5. <u>Bact. schotmulleri</u>, <u>Bact. aertrycke</u> and other closely related forms can not be differentiated on the basis of the digestion of pectin and the methylated glucoses used.

6. It is suggested that an agar medium containing only 3-monomethyl glucose may be useful for the isolation of <u>Bact. cloacae</u>.

BIBLIOGRAPHY

1. Bourquelot, E. (1915). La synthese biochimique des d-glucosides d'alcools monovalents. II. -Alcool-d-glucosides- . Ann. de Chim. (Paris) 3: 287-337.

2. Freudenberg, K., and Hixon, R.M. (1923). Zur Kenntnis der Aceton-Zucker IV: Versuche mit Galaktose und Mannose. Ber. deut. chem. Gesell. 56: 2119-2128.

3. Haworth, W.N. (1915). A new method of preparing alkylated sugars. Jour. Chem. Soc. (London) 107: 8-16.

4. Irvine, J.C., and Hogg, T.P. (1914). Partially methylated glucoses. Part III. Monomethyl glucose. Jour. Chem. Soc. (London) 105: 1386-1396.

5. Weldin, J.C., and Levine, Max. (1923). An artificial key to the species and varieties of the colon-typhoid or intestinal group of bacilli. Proc. Amer. Soc. of Bact., Bact. Abst. 7: 13-15.

PART II- THE STRUCTURE OF CHLORALOSE DERIVATIVES

THE STRUCTURE OF CHLORALOSE DERIVATIVES

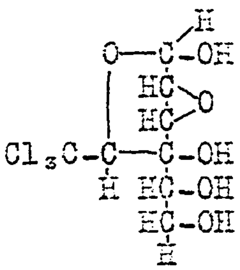
Introduction

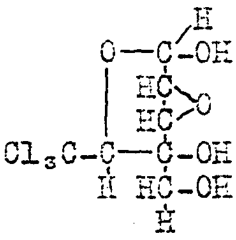
The main difficulty in the study of carbohydrates is the preparation of definite derivatives, the preparation of the desired derivatives being rendered more difficult due to the apparently equal reactivity of the various hydroxyl groups. In recent years, advantage has been taken of the formation of acetal compounds with aldehydes and ketones for muzzling certain groupings and leaving others free for chemical study. These compounds date from the study of Schiff (1888), but their application for the purpose indicated is the work of Fischer, Irvine, Freudenberg, Levene and others, using primarily the acetone hexoses. The scope of such work is limited by the instability of this particular acetal linkage.

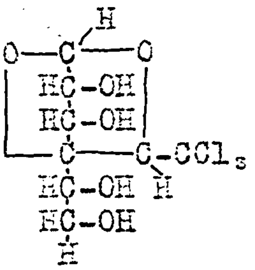
These unions vary greatly in stability. The camphor glucose is hydrolyzed by water (Schiff, 1888, Pg. 21), the acetone glucose is stable to concentrated alkalies, but is hydrolyzed by extremely dilute acids. The chloral glucose is so stable to acids (Pictet and Reichel, 1923, page 625) that it can be oxidized with the loss of carbon dioxide to a pentose with concentrated nitric acid, the derivative still retaining the chloral group. Because of this stability, it was concluded in the older literature that the chloral derivatives must have the chloral group joined to the sugar by a carbon to carbon linkage. From other studies in this laboratory, it seemed probable that this was not the case, but that the chloral condensation paralleled that of the other carbonyl groupings. Evidence of the structure of β-glucochloralose and β-xylochloralose is presented in this paper.

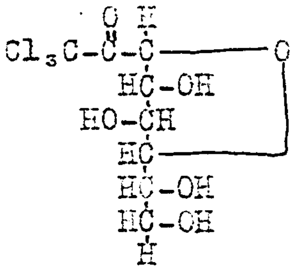
Historical Survey

Two crystalline chloral derivatives of glucose were reported by Heffter (1889) but were not named, and were further studied by Meunier (1906). A detailed study of the chloral derivatives of the various carbohydrates was made by Hanriot (1909) and by Hanriot and Richet (1892 and following papers) and formula I proposed for beta-glucochloralose and formula II for beta-xylochloralose. These are the formulae given in such handbooks as Meyer and Jacobson, Tollens, and von Lippmann.


I
β-glucochloralose


II
β-xylochloralose


III
β-glucochloralose


IV
β-glucochloralose

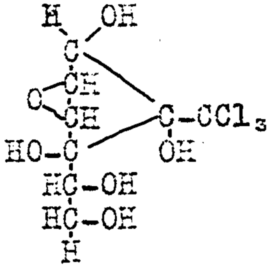
The position of the oxygen linkage was changed by Petit and Polonowski (1894) as shown in formula III. The possibilities of stereo-isomerism in this group of compounds were suggested by M. Combes during the reading of Hanriot and Richet's (1893) paper. Quite recently, Pictet and Reichel (1923) have obtained alpha-glucochloralose and beta-glucochloralose by the addition of chloral to levo-glucosan and glucosan respectively, and they advance formula IV for the beta-glucochloralose. This is the formula given by Pringsheim (1925).

Discussion of Structure of Beta-glucochloralose

Since chloralose, beta-chloralose and all their derivatives contain the three chlorine atoms of the chloral, it is evident that the chloral unites by its aldehyde group with glucose, and that the chloralose has included the group $>\overset{H}{C}—CCl_3$. On the other hand, the absence of reducing properties, the inability to combine with hydroxylamine and phenylhydrazine, and the resistance to hydrogenation point to the fact that chloralose compounds do not contain the free aldehyde group of the glucose nor of the chloralose. The two aldehyde groups of chloral and of glucose have therefore been modified in this union.

This fact was recognized by Hanriot and co-workers (1893,c) but they could not reconcile the stability and the volatility of the chloralose with a structure showing the compound as an ether of glucose, and for this reason assigned it the carbon to carbon linkage shown in formula I. This structure, however, seems out of the question since the ethylene oxide union would not be stable to acids and the condensation of chloral as represented would bear no analogy with Fischer's conclusions on such condensations of aldehydes and ketones with the carbohydrates.

Beta-chloralose forms a tetra-substituted ester, and even though Hanriot and Richet employed a great excess of acid chloride and, in addition, zinc chloride, they were unable to secure a pento-substituted ester. This fact renders a formula such as that represented by formula V as very improbable.

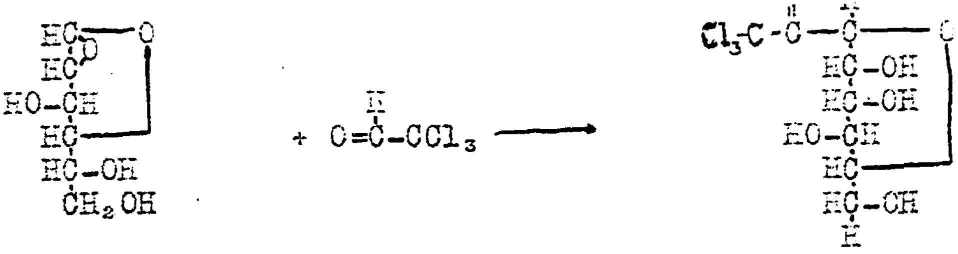


V

β -glucochloralose

Furthermore, the anhydric oxygen is not located on carbons 5 or 6 for, on oxidation, the $CH_2OH-CHOH$ is destroyed, giving the same acid that is secured by oxidation of beta-xylochloralose, and the acid formed still contains the anhydric oxygen.

Previous to the work of Pictet and Reichel (1923) it was thought that glucose and chloral first formed an addition product which finally lost a molecule of water, but these authors showed that this interpretation is no longer supported when they prepared the two chloraloses (α and β) by the direct addition of chloral to the two anhydrides of glucose: i.e., glucosan and levo-glucosan. According to the conclusions of Pictet and Reichel, the glucosan adds to the chloral, breaking easily the ethylene oxide ring which it contains. This is shown as follows:



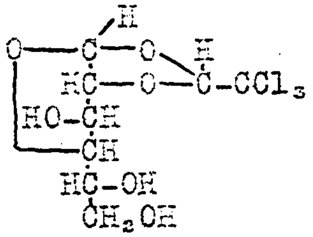
Glucosan

VI
β-glucochloralose

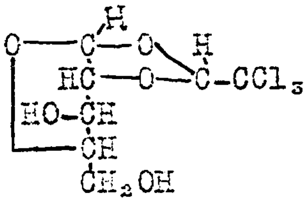
It seems more probable that the extraordinary stability of this particular acetal linkage is induced by the negativity of the three chlorine atoms of the chloral residue. This assumption is supported by the action of hydrogenated beta-glucochloralose toward oxidizing agents, for if the chlorine atoms are replaced by hydrogen by the action of amalgam, the linkage is weakened, and when two chlorine atoms are removed (so-called bidechloroglucochloraloses), treatment with concentrated nitric acid produces appreciable quantities of saccharic acid, the chloral group having been hydrolyzed off. Furthermore, if only one chlorine atom is removed in beta-galactochloralose, and the resulting compound (dechlorogalactochloralose) is treated with concentrated nitric acid, mucic acid is obtained as the principal product.

With the assumption that unusual valences are responsible for the stability of the chloraloses, the chloral condensation, from analogy with the acetone reaction, may be considered as the same type of reaction, and the formulae considered as shown in formulae VII and VIII.

The formula as here given (VII) would not allow the formation

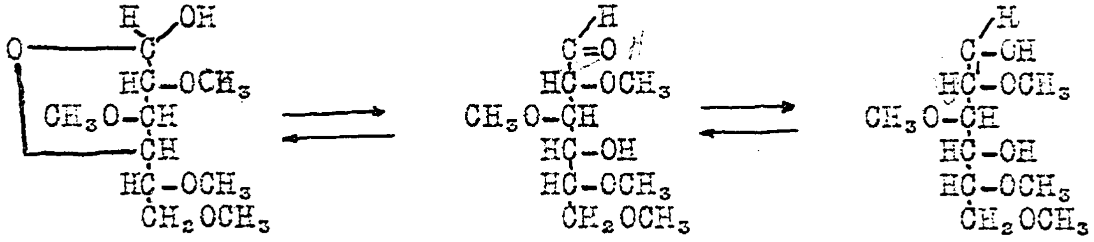


VII
β-glucochloralose



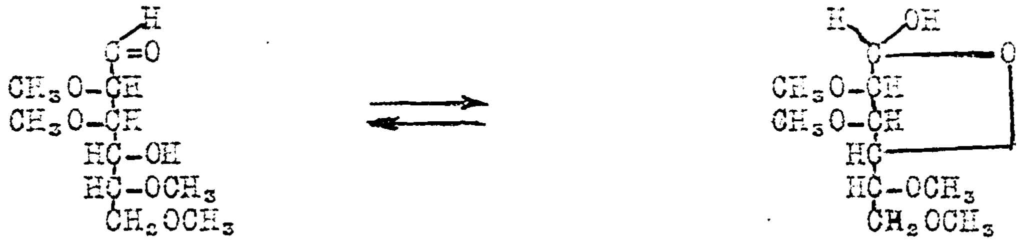
VIII
β-xylochloralose

of a tetra-substituted chloralose, and it is necessary to show the possibilities of keto-enol isomerism in order to account for these well-defined tetra-acetates and tetra-benzoates. That there is a possibility of this isomerism is shown by the pionner work of Lobry de Bruyn and van Ekenstein (1896 and 7) in which it was shown that if glucose is placed in water with a little sodium hydroxide, traces of fructose and mannose are found, and if fructose is placed in water, glucose and mannose can be found. Corroboration is given by the recent work of W. Lee Lewis and co-workers (1926), in which the presence of a relatively stable enolic compound was demonstrated in the equilibrium mixture during the interconversion of normal tetramethyl glucose to normal tetramethyl mannose. This common enol form is present to the extent of 34%. This reaction may be written:



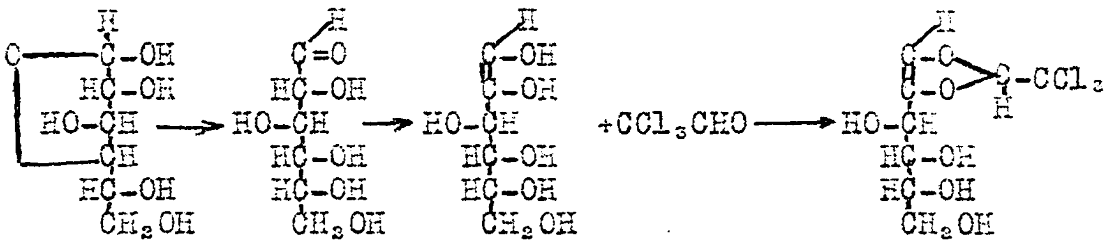
Tetramethyl glucose

Common monomethyl ene-diol



Tetramethyl mannose

The reaction, then, for the formation of the beta-glucochloralose can be written showing the intermediate formation of an enol form.

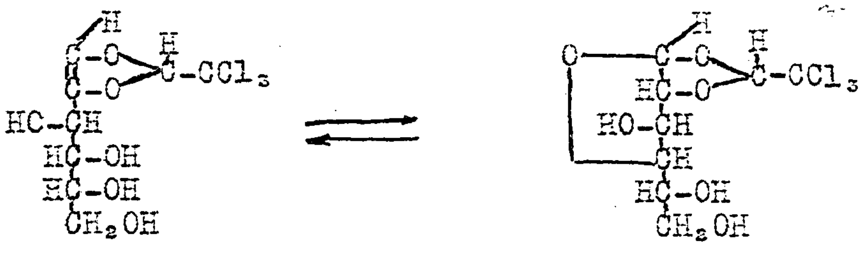


IX

Butylene-oxide form
Aldehyde form
Enol form
Enol form

α-d-glucose
β-glucochloralose

The enol form (IX) would then give a tetrasubstituted benzoyl or acetyl derivative. It would be in equilibrium with the keto form as follows:



X

enol form
keto form

It appears from the fact that in most cases tetra-benzoyl and tetra-acetyl compounds have been obtained that the enol form is the stable form. Under the correct conditions it should be possible to get tri-substituted esters. In fact, not only has a tri-benzoyl beta-galactochloralose been described (Hanriot and Richet, 1896,b), but also it has been impossible to obtain the corresponding tetrabenzoyl derivatives.

Outline of Research

The present research was undertaken with the view of showing that the chloral condensation is analogous to the acetone condensation in that the aldehyde functions of both sugar and aldehyde are lost with the formation of ether linkages and that carbon to carbon linkages were not formed. It was believed that the methylation process offered the best means of determining whether this was true, and it was hoped to carry out the reactions represented by the scheme:

1,2-monochloral glucose (beta-glucochloralose) (XI)

XII $\big\downarrow$ $(CH_3)_2SO_4$ NaOH

3,5,6-trimethyl-1,2-monochloral glucose

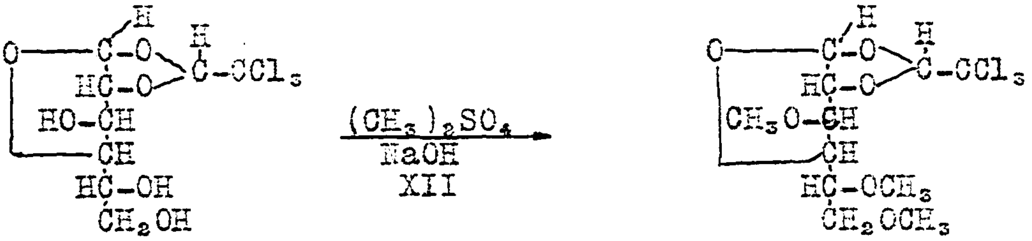
$\big\downarrow$ Na-Hg amalgam

3,5,6-trimethyl bidechlorobetaglucochloralose (XIII)

$\big\downarrow$ H_2SO_4

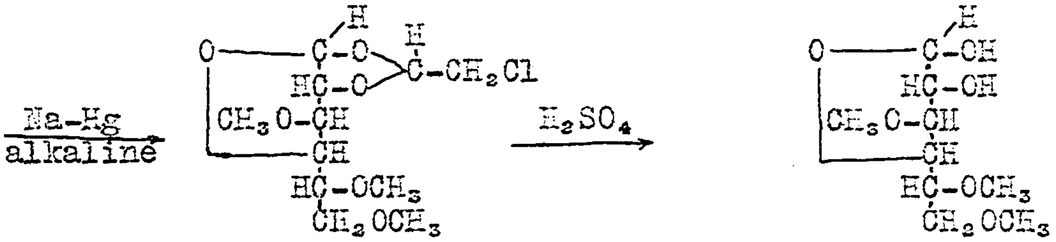
3,5,6-trimethyl glucose (XIV)

The reactions are written out in full as follows:



XI
beta-glucochloralose

trimethyl beta-glucochloralose



XIII
trimethyl bidechloro-betaglucochloralose

XIV
3,5,6-trimethyl glucose

Experimental Part

Preparation of Beta-glucochloralose (XI)

The method essentially followed was that of Ross and Payne (1923) who stirred cotton or glucose into a mixture of chloral hydrate and concentrated sulfuric acid contained in a beaker cooled with snow. After mixture, the mass was allowed to stand for twelve hours, and the liquid poured into a large volume of water. The mixture of dichloral glucoses separated out. The filtrate was boiled on the steam bath and precipitated the beta-chloralose. It was found in our work that the yield of beta-chloralose can be increased if the mixture is not allowed to stand over six hours. It is probable that the monochloral glucose is formed first: the condensation with a second molecule of chloral then taking place. This is borne out by the fact that a dichloral glucose (m.p. 268°) described by Ross and Payne was prepared in this laboratory by treating beta-chloralose with chloral hydrate and sulphuric acid, a preparation which is contrary to the conclusion of these authors (ibid, p. 2365), who express the opinion that the monochloralglucoses have four free hydroxyls which points to aldol condensation; while the dichloral glucoses possess but one free hydroxyl, thus indicating acetal condensation throughout. Therefore, "monochloral glucoses could not give rise to dichloral glucoses by condensation with a second mole of chloral".

Methylation of Beta-glucochloralose (XII)

Beta-glucochloralose was methylated by the use of dimethyl sulfate and sodium hydroxide. However, because of the insolubility of the beta-glucochloralose, the method as originally worked out by Haworth (1915) was greatly changed. Therefore, a detailed description of the preparation will be given.

Five grams of the beta-glucochloralose, very finely powdered, are placed in a round-bottomed flask equipped with a Hopkins condenser and mechanical stirrer. One hundred cubic centimeters of dimethyl sulfate are added, the temperature brought up to 55-60° and the mixture stirred until the most of the beta-glucochloralose has gone into solution. Solid, powdered sodium hydroxide is added in small amounts while stirring and as the mixture becomes too thick for stirring some distilled water is added. The addition of the sodium hydroxide is carried out until the reaction becomes alkaline. The solution is cooled, and extracted repeatedly with chloroform, which extract is dried over calcium oxide, and the chloroform then taken off under reduced pressure. The chloroform residue consists of a syrup which is taken up in pure methyl alcohol and filtered from any insoluble material (probably incompletely methylated chloralose). Water is added cautiously to the methyl alcohol filtrate until it is cloudy, and on standing a white flocculent precipitate settles out. This may be filtered, taken up again in petrolic ether, filtered from any insoluble residue, evaporated to a syrup, taken up in methyl alcohol and precipitated cautiously by the addition of water. The crystals show a melting point of 109-109.5° (uncorr.), are soluble in most of the common organic solvents and are obtained in yields of from 30-35%. The analysis showed them to be the trimethyl beta-glucochloralose:

Analysis. Calc. for $C_{11}H_{16}O_6Cl_3$: Cl, 30.26. Found 30.44;, 30.42.

Comparison of the percent chlorine of trimethyl beta-glucochloralose with other methyl derivatives is given in Table I. The chlorine content of the trimethyl derivative just mentioned differs greatly from that of any other methylated compounds.

Table I

Percent Chlorine in Methylated Chloralose Derivatives

Name of Derivative	Percent Cl
Beta-glucochloralose	34.37
Dimethyl chloralose	31.52
Monomethyl chloralose	32.88
Trimethyl dechloralose	22.37
TRIMETHYL BETA-GLUCOCHLORALOSE	30.25
Tetramethyl chloralose	29.10
Tetramethyl dechloralose	21.41
Tetramethyl methyl dechloralose	25.42
Trimethyl methyl chloralose	21.41

The trimethyl beta-glucochloralose showed little or no rotation in methyl alcohol (Conc. =2.2%) in a decimeter tube. The crystals are soluble in hot water, glacial acetic acid, and ethyl acetate.

Hydrogenation of Trimethyl Beta-glucochloralose

The hydrogenation of the trimethyl beta-glucochloralose was carried out with sodium-mercury amalgam in a manner essentially that of Hanriot and Kling (1911 and 1913). The reaction was carried out in an alkaline solution so as to avoid any hydrolysis of partially or completely dechlorinated compounds. Such compounds undoubtedly would hydrolyze very readily, even when only a small amount of acid is present. This would apply especially to the 3,5,6-trimethyl 1,2-acetal glucose.[1] For these reasons the solution was kept alkaline during the reduction. This dechlorination was carried out at a temperature of around 50° for about four hours, two grams of trimethyl beta-glucochloralose being dechlorinated at a time. The alkaline solution was filtered from the mercury, and neutralized with sulfuric acid, taking care to keep the solution slightly alkaline. This solution was extracted with ether, and on evaporation of the ether, a small amount of crystals was obtained, m.p. 105-6°, for which a sodium fusion showed no chlorine present. They did not reduce Fehlings or alkaline potassium permanganate and were very slowly soluble in water , methyl alcohol, ether, and glacial acetic acid, and were quickly soluble in ethyl acetate, chloroform and petrolic ether. The quantity was too small for analysis, but the crystals were believed to be trimethyl glucochloralose with all three chlorine atoms replaced by hydrogen (or 3,5,6-trimethyl 1,2-acetal glucose). Hydrolysis with dilute sulfuric acid gave a syrup which had characteristics indicative of 3,5,6-trimethyl glucose.

Along with the crystals just described was obtained a small amount of syrup, insoluble in water, but soluble in ether, methyl alcohol and chloroform. The syrup contained chlorine, and the

---

[1] Would be called 3,5,6-trimethyl tridechloro-betaglucochloralose, according to the older unsatisfactory nomenclature.

chlorine was not precipitated with alcoholic silver nitrate. It distilled under high vacuum, using Pregl's micro method (1917, p. 179) to give a colorless syrup, still containing chlorine, and having the characteristics already mentioned. It was hydrolyzed with difficulty, giving a small amount of syrup, having the characteristics of 3,5,6-trimethyl glucose. This syrup is believed to be a mixture of trimethyl dechlorobetaglucochloralose and trimethyl bidechlorobetaglucochloralose.

Isolation of 3,5,6-Trimethyl Glucose (XIV)

The solution, after reduction, was neutralized with sulfuric acid, and extracted with ether. The residue syrup left on evaporation of the ether was treated with water, and any 3,5,6-trimethyl glucose present dissolved, leaving partially dechlorinated methylated beta-glucochloraloses. Additional 3,5,6-trimethyl glucose may be secured by refluxing this syrup with dilute sulfuric acid. The water filtrate was evaporated, leaving a slightly colored syrup, which can be distilled, using Pregl's micro method, at 130-140° (paraffin bath temperature) and 0.4 mm. pressure, a colorless syrup being obtained. A determination of its rotation was made in alcohol solution:

$$[\alpha]_D^{22} \ (CH_3OH) = \frac{-1.75^\circ \times 0.7402}{1 \times 0.852 \times 0.1483} = -10.24^\circ$$

Levene and Meyer (1926) state the rotation of 3,5,6-trimethyl glucose to be -8° to -11°; Levene and Meyer (1921) give it as -10.95° (initial) and -14.6° (final) in water; and Irvine and

Patterson (1922) give it as -57.3° (ethyl alcohol) and -15.7° (water). It is noteworthy that the 3,5,6-trimethyl glucose is the only trimethyl glucose so far described in the literature, which has a negative rotation. A micro tube with a small bore was used in making the determination of the rotation, and undoubtedly some error was introduced due to this fact.

The syrup reduces Fehlings solution but not in the cold, and alkaline potassium permanganate very strongly in the cold. Levene and Meyer, (1926) state that it should not do this if absolutely pure. It gives a dark green solution and precipitate in the orcinol test; Levene and Meyer (1926) report a dark blue solution and precipitate but do not give directions for making the test. It was very soluble in methyl alcohol and chloroform and less soluble in water. An osazone (m.p. 163-4°) has been described by Cramer and Cox (1922) for 3,5,6-trimethyl glucose, but this has as yet not been obtained due to small yields of the syrup.

Miscellaneous Tests with Beta-glucochloralose

Treatment with Toluolsulfochloride

The beta-glucochloralose, finely powdered,(one gram) and the toluol sulfochloride (1.5 grams) were mixed in a large test-tube and 20 cubic centimeters of pyridine were added. The solids went into solution very quickly, and considerable heat was developed. The test-tube and contents were kept at 45-50°for 18 hours. Water was gradually added and then in large amounts, but no precipitate or oil of a sulfo-derivative was visible even when an equal volume of water was added. On standing, the beta-glucochloralose was obtained unchanged.

Treatment with thionyl chloride

One-half gram of beta-glucochloralose (m.p. 227°), very finely powdered, was placed in a soil flask with 25 cubic centimeters of dry thionyl chloride. It was then refluxed with occasional shaking on a hot plate, moisture being excluded. At the end of 45 minutes, the beta-glucochloralose had gone completely into solution. The refluxing was continued for a total of two hours, and the yellow liquid evaporated under reduced pressure almost to dryness. The residue remaining was taken up with chloroform, which,on evaporation, left crystals having a melting point of 190-2°,organic in nature, burning with a luminous flame and with a peculiar odor. The crystals were washed with ether. On standing, they decompose giving off an odor of sulfur dioxide.

Treatment with chloral

One-half gram of beta-glucochloralose (m.p. 227°) was added to a mixture of ten grams of chloral hydrate and ten cubic centimeters of concentrated sulfuric acid in a beaker. The chloralose went completely into solution in fifteen minutes. It was then allowed to stand at room temperature for seven hours, and then poured into 250 cubic centimeters of ice water. A white precipitate which formed was filtered, washed repeatedly with water, and repeatedly recrystallized from methyl alcohol, washing each time with chloroform. Crystals melting at 262° were obtained. Ross and Payne (1923) report a dichloral glucose of melting point 264° . It seems possible therefore, to prepare a dichloral glucose from monochloral glucose (beta-glucochloralose).

Treatment with sulfuryl chloride

One gram of beta-chloralose and 20 cubic centimeters of pure sulfuryl chloride were placed in a soil flask, and refluxed excluding moisture for a total of two hours. The beta-glucochloralose dissolved completely in about 15 minutes. Evaporation of the sulfuryl chloride by vacuum distillation and treatment of the residue with chloroform yielded crystals, which were not beta-glucochloralose, and decomposed when moist.

Treatment with dry pyridine

One-half gram of beta-glucochloralose was dissolved in 20 cubic centimeters of dry pyridine and refluxed for four hours. Water was added to the solution, but no precipitate was produced until it had stood for some time. The precipitate was unchanged beta-glucochloralose. The filtrate from the crystals showed no reduction with Fehlings solution.

Treatment with zinc chloride

One gram of beta-gluco-chloralose, finely powdered, was intimately mixed with 5 grams of anhydrous zinc chloride, and placed in a drying oven at 100° for ten hours. The material was very brown when removed, and considerable of the residue went into solution in water. Extraction of the water solution with chloroform gave crystals which were not further investigated.

Orcin-Hydrochloric Acid Test

An attempt was made, by the use of color tests, to determine the possibility of identifying beta-glucochloralose from the different dichloral glucoses, since their solubilities are very near alike, and the melting point of one dichloral glucose is only two degrees less (225° C) than that of beta-glucochloralose (227° C).

One porcelain spoonful (small spatula) of the substance being tested was placed in a test-tube with one spoonful of orcin. Two cubic centimeters each of water and concentrated hydrochloric acid were added, and the mixture immersed in a boiling water bath for five minutes, shaking continuously. The color was observed, after cooling, by reflected light, and after allowing the precipitate to settle. The tests were repeated several times, and the following results were always obtained:

Beta-glucochloralose (m.p. 227° C)

Pinkish yellow tinge with pink color most pronounced. Substance in solution precipitates out on cooling.

Dichloral glucose (m.p. 225° C)

Faint pink tinge but not nearly as distinct as the color produced by the 264° dichloral glucose. No flocculent precipitate.

Dichloral glucose (m.p. 264° C)

Strong pink color. Clear liquid, no flocculent precipitate.

As carried out, the orcin-hydrochloric acid test does not offer a good means of distinguishing between the chloral glucoses.

Resorcinol-Hydrochloric Acid Test

This test, carried out as given above in the case of the orcin test, will not offer a means of differentiating between the chloral glucoses.

Attempt to prepare Beta-glucochloralose by Method used in preparing Acetone Glucose

One hundred grams of chloral hydrate, 50 grams of glucose and 500 cubic centimeters of dry acetone were placed in a one liter bottle and ten drops of concentrated sulfuric acid were added. The bottle was sealed and shaken intermittently for three weeks. The mixture was colorless. No alpha or beta-glucochloralose were found present.

Fifty grams of glucose and 500 cubic centimeters of freshly distilled acetone were placed in a liter bottle with 100 grams of freshly distilled chloral. Heat was developed, and the solution became dark-colored. After shaking for three weeks, the contents were removed, but no chloralose compounds were found.

Solubility Tests with Beta-gluco-chloralose

For convenience, the solubilities of beta-glucochloralose are here tabulated.

Table II

Solubilities of Beta-glucochloralose

Solvent	Cold	Hot
Water	Insoluble	s.soluble
Toluene	"	Insoluble
Carbon tetrachloride	"	"
Dimethyl sulfate	"	Soluble

Table II

(Continued)

Solvent	Cold	Hot
Petrolic ether	Insoluble	Insoluble
Ethyl acetate	s.Soluble	Soluble
Chloroform	Insoluble	Insoluble
Nitrobenzene	s.soluble	s.soluble
Dichloroethylene	Insoluble	Insoluble
Tetrachloroethylene	"	"
Diethyl oxalate	"	Soluble
Trimethylene glycol	"	"
Methyl iodide	s.soluble	-----
Acetone	Insoluble	-----
Thionyl chloride	s.soluble	Soluble
Sulfuryl chloride	s.soluble	Soluble
Pyridine	Soluble	"

Discussion of Results

The methylation prosess as given is carried out in an acid medium until the dimethyl sulfate is neutralized. By the use of solid sodium hydroxide at first, methylation takes slowly and completely, and precipitation of any beta-glucochloralose by the addition of the water is avoided. The process is necessarily quite a violent means of methylation, which probably accounts for the low yields of trimethyl beta-glucochloralose.

The formation of trimethyl glucose on reduction with sodium amalgam was rather unexpected. No more than two chlorine atoms were reported in the literature as being removed by the amalgam. It was to be expected that a mixture of partially reduced methylated beta-glucochloraloses would be obtained.

It seems, therefore, from the experimental work that the methylated glucochloralose is stable toward hydrolytic agents. This stability is lessened by removal of two or more chlorine atoms, after which the chloral residue can be hydrolyzed off. Assuming that the beta-glucochloralose contains the butylene oxide ring, it seems probable that the chloral group is joined to the sugar residue on carbons 1 and 2 by oxygen linkages.

Summary

Condensation of chloral with glucose takes place in a manner analogous to the condensation of acetone with glucose. The condensation, assuming no shifting of linkages or groups, takes place on carbons 1 and 2 of the glucose, 3,5,6-trimethyl glucose being obtained on hydrolysis of the chloral group.

The condensation should be represented by an acetal linkage, and not by a carbon to carbon linkage as given in previous formulae for beta-glucochloralose.

The formula best representing the structure of beta-glucochloralose is that shown by formula VII in the text of this thesis.

The possibility of keto-enol isomerism, indicating the presence of two forms of beta-glucochloralose, is suggested.

A dichloral glucose can be prepared from a monochloral glucose contrary to the views of Ross and Payne.

Inasmuch as xylose has the same position in the pentose series as glucose holds in the hexose series, the conclusions here given apply to beta-xylochloralose and, therefore, the formula best representing the structure of beta-xylochloralose is that shown in formula VIII in the text of this thesis.
The conclusions given for beta-glucochloralose must apply to the xylose derivative, since on oxidation of beta-glucochloralose with nitric acid, the same chloralic acid is obtained as is prepared from beta-xylochloralose by oxidation with sulfuric acid.

BIBLIOGRAPHY

de Bruyn, C.A. Lobry, and v. Ekenstein, A. (1896). Action des alcalis sur les sucres. III. Transformation des sucres sous l'influence de l'hydroxyde de plomb. Rec. Trav. Chim. des Pays-Bas. 15:92-96.

de Bruyn, C.A. Lobry, and v. Ekenstein, A. (1897). Action des alcalis sur les sucres. IV. Remarques generale. Rec. Trav. Chim. des Pays-Bas. 16:241-4.

de Bruyn, C.A. Lobry, and v. Ekenstein, A. (1897). Action des alcalis sur les sucres. V. Transformation de la galactose. Les tagatose, et la galtose. Rec. Trav. Chim. des Pays-Bas. 16:244-55.

de Bruyn, C.A. Lobry, and v. Ekenstein, A. (1897). Action des alcalis sur les sucres. VI. La glutose et la pseudo-fructose. Rec. Trav. Chim. des Pays-Bas. 16:256-63.

de Bruyn, C.A. Lobry, and v. Ekenstein, A. (1897). Action des l'eau bouillante sur la fructose. Rec. Trav. Chim. des Pays-Bas. 16:264.

Cramer, M., and Cox, E.H. (1922). Sur la constitution de la glucosan. Helv. Chim. Acta. 5:884-8.

Hamriot, M., and Richet, Ch. (1892). D'une substance derivee du chloral ou chloralose et de ses effets physiologiques et therapeutiques. Compt. Rend. Acad. Sci. 116:63-65.

Hanriot, M., and Richet, Ch. (1893,a). Sur le chloralose. Bull. Soc. Chim. (Paris) 3 9:947.

Hanriot, M., and Richet, Ch. (1893,b). Sur le chloralose. Compt. Rend. Acad. Sci. 117:734-7.

Hanriot, M., and Richet, Ch. (1894). Sur le chloralose. Bull. Soc. Chim. 3 11:37-44.

Hanriot, M., and Richet, Ch. (1894). Sur le chloralose. Bull. Soc. Chim. (Paris) 3 11:258.

Hanriot, M., and Richet, Ch. (1894). Sur le chloralose. Bull. Soc. Chim. (Paris) 3 11:303-5.

Hanriot, M., and Richet, Ch. (1896). Sur les chloraloses. Compt. Rend. Acad. Sci. 122:1127-9.

Hanriot, M. (1909). Sur les chloraloses, Ann. Chim. et Phys., 18:466-502.

Hanriot, M. and Kling, A. (1911,a). Action des alcalis sur les chloraloses. Compt. Rend. Acad. Sci., 152:1398-9.

Hanriot, M. and Kling, A. (1911,b). Action de l'ammoniaque sur les chloraloses. Compt. Rend. Acad. Sci., 152:1596-9.

Hanriot, M. and Kling, A. (1913). Action des reducteurs sur les chloraloses. Compt. Rend. Acad. Sci., 156:1380-2.

Haworth, W.N. (1915). A new methos of preparing alkylated sugars. Jour. Chem. Soc., (London) 107:8-16.

Heffter, A. (1889). Ueber die Einwirkung von Chloral auf Glucose. Ber. deut. chem. Gesell., 22:1050.

Irvine, J.C. and Patterson, J. (1922). The constitution of acetone derivatives of glucose and fructose. Jour. Chem. Soc., (London) 121:2146-61.

Levene, P.A. and Meyer, G.M. (1921). Phosphoric esters of some substituted glucoses and their rate of hydrolysis. Jour. Biol. Chem., 48:233-48.

Levene, P.A. and Meyer, G.M. (1926). Diacetone glucose. III. Methylated methyl glucosides prepared from monoacetone glucose. Jour. Biol. Chem., 70:343-10.

Lewis, W. Lee, Wolfram, M.L. and Greene, R.D. (1926). Studies in the reactivity of the methylated sugars. II. The Lobry de Bruyn interconversion. Organic Division Abstracts, A. C.S., Philadelphia Meeting.

Meunier, M.J. (1906). Sur les composes que la mannite et la sorbite forment avec les aldehydes. Ann. Chim. et Phys., (6) 22:413-32.

Petit, A. and Polonowski, M. (1894). Sur le chloralose. Bull. Soc. Chim., (Paris) (3) 11:125-33.

Pictet, A. and Reichel, F.H. (1923). De l'action du chloral sur les glucosanes. Helv. Chim. Acta, 6:621-27.

Pregl, F. (1917). Die Quantitative Organische Mikro-analyse. Julius Springer, Berlin.

Ross, J.H. and Payne, J.M. (1923). The action of chloral upon cellulose. Jour. Amer. Chem. Soc., 45:2363-6.

Schiff, H. (1888). Verbindungen von Zuckerarten mit aldehyden und Acetonen. Liebig's Annalen der Chemie 244:19-28.

ADDITIONAL BIBLIOGRAPHY ON CHLORALOSES

Besides the references already listed, the following papers include the chloraloses in their discussion:

Binet, M., and Collazo, J.A. (1925). Lactic acid of blood under respiration. Medicine (Paris) 6:924.

Cardot, H.,and Laugier, H. (1922). Anesthésie et réflexe linguo-maxillaire. Compt. Rend. Soc. Biol., 87:215-9.

Cardot, H.,and Laugier, H. (1922). Le réflexe linguo-maxillaire. Compt. Rend. Acad. Sci., 174:1368-9.

Chevalier, J., and Cherbuliez, A. Chloralose et parachloralose. Leur action sur le système nerveux bulbo-médullaire. Compt. Rend. Soc. Biol., 91:642-4.

Desch, C.H., and Lapworth, A. (1909). Carbohydrates. Ann. Reports Prog. of Chem., 6:80.

Gautrelet, J., (1918). Contribution à l'étude physiologique du chloralose. Compt. Rend. Soc. Biol., 81:369-41.

Gautrelet, J., Bargy, R., and Vechiu, Mme. (1926). Action du chloralose sur le système nerveux vegetatif. Compt. Rend. Acad. Sci., 182:1048-50.

Hanriot, M., and Richet, Ch. (1893). De l'action physiologiques du parachloralose. Soc. de Biologie. 45:614-5.

Hanriot, M., and Richet, Ch. (1893). Effets physiologique du chloralose. Soc. de Biologie. 45:129-33.

Hanriot, M., and Richet, Ch. (1893). Effets psychiques du chloralose sur les animaux. Soc. de Biologie. 45:109-13.

Hanriot, M., and Richet, Ch. (1893). Effets physiologiques du chloralose. Mém. Soc. de Biol., 5:1-16.

Hanriot, M., and Richet, Ch. (1894). Des effets hypnotiques de l'arabinochloralose. Soc. de Biologie. 46:791-2.

Hanriot, M. (1895). Combinaisons du chloral avec xylose et arabinose. Bull. Soc. Chim., (Paris) 13:227.

Hanriot, M. (1895). Sur l'arabinochloral et le xylochloral. Compt. Rend. Acad. Sci., 120:153-5.

Hanriot, M. (1896). Combinaisons du chloral et bromal avec divers sucres. Bull. Soc. Chim., (Paris) 15:626.

Hanriot, M. (1909). Sur les acides chloraliques. Bull. Soc. Chim., (Paris) (4) 5:819-26.

Hanriot, M. (1909). Sur les acides chloraliques. Compt. Rend. Acad. Sci., 148:487-9.

Hanriot, M., and Kling, A. (1912). Action des alcalis sur les chloraloses. Bull. Soc. Chim., (Paris) (4) 11:207.

Hanriot, M., and Kling, A. (1919). Sur les chloraloses. Ann. Chim. et Phys., 12:129-50.

Kshishkowski, K. (1911). Chloralose als narcoticum bei niederen Tieren. Zentr. Physiol., 25:8-11.

Kshishkowski, K. (1912). Chloralose als narcoticum bei niederen Tieren. Zentr. Physiol., 26-525-8.

Meunier, M.J. (1896). Sur le dichloral glucose et sur le monochloral glucosane. Compt. Rend. Acad. Sci., 122: 142-4.

Meunier, M.J. (1896). Ueber dichloral glucose und monochloral glucose. Ber. deut. chem. Gesell., 29: Referate 177.

Meunier, M.J. (1896). Sur le dichloral glucose et sur le monochloral glucosane. Bull. Soc. Chim., (Paris) (3) 15:631-3.

Raymond-Hamet, M. (1926). Inversion of the normal action of adrenaline. Compt. Rend. Acad. Sci., 183-485-6.

Regnier, M., and Tiffeneau, M. (1913). Étude physiologique des chloraloses mono- et bidechlores. Compt. Rend. Soc. Biol., 74:874-7.

Richet, C. (1895). Anesthesie et Anesthesiques. Dict. de Physiologie, Paris, Alcau, 1:536.

Richet, C., and Lassabliere, P. (1926). Effets protecteurs du chloralose sur l'anesthesie chloroformique. Compt. Rend. Acad. Sci., 183:175-6.

Tiffeneau, M. (1915). Sur la destinee du chloralose dans l'organisme et ses rapports avec la conjugaison glycuronique. Compt. Rend. Acad. Sci., 160:38-41.

BIBLIOGRAPHY ON 3,5,6-TRIMETHYL GLUCOSE

Cramer, M. and Cox, E.H. (1922). Sur le constitution de la glucosan. Helv. Chim. Acta 5:884-8.

Haworth, W.N. and Sedgwick, W.G. (1926). New crystalline forms of trimethyl glucose and dimethyl glucose. Jour. Chem. Soc., (London) 129:2573-80.

Irvine, J. C. and Scott, J.R. (1913). ζ-monomethyl and γ, ε, ζ-trimethyl glucoses. Jour. Chem. Soc., (London) 103:564-75.

Irvine, J.C., Fyfe, A.W. and Hogg, T.P. (1915). Derivatives of a new form of glucose. Jour. Chem. Soc., (London) 107:524-41.

Irvine, J.C. and McDonald, J.L.A. (1915). The formation and preparation of glucose monoacetone. Jour. Chem. Soc., (London) 107:1701-10.

Irvine, J.C. and Patterson, J. (1922). The constitution of acetone derivatives of glucose and fructose. Jour. Chem. Soc., (London) 121:2146-61.

Levene, P.A. and Meyer, G.M. (1921). Phosphoric esters of some substituted glucoses and their rate of hydrolysis. Jour. Biol. Chem., 48:233-48.

Levene, P.A. and Simms, H.S. (1925). Lactone formation from mono- and di-carboxylic sugar acids. Jour. Biol. Chem., 65:31-47.

Levene, P.A. and Meyer, G.M. (1926). Diacetone glucose. III. Methylated methyl glucosides prepared from monoacetone glucose. Jour. Biol.Chem., 70:343.

Levene, P.A. and Simms, H.S. (1926). Lactone formation from gluconic acids and the structure of glucose. Jour. Biol. Chem., 68:737-49.

Sobotka, H. (1926). Oxidation of methylated glucoses. Jour. Biol. Chem., 69:267-75.