

SOME DERIVATIVES OF GLUTAMIC AND GLUCOSE PHOSPHORIC ACIDS

by

John Albert Davis Sc.B. Ottawa University 1927

Submitted to the Department of
Chemistry and the Faculty of the
Graduate School of the University
of Kansas in partial fulfillment
of the requirements for the de-
gree of Master of Science.

Approved by:



Instructor in charge



Head or Chairman of Dept.

May 15, 1928.

I wish at this point to
thank Dr. R. Q. Brewster
for his interest and many
helpful suggestions in
directing this work.

John A. Davis

INDEX

PART I

GLUCOSE PHOSPHORIC ACID

	Page
1 INTRODUCTION	1
2 CALCIUM SALT OF GLUCOSE PHOSPHORIC ACID	3
3 BARIUM SALT OF GLUCOSE PHOSPHORIC ACID	4
4 FREE GLUCOSE PHOSPHORIC ACID	9
5 DERIVATIVES OF GLUCOSE PHOSPHORIC ACID	10
6 ANILINE SALT OF GLUCOSE PHOSPHORIC ACID	11
7 GUANIDINE SALT OF GLUCOSE PHOSPHORIC ACID	12

PART II

GLUTAMIC ACID DERIVATIVES

1 DIETHYL GLUTAMATE	14
2 ETHYL SULFATE PLUS BARIUM GLUTAMATE	17
3 E. FISHER'S METHOD FOR PREPARATION OF DIETHYL GLUTAMATE	18
4 ATTEMPT TO PREPARE DIETHYL GLUTAMATE USING SULFURIC ACID	20
5 GUANIDINE GLUTAMATE	21
6 CONCLUSION	24

PART IGLUCOSE PHOSPHORIC ACIDINTRODUCTION

Phosphorous oxychloride which may be considered as the chloride of phosphoric acid, reacts with water in such a way that hydrogen chloride is split off and the remainder of the two moles unite. When three moles of water and one of phosphorous oxychloride are used, three moles of hydrogen chloride and one of phosphoric acid are produced. The question naturally arises, will not other substances containing hydroxyle groups, such as glucose for example, behave in a similiar manner under suitable conditions? In other words, will not the hydrogens of one or more hydroxyl groups unite with chlorine of the phosphorous oxychloride to form hydrogen chloride and a substituted phosphoric acid? Under suitable conditions might not one of the chlorines unite with one of the hydrogens in glucose and the other two chlorines be replaced by hydroxyl groups, thus giving a substituted phosphoric acid which should be dibasic?

Neuberg and Pollok ¹prepared such a compound but

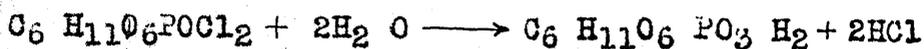
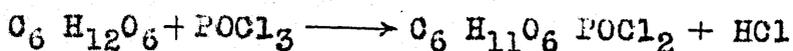
Their yield was small. P. A. Levene and C. M. Meyer² prepared α methyl glucose phosphoric acid by a different method and obtained nine grams of the barium salt of the acid from ten grams of α methyl glucoside.

This immediately suggests a better method of preparing glucose phosphoric acid for if their method works well with the methyl glucoside, should it not also work in the preparation of the unsubstituted glucose phosphoric acid? This method was tried out as part of this piece of research.

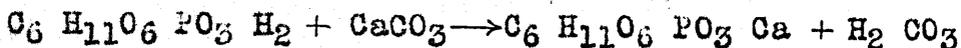
As phosphorous and nitrogen seem to be essential to the growth of bacteria, it seems probable that by preparing glucose phosphoric acid and using it in the preparation of other compounds, substances will be found upon which bacteria will thrive. A study of the nature of these compounds and the way in which bacteria react with them should throw light upon the way in which bacteria act upon organic compounds.

EXPERIMENTALTHE CALCIUM SALT OF GLUCOSE PHOSPHORIC ACID

In the preparation of the calcium salt of glucose phosphoric acid, the method of Neuberg and Pollok¹ was used. Two hundred fifty grams of glucose was dissolved in one and five tenths liters of water and well cooled with ice. The mixture was stirred mechanically while a solution of seventy-seven grams of phosphorous oxychloride in two hundred fifty cubic centimeters of chloroform was gradually added. The mixture was stirred for ten hours while the phosphorous oxychloride was being added, after which it was allowed to stand over night.



Glucose phosphoric acid



Calcium salt

The $CaCO_3$ and $Ca_3(PO_4)_2$ was then filtered off and carefully washed several times with a little cold water. The filtrate was concentrated to 500 c.c. in a vacuum

¹ Ber. 43 (1910) 2061

at 40°C. It was then filtered clear and four volumes of alcohol added. The calcium glucose phosphate was precipitated along with a little calcium chloride. It was filtered out and dried and purified by dissolving in a little water and reprecipitating with alcohol. This process was repeated until portions of the salt no longer gave a chloride test with silver nitrate solution. The yield was very small (3.5 grams).

The product gave no precipitate with lead acetate but gave a good precipitate with lead acetate plus ammonium hydroxide. It gave no reaction with ammonium molybdate. After oxidation with nitric acid it gave the phosphate test with ammonium molybdate.

THE BARIUM SALT OF GLUCOSE PHOSPHORIC ACID

In the preparation of the barium salt of glucose phosphoric acid the method of P. A. Levene and G. M. Meyer² was followed, using glucose in place of the α methyl glucoside.

Ten grams of anhydrous glucose was suspended in 50 c.c. of water-free pyridine and the solution cooled to -20°C . To it was added in small quantities 7.8 g. of POCl_3 in 20 c.c. of pyridine, also cooled to -20°C . The POCl_3 in pyridine being well cooled before mixing.

The glucose was dried for three days in a vacuum dessicator over phosphorous pentoxide at 110°C . under a pressure of about 20 mm. The pyridine was dried over solid sodium hydroxide for twenty-four hours. The pyridine layer was removed and dried over solid potassium hydroxide for two days. A portion of the pyridine layer was then removed and allowed to stand over metallic sodium for three days. During this time a considerable quantity of a black tarry substance separated out. The pyridine layer was then decanted off and fractionally distilled, the fraction 118° - 123° being retained.

In a later preparation, pyridine which had stood with occasional shaking for five days over solid potassium hydroxide was used. The pyridine was decanted off and fractionally distilled. The fraction 118° - 128° was retained.

After the POCl_3 in the pyridine had been added to the glucose, the reaction mixture was allowed to stand for several hours at -20° , during which time a considerable quantity of pyridine hydrochloride separated out. The mixture was then diluted with 20 c.c. of ice water and allowed to come to room temperature. Then it was diluted by pouring into 200 c.c. of cold water. An excess of barium hydroxide (100 g.) was added and the pyridine evaporated under reduced pressure at a temperature of about 30°C .



When the pyridine had been removed the solution was made just acid to Congo red paper and filtered. Hydrochloric acid was then removed by the addition of 30 g. of silver sulfate. After stirring for one half hour the silver chloride was filtered off and the excess silver removed with hydrogen sulfide. The silver sulfide was filtered off and the excess hydrogen sulfide removed with a current of air.

An excess of barium hydroxide was then added and carbon dioxide passed in until the solution was neutral to litmus. The solution was filtered and concentrated

under reduced pressure at a temperature of 45° until the volume was reduced to one half the original and the solution was again filtered. The filtrate was then concentrated as above to a small volume which was poured into a large volume of alcohol. The precipitate so obtained was purified by dissolving in a small quantity of water and reprecipitating with alcohol. The precipitate was filtered from the alcohol and dried on a porous plate, giving a brownish-white powder. Yield 8 g. of the barium salt from 30 g. of glucose.

The substance so prepared is the barium salt of glucose phosphoric acid. It is stable to carbon dioxide but gives a good precipitate of barium sulfate with dilute sulfuric acid. It does not give a precipitate with ammonium molybdate.

ANALYSIS OF THE SALT.

The barium salt was decomposed with sulfuric acid and the resulting barium sulfate weighed and from this the percentage of barium was calculated.

Weight of sample	.7000 g.	.7000 g.
Wt. of BaSO ₄ + Crucible	15.7241	16.1283
Wt. of Crucible	15.3164	15.7241
	<hr/>	<hr/>
Wt. of BaSO ₄	0.4077 g.	.4042 g.
Percentage of barium (experimental)	34.28%	34.00%
Percentage of barium (theoretical) based on the formula C ₆ H ₁₁ O ₉ PBa = 34.59%		

Therefore the product obtained was the barium salt of glucose phosphoric acid.

FREE GLUCOSE PHOSPHORIC ACID

The free glucose phosphoric acid was prepared from its barium salt. The barium salt was dissolved in a little water and dilute sulfuric acid (about 5% H₂SO₄) was added as long as a precipitate formed.

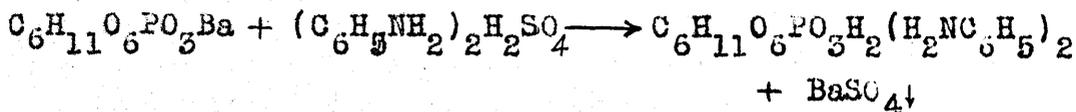


The barium sulfate was filtered off and the filtrate concentrated to a small volume under reduced pressure at 45° C. Approximately 600 c.c. of alcohol was then added and the free acid filtered off. It was dried on the porous plate and then in the dessicator over sulfuric acid. The resulting product did not give a precipitate with sulfuric acid or ammonium molybdate. When the glucose phosphoric acid was oxidized with concentrated nitric acid and then ammonium molybdate added, a good phosphate test was obtained.

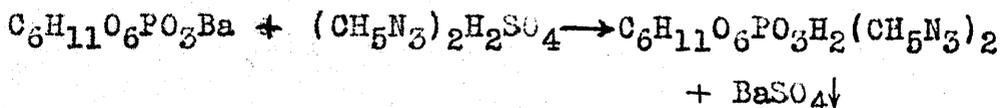
DERIVATIVES OF GLUCOSE PHOSPHORIC ACID

Since glucose phosphoric acid is a dibasic acid, it should unite with two moles of mono-acidic bases to form salts. Thus with aniline it should form the corresponding aniline salt and with guanidine it should give the corresponding guanidine salt.

With aniline sulfate and the barium salt of glucose phosphoric acid in aqueous solution, double decomposition should take place and barium sulfate should be precipitated out while the aniline salt of glucose phosphoric acid should be left in solution. This reaction should be as follows:



A similar reaction should take place with guanidine sulfate and the barium salt of glucose phosphoric acid and barium sulfate should be precipitated while ^{the} guanidine salt of glucose phosphoric acid would remain in solution.



ANILINE SALT OF GLUCOSE PHOSPHORIC ACID

Equal molecular quantities of the barium salt of glucose phosphoric acid and aniline sulfate were dissolved in water and warmed slightly. The precipitate of barium sulfate which settled out was filtered off and the filtrate evaporated to dryness on the water bath. The residue was washed with cold and then hot alcohol and then dried on a porous plate and then in a dessicator over sulfuric acid.

The resulting product is soluble in water but insoluble in ether. It decomposes at about 110°C .

Analysis of the product was made by the Kjeldahl method for nitrogen. (Only enough of the product was obtained for one analysis)

Wt. of sample	0.1784 g.
c.c. of standard acid	30. c.c.
c.c. of standard base	30.7 c.c.
1 c.c. base =	0.7444 c.c. acid
1 c.c. acid =	0.001874 grams of nitrogen

Percentage of nitrogen (experimental) 6.25%

Percentage of nitrogen (theoretical) based on

formula $C_6H_{11}O_6PO_3H_2(NH_2C_6H_5)_2 = 6.28\%$

Therefore the compound was the aniline salt of glucose phosphoric acid.

GUANIDINE SALT OF GLUCOSE PHOSPHORIC ACID

Equal molecular quantities of the barium salt of glucose phosphoric acid and guanidine sulfate were dissolved in water and warmed slightly. The precipitate of barium sulfate which settled out was filtered off and the filtrate evaporated to dryness on the water bath. The residue was then dissolved in as small a portion of water as possible and alcohol added as long as a precipitate formed. Upon the addition of the alcohol the product separated out as a thick gummy liquid from which the alcohol was decanted. The resulting product gradually solidified upon drying in a dessicator. It was soluble in water but insoluble in ether. Upon exposure to the air it rapidly took up moisture, becoming gummy.

Nitrogen was then determined by the Kjeldahl method.

ANALYSIS

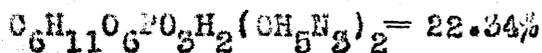
Weight of sample	0.2084 g.	0.2070 g.
c.c. of standard acid	50 c.c.	50 c.c.
c.c. of standard base	35.6 c.c.	35.6 c.c.

1 c.c. base = 0.7444 c.c. acid

1 c.c. acid = 0.001874 grams of nitrogen

Percentage of nitrogen (experimental) 22.47% 22.7%

Percentage of nitrogen calculated from the formula

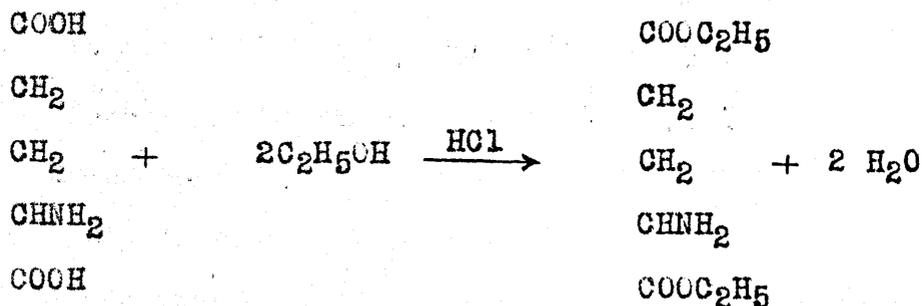


Therefore the product is the guanidine salt of glucose phosphoric acid.

PART TWOGLUTAMIC ACID DERIVATIVESDIETHYL GLUTAMATE

Glutamic acid is readily attacked by molds. This suggests that a study of the derivatives of glutamic acid be made, to determine if these derivatives are also attacked by molds and what action, if any, bacteria would have upon these compounds.

Glutamic acid being a dicarboxy acid should form a diethyl ester. One would expect the following reaction to take place.



Several unsuccessful attempts were made to prepare diethyl glutamate in the following way: Ten grams of glutamic acid (purified by boiling with animal charcoal

and recrystallized from hot water) was suspended in 75 c.c. of absolute alcohol and the mixture completely saturated with hydrogen chloride gas. Seventy-five more c.c. of absolute alcohol was then added and the mixture refluxed on the water bath for three hours. The HCl was then neutralized with K_2CO_3 and the solution filtered. The alcohol was evaporated with vacuum and an attempt made to vacuum distill the residue.

E. Fisher³ gives the boiling point of diethyl glutamate as 139°-140° C. under a pressure of 10 mm. A very small quantity of a thick tarry smelling liquid distilled over at about 180° C. under a pressure of about 25 mm. This was at first thought to be impure diethyl glutamate but after standing several days, a good many white crystals had formed in the liquid. These crystals were soluble in water, alcohol, ether, chloroform, gasoline and benzene. They were purified by dissolving in ether and recrystallized by evaporating off most of the ether. They were then dried on a porous plate. Their melting point was 59° C.

³ Ber. 34 (1901) 453

Nitrogen in these crystals was determined by the Kjeldahl method.

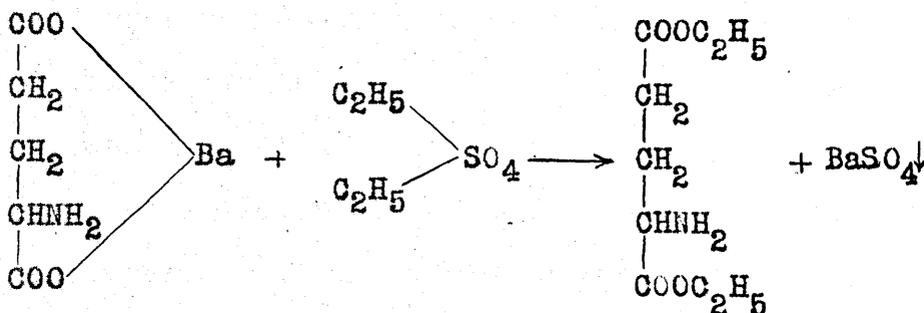
Wt. of sample	0.3068 g.	.2000 g.
c.c. of standard acid used	40. c.c.	40 c.c.
c.c. of standard base used	31.8 c.c.	40 c.c.
1 c.c. base = .7444 c.c. acid		
1 c.c. acid = 0.001874 grams of nitrogen		
Percentage of nitrogen (experimental)	9.97%	9.55%
Percentage of nitrogen in diethyl glutamate is 6.09%		
based on the formula $C_9H_{17}O_4N$		

This shows that the crystals are not diethyl glutamate. Time did not permit of a determination of their exact composition.

An attempt was made to prepare the ester using a four normal solution of hydrogen chloride in alcohol instead of the completely saturated solution of hydrogen chloride in alcohol, but the results were the same as those using a saturated solution.

ETHYL SULFATE PLUS BARIUM GLUTAMATE.

A general method of preparing the ethyl ester of an acid is to treat the barium salt of the acid with ethyl sulfate. One would thus expect the following action to take place, in which barium sulfate is precipitated out and the ester is formed.



25 grams of glutamic acid was suspended in a little water and barium carbonate was slowly added until carbon dioxide ceased to be given off. The excess of barium carbonate was filtered off and the solution evaporated to a paste on a water bath. The barium glutamate was then heated to dryness in an oven at 110 C. The salt was then powdered and mixed with slightly more than the theoretical amount of freshly distilled ethyl sulfate using m-xylene as a solvent. The mixture was then refluxed for three hours. A large amount of tar separated

out during the process, but no barium sulfate appeared to be formed and upon vacuum distillation of both the liquid and the tar no diethyl glutamate was obtained.

E. Fisher³ prepared diethyl glutamate as follows: Ten grams of glutamic acid was suspended in 75 c.c. of absolute alcohol and the mixture saturated with HCl. 150 c.c. more alcohol was then added and the mixture refluxed for three hours. Most of the alcohol was then distilled off in a vacuum from a water bath. The solution was then diluted with ether and neutralized with K_2CO_3 in an ice bath. The K_2CO_3 and KCl mixture was then filtered off and the solution dried over sodium sulfate. The liquid was decanted from the drying agent and the ether distilled off. The ester was then distilled off in vacuum. Boiling point 139° - 140° C. at 10 mm.

Fisher's method was tried giving the following results: The neutralization of the hydrogen chloride with potassium carbonate took place very slowly at the low temperature of the ice bath. Upon distillation a

little brown thick liquid was obtained which was similar in appearance to that produced in previous attempts to prepare the ester. However, it did not crystallize out upon standing so it may have been the impure ester, though time did not permit of its proof.

ATTEMPT TO PREPARE DIETHYL GLUTAMATE USING SULFURIC ACID

In the formation of the ester water is also formed. The presence of this water would tend to hydrolyse the ester as fast as formed so an attempt was made to prepare the ester using sulfuric acid as a dehydrating agent.

In one attempt twenty grams of glutamic acid was dissolved in 150 c.c. of absolute alcohol and 25 c.c. of concentrated sulfuric acid added and the solution refluxed for three hours on a water bath. A second attempt was carried out as above but the solution was refluxed for six hours instead of three hours. In a third attempt 20 g. of glutamic acid was dissolved in 150 c.c. of absolute alcohol and 10 c.c. of concentrated sulfuric acid added. This solution was then refluxed for three hours.

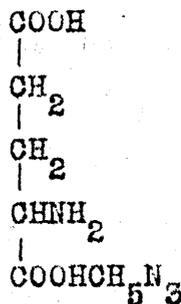
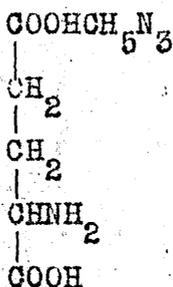
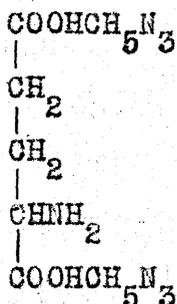
An excess of barium carbonate was added to each of these solutions as soon as they became cool. Neutralization of the acid was very slow so after standing a week with occasional stirring, about 5 grams of powdered barium hydroxide was added to neutralize

the last traces of acid. The solutions were then filtered and the filtrate distilled under reduced pressure but no diethyl glutamate was obtained.

GUANIDINE GLUTAMATE

Guanidine carbonate, being a salt of a very weak acid should be decomposed by an acid which is stronger than carbonic acid, to give carbonic acid and the guanidine salt of the acid used.

In the case of glutamic acid there should theoretically be three guanidine glutamates. One diguanidine glutamate and two isomeric monoguanidine glutamates, depending upon whether both carboxy groups have united with the base to give the diguanidine compound or upon which one of the carboxy groups has united with the base to give the monoguanidine compound. These compounds would have the following structures:



A

B

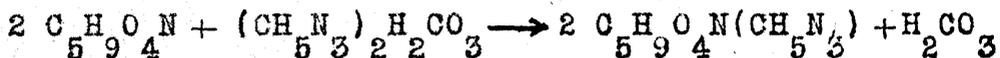
I

II

Digu^anidine Glutamate

Monoguanidine Glutamates

Guanidine carbonate was dissolved in a little warm water and glutamic acid added slowly as long as carbon dioxide was given off.



The resulting solution was then evaporated almost to dryness on the water bath and the guanidine glutamate allowed to crystallize out. These crystals were powdered and dried in a dessicator. They were not soluble to any extent in alcohol, ether, chloroform, gasoline, benzene, acetone, or pyridine but were slightly soluble in hot methyl alcohol. Their melting point was 150°C.

Nitrogen in this compound was determined by the Kjeldahl method.

Weight of sample	0.2000 g.	0.2000 g.
c.c. of standard acid used	50 c.c.	50 c.c.
c.c. of standard base used	28.6	28.3

1 c.c. base = .7444 c.c. acid

1 c.c. acid = 0.001874 grams of nitrogen

Percentage of nitrogen (experimental) 26.89% 27.09%

Percentage of nitrogen (theoretical) as calculated from the formula $C_5H_9O_4N(CH_5N_3)$ for the monoguanidine glutamate is 27.18% while that of the diguanidine glutamate is 37%. This shows that the product obtained is a monoguanidine glutamate. Whether its formula is represented as that listed above as A or whether it is the one listed as B or perhaps a mixture of these two I have no way of proving.

CONCLUSIONS

Only a very few of the possible derivatives of glucose phosphoric acid were prepared, owing to the difficulty with which glucose phosphoric acid may be obtained. In the first place the yield of glucose phosphoric acid is not very good and the process by which it is prepared is very time consuming, due to the large amount of evaporation which must be carried out at a low temperature in order not to decompose the glucose phosphoric acid.

The method of Neuberg and Pollok¹ may be used to prepare glucose phosphoric acid, but it was found that a better yield could be obtained if the method used by Levene and Meyer² in preparing α -methyl glucose phosphoric acid was applied to the preparation of glucose phosphoric acid.

Attempts to prepare diethyl glutamate were unsuccessful, due probably to the ease with which the

¹ Ber. 43 (1910) 2061

² J. Biol. Chem. 48 (1921) 235

ester is decomposed. The preparation of this ester is a problem which needs further investigation. Likewise the chemical nature of the crystalline compound obtained in the unsuccessful attempts to prepare diethyl glutamate suggests a problem for further research.

A study of the action of bacteria upon the compounds prepared is beyond the scope of this work as this work is concerned chiefly in the preparation of the compounds, so that others may use these compounds and study the action of bacteria with these substances.