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Reduction Products of D-Glucoheptulose

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REDUCTION PRODUCTS OF d-GLUCOHEPTULOSE

by

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V I T A

I was born in Poland June 4, 1912. In 1921 I came to America and attended St. Adalbert's Parochial school in Staten Island, N. Y. from which I was graduated in 1927. In 1930 I was graduated from Port Richmond High School and in the fall of the same year I registered in the City College of the College of the City of New York. In 1934 I received the Bachelor in Science degree from the City College and registered in the Loyola School of Medicine which I attended for two years. During the next year I was a holder of a Teaching Fellowship in Physiological Chemistry in the Loyola Medical School.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. W. R. Tweedy, head of the department, for the many helpful suggestions during the course of this research.

To Dr. F. L. Humoller, under whose direction this research was carried on, I wish to express my deepest appreciation. His untiring discussions which resulted in most valuable suggestions, were a source of great encouragement during times of disappointment. It has been a privilege and a rare pleasure to have worked under Dr. Humoller's supervision for the past year.

Acknowledgement is also due Mr. J. M. Dudek for his technical assistance.

In 1928 Bertrand and Nitzberg (1) prepared l-glucoheptulose (referred to by them in their papers as a-glucoheptulose)* by the action of bacterium Xylinum on a-glucoheptitol. When they reduced the l-glucoheptulose with sodium amalgam in an effort to demonstrate its ketonic nature they obtained a-glucoheptitol and instead of b-l-glucoheptitol, an unexpected alcohol which they called a-glucoheptulitol (since it was a derivative of a-glucoheptulose according to their nomenclature). This new alcohol melted at 144°C. and had a specific rotation of -2.24°. They assumed, though not without reservation, that this a-glucoheptulitol was either b-d-glucoheptitol or b-l-glucoheptitol (2) (3).

Later Y. Khouvine and G. Nitzberg (4) repeated the work of G. Bertrand and G. Nitzberg on the reduction of l-glucoheptulose and likewise obtained a-glucoheptitol and a-glucoheptulitol but from their crystallographic studies came to the conclusion that a-glucoheptulitol could not be identical with the b-glucoheptitols. They were unable however, to offer a structure for a-glucoheptulitol.

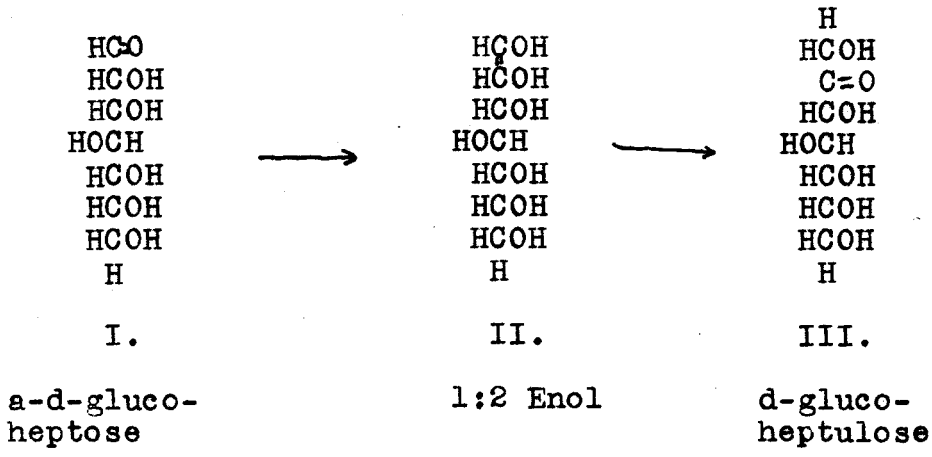
In 1930 W.C. Austin prepared d-glucoheptulose (which substance was proved to be the enantimorph of Bertrand's a-glucoheptulose i.e. l-glucoheptulose) by rearrangement of a-d-glucoheptose in calcium hydroxide solution and assigned to this new ketose a structural formula based on the following evidence:-

1. Method of preparation.

According to the Lobry de Bruin rearrangement if an aldose is

* "a" and "b" have been used in this thesis to designate the Greek characters alpha and beta respectively.

subjected to dilute alkaline treatment it undergoes a series of changes which may be illustrated with the compounds under consideration as shown below:-



I. a-d-gluco-heptose

II. 1:2 Enol

III. d-gluco-heptulose

2. Identity of osazone of d-glucoheptulose with that of a-d-glucoheptose.

It is evident that if d-glucoheptulose (III) has the structure represented, it should yield the same osazone as a-d-glucoheptose (I) because carbons 3-6 in both compounds I and III have the same spacial configuration. The identity of the osazone of d-glucoheptulose with that of a-d-glucoheptose was established by W. C. Austin on the following facts:-

1. Both had identical melting points.
2. Melting point of a mixture of the two osazones was the same as of either separately.
3. Both had identical optical rotation and mutarotation.
4. Mixed solubilities of the osazones proved them to be

identical,

Theoretically reduction of d-glucoheptulose should yield two alcohols, a-glucoheptitol and b-d-glucoheptitol of configurations:-

a-glucoheptitol	b-glucoheptitol
H	H
HCOH	HCOH
HCOH	HOCH
HCOH	HCOH
HOCH	HOCH
HCOH	HCOH
HCOH	HCOH
HCOH	HCOH
H	H

When Khouvine and Nitzberg (6) reduced Austin's d-glucoheptulose in an effort to see how this d-enantiomorph of their ketose would act when subjected to hydrogenation, they likewise obtained a-glucoheptitol and instead of the expected b-d-glucoheptitol an alcohol which they called a-d-glucoheptulitol since it was derived from a-d-glucoheptulose (d-glucoheptulose in the American nomenclature.) This substance had a melting point of 143-143.5°C and a specific rotation of +2.10°. It was the optical antipode of the heptulitol obtained from the reduction of l-glucoheptulose. (The a-d-glucoheptulitol from here on in this thesis shall be referred to as the d-glucoheptulitol.)

These results led Armstrong (7) to suggest that perhaps an error in the assignment of structure to d-glucoheptulose has been made. In view of the above facts and the assertion of Armstrong it seemed interesting to investigate the following possibilities:-

1. Since the properties of b-d-glucoheptitol described in the literature were those of a substance prepared from sirupy b-d-glucoheptose, it was thought imperative that a similar study should be made on b-d-glucoheptitol prepared by the reduction of crystalline b-d-glucoheptose in order to rule out the possibility that Khouvine's d-glucoheptulitol might actually be b-d-glucoheptitol in a higher state of purification than thatobtained by Philippe and described in the literature (8) (9).

2. The d-glucoheptulitol might be a polysaccharide formed by the condensation of two or more molecules of either of the two alcohols or a molecule of an alcohol with a molecule of the ketose, since it could not be a physical mixture of the ketose and an alcohol because it does not reduce Fehling's solution.

3. There might be some unexpected rearrangement during the reduction of the ketose. If so it was considered imperative to characterize this d-glucoheptulitol more thoroughly than Khouvine had done.

The method of attack, in brief, consisted of preparing both crystalline glucoheptoses; reducing them to their respective alcohols; preparing d-glucoheptulose and comparing its reduction products with the two alcohols obtained from their sugars. Since d-glucoheptulitol was again obtained its study was undertaken.

b-d-Glucoheptonic acid was isolated from a crude sirup by means of its insoluble brucine salt. This was then changed to the barium salt and finally to the lactone by means of sulfuric

acid. a-d-Glucoheptonic acid lactone was available in the crystalline form and was recrystallized.

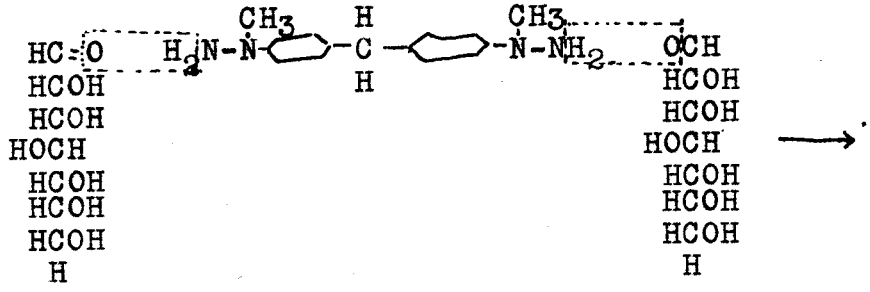
Both of these lactones were reduced to the respective aldoses which were crystallized and purified by recrystallization. Portions of each of these were then reduced to the corresponding alcohols, the physical and chemical properties of which agreed with the properties cited in the literature. (8) (9) (10)

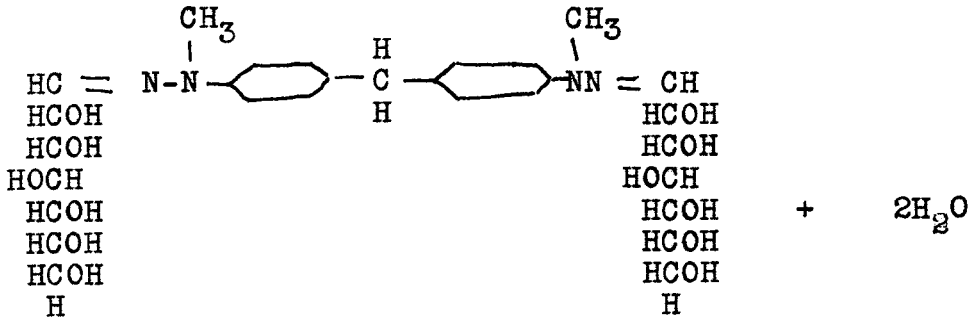
	a-d-Glucoheptitol		b-d-Glucoheptitol	
	Found	Reported in literature	Found	Reported in literature
M.P.	129°	129°	129°	129°
Sp. rot.	0°	0°	+0.6°	+0.8°
Character	needles	needles	rectangles	rectangles

d-Glucoheptulose was prepared from a-d-glucoheptose by the Lobry de Bruin (11) rearrangement. This consisted of treating the aldose with a saturated calcium hydroxide solution at 35° for seven days. The progress of the rearrangement was followed polarimetrically. The calcium was then removed by means of oxalic acid and the system was worked up to yield several crops consisting of variable mixtures of aldose and ketose.

In his work W.C. Austin (5) separated d-glucoheptulose from the aldose-ketose mixture by the method of Hudson and Isbell (12) whereby the aldose is oxidized with bromine water containing barium benzoate and the ketose is largely unaffected. The hept-

onic acid is then removed as its barium salt. Though this procedure is effective it is tedious and entails some loss of the ketose due to the many manipulations involved. In the course of this work advantage was taken of the fact that when a solution of di-phenyl-methane-di-methyl-di-hyrazine in 95% alcohol is mixed with an equal volume of an aqueous solution of d-glucoheptose, the heptose is precipitated quantitatively in a few hours as the dihydrazone, while under the same condition di-phenyl-methane-di-methyl-di-hydrazine will not react with the ketose even if allowed to remain in contact with it for days. The quantity of ketose isolated from the ketose-aldose mixture after removal of the aldose by this method was 100% of that calculated. Braun (13) reports that di-phenyl-methane-di-methyl-di-hydrazine forms insoluble hydrazones with all aldose sugars in which the two carbon atoms next to the carbonyl group have their hydroxyl groups in the Cis position. This work therefore is a confirmation of Braun's contention. The reaction is represented below:-





When d-glucoheptulose was reduced with sodium amalgam and the system worked up it yielded two crops of material. Crop I melted at 140° and had specific rotation of + 2.04°. Obviously this substance is d-glucoheptulitol (4) From crop II by a method of fractional crystallization a pure substance was isolated which is b-d-glucoheptitol on the following evidence:-

1. It crystallizes as squares aggregating in star like heaps.
2. It melts at 129°C.
3. Mixed with b-d-glucoheptitol prepared from crystalline b-d-glucoheptose it melts at 129°C.
4. Its specific rotation is + 0.62° (room temp.)

In the second reduction of d-glucoheptulose three pure compounds were isolated:- 1. a-glucoheptitol was obtained as crop I. It melted at 129°C. When mixed with a-glucoheptitol obtained by the reduction of a-d-glucoheptose it melted at 129° C. It was optically inactive. 2. From the mother liquors of recrystallizations of crop I was obtained d-glucoheptulitol. It melted at 140°C. When mixed with d-glucoheptulitol obtained from the first reduction it melted at 140°C. It had a specific

rotation of + 1.95°. 3. From the original mother liquors of crop I b-d-glucoheptitol was obtained again by means of fractional crystallization. It melted at 129°. Its mixed melting point with b-d-glucoheptitol obtained by the reduction of crystalline b-d-glucoheptose was 129°. It had a specific rotation of + 0.57°

A third reduction of the ketose yielded exactly the same products as the system of the first reduction viz., d-glucoheptulitol and b-d-glucoheptitol. Subsequently four more reductions of 20 g. portions were carried out in order to accumulate a supply of d-glucoheptulitol for its study. A thorough consideration of the conditions of the reductions failed to disclose a single suspicion as to the reason why the greatest portion of ketose in the second reduction went over into a-glucoheptitol, whereas in the first and all subsequent ones into d-glucoheptulitol. It should be recalled at this point that Khouvine's reductions yielded consistently d-glucoheptulitol and a-glucoheptitol. In view of these results and the fact that this work yielded d-glucoheptulitol, a-, and b-glucoheptitols, there should be no doubt as to the correctness of the formula assigned to d-glucoheptulose by W. C. Austin. Additional confirmation of this formula will be given below in the study of d-glucoheptulitol.

d-Glucoheptulitol does not lose weight in high vacuum at 100°C in the presence of P_2O_5. It does not give a test for desoxy carbohydrates (14). Treatment with 0.1N sulfuric acid at

room temperature and at 100°C for half an hour leaves it uneffected. It is stable to 10% barium hydroxide treatment for one and a half hours at boiling temperature. However when subjected to boiling 10% sulfuric acid for one and a half hours it is partialy converted to a-glucoheptitol as shown by the optical rotation, and mixed melting point of the material isolated. Its molecular weight determined by the lowering of the freezing point method is 192.3. Consequently d-glucoheptulitol must be a derivative of a monosaccharide. Since it is changed to a-glucoheptitol by 10% sulfuric acid, d-glucoheptulitol must contain 7 carbon atoms attached to each other in a straight chain i.e., there cannot be a carbon side chain. From the behavior of the d-glucoheptulitol toward 10% sulfuric acid and from its molecular weight one would be tempted to assume that d-glucoheptulitol is the anhydride of a-glucoheptitol crystallizing as 4 molecules of the anhydride with 3 molecules of water. This monoanhydride should have a molecular weight of 198. It should contain 40.47% C and 7.58% H. Combustion studies on the d-glucoheptulitol show it to contain 40.51% C and 7.40% H. Since d-glucoheptulitol fails to yield any water of crystallization at 100°C in high vacuum over P_2O_5 such a state of affairs cannot be assumed definitely.

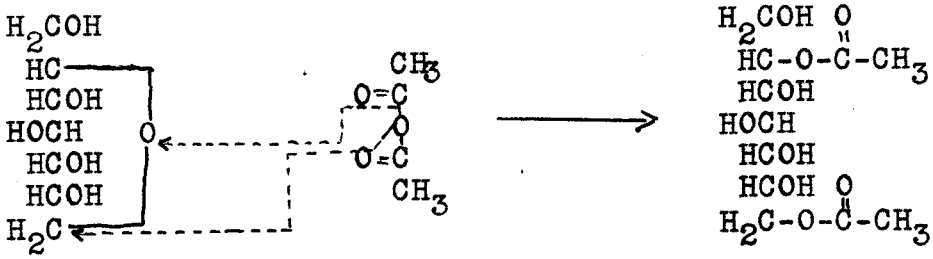
It was next thought desirable to determine the number of alcoholic OH groups in d-glucoheptulitol by acetylation studies. However it was considered necessary to prepare first the heptacetate of a-glucoheptitol. This was done by treating a-glucohepti-

tol with acetic anhydride in the presence of fused zinc chloride. The product obtained had the same constants as those reported by E. Fischer (15). It crystallized in the form of diamonds, and melted at 115°C.

When the acetate of the d-glucoheptulitol was prepared by the same method it proved to be identical with the acetate of a-glucoheptitol. It had the same characteristic appearance; it melted at 115°C; when mixed with the heptacetate of a-glucoheptitol, it melted at 115°C. Analysis showed:-

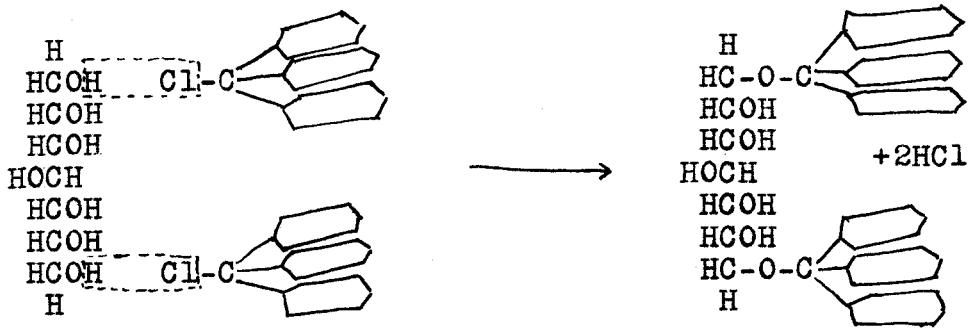
	Acetate of d-glucoheptulitol (Found)	Acetate of a-glucoheptitol (Calculated)
C	49.65%	49.74%
H	6.08%	5.97%

Since the more drastic method of forming the acetate of d-glucoheptulitol yielded only the acetate of a-glucoheptitol an attempt was made to form the acetate by means of the milder pyridine method. Again a compound was isolated which was identical with the acetate of a-glucoheptitol in every respect. It is known that an anhydride may give the heptacetate of d-glucoheptulitol with acetic anhydride under totaly anhydrous conditions. The mechanism is represented below:-



Then the remaining 5 OH groups are acetylated in the usual manner.

Next an attempt was made to determine the number of primary alcohol groups in the d-glucoheptulitol molecule by preparing its trityl derivative. Since its acetate proved to be identical with the acetate of a-glucoheptitol it was considered important to prepare first the di-trityl derivative of a-glucoheptitol. This was accomplished by treating the a-glucoheptitol in pyridine solution with tri-phenyl-chloro-methane. The reaction is represented below:-



a-glucoheptitol

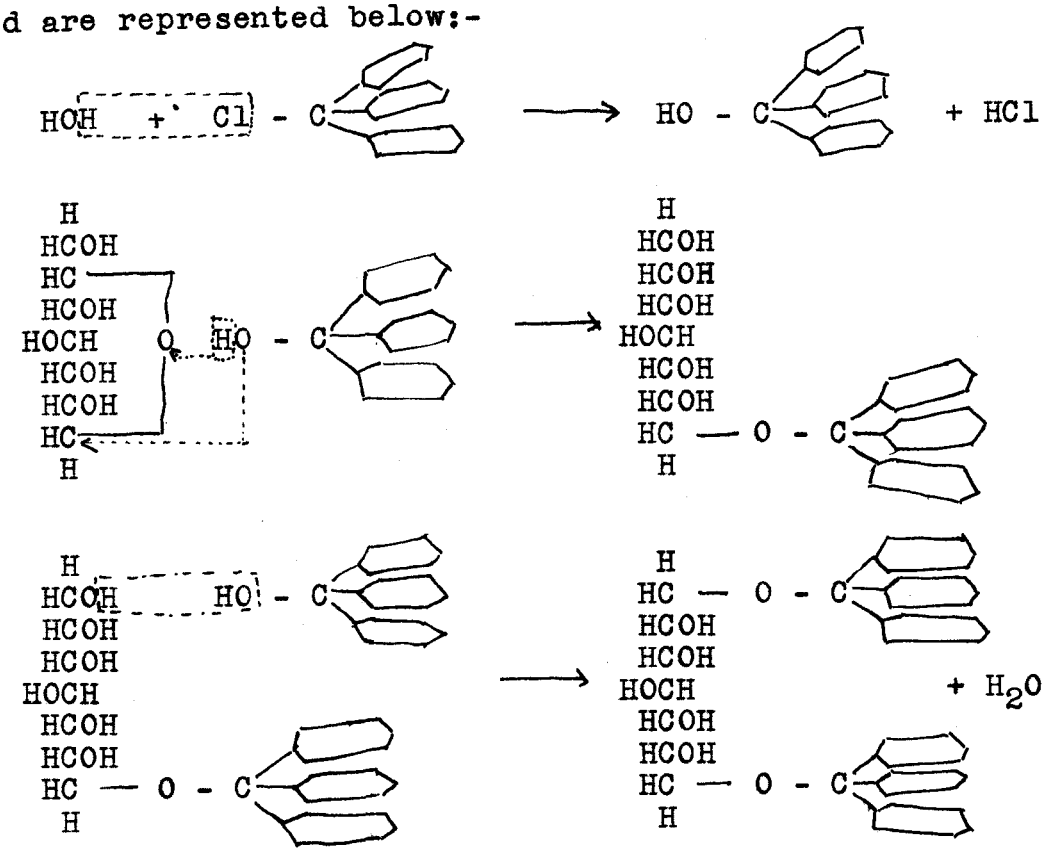
di-trityl-
a-glucoheptitol

The compound when purified melted at 143-144°C. Analysis showed:-

	Calculated	Found	Difference
C	77.57%	77.82%	0.25%
H	6.37%	6.48%	0.11%

The trityl derivative prepared from glucoheptulitol by the same method proved to be identical with the di-trityl of a-glucoheptitol. It melted at 142-143°C; mixed with di-trityl a-gluco-

heptitol it melted at 142-143°C. Unfortunately the method of preparing trityl derivatives is such that absolutely anhydrous conditions cannot be maintained in the system up to the point of actual isolation of the derivative. Consequently there is some hydrolysis of the tri-phenyl-chloro-methane to yield tri-phenyl-methyl carbinol which then reacts with the anhydride to yield the di-trityl derivative of a-glucoheptitol. The mechanisms involved are represented below:-



It is not to be understood that all of the tri-phenyl-chlor-methane must undergo a hydrolysis before it can react with the anhydride. This is very improbable. However, sufficient hydro-

lysis occurs to split the anhydride ring. Once this happens the remaining primary alcohol group can enter in a reaction with the tri-phenyl-chloro-methane. It is very improbable that the heptulitol is hydrolyzed first and then reacts with the tri-phenyl-chloro-methane since this anhydride does not hydrolyze in water even at boiling temperature. It requires 10% sulfuric acid for its hydrolysis.

Thus every attempt at preparing derivatives of d-glucoheptulitol yielded derivatives of a-glucoheptitol adding further weight to the assumption that d-glucoheptulitol is an anhydride of a-glucoheptitol. Though the anhydride structure cannot definitely be assumed nevertheless certain vital conclusions can be drawn at this point.

1. Since the heptulitol is so readily converted to a-glucoheptitol, its isolation from the reduction system is not a hinderance to, as implied by Armstrong, but an actual proof of the structure of d-glucoheptulose as determined by W. C. Austin. There is no doubt that Khouvine would have arrived at the same conclusion if she had worked up her mother liquors to isolate all three substances and then had studied the properties of d-glucoheptulitol.

2. a-Glucoheptitol is optically inactive by internal compensation. Since d-glucoheptulitol rotates polarized light it cannot be a derivative of a-glucoheptitol since such a derivative would of necessity be a racemic mixture and consequently

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EXPERIMENTAL PART

Isolation of b-Glucoheptonic acid.

b-Glucoheptonic acid was isolated from a crude sirup left by W.C. Austin, by means of its insoluble brucine salt. This was changed to the barium salt by means of caustic baryta and finally to the heptonic acid lactone by means of sulfuric acid. After purification the lactone melted at 151°C and had a specific rotation of - 78.3° (10 min. after solution, room temp.). In 15 min. the specific rotation reached - 75.0° and in 24 hrs. - 64.0°.

Reduction of a-d-Glucoheptonolactone.

Hundred g. of a-d-glucoheptonolactone were dissolved in 800 cc. of water and volume was made up to 1000 cc. The solution was chilled to 0°C. and kept within 3° of this temperature during the entire procedure. Four percent sodium amalgam was added at the rate of 100 g. per 30 min. and the system was stirred vigorously. The reaction was kept just on the acid side of Congo Red by the addition of 20% sulfuric acid. The progress of reduction was followed by Goebel's Iodimetric aldose titrations(16). When the aldose content reached 82 g. the reduction system was brought to room temperature. The mercury was then separated and the solution was neutralized and concentrated in vacuum up to the point of crystallization of sodium sulfate. The concentrate was then poured into 8 volumes of boiling 95% ethanol and allowed to stand for about 2 hrs. The sodium sulfate was filtered off. It

was negative to Fehling's solution. In other experiments when the sulfate was positive it was extracted with 95% ethanol until the test was negative. The filtrate after concentration readily crystallized to give 47.5 g. of a-d-glucoheptose melting at 193° C. When the mother liquors were again concentrated they yielded 8 g. more of the aldose melting at 187.5°C.

Subsequently 822 g. of a-d-glucoheptonolactone were reduced to yield 456 g. crystalline a-d-glucoheptose. The crude heptose when recrystallized from water gave 410 g. of pure material which melted at 191°C and had a specific rotation of - 24° (room temp., 20 min. after sol.).

Reduction of b-d-Glucoheptonolactone.

Seventy-nine and a half g. of b-d-glucoheptonolactone were dissolved in 750 cc of water. The reduction was accomplished in the same manner as described for the a-lactone. When the total aldose reached 65.8 g. the reduction was stopped, and the mercury was separated. The solution was neutralized and concentrated to the point of crystallization of sodium sulfate. The concentrate was then poured into enough 95% ethanol to make the final strength 85% and allowed to stand overnight. The next morning the sulfate was filtered off, and extracted once more with 85% ethanol for b-d-glucoheptose. The combined alcoholic extracts were concentrated to a sirup and allowed to stand in a vacuum desiccator over the week end. No crystals appeared. It was therefore taken up in 150 cc. of 95% ethanol and placed in the ice chest. Crystalli-

zation failed to set in after 3 weeks of standing, the sugar simply separating as a sirup on the bottom of the flask. The system was therefore decanted; the decantate was concentrated to a sirup and taken up in absolute ethanol. Overnight a sirup separated again but the system was allowed to remain in the ice chest for 6 weeks at the end of which time there appeared two foci of crystalline material on top of the sirup. The supernatent fluid was decanted; the residue was stirred up and it crystallized, though exceedingly slowly. The decanted fluid was concentrated and seeded and it too crystallized. After five days of crystallization both systems were filtered off. Thus 7.5 g. of crystals were obtained as well as a thin sirup. The crystals after one recrystallization melted at 120°C and weighed 4.5 g. Their mutarotation in a 4% solution corresponded to the values reported by Isbell (17).

Mutarotation of b-d-Glucoheptose

5 min.	after	solution	- 2.87°
7 "	"	"	- 4.00°
8 "	"	"	- 4.38°
11 "	"	"	- 5.00°
15 "	"	"	- 2.38°
30 "	"	"	- 0.37°
24 hrs.	"	"	- 0.12°

Reduction of a-d-glucoheptose.

Ten g. of a-d-glucoheptose were dissolved in 100cc. of water. Sulfuric acid was added to produce an acid reaction and 100 g. 4% amalgam were added. The system was kept at room temperature and under constant agitation in a shaking machine. The

amalgam from then on was added in 100 g. portions and acid was added from time to time to keep the system just acid to litmus. After the addition of 500 g. of amalgam 15 drops of the system reduced Fehling's solution slightly. An additional 100 g. amalgam was added and the reduction was pushed to the end in an alkaline medium.

Sodium sulfate was removed in the usual manner by the aid of ethanol and the alcoholic solution was then concentrated to about 25 cc. The concentrate was taken up in 100 cc of absolute ethyl alcohol and after standing overnight in the ice chest it yielded 8 g. of material melting at 127°C. After two recrystallizations it melted at 129°C, and was optically inactive.

Analysis showed:-

	Found	Calculated
C	39.37%	39.61%
H	7.69%	7.60%

Reduction of Crystalline b-d-glucoheptose.

Three and a half g. of crystalline b-d-glucoheptose, were reduced in the same manner as that described for the a-d-glucoheptose, to yield 2.6 g. of crystalline b-d-glucoheptitol. After two recrystallizations this alcohol reached a constant melting point of 129°C. and crystallized as small rectangles aggregating in rosettes. The b-d-glucoheptitol obtained by the reduction of sirupy b-d-glucoheptose melted at the same temperature as did the b-d-heptitol obtained from the crystalline aldose. A melting

point determination of a mixture of the two samples of b-d-glucoheptitol showed no depression, and both substances had the same crystalline appearance. This proved that the b-d-glucoheptitol obtained from sirupy b-glucoheptose and described by Philippe was the true b-d-glucoheptitol.

Rearrangement of a-d-Glucoheptose to the d-Glucoheptulose.

Two hundred g. of a-d-glucoheptose, M.P. 190°C, Sp. Rot. - 24.0° (20 min. after sol.) were dissolved in 2 liters of saturated lime water. A sample of the solution was filtered through a carbon pad and its rotation (25 min. after sol.) was - 14.7°. The solution was placed in the incubator at 35°C. After 4 hrs. it reached a specific rotation of - 10.2° and after 7 days +35.0°. At the end of this time the calcium was precipitated by adding the required amount of oxalic acid. After filtering through carbon a nearly colorless solution was obtained. This was concentrated to about 200 cc. and 200 cc. of methyl alcohol were added to it. On standing in the ice chest overnight 96 g. of material crystallized. M.P. 158-170°C., Sp. Rot. + 32.5°(8 min. after sol.). The mother liquors were returned to the ice chest and 30 g. more material separated out, M.P. 160°C, Sp. Rot. +59.5° (9 min. after sol.) The mother liquors of this crop yielded 26g. more crystals.

In another experiment a solution of 200 g. of a-d-glucoheptosein saturated calcium hydroxide reached a final specific rotation of +38.6°; and after the system was worked up it yielded

145 g. crystalline material.

Isolation of d-Glucoheptulose from the Aldose-Ketose Mixture by the Aid of Di-Phenyl-methane-di-methyl-di-hydrazine.

Since this method had not been employed by anyone for the separation of d-glucoheptulose from the aldose-ketose mixture it was necessary first to carry out preliminary experiments on the pure d-glucoheptulose and a-d-glucoheptose.

One g. of a-d-glucoheptose was dissolved in 60 cc. 45% ethanol and to it was added 2 g. of di-phenyl-methane-di-methyl-di-hydrazine in 15 cc. 95% ethanol. After standing some time a cloudiness appeared and within an hour the entire system became a gel-like solid. The crystals were a light orange in color. After drying they assumed a dark brown appearance and melted at 160-165°C (with decomposition). They were the di-hydrazone of d-glucoheptose.

To 0.5 g. of d-glucoheptulose in 30 cc. of 45% ethanol was added 1.0 g. of the dihydrazine. After standing over week-end the system showed no precipitate. It was therefore diluted with water and a light yellow precipitate appeared. This was soluble in glacial acetic acid and melted at 75-80°C. It was therefore the original di-hydrazine which was caused to precipitate by the addition of water . The remainder of the di-hydrazine was then precipitated by the addition of 50 cc. of 37% formaldehyde. A polarimetric estimation of the ketose in the filtrate showed .44g; there was therefore a loss of only 0.06 g. of the original ketose

by the manipulations.

After a pilot experiment on 20 g. of the aldose-ketose mixture, 278 g. of material were dissolved in enough water to make the final volume 1500 cc.. Two 2 cc. samples were then removed for iodimetric aldose estimations. Twenty four percent (67.55 g.) of the 278 g. was found to be aldose. This would have required 41.4 g. of di-phenyl-methane-di-methyl-di-hydrazine to precipitate all the aldose under ideal conditions. Fifty-eight g. of the di-hydrazine or an excess of 17 g. were dissolved in 1500 cc. ethanol and the sugar mixture was then poured into the alcoholic hydrazine solution and the system was allowed to stand overnight. The next morning the fine precipitate of di-hydrazone of a-d-glucoheptose was filtered off and washed two times with 100 cc portions of ethyl alcohol. When dried it weighed 52 g. and melted at 167°C. To the filtrate was added an excess of formaldehyde to precipitate the uncombined di-hydrazine and the system was allowed to stand overnight. The next morning the formaldehyde-di-hydrazine was filtered off and the filtrate, charcoaled and concentrated under reduced pressure. The sirup was rediluted with water and concentrated again in vacuum. This was repeated several times to remove all the formaldehyde. Finally the formaldehyde free sirup was taken up in 300 cc of methyl-ethyl alcohol in the proportion of 7:3. Upon seeding and cooling a fine precipitate began to separate out and in a short time the entire system solidified. The precipitate, when dry, weighed 177 g. and

melted at 163°C. When the mother liquors were worked up they yielded 35 g. more material which melted at 156°C. A total of 212 g. of crude ketose was thus obtained. Since the theory calls for 211 g., it can be said that this method of separation yielded practically quantitative results. After appropriate recrystallization 140 g. of d-glucoheptulose was obtained which had a constant melting point of 169°C. and 54 g. of less pure material melting at 161°C.

Reduction of d-Glucoheptulose.

Three reduction systems were worked up completely. Four other systems were worked up merely for the purpose of accumulating a supply of d-glucoheptulitol. All reductions were accomplished under the same conditions as those described above for a-d-glucoheptose.

Reduction No. 1:- Twenty grams of d-glucoheptulose were reduced. After separation of the sulfate the system was concentrated in vacuum to a thin sirup and taken up in 100 cc. boiling absolute ethyl-alcohol. Upon cooling, a precipitate consisting of fine needles aggregating in rosettes began to come down. The next morning the precipitate was filtered off to give 10 g. of material as C_{r}op I. It melted at 139°C. The mother liquors were concentrated and taken up in 50 cc. of boiling absolute ethyl alcohol to yield 4.0 g. of material cropII. This crop melted at 116°C and consisted of small slender rectangles and fine needles. A third crop of 1 g. melted at 107°C, appeared

amorphous and was of no consequence.

Crop I was dissolved in 20 cc. of distilled water filtered through hardened paper, concentrated to a small volume and taken up in 100 cc absolute ethanol. Nine grams material were recovered which melted at 141°C. Three subsequent recrystallizations failed to alter themelting point of this material. Its specific rotation at 28°C. was + 2.04°. No doubt remains that this material is the same d-glucoheptulitol as obtained by Y. Khouvine. (6)

Crop II was dissolved in a small quantity of water, filtered and taken up in hot absolute ethyl alcohol. Overnight there separated 3 g. material which melted at 116°C. Another such recrystallization brought the melting point down to 115°C. The material was then dissolved in the smallest quantity of boiling methyl alcohol and allowed to crystallize. Its melting point reached 119°C. After another recrystallization from absolute methanol it melted at 125°C. and still consisted of a mixture of squares and needles. At this point fractional crystallization from methyl alcohol was attempted. The material was dissolved in hot methyl alcohol and upon cooling four separate crops were obtained:-

Crop a. melted at 129°C.

Crop b. " " 128°C.

Crop c. " " 126°C.

Crop d. " " 126°C.

Crop a. when mixed with b-d-glucoheptitol melted at 128°C., with

a-glucoheptitol at 114°C., with d-glucoheptulitol at 120°C. Crops a. and b. were therefore combined and again recrystallized from absolute methanol. After this procedure the precipitate melted at 129°C. Crop c. was recrystallized separately from absolute methanol and it melted at 128°C. Another recrystallization brought the melting point up to 129°C. This was therefore combined with the material obtained from crop a. and b. (M.P. 129°) and recrystallized again to yield 1 g. of material crystallizing as small rectangles which melted at 129°C and had a specific rotation of + 0.62°. When mixed with b-d-glucoheptitol obtained from the reduction of crystalline b-d-glucoheptose it melted at 129°C. This substance therefore was b-d-glucoheptitol. Crop d. when mixed with b-glucoheptitol melted at 115°C. It was therefore discarded.

An attempt was made to isolate a-glucoheptitol from mother liquors of recrystallization in this experiment but this could not be done on account of the small quantities of material that one had to work with. By way of summary, reduction No.1 yielded d-glucoheptulitol and b-d-glucoheptitol.

Reduction No. 2:-

Since in the reduction No. 1 d-glucoheptulitol was obtained as crop one in a yield of 50%, it seemed desirable to work up a supply of this material for subsequent study. Being interested primarily in this fact, a less pure stock of d-glucoheptulose was used. This melted at 160-161°C and weighed 54 g. Estima-

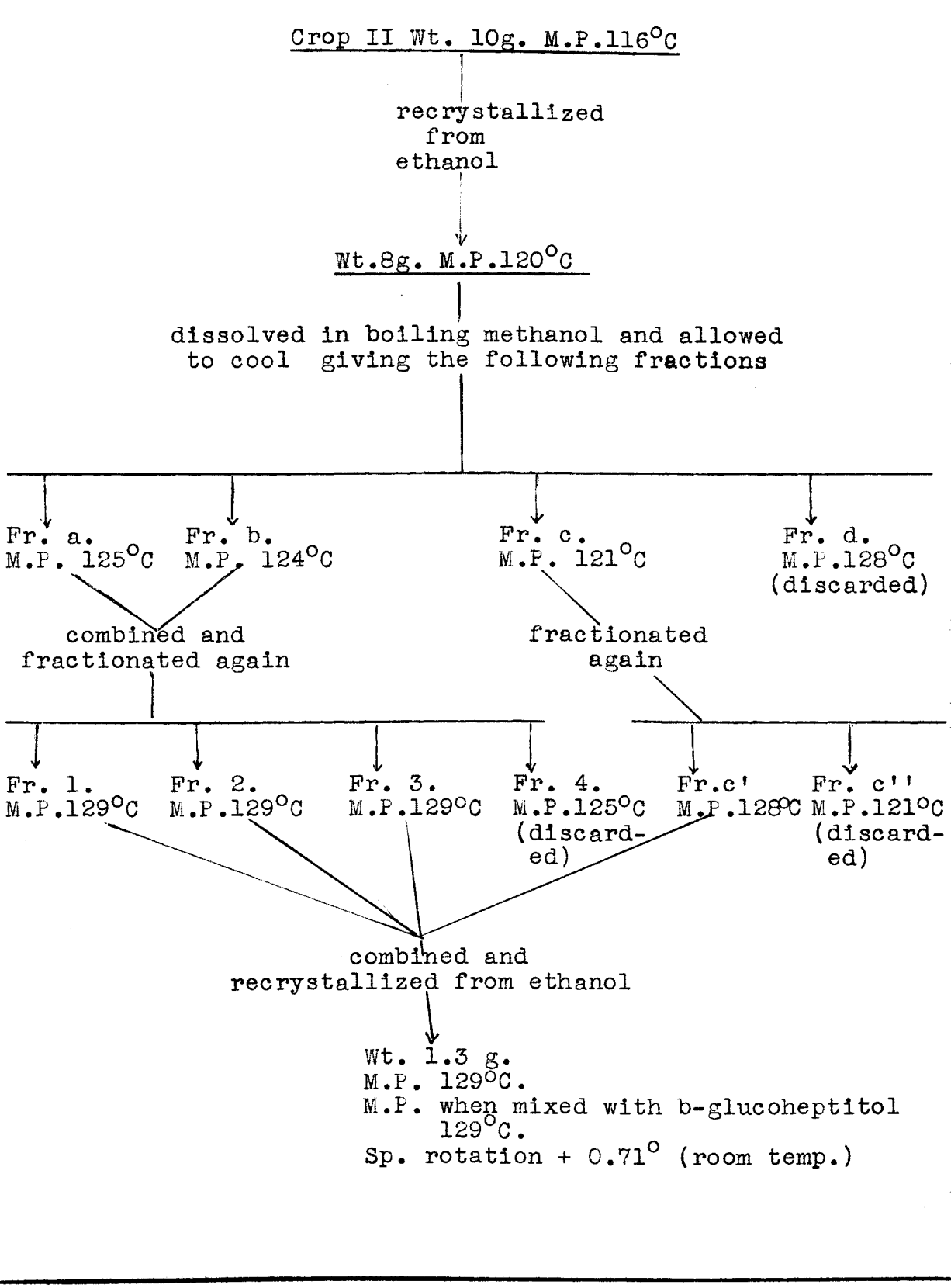
tions by polarimetry showed this material to contain 18.36% aldose, by iodimetry 18.00%. Accordingly the 54 g. of material contained 9.72 g. of a-d-glucoheptose and 44.28g. d-glucoheptulose. Reduction was accomplished under conditions which were strictly identical with those of the first reduction. The mercury and sulfate were removed and the system was concentrated to a sirup and taken up in 300 cc. of 95% ethanol. Overnight there separated 25 g. of material as crop I. This crystallized as slender rods aggregating in rosettes, weighed 25 g. and melted at 124° C. The mother liquors of crop I were charcoaled, concentrated to sirup and taken up in 1500 cc absolute ethanol to yield a crop II of material weighing 10 g. and melting at 116°C. Mother liquors of crop II yielded a crop III of 1.5 g. This crop proved to be of no significance.

Crop I after three recrystallizations reached a constant melting point of 128°C. It weighed 18 g. and was optically inactive. It is obvious therefore that this material is a-glucoheptitol. Since there was only 9.0 g. of a-d-glucoheptose in the material reduced it can safely be said that at least one-half of this a-glucoheptitol came from the reduction of d-glucoheptulose.

The mother liquors of recrystallizations of crop I were concentrated and treated with ethanol to yield 2.3 g. of material which melted at 137°C. After two recrystallizations it reached a constant melting point of 140-141°C. Its specific rotation at 25°C was +1.95°. When mixed with the d-glucoheptulitol obtained

in Reduction No. 1, it melted at 140-141°C. Consequently this material is d-glucoheptulitol.

Crop II was dissolved in a small quantity of water and thrown into enough boiling absolute alcohol to obtain a permanent turbidity. After one hour crystals appeared which were almost perfect squares. Upon stirring, a voluminous mass of a precipitate appeared which consisted of fine needles. It was obvious that crop II at this point contained at least two different compounds. After crystallization was complete the precipitate was filtered off and dried. It weighed 8 g. and melted at 120°C. The eight g. of material were dissolved in the smallest quantity of boiling methanol and upon cooling, again crystals began to appear which took the form of small squares aggregating in heaps or stars. Fractional crystallization was therefore made use of to separate these and the steps are indicated in the following flow sheet:-


Crop II Wt. 10g. M.P.116°C
recrystallized from ethanol
Wt.8g. M.P.120°C
dissolved in boiling methanol and allowed to cool giving the following fractions
Fr. a. M.P. 125°C
Fr. b. M.P. 124°C
Fr. c. M.P. 121°C
Fr. d. M.P.128°C (discarded)
combined and fractionated again
fractionated again
Fr. 1. M.P.129°C
Fr. 2. M.P.129°C
Fr. 3. M.P.129°C
Fr. 4. M.P.125°C (discarded)
Fr.c' M.P.128°C
Fr. c'' M.P.121°C (discarded)
combined and recrystallized from ethanol
Wt. 1.3 g.
M.P. 129°C.
M.P. when mixed with b-glucoheptitol 129°C.
Sp. rotation + 0.71° (room temp.)

Thus it is evident that the substance isolated from crop II in this reduction is the same as that isolated from crop II of the first reduction namely, b-d-glucoheptitol. To summerize, reduction No. 2 yielded a-glucoheptitol, b-d-glucoheptitol and d-glucoheptulitol.

A third reduction of purest d-glucoheptulose yielded the same products as reduction No. 1, viz. d-glucoheptulitol, and b-d-glucoheptitol.

Subsequently 80 g. more of purest d-glucoheptulose were reduced in 20 g. portions to accumulate a supply of d-glucoheptulitol. In each of these reductions like in reduction No. 1 a 50% yield of d-glucoheptulitol was obtained.

Treatment of d-Glucoheptulitol with 0.1N Sulfuric Acid.

1 g. of d-glucoheptulitol was dissolved in 25 cc. of 0.1N sulfuric acid. Its specific rotation at 25°C was + 1.96°. The solution did not undergo a change in rotation after twenty four hours. At the end of this time it was concentrated under vacuum and about 1 g. of substance was recovered which was identical with the d-glucoheptulitol before it was put in solution. Thus d-glucoheptulitol was found to be stable to 0.1N sulfuric acid at room temperature. Similarly when 1 g. of d-glucoheptulitol was subjected to boiling 0.1N sulfuric acid for 30 minutes, it was recovered unchanged.

Treatment of d-Glucoheptulitol with 10% Barium Hydroxide.

Four g. of d-glucoheptulitol was treated with 10% barium

hydroxide at boiling temperature for 1 hr. and 40 minutes. The barium was then quantitatively precipitated with sulfuric acid and filtered off. The filtrate was concentrated and taken up in absolute ethyl alcohol to yield crop I, Wt. 2.8 g., M.P. 139°C. The mother liquors were concentrated and taken up in 95% ethyl alcohol, and a few drops of ether were added to the system to produce a permanent turbidity. Overnight a small amount of amorphous matter separated out. Hence the system was again concentrated and taken up in absolute ethyl alcohol to give a crop II Wt. 0.3 g. M.P. 139°C. Thus boiling barium hydroxide was without effect on d-glucoheptulitol.

Treatment of d-Glucoheptulitol with 10% Sulfuric Acid at Boiling Temperature.

Six grams of d-glucoheptulitol were dissolved in 30 cc. of 10% sulfuric acid and heated at boiling temperature over an oil bath for 1¼ hrs.. At the end of this time the system showed only a slight discoloration. It was charcoaled and read in the polariscope. Its specific rotation at 25°C was - 1.83°.

The sulfate was precipitated with barium hydroxide and filtered off. The filtrate was concentrated and taken up in 85 cc. of boiling absolute ethanol. After cooling a few crystals separated out on the bottom of the flask. The system was stirred and in five minutes a good crop of precipitate separated out leaving the supernatent liquid clear. After another 10 minutes the supernatent liquid began to get cloudy. Hence the precipi-

tate was filtered off. It weighed 3 g. and melted at 125.5°C. After two recrystallizations it melted at 129°C and was optically inactive. When mixed with a-glucoheptitol it melted at 129°C. The mother liquors of crop I were concentrated and taken up in hot absolute ethanol to yield on cooling 0.5 g. of material as crop II. This melted at 138°C. After one recrystallization it melted at 138.5°C and had a specific rotation of + 2.4° (room temp.) The mother liquors of crop II were concentrated to dryness, weighed, and dissolved in water. They showed a specific rotation of - 9.4°. (room temp.) The high levorotation in these residues apparently is due to decomposition products of acid treatment.

Determination of the Molecular Weight of d-Glucoheptulitol.

The molecular weight of d-glucoheptulitol was determined by the cryoscopic method. Distilled water was used as the solvent The freezing point of the pure solvent was:-

2.290	(Beckman Scale)
2.293	
2.293	
2.293	
2.293	
2.293	(Average)

Then 1.0009 g. of d-glucoheptulitol (M.P. 140°C.) were dissolved in 25 cc. of the above solvent at 18-19°C. The freezing point of the solution was:-

1.905	(Beckman Scale)
1.910	
1.904	
1.904	
1.906	(Average)

Hence the average depression was 0.387°C. The density of water at 18-19°C is 0.998. Therefore the total volume of water was 24.950 and the molecular weight:-

$$M.\ W. = 1860 \times \frac{1.0009}{24.95 \times 0.388} = 192.3$$

Preparation of Heptacetate of a-Glucoheptitol.

Two grams of a-glucoheptitol were dissolved in 10 cc. acetic anhydride containing a small amount of freshly fused zinc chloride and the solution was heated under reflux for 1 hr.

Acetic anhydride was removed by heating the solution in vacuum. Then to the sirupy residue absolute ethanol was added to convert the remaining anhydride to the ethyl ester. The latter was removed by distillation under vacuum. The process was repeated until the system showed no perceptible odor of ethyl acetate. The residue was then taken up in absolute alcohol and filtered through carbon.

On cooling the solution began to crystallize. The crystals took the shape of rhombic plates and after recrystallization from water and then from ethanol they reached a constant melting point of 115°C.

Preparation of Acetate of d-Glucoheptulitol.

1. With zinc chloride as the catalyst.

Four grams of d-glucoheptulitol and 40 g. of acetic anhydride containing 1 g. of freshly fused zinc chloride were mixed. Not all of the d-glucoheptulitol went into solution but

on gentle warming it dissolved. The solution was heated on the boiling water bath for 3 hrs. At the end of this time it was slightly brown in color. The acetic anhydride was removed in the same way as described under the preparation of heptacetate of a-glucoheptitol and the residue was taken up in about 50 cc. of chloroform and extracted with an equal volume of water to remove the zinc chloride. The chloroform layer was then filtered through carbon and dried with anhydrous calcium sulfate. The chloroform was driven off in vacuum. The residue was taken up in about 50 cc. of absolute ethanol. Crystallization soon set in and it was allowed to go to completion over the week end. The precipitate consisting of rhombic plates was filtered off, washed and dried in vacuum. Yield, 6.0 g.M.P., 113-114°C. After recrystallization from 60 cc. of ethanol the crystals melted at 115°C. When mixed with the heptacetate of a-glucoheptitol they melted at 115°C. Like the heptacetate of a-glucoheptitol they showed no optical rotation in chloroform. Consequently the two substances are identical.

2. With pyridine as the catalyst.

Since the more drastic method of forming the acetate of d-glucoheptulitol yielded only an acetate of a-glucoheptitol, an attempt was made to form the acetate of d-glucoheptulitol by means of the milder pyridine method.

Two grams of d-glucoheptulitol were suspended in 15 cc. of redistilled anhydrous pyridine and 10 cc. of acetic anhydride was

added. The mixture was tightly stoppered and kept at zero degrees centigrade over night. The next morning not all the material had gone into solution. However the mixture was treated with 100 cc. of water and then extracted with three 30 cc. portions ether. The combined ether extracts were extracted once with 20 cc. of water. The ether was then evaporated off under reduced pressure, and the residual sirup, dissolved in about 20 cc. absolute ethanol. Crystallization soon began and it was allowed to go to completion. A microscopic examination revealed the crystals to be identical with the acetate prepared by means of zinc chloride. They also had the same melting point and were optically inactive.

Preparation of the Trityl Derivative of a-Glucoheptitol.

Two grams of a-glucoheptitol were dissolved in about 25 cc. of pyridine. To it were added 6 g. of triphenyl-chloro-methane. The suspension of the heptitol was shaken from time to time and kept at room temperature for two days. At the end of which time it went into solution. The solution was then chilled at0°C for two hours and water was added drop by drop until a permanent cloudiness was obtained. It was then poured into a liter of ice with ice water whereupon a heavy oily precipitate appeared. The mixture was allowed to digest over night.

The next morning the water was decanted as thoroughly as possible and the oil, dissolved in 150 cc. of hot methanol. On cooling copious crystals came down. The next morning the pre-

cipitate consisting of fine needles was filtered off, washed wit methyl alcohol and dried in vacuum at 50°C. After three re-crystallizations and drying over phosphorous pentoxide it melte at 143-144°C.

Preparation of the Trityl Derivative of d-Glucoheptulitol.

Two grams of d-glucoheptulitol were treated with 6 g. of triphenyl-chloro-methane and 25 cc of pyridine under thesame conditions as those described for a-glucoheptitol above. At the end of the operation a copious precipitate was obtained which consisted of needles melting at 142-143°C after purification. A mixture of this compound with the di-trityl derivative of a-glucoheptitol melted at 142-143°C. Analysis showed:-

	Di-trityl of d-glucoheptulitol Found	Di-trityl of a-glucoheptitol Calculated
C	77.82%	77.57%
H	6.48%	6.37%

Thus again an attempt at preparing a derivative of d-glucoheptulitol yielded a derivative of a-glucoheptitol.

Summary

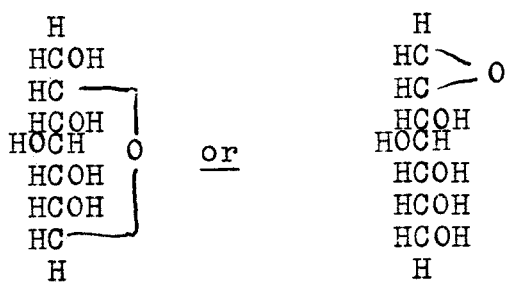
1. Reduction of crystalline b-glucoheptose yielded b-d-glucoheptitol with physical constants corresponding to those of the b-d-glucoheptitol which Philippe obtained by the reduction of sirupy b-d-glucoheptose.

2. A new method for the separation of d-glucoheptulose from a-d-

glucoheptose was presented. The method employs di-phenyl-methane-di-methyl-di-hydrazine. This reagent precipitates the aldose as the di-hydrazone and leaves the ketose in solution. The method is less tedious than that of Hudson and Isbell whereby the aldose was first oxidized and then removed as the barium salt of the acid, and gives yields of the ketose which are quantitative.

3. Reduction of d-glucoheptulose in accordance with the classical theory yielded a-glucoheptitol and b-d-glucoheptitol thus confirming the structure assigned to this ketose by W. C. Austin In addition it yielded d-glucoheptulitol which substance by virtue of the ease with which it is converted to a-glucoheptitol serves not to discredit but to substantiate the structure of d-glucoheptulose as assigned by W. C. Austin.

4. From the molecular weight, hydrolysis studies, optical rotation, acetate and trityl derivatives a tentative structure was assigned to d-glucoheptulitol which isthat of an anhydride:-



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