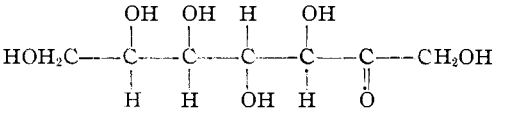
[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

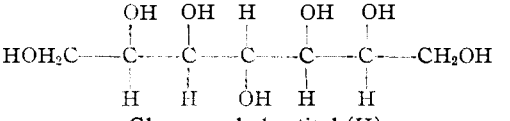
Some Studies on L-Glucoheptulose

BY W. DAYTON MACLAY, RAYMOND M. HANN AND C. S. HUDSON

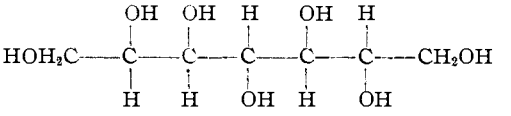
The biochemical oxidation of a number of polyatomic alcohols to ketoses by *Acetobacter suboxydans* has been reported[1] in previous publications from this Laboratory. The present article describes the preparation of L-glucoheptulose through the biochemical oxidation of D-gluco-D-gulo-heptitol[2] (α-glucoheptitol) by the procedure previously found to be successful for the oxidation of D-manno-D-gala-heptitol (perseitol) to L-galaheptulose. The L-glucoheptulose was obtained in a yield of 88%; the pure ketose melted at 172–173° (cor.) and exhibited a specific rotation $[\alpha]^{20}D$ of $-67.8°$ in water without mutarotation; these constants agree with those reported (m. p. 173.5°; $[\alpha]^{20}D$ $-67.1°$) by Bertrand and Nitzberg[3] for the "α-glucoheptulose" which they obtained by the biochemical oxidation of D-gluco-D-gulo-heptitol with *Bacterium xylinum* (syn. *Acetobacter xylinum*). Austin[4] has shown that "α-glucoheptulose" is L-glucoheptulose since it is the enantiomorph of D-glucoheptulose (m. p. 171–174°; $[\alpha]^{20}D$ $+67.7°$) prepared by the Lobry de Bruyn rearrangement of D-gluco-D-gulo-heptose (D-α-glucoheptose). He prepared the D-glucoheptose phenylosazone that is common to D-gluco-D-gulo-heptose and D-glucoheptulose and showed that it mutarotates in absolute alcohol–pyridine solution from $-5.3°$ to $+35.0°$; he pointed out that the phenylosazone of "α-glucoheptulose," which had been described by Bertrand and Nitzberg as identical with D-glucoheptose phenylosazone, must in reality have been the enantiomorphous L-gluco-heptose phenylosazone. Bertrand and Nitzberg did not report its rotation; Austin did not have the L-form of glucoheptulose available for the re-preparation of its phenylosazone. We have now confirmed the observation of Austin on the rotation of D-glucoheptose phenylosazone; we have also prepared the enantiomorphous L-glucoheptose phenylosazone from L-glucoheptulose and find that it mutarotates from $+6.0°$ to $-35.3°$ in absolute alcohol–pyridine solution, proving that the phenylosazones are enantiomorphs, as was concluded by Austin. Confirmation of the configuration of L-glucoheptulose (I) was obtained also through its reduction with hydrogen and Raney nickel to form a mixture of the inactive L-gluco-L-gulo-heptitol (syn. D-gluco-D-gulo-heptitol, α-glucoheptitol) (II) and the active L-gluco-L-ido-heptitol (syn. L-β-glucoheptitol) (III).



L-Glucoheptulose (I)



L-Gluco-L-gulo-heptitol (II)
(syn. D-Gluco-D-gulo-heptitol, α-Glucoheptitol)



L-Gluco-L-ido-heptitol (III)
(syn. L-Ido-D-gulo-heptitol, L-β-Glucoheptitol)

The alcohols were separated by conversion into their respective heptaacetates, that of L-gluco-L-gulo-heptitol crystallizing readily while that of L-gluco-L-ido-heptitol remained dissolved in the acetylating solution. The L-gluco-L-gulo-heptitol heptaacetate was optically inactive, melted at 118–119° (cor.) and gave a quantitative yield of the inactive L-gluco-L-gulo-heptitol upon deacetylation. The L-gluco-L-ido-heptitol heptaacetate was obtained as a sirup, which upon deacetylation yielded crystalline L-gluco-L-ido-heptitol, melting at 129–130° (cor.) and exhibiting a specific rotation $[\alpha]^{20}D$ of $-0.8°$ in water. The recorded values[5] of these constants for the enantiomorphous D-gluco-D-ido-heptitol are 130–131° and $+0.8°$, respectively, and we also have observed a melting point of 129–130° (cor.) and a specific rotation $[\alpha]^{20}D$ of $+0.7°$ in water on a sample of D-gluco-D-ido-heptitol prepared by the catalytic reduction of D-gluco-D-ido-heptose with hydrogen and Raney nickel. The L-gluco-L-ido-

(1) Hann, Tilden and Hudson, THIS JOURNAL, **60**, 1201 (1938); Hann and Hudson, *ibid.*, **61**, 336 (1939); Tilden, *J. Bact.*, **37**, 629 (1939).

(2) Concerning this nomenclature, see Hudson, THIS JOURNAL, **60**, 1537 (1938).

(3) Bertrand and Nitzberg, *Compt. rend.*, **186**, 925, 1172 (1928).

(4) Austin, THIS JOURNAL, **52**, 2106 (1930).

(5) Phillipe, *Compt. rend.*, **147**, 1481 (1908).

heptitol was further characterized by conversion to its crystalline heptabenzoate and this compound was found to have the same melting point, namely, 181–182° (cor.) as authentic D-gluco-D-ido-heptitol heptabenzoate; the specific rotation of the heptabenzoate in chloroform solution (−25.3°) was equal in magnitude, but opposite in sign, to that of the D-isomer. The data from the reduction therefore confirm the accepted configuration of L-glucoheptulose (I). They are also in agreement with the results obtained by Khouvine,[6] who has shown that D-glucoheptulose is reduced by hydrogen and Raney nickel to form D-gluco-D-gulo-heptitol and D-gluco-D-ido-heptitol, although reduction with sodium amalgam yielded D-gluco-D-gulo-heptitol and a substance designated as α-D-glucoheptulitol, which Humoller, Kuman and Snyder[7] regard as mixed crystals of D-gluco-D-gulo-heptitol and a small amount of an optically active impurity of undetermined identity.

We express our appreciation to Mr. L. B. Lockwood, Dr. Evelyn B. Tilden and Dr. W. T. Haskins for assistance in parts of this work.

Experimental

L-Glucoheptulose (I) from **D-Gluco-D-gulo-heptitol.**—A medium for inoculating the solution employed in the biochemical oxidation was prepared as follows: a solution of 10 g. of D-gluco-D-gulo-heptitol, 1.0 g. of Difco yeast extract, 0.6 g. of potassium acid phosphate and 0.1 g. of glucose in 200 cc. of water was placed in a 500-cc. Jena glass gas-washing bottle; following sterilization, a bacterial suspension of *Acetobacter suboxydans* was added, and the bottle was placed in a 30° constant temperature room and sterile air was passed through the suspension at a rate of 200 cc. per minute; after twenty-four hours the oxidation of the alcohol to ketose was complete and the inoculum was ready for use. A solution of 400 g. of D-gluco-D-gulo-heptitol, 20 g. of Difco yeast extract, 12 g. of potassium acid phosphate, 2 g. of glucose and 0.5 g. of octadecyl alcohol in 4000 cc. of water was sterilized in a 10-liter Pyrex bottle suitably equipped with an air intake and dispersion unit. After introduction of the previously described inoculum the bottle was placed in a 30° constant temperature room and sterile air was passed through the culture at a rate of 12 liters per minute. Subsamples were analyzed for reducing value at the expiration of 24, 48, 96, 120 and 144 hours and the analyses indicated that the oxidation was 9, 28, 80, 93 and 100% complete at these periods. The solution was clarified by filtration and successive treatments with lead acetate and hydrogen sulfide, and the final filtrate was concentrated *in vacuo* at 60° to a volume of 400 cc.; the sirup was diluted with 700 cc. of warm alcohol and as the solution cooled it deposited the crystalline ketose in a yield of 349 g. (m. p. 166–167°). A second crop of 6 g. was obtained by concentrating the filtrate to a volume of 55 cc. and adding 285 cc. of warm alcohol, and the final mother liquor, upon treatment with *p*-bromophenylhydrazine, gave a yield of L-glucoheptose *p*-bromophenylosazone equivalent to 12 g. of ketose. The yield of crystalline ketose was therefore 355 g. or 88%, and the over-all yield was 367 g. or 91%. The compound was recrystallized by solution in 1 part of water and addition of 5 parts of alcohol; it formed large rhombs which melted at 172–173° (cor.) and showed a specific rotation of −67.8° (*c*, 2.5; *l*, 4) in water, without detectable mutarotation. Bertrand and Nitzberg[3] reported a melting point of 173.5° and a specific rotation of −67.1° in water for "α-glucoheptulose" and Austin[4] reported a melting point of 171–174° and a specific rotation of +67.7° in water for D-glucoheptulose.

Mr. G. L. Keenan, of the Food and Drug Administration of the Federal Security Agency, has examined the L-glucoheptulose and finds its optical-crystallographic properties to be identical with those of authentic D-glucoheptulose, as would be expected. *In ordinary light*, the powdered L-glucoheptulose consists of angular fragments, showing by the immersion method in oily organic liquids refractive indices, $n_\alpha = 1.545$, n_β indeterminate and $n_\gamma = 1.560$, both ±0.002; *in parallel polarized light* (*crossed nicols*) the birefringence is moderate and the fragments extinguished sharply with crossed nicols, only an occasional fragment being found which remains bright; *in convergent polarized light* (*crossed nicols*), a partial biaxial interference figure is only rarely observed.

A mixture of 1.0 g. each of pure L- and D-glucoheptulose was recrystallized from 15 parts of warm alcohol. The crystals which were deposited as the solution cooled melted at 150–152° (cor.) and this melting point was unchanged by further recrystallization. This D,L-glucoheptulose showed no optical rotation in aqueous solution. An optical-crystallographic examination by Mr. Keenan disclosed that the indices of refraction of the crystals were identical with those of the optically active forms of the ketose; the crystals form therefore a mixture of the D- and L-modifications rather than a true racemate.

L-Glucoheptose Phenylosazone.—A solution of 10.0 g. of L-glucoheptulose in a mixture of 25 cc. of phenylhydrazine 12.5 cc. of acetic acid and 100 cc. of water was heated on the steam-bath for two hours. The osazone, which separated as an oil as the reaction progressed, crystallized readily as the suspension was cooled. The yield was 18.1 g. (98%). The compound was recrystallized from 25 parts

TABLE I

MUTAROTATION OF D- AND L-GLUCOHEPTOSE PHENYLOSAZONES

Concentration 1.6 g. in 100 cc.; tube length 1 dm.; temp. 20 ± 0.5°.

Time after making soln., hr.	D-Form	L-Form
0.25	− 5.6°	+ 6.0°
3	+ 9.0	− 9.6
6	+15.5	−15.7
24	+30.2	−31.0
48	+32.7	−34.2
96	+34.9	−35.3
120 (final)	+34.9	−35.3

(6) Khouvine, *Compt. rend.*, **204**, 983 (1937).

(7) Humoller, Kuman and Snyder, THIS JOURNAL, **61**, 3370 (1939).

of boiling alcohol in the form of yellow needles which melted with decomposition at 181–182° (cor.). The specific rotation was determined in a mixture of 2 parts of pyridine and 3 parts of absolute alcohol and, as shown in the following summary, it was equal in magnitude, but opposite in sign to that of a sample of authentic D-gluco-heptose phenylosazone.

A mixture of 1.0 g. of each of the enantiomorphous osazones was dissolved by refluxing in 250 cc. of alcohol and, upon cooling, the solution deposited yellow needles which melted at 176–177° (cor.). This melting point was not changed by recrystallization from alcohol and a pyridine–alcohol solution of the recrystallized product showed no rotation. Since the melting point of the compound is lower than that of its components it may be designated at the present time only as D,L-glucoheptose phenylosazone.

Anal. Calcd. for $C_{19}H_{24}O_5N_4$: C, 58.75; H, 6.23. Found: (for L-form) C, 58.78; H, 6.14; (for D,L-form) C, 58.13; H, 6.18.

Reduction of L-Glucoheptulose.—A solution of 50.0 g. of L-glucoheptulose in 250 cc. of water was heated for six hours at 100° under a pressure of 2500 pounds (167 atm.) of hydrogen in the presence of 10.0 g. of Raney nickel. Following removal of the catalyst, the solution was concentrated *in vacuo* at 60° to a thick sirup, which was thinned with 25 cc. of water and then mixed with 300 cc. of warm alcohol. As the solution cooled it deposited 47.5 g. of a mixture of plates and needles (m. p. 116–119°) and an additional 2.6 g. of crystalline material (m. p. 116°) was obtained from the mother liquor. The total yield was therefore 50.1 g. (99%). It was found impractical to separate the mixture of crystalline heptitols by fractional crystallization, and accordingly, 45.0 g. of the mixed alcohols was acetylated by heating for two hours on the steam-bath with 180 cc. of acetic anhydride and 11.3 g. of fused sodium acetate. The acetylation mixture was poured upon crushed ice and the precipitated L-gluco-L-gulo-heptitol heptaacetate (41 g., 38%) was removed by filtration. The acetate, after recrystallization from 4 parts of alcohol, melted at 118–119° (cor.) and this melting point was not depressed upon admixture with authentic L-gluco-L-gulo-heptitol heptaacetate. A chloroform solution of the substance showed no optical activity. Upon deacetylation the heptaacetate gave a quantitative yield of L-gluco-L-gulo-heptitol.

Anal. Calcd. for $C_{21}H_{30}O_{14}$: C, 49.79; H, 5.97; CH_3CO, 59.5. Found: C, 49.68; H, 5.89; CH_3CO, 59.2.

The dilute acetic acid mother liquor remaining, after removal of the crystalline L-gluco-L-gulo-heptitol heptaacetate, was neutralized with sodium bicarbonate and extracted with chloroform. The washed extract was concentrated to a dry sirup (59.5 g., 55%) which was dissolved in 500 cc. of absolute methanol and deacetylated with barium methylate in the usual manner. The solution was freed of barium ion by balancing out with sulfuric acid, and after concentration to a sirup was dissolved in 100 cc. of boiling methanol. The alcoholic solution upon cooling deposited L-gluco-L-ido-heptitol in a yield of 21.8 g. (48%). The substance was recrystallized by solution in 0.5 part of water and the addition of 10 parts of warm methanol; it formed plates which melted at 129–130° (cor.) and showed a specific rotation $[\alpha]^{20}D$ of −0.8° (*c*, 4.0; *l*, 4) in water. Pure D-gluco-D-ido-heptitol, prepared by the catalytic reduction of D-gluco-D-ido-heptose, melted at 129–130° (cor.) and showed a specific rotation $[\alpha]^{20}D$ of +0.7° in water in agreement with the recorded value[5] (m. p. 130–131°; $[\alpha]^{20}D$ +0.8°) of Phillipe. An examination by Mr. G. L. Keenan showed that the L- and D-forms of the alcohol possessed identical optical crystallographic properties. *In ordinary light* they consist of thin quadratic plates showing refractive indices, $n_\alpha = 1.552$, n_β indeterminate and $n_\gamma = 1.561$, both ±0.002; *in parallel polarized light (crossed nicols)* the birefringence is weak and low order polarization colors are invariably shown; no interference figures were observed *in convergent polarized light (crossed nicols)*.

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.60; H, 7.60. Found: C, 39.66; H, 7.54.

L-Gluco-L-ido-heptitol Heptabenzoate (L-β-Glucoheptitol Heptabenzoate).—A mixture of 1.0 g. of L-gluco-L-ido-heptitol, 5 cc. of pyridine, and 6 cc. (10.5 molecular equivalents) of benzoyl chloride was heated in a boiling water-bath for two hours. The solid reaction products were dissolved in a mixture of chloroform and water and the chloroform layer was separated, washed, dried over anhydrous sodium sulfate and concentrated to a sirup; the sirup was dissolved in 475 cc. of boiling alcohol and as the solution cooled it deposited the heptabenzoate in a yield of 3.2 g. (72%). The compound was recrystallized from 150 parts of boiling alcohol and was obtained in the form of needles which melted at 181–182° (cor.) and exhibited a specific rotation $[\alpha]^{20}D$ of −25.3° (*c*, 2.0; *l*, 4) in chloroform. D-Gluco-D-ido-heptitol heptabenzoate, prepared from D-gluco-D-ido-heptitol, obtained by the catalytic reduction of D-gluco-D-ido-heptose, showed the same melting point and its rotation, +25.1°, was the same in magnitude, but opposite in sign. Phillipe[5] recorded the melting point of D-gluco-D-ido-heptitol heptabenzoate as 182°, but did not measure its rotation.

Anal. Calcd. for $C_{56}H_{44}O_{14}$: C, 71.48; H, 4.71; C_6H_5CO, 78.2. Found: C, 71.55; H, 4.65; C_6H_5CO, 77.7.

Racemic Gluco-ido-heptitol Heptabenzoate (Racemic β-Glucoheptitol Heptabenzoate).—A mixture of 0.4 g. each of D-gluco-D-ido- and L-gluco-L-ido-heptitol heptabenzoates was dissolved in 640 cc. of boiling 95% alcohol and as the solution cooled it deposited the racemate in the form of small needles. The pure compound may be recrystallized from 750 parts of alcohol; it was devoid of optical activity in chloroform solution. Its melting point of 193–194° (cor.) is considerably higher than that of its components, which shows that it is a true racemate.

Anal. Calcd. for $C_{56}H_{44}O_{14}$: C, 71.48; H, 4.71; C_6H_5CO, 78.2. Found: C, 71.54; H, 4.63; C_6H_5CO, 78.0.

Racemic Gluco-ido-heptitol (Racemic β-Glucoheptitol).—A mixture of 1.0 g. each of pure D-gluco-D-ido-heptitol and L-gluco-L-ido-heptitol was dissolved in 1 cc. of water and the aqueous solution diluted with 50 cc. of hot absolute methanol. As the solution cooled, racemic gluco-ido-heptitol was deposited in a yield of 1.85 g. (92%). The substance was recrystallized by solution in 1 part of water and the addition of 30 parts of methanol and formed small plates which melted at 114–115° (cor.) and showed no ro-

tation in an aqueous solution. An optical-crystallographic examination of the compound by Mr. Keenan showed that *in ordinary light* the refractive indices were $n_\alpha = 1.552$, n_β indeterminate and $n_\gamma = 1.555$, both ± 0.002; *in parallel polarized light (crossed nicols)* the birefringence is weak and low order polarization colors are usually seen; no interference figures were observed *in convergent polarized light*. The differences in these optical-crystallographic properties from those of L-gluco-L-ido-heptitol indicate that the substance which is here described is a true racemate.

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.60; H, 7.60. Found: C, 39.66; H, 7.72.

Summary

L-Glucoheptulose has been prepared in high yield through the biochemical oxidation of α-glucoheptitol with *Acetobacter suboxydans*. The phenylosazone of the ketose has been shown to possess a specific rotation equal in magnitude, but opposite in sign, to that of D-glucoheptose phenylosazone. Catalytic reduction of L-glucoheptulose by hydrogen and Raney nickel produces L-gluco-L-gulo-heptitol and L-gluco-L-ido-heptitol and thus provides supporting evidence for the accepted configuration of the ketose. New substances which have been described are D,L-glucoheptulose, L-gluco-L-ido-heptitol (L-β-glucoheptitol), L-gluco-L-ido-heptitol heptabenzoate, racemic gluco-ido-heptitol heptabenzoate, and racemic gluco-ido-heptitol.

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The Oxidative Degradation of L-Glucoheptulose

BY NELSON K. RICHTMYER AND C. S. HUDSON

In an earlier communication from this Laboratory[1] we reported the isolation of potassium L-galactonate in a 45% yield from the degradation of perseulose (L-galaheptulose) in alkaline solution by oxygen, according to the procedure developed by Spengler and Pfannenstiel.[2] Having available a considerable amount of L-glucoheptulose from another research,[3] we had planned to degrade it in similar fashion to potassium L-gluconate, and thus to obtain, by reduction of the lactone, the difficultly accessible L-glucose.[4] Unfortunately for our purpose the oxidation could not be controlled sufficiently to yield L-gluconic acid as the principal degradation product. Instead there was formed one of those complex mixtures of acids such as have been obtained, by a succession of enolizations and cleavages, from the oxidation of other sugars in alkaline solution by air.[5]

When the oxidation of L-glucoheptulose was carried out in the manner described previously for L-galaheptulose, the first product to be isolated was the readily crystallized potassium L-arabonate, in a yield of 31%. It was identified by its rotation[6] of +5.2° in water as compared to −5.0° for potassium D-arabonate, and by conversion to the benzimidazole derivative described by Moore and Link.[7] The remainder of the oxidation products was converted to a mixture of brucine salts. Crystallized from water, the least soluble fraction, about 14% of the theoretical yield, consisted of approximately equal amounts of the brucine salts of L-gluconic and L-erythronic acids. Brucine L-gluconate was identified by comparison of its constants with those recorded in the literature, by conversion to potassium L-gluconate rotating −11.3° in water as compared to +11.3° for potassium D-gluconate, and by conversion to 2-[L-*gluco*-pentahydroxyamyl]-benzimidazole. This last-named compound has the same melting point as the D-derivative described by Moore and Link,[8] while the rotation, −9.0° in citric acid solution, is about equal in magnitude but is opposite in sign to that recorded for its better-known antipode.

Brucine L-erythronate was identified by analy-

(1) Richtmyer, Hann and Hudson, THIS JOURNAL, **61**, 340 (1939).

(2) Spengler and Pfannenstiel, *Z. Wirtschaftsgruppe Zuckerind.*, **85**, Tech. Tl. 547 (1935).

(3) Maclay, Hann and Hudson, THIS JOURNAL, **64**, 1606 (1942).

(4) Fischer, *Ber.*, **23**, 2618 (1890); for the crystalline L-gluconolactone, see Kiliani, *ibid.*, **58**, 2349 (1925); Upson, Sands and Whitnah, THIS JOURNAL, **50**, 519 (1928). Although L-glucose has been reported to occur in *Grindelia robusta* [Power and Tutin, *Pub. Wellcome Chem. Research Lab.*, **57**, 1 (1905)], in capsularin, a glycoside from jute (*Corchorus capsularis*) [Saha and Choudhury, *J. Chem. Soc.*, **121**, 1044 (1922)], and collagen [Beek, THIS JOURNAL, **63**, 1483 (1941); *J. Research Natl. Bur. Standards*, **27**, 507 (1941)], the evidence is not conclusive in any case.

(5) See, for example, the degradation of D-glucose [Power and Upson, THIS JOURNAL, **48**, 195 (1926)].

(6) Throughout the article the rotations are specific rotations at 20° for sodium light; *c* designates concentration in grams per 100 cc. of solution, and *l* the length of the tube in decimeters.

(7) Moore and Link, *J. Biol. Chem.*, **133**, 300 (1940).

(8) Moore and Link, ref. 7; see also Haskins and Hudson, THIS JOURNAL, **61**, 1267 (1939).