

[CONTRIBUTION FROM THE POLARIMETRY SECTION, BUREAU OF STANDARDS, UNITED STATES DEPARTMENT OF COMMERCE]

## RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XXVII. SYNTHESIS OF A NEW DISACCHARIDE KETOSE (LACTULOSE) FROM LACTOSE<sup>1</sup>

BY EDNA M. MONTGOMERY AND C. S. HUDSON

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The comparison of the rotation of sorbose with that of methylsorbose,<sup>2</sup> which indicated that sorbose may possibly exist in solution in only one form, namely, as  $\alpha$ -sorbose, opened a line of thought which has led us to undertake the preparation of new ketoses and ketose derivatives. If the fact that several of the known ketose sugars (sorbose, tagatose, mannoketoheptose and  $\alpha$ -glucoheptulose)<sup>3</sup> do not show mutarotation is to be ascribed to the existence of these sugars in only one form in solution, one must inquire why these ketoses are unable to undergo the tautomeric balanced reactions which cause the mutarotation of aldoses. In sharp contrast to the behavior of these four ketoses stands the observation that fructose, turanose<sup>4</sup> (which is glucosido-fructose) and perseulose (which is very similar in configuration to fructose)<sup>5</sup> exhibit mutarotation. If the four non-mutarotating ketoses do not change partially in solution from alpha to beta forms of like ring structure, is the mutarotation of the other three due to such a change or may it not rather involve a shifting of the oxygen ring? The fact that fructose mutarotates but its tetra-acetate does not, whereas both glucose and glucose tetra-acetate mutarotate, is indeed evidence that the mutarotation of fructose is caused by ring-shifting; its tetra-acetate cannot shift the ring except by an improbable acetyl migration. With these questions and ideas in mind we have begun the collection of further experimental data on ketoses and their derivatives.

Few ketoses are known in comparison with the large number of aldoses that are recognized. The number may be increased either by the discovery of new naturally occurring ketoses or by way of laboratory synthesis. The discovery of mannoketoheptose in the avocado<sup>5</sup> and of sedoheptose in the stonecrop<sup>6</sup> indicates that a systematic search for other new ketoses in natural products might be well repaid, but for the present the

<sup>1</sup> Publication authorized by the Director of the Bureau of Standards. Article XXVI was published in *THIS JOURNAL*, **52**, 1707 (1930). The synthesis of lactulose was reported by the authors at the April, 1929, meeting of the National Academy of Sciences [*Science*, **69**, 556 (1929)]. The name lactoketose was then used for the sugar but it has been decided subsequently to name it lactulose.

<sup>2</sup> Hudson, *THIS JOURNAL*, **47**, 278 (1925).

<sup>3</sup> Bertrand and Nitzberg, *Compt. rend.*, **186**, 925 (1928).

<sup>4</sup> Hudson and Pacsu, *Science*, **69**, 278 (1929).

<sup>5</sup> La Forge, *J. Biol. Chem.*, **28**, 511 (1917).

<sup>6</sup> La Forge and Hudson, *ibid.*, **30**, 61 (1917).

path of synthesis appears to offer prospect of larger or at least more rapid returns.

The conversion of aldoses to ketoses is a rich and only slightly explored field. The first synthesis of this kind was performed by Emil Fischer<sup>7</sup> in the following two ways: (1) glucose  $\longrightarrow$  glucose osazone  $\longrightarrow$  isoglucosamine  $\longrightarrow$  fructose; (2) glucose  $\longrightarrow$  glucose osazone  $\longrightarrow$  glucosone  $\longrightarrow$  fructose. Although Fischer was hopeful that these reactions would prove of general application, such has not been the case and no other ketose has been synthesized by them. Philippe's<sup>8</sup> attempt to prepare a ketose from  $\alpha$ -glucoheptose through Fischer's methods was unsuccessful. New methods of ketose synthesis, introduced by Bertrand and by Lobry de Bruyn, have led to the discovery of perseulose,<sup>9</sup>  $\alpha$ -glucoheptulose,<sup>3</sup> tagatose and *d*-sorbitose,<sup>10</sup> the antipode of natural *l*-sorbitose. Bertrand has utilized the sorbitose bacterium to oxidize alcohols of the sugar group to ketoses but the application of this biological method seems to be considerably restricted by the specificity of the bacterium. The Lobry de Bruyn rearrangement of aldoses and ketoses in alkaline solution appears at the present time to offer the best prospect for synthesizing new ketoses from the large number of known aldoses. The researches of Lobry de Bruyn, Van Ekenstein, and Nef<sup>11</sup> have shown that the important factors for the success of the synthesis are (1) the obtaining of conditions of alkaline concentration, time and temperature which give suitable rearrangement of the aldose with a minimum of side reactions and (2) a separation of the ketose from the mixture of sugars and decomposition products which results from the alkaline treatment of an aldose. The success which Wolfrom and Lewis<sup>12</sup> have reported in the rearrangement of tetramethylglucose and tetramethylmannose at 35° in saturated limewater has led us to select these conditions for the initial step in ketose synthesis. In carrying out the second step the procedure of Nef<sup>11</sup> in a modified form has been used and aldoses have been removed from ketoses by oxidizing them with bromine water under the mild conditions of acidity that result from the presence of a buffering salt such as barium benzoate, as recommended lately by Hudson and Isbell.<sup>13</sup> By applying these reactions to lactose it has been possible to transform it to a new crystalline disaccharide ketose, to which the name lactulose is given because of its origin, and in the following article there is described by our colleague, W. C. Austin, the preparation in similar manner of a new crystalline ketose from *d*- $\alpha$ -glucoheptose, to which he has given the name *d*-

<sup>7</sup> Fischer, *Ber.*, **22**, 87 (1889); Fischer and Tafel, *ibid.*, **20**, 2566 (1887).

<sup>8</sup> Philippe, *Ann. chim. phys.*, **26**, 289 (1912).

<sup>9</sup> Bertrand, *Compt. rend.*, **147**, 201 (1908).

<sup>10</sup> Lobry de Bruyn and Van Ekenstein, *Rec. trav. chim.*, **16**, 262 (1897); **19**, 1 (1900).

<sup>11</sup> Nef, *Ann.*, **403**, 204 (1914).

<sup>12</sup> Wolfrom and Lewis, *THIS JOURNAL*, **50**, 837 (1928).

<sup>13</sup> Hudson and Isbell, *ibid.*, **51**, 2225 (1929).

glucoheptulose. These results indicate that the directions which we have worked out are probably of general applicability for synthesizing many new ketoses from the large number of aldoses that are known. Work in this field is being continued by the authors and at present the synthesis of *d*-mannoketoheptose is being attempted.

**Behavior of Lactose in Weak Calcium Hydroxide Solution.**—A solution of 180 g. of  $\alpha$ -lactose monohydrate in one liter of clear limewater of 0.043 normality, which had been prepared by saturating distilled water with slaked lime at 35°, was kept at 35° until the change of rotation that results from the Lobry de Bruyn rearrangement was complete. The initial mutarotation of lactose in such an alkaline solution is very rapid and the first reading, which was made after the lapse of four minutes, showed the equilibrium rotation of lactose,  $[\alpha]_D +52.5$ . The rearrangement became noticeable at the end of half an hour, when the rotation was +50.7. After twenty-four hours it was +37.7, after thirty-six hours +31.6 and after forty-eight hours +31.5. The time required for the equilibration of lactose, thirty-six hours, is much shorter than that observed by Wolfrom and Lewis for *d*-glucose under like conditions, which was ten days. The addition of more lime to samples of the equilibrated lactose solution caused no further change of rotation. When the equilibrated solution was analyzed for aldoses by alkaline hypiodite, using the directions of Goebel,<sup>14</sup> a high result indicating the absorption of more iodine than could be accounted for by all the lactose originally present, was obtained. This result conforms with the observation of Wolfrom and Lewis in the case of the glucose rearrangement and is presumably due, as they have suggested, to the presence of an unsaturated enolic derivative of the aldose. The equilibrated lactose solution was acidified at 10° with sulfuric acid to a normality of 2.0 and kept at 35° for two hours, as recommended by Wolfrom and Lewis, to accomplish de-enolization. Its analysis then by iodine oxidation indicated a mixture of approximately 70% aldose and 30% ketose. Experience in preparing the crystalline ketose from a considerable number of equilibrated solutions indicates that the yield is somewhat increased by the introduction of the step of de-enolization but that the increase is hardly worth the extra labor involved.

**Isolation of a New Ketose from the Equilibrated Solution.**—If one employs a de-enolized solution, the sulfuric acid is neutralized with calcium carbonate without heating and the filtrate and washings are concentrated under reduced pressure to a sirup. When de-enolization is omitted, the equilibrated solution is reduced similarly to a sirup. In either case the sirup resulting from the treatment of 180 g. of lactose is dissolved in 500 cc. of 95% alcohol. Lactose crystallizes abundantly (about 110–125 g.) and is filtered off after several days' standing at low temperature. The mother liquor and alcoholic washings are concentrated to a sirup which is then mixed with six volumes of 95% alcohol and the precipitated calcium salts are removed. The filtrate is concentrated again, the sirup obtained is dissolved in 50 cc. of absolute methyl alcohol and allowed to crystallize slowly in a desiccator, yielding a second crop of lactose; repetition of this treatment usually gives a third small crop of lactose, bringing the total recovery to about 120–135 g. Another repetition yields by a slow crystallization, which is much aided by seeding with crystals of lactulose, a crop of about 14 g. of impure ketose of  $[\alpha]_D -32$  (final) when a de-enolized solution has been employed, or about 11 g. showing  $[\alpha]_D -23$ , when de-enolization has been omitted. The impure sugar is a mixture of lactulose and presumably lactose; the pure ketose can be obtained from it through the process of oxidizing the aldose with bromine water in the presence of a buffering salt. The amount of aldose present, calculated as lactose, is first estimated

<sup>14</sup> Goebel, *J. Biol. Chem.*, **72**, 801 (1928).

by hypiodite; it is usually about 15% of the total dry matter. Bromine to the extent of 1.1 moles (Br<sub>2</sub>) for each mole of aldose present is added to a 10% aqueous solution of the impure ketose containing an excess of barium benzoate (1.25 mole) and the solution is kept at room temperature in the dark for two days. The excess bromine, the barium and the benzoic acid are then removed as described by Hudson and Isbell and the aldobionic acid is converted to a salt (calcium, barium or lead) without heating. The solution is concentrated to a sirup and the salts are precipitated by adding 6 volumes of 95% alcohol, filtering, reconcentrating the filtrate, dissolving it in 250 cc. of absolute alcohol, refiltering and reconcentrating. A solution of the last sirup in an equal volume of absolute methyl alcohol yields by a slow crystallization (seven days) about 65% of the ketose that was present in the impure product; its  $[\alpha]_D^{25}$  value is  $-51.5$  (final) and the rotation is not changed by recrystallization from 50% methyl alcohol. The yield of pure lactulose is about 6 to 7.5 g. from 180 g. of lactose or 10 to 15% of the lactose that underwent rearrangement. We have not investigated the other products that are apparently formed in considerable quantity. While the yield of lactulose leaves much to be desired, it is probable that the general application of the method will show better results with many other aldoses because lactulose is very difficult to crystallize; in support of this expectation it is mentioned that Austin (see next article) has obtained much higher yields of *d*-glucoheptulose from *d*- $\alpha$ -glucoheptose by the method.

**Properties of Lactulose.**—Lactulose crystallizes in hexagonal, colorless plates often grouped in clusters. It melts at 158°, gives the Seliwanoff test for ketoses, is not oxidized by hypiodite, reduces Fehling's solution on heating and is sweeter than lactose but not as sweet as sucrose. It is an anhydrous sugar of the formula C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>. Its combustion shows C, 41.90% and H, 6.50%, whereas the calculated values from this formula are C, 42.09% and H, 6.43%; the freezing-point method indicates its mol. wt. to be 340–352, agreeing with the formula. It is very soluble in water. Lactulose exhibits rapid mutarotation. In aqueous solution at 22° its  $[\alpha]_D$  value is  $-23.8$  two minutes after solution and the final value is  $-51.5$ . The course of the mutarotation is shown in the table. It follows the unimolecular order and the extrapolated initial rotation is about  $[\alpha]_D^{25} - 5$ . Since the change is toward the negative and the mutarotating component (fructose, see later) is a *d*-sugar, the crystalline form of the disaccharide is to be designated  $\alpha$ -lactulose. The rate of its mutarotation (0.114) at 22° is approximately the same as that of fructose<sup>15</sup> (0.082 at 20°).

TABLE I  
MUTAROTATION OF ALPHA LACTULOSE AT 22° IN DILUTE AQUEOUS SOLUTION

Time, minutes	$[\alpha]_D^{25}$	$(k_1 + k_2) = \frac{1}{t} \log \frac{r_0 - r_\infty}{r - r_\infty}$
0	$[-5]$	..
2	$-23.8$	0.112
2.6	$-27.9$	.113
3.6	$-32.7$	.109
4.3	$-36.9$	.117
5.7	$-41.6$	.118
7.1	$-44.5$	.116
8.7	$-46.7$	.113
24 hours	$-51.5$ (constant)	..

**Preparation of Lactone Osazone and its Anhydride from Lactulose.**—The phenyl-osazone of lactulose was not distinguishable from lactose osazone and it showed the unique characteristic of the latter in changing to a crystalline anhydride on heating its

<sup>15</sup> Hudson and Yanovsky, THIS JOURNAL, 39, 1013 (1917).

solution in dilute sulfuric acid.<sup>16</sup> This anhydride is particularly suitable for the characterization of lactose osazone because it shows a definite rotatory power and does not exhibit mutarotation. We find that lactose osazone, which exhibits mutarotation, shows varying final values and by successive recrystallizations it was not possible to obtain a substance of constant final rotation. The anhydro-osazone from lactose melted at 226° (uncorr.) with decomposition and showed  $[\alpha]_D^{20} -174$  in a mixture of four parts of pyridine and six parts of alcohol<sup>17</sup> (concentration, 2 g. of anhydro-osazone/100 cc.). The anhydro-osazone from lactulose melted at 226 with decomposition and showed  $[\alpha]_D^{20} -174$ . A mixture of the two products melted at 225–226 with decomposition. The nitrogen content of the anhydro-osazone from lactulose by the Dumas method was 11.06%; theory requires 11.15%. Finally, the identity of the anhydro-osazones from the two sugars was conclusively established by solubility measurements of the two substances in acetone at 5°. The solubility of the anhydro-osazone from lactose was 0.1484 g./100 cc. from supersaturation and 0.1476 from undersaturation, and the corresponding values for the anhydro-osazone from lactulose were 0.1492 and 0.1480. The possibility that the anhydro-osazones might be different compounds of closely agreeing solubilities and other properties was tested by a measurement of the solubility of a mixture of equal quantities of the two anhydro-osazones, which gave the values 0.1484 and 0.1492 from under- and supersaturation; these data make it certain that the two products are the same chemical substance because if such were not the case the solubility of the mixture would be the sum of the individual solubilities, approximately 0.297 g. This method of testing the identity of pure substances is somewhat like the method of "mixed melting points" and may well be designated a "mixed solubility" method. It is very sensitive and is recommended in cases where the mixed melting-point method is of questionable significance because of decomposition of the substance.

**Identification of the Component Hexoses of Lactulose.**—A solution of 6 g. of lactulose in 120 cc. of 1% sulfuric acid was kept at 100° for eight hours to accomplish hydrolysis. Exact removal of the acid with barium hydroxide and concentration of the solution under reduced pressure to a thick sirup, which was taken up in very little methyl alcohol, yielded 1.5 g. of a crystalline sugar. After one recrystallization from this solvent, 1.0 g. of the sugar, dried at 50° for six hours, was obtained, showing  $[\alpha]_D^{20} +81$  (final) in aqueous solution; this value agrees with the known rotation of *d*-galactose. Fructose was isolated from the mother liquor in the form of its crystalline compound with calcium hydroxide, which was then decomposed with the exact equivalent of oxalic acid. Evaporation of the filtrate to a sirup, solution of this in alcohol and slow evaporation in a desiccator yielded 0.36 g. of crystalline sugar, of  $[\alpha]_D^{20} -91.5$  (final), which agrees with the known rotation of *d*-fructose.

**The Structure of Lactulose.**—Lactulose shows the composition and molecular weight of a disaccharide, C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>, as has been mentioned. Its reduction of Fehling's solution, the positive Seliwanoff test and the fact that it is not oxidized by bromine or by hypiodite show that its molecule contains a ketose with a free reducing group. The fact that its anhydro-osazone is identical with lactose anhydro-osazone shows that its structure is to be expressed as *d*-galactosido-4-*d*-fructose, because lactose is known to be *d*-galactosido-4-*d*-glucose<sup>18</sup> and lactose anhydro-osazone can result from no ketose of any other structure. Since lactulose crystallizes as an alpha form, as shown by the direction of its mutarotation, and since lactose is a beta galactosido disaccharide as shown from its hydrolysis by emulsin and from its rotation (see article XXVI<sup>19</sup> of this

<sup>16</sup> Fischer, *Ber.*, 20, 821 (1887).

<sup>17</sup> Neuberg, *ibid.*, 32, 3386 (1899).

<sup>18</sup> Zemplén, *ibid.*, 60, 1309 (1927).

<sup>19</sup> Hudson, *THIS JOURNAL*, 52, 1707 (1930).

series), its structure may be further specified by the designation alpha 4- $\beta$ -*d*-galactosido (1,4)- $\alpha$ -*d*-fructose, the 1,4-ring being assigned to the galactose portion because of the ring structure of lactose shown in article XXVI. The ring of the fructose component remains unknown, but it obviously cannot be of the 2,4-type. The isolation of *d*-galactose and *d*-fructose by the acid hydrolysis of lactulose agrees with the assigned composition.

### Summary

As an experimental introduction to the extension of the Lobry de Bruyn rearrangement for the synthesis of new ketoses, conditions for the conversion of lactose to a disaccharide ketose have been studied and a new ketose named lactulose has been isolated in pure crystalline condition. Lactulose is shown to be 4- $\beta$ -*d*-galactosido(1,4)-*d*-fructose and the crystalline sugar is an alpha form.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE POLARIMETRY SECTION, U. S. BUREAU OF STANDARDS]

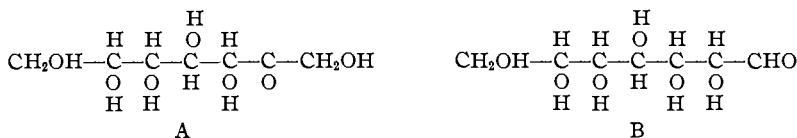
## RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XXVIII. THE CONVERSION OF *d*-ALPHA-GLUCOHEPTOSE TO A NEW KETOSE, *d*-GLUCOHEPTULOSE<sup>1</sup>

BY W. C. AUSTIN

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It is to be expected on structural grounds that a new ketose of configuration (A) may be prepared in some suitable way from the known *d*- $\alpha$ -glucoheptose, of configuration<sup>2</sup> (B). The osazone of this hypothetical



ketose should be identical with *d*-glucoheptose osazone, which has been prepared by Fischer<sup>2</sup> through the action of phenylhydrazine on either *d*- $\alpha$ -glucoheptose or *d*- $\beta$ -glucoheptose. This ketose, to which the name *d*-glucoheptulose might well be given, should bear the same configurational relationship to the epimeric aldoses, *d*- $\alpha$ - and *d*- $\beta$ -glucoheptose, as does *d*-fructose to *d*-glucose and *d*-mannose. The properties of *d*-glucoheptulose should apparently be of considerable interest because all the asymmetric

<sup>1</sup> Publication approved by the Director of the U. S. Bureau of Standards. The author was a National Research Fellow in Medicine, 1927-1928. He has arranged with Dr. C. S. Hudson, under whose direction the research was carried out, that it will be included as No. XXVIII in the series entitled "Relations between Rotatory Power and Structure in the Sugar Group." No. XXVII was published in THIS JOURNAL, 52, 2101 (1930).

<sup>2</sup> Fischer, *Ann.*, 270, 64 (1892).