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# RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XVII.[2] THE STRUCTURE OF NEOLACTOSE

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When the conversion of lactose octa-acetate by active aluminum chloride to the chloro-acetyl derivative of another disaccharide, neolactose, was observed[3] it was thought that neolactose might be identical with Bergmann's 5-galactosido-mannose[4] since epimerizations at Carbon 2 are often met in sugar reactions. Experiment soon showed, however, that neolactose is a new sugar. The phenylosazone of neolactose proved to be very different from the easily recognized lactose osazone, a substance that is also the osazone of 5-galactosido-mannose, just as glucose and mannose give glucose osazone. Neolactose was obtained as a sirup by the de-acetylation of its octa-acetate and its aqueous solution was dextrorotatory, but after hydrolysis of the sugar with hydrochloric acid the products were levorotatory. This sharply distinguishes neolactose from 5-galactosido-mannose, both the component hexoses of which are dextrorotatory. It thus became evident that the structural change in the lactose molecule which results from the action of aluminum chloride upon lactose octa-acetate is not a simple epimerization at Carbon 2, as first suspected, and accordingly a detailed study of the nature of the component hexoses of neolactose was undertaken. Oxidation of the sugar by nitric acid yielded mucic acid in about the same proportion that is obtainable from lactose, which showed that one of the hexoses is a galactose, though whether it is *d*- or *l*-galactose was not evident because both forms yield mucic acid. Neolactose was then oxidized by bromine to its monobasic neolactobionic acid, which was obtained as a sirup free from substances that reduce Fehling's solution. Its hydrolysis by hydrochloric acid gave a good yield of a crystalline hexose which was readily identified as *d*-galactose, and an hexonic acid which gave

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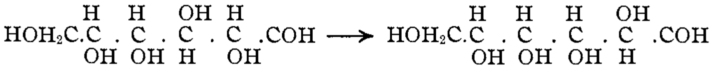
[1] Published by permission of the Director of the Bureau of Standards.

[2] Article XVI was published in THIS JOURNAL, **48,** 2002 (1926). Occasion is here taken to correct an error in Article XIII [THIS JOURNAL, **48,** 1434 (1926)], where it was stated that Irvine and Burt had observed a rearrangement of tetramethyl-$\gamma$-methyl-mannoside to tetra-methyl-$\alpha$-methyl mannoside. Their observation was, however, that $\gamma$-methyl-mannoside readily rearranges to $\alpha$-methyl-mannoside, a change which appears to consist, as they state, in a shifting of the oxygen ring while the reducing group remains substituted. A shifting of a methyl group does not take place in this change. In Article XIV [THIS JOURNAL, **48,** 1437 (1926)], $-y$ was misprinted for $+y$ in the line above Table II and y for Y in Equation 8.

[3] Kunz and Hudson, *ibid.*, **48,** 1978 (1926).

[4] Bergmann, *Ann.*, **434,** 79 (1923).

a crystalline brucine salt that showed the melting point and rotation that Levene and Meyer[5] have observed for brucine *d*-altronate. The melting point of the brucine salt was not affected by mixing the substance with a specimen of Levene and Meyer's original preparation and it became almost certain, therefore, that the second hexose component of neolactose is *d*-altrose. Conclusive proof that such is the case was then obtained by hydrolyzing neolactose, obtaining crystalline *d*-galactose in good yield and an accompanying sirupy sugar which yielded crystalline *d*-altrose phenylosazone and, on oxidation with nitric acid, gave crystalline *d*-talomucic acid. The structure of neolactose is thus *d*-galactosido-*d*-altrose and its *d*-altrose component owes its origin to the action of aluminum chloride upon the acetylated structure of *d*-glucose which is present in the molecule of lactose octa-acetate or chloro-hepta-acetate. This is a new type of isomerization in the sugar group and a remarkable stereochemical change, since both Carbon atoms 2 and 3 of the glucose structure are isomerized.



$$\mathrm{HOH_2C.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ .\ \overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ .\ \overset{\mathrm{OH}}{\underset{\mathrm{H}}{\mathrm{C}}}\ .\ \overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ \mathrm{.COH} \longrightarrow \mathrm{HOH_2C.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ .\ \overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ .\ \overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\ .\ \overset{\mathrm{OH}}{\underset{\mathrm{H}}{\mathrm{C}}}\ \mathrm{.COH}$$

*d*-glucose *d*-altrose

Having in hand a sufficient supply of a crystalline derivative of *d*-altrose (that is, $\alpha$-neolactose octa-acetate) from which this hexose could be obtained by the reactions of de-acetylation and acid hydrolysis, which run smoothly and nearly quantitatively, it was sought to learn the rotation, hitherto unmeasured, of this very rare hexose. It was found that the equimolecular mixture of *d*-galactose and *d*-altrose that results from the acid hydrolysis of neolactose shows $[\alpha]_D$ = —8.95 in water, from which it follows, since *d*-galactose shows $[\alpha]_D$ = +80, that the $[\alpha]_D$ value of *d*-altrose is approximately —98 in water. It is evidently a very strongly levorotatory sugar and of about the same rotation as *d*-arabinose ($[\alpha]_D^{20}$ = —105, final), a pentose of closely similar configuration. In like manner, the $[\alpha]_D$ value of neolactose has been found to be approximately + 35° in water.

Thanks are expressed to Dr. P. A. Levene for supplying the specimen of brucine *d*-altronate. One of the authors (A. K.) expresses his thanks to the International Education Board for a grant that enabled him to take part in this research.

## Experimental Part

**Phenylhydrazine Derivative of Neolactose.**—Two g. of $\alpha$-neolactose octa-acetate was de-acetylated by mixing the solution in 50 cc. of absolute chloroform with 33 cc. of absolute methyl alcohol in which 0.33 g. of metallic sodium had been dissolved.[6] Both solutions were previously cooled in an ice- and salt-bath and the mixture was kept therein for one-half hour. Five cc. of ice water was then added and after a further hour's standing the solution was made slightly acid with acetic acid and evaporated under re-

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[5] Levene and Meyer, *J. Biol. Chem.*, **26**, 363 (1916).

[6] Zemplén, *Ber.*, **59**, 1254 (1926). Zemplén and Kunz, *Ber.*, **56**, 1705 (1923).

duced pressure to a sirup which was dissolved in 30 cc. of water containing 1.5 g. of phenylhydrazine hydrochloride. The mixture was heated on the steam-bath one hour and a half, and as it cooled small, rather dark colored crystals separated which were recrystallized from 30 cc. of hot water; yield, 0.3 g. The substance, which is presumably neolactose phenylosazone, consists of yellow crystals that melt at 195° with decomposition. It is more soluble in water than lactose phenylosazone and does not form an insoluble anhydride derivative, as does the latter, when heated with dil. sulfuric acid. Certainly, neolactose does not yield lactose phenylosazone and therefore cannot be Bergmann's 5-galactosido-mannose.

**Neolactobionic Acid.**—Fischer and Meyer's directions[7] for preparing lactobionic and maltobionic acids have been followed. Forty g. of α-neolactose octa-acetate was de-acetylated in the manner already described and the resulting neolactose sirup was dissolved in 150 cc. of water and allowed to react with 20 g. of bromine for two days at room temperature. The excess of bromine was removed by a stream of air, the hydrobromic acid by silver carbonate and the excess of silver by hydrogen sulfide and the solution was concentrated under reduced pressure to 50 cc. To 45 cc. of this solution, a filtered hot solution of basic lead acetate was added until no further precipitate formed. The amorphous precipitate was filtered off, washed with water, alcohol and ether and dried. The weight was 52 g. This lead salt was decomposed with hydrogen sulfide, the filtered solution evaporated under reduced pressure to dryness, the residue dissolved in hot alcohol and then poured into absolute ether. The clear ether was decanted from the viscous sirup which was then dissolved in hot alcohol and evaporated to dryness under reduced pressure. This operation was repeated and lastly the evaporation was made with absolute ether. The yield was 6 g. of almost colorless sirup that did not reduce Fehling's solution. Neolactobionic acid closely resembles lactobionic and maltobionic acids. Like them it is amorphous and no crystalline salt of it has so far been obtained.

**Hydrolysis of Neolactobionic Acid.**—A solution of 6 g. of neolactobionic acid in 30 cc. of 5% sulfuric acid was heated on the steam-bath for one hour, neutralized with barium carbonate, filtered and the filtrate evaporated under reduced pressure to a sirup, which was dissolved in a small amount of water and poured into hot absolute alcohol. The barium salt that precipitated was subjected again to the same operation, after which it was found to be free from reducing sugars by the Fehling test. The two alcoholic solutions were united and evaporated under reduced pressure to dryness and this residue was dissolved in a small volume of hot methyl alcohol, from which solution a sugar readily crystallized. Its rotation ($[\alpha]_D^{22} = +80.7$, final, in water) and its conversion to mucic acid showed it to be *d*-galactose. The sugar-free barium salt mentioned above was found to reduce Fehling's solution after heating with acid, which indicated that it contained some barium neolactobionate due to incomplete hydrolysis. A solution of 3 g. of it in 25 cc. of 5% sulfuric acid was accordingly heated on the steam-bath for two hours and then neutralized with barium carbonate and the barium salt separated from sugar as already described. The yield was 2.2 g. of barium salt and a second portion of crystalline *d*-galactose.

Two g. of the barium salt was dissolved in water, the barium was removed quantitatively as sulfate and the filtrate was evaporated under reduced pressure to a sirup which was dissolved in a little methyl alcohol and the solution poured into 50 cc. of ethyl acetate to precipitate impurities. The filtered solution was then evaporated to a thick sirup; yield, 0.8 g. This material did not reduce Fehling's solution. It was decidedly dextrorotatory; a solution of it in 10 cc. of water in a 1-dcm. tube rotated the sodium ray 1.36° to the right. This solution was heated on the steam-bath with 2 g. of brucine

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[7] Fischer and Meyer, *Ber.*, **22**, 361, 1941 (1889).

for one-half hour, the excess of brucine was extracted with chloroform and the solution evaporated to dryness. The residue crystallized readily from alcohol. After three recrystallizations the substance showed $[\alpha]_D = +23.7$ in water; m. p., 158°, with decomposition. These are characteristic properties of brucine *d*-altronate[5] and, through the kindness of Dr. Levene, a mixed-melting-point measurement was made by the use of some brucine *d*-altronate that had been prepared from *d*-altronic acid obtained from *d*-ribose;[8] the melting point was the same, 158°.

**The Hydrolysis of Neolactose.**—A solution of 20 g. of α-chloro-acetyl neolactose in 200 cc. of acetone and 10 cc. of water was refluxed for two hours with 10 g. of freshly prepared silver carbonate, the silver chloride and excess of carbonate were filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was hydrolyzed by heating it at 98° with 200 cc. of *N* hydrochloric acid for two and one-half hours. The hydrochloric acid was removed with silver carbonate, the excess of silver with hydrogen sulfide, and the solution evaporated under reduced pressure to a thick sirup. The solution of this in 30 cc. of methyl alcohol and the addition of 15 cc. of ether resulted in a rapid crystallization of *d*-galactose which was complete in 24 hours in the ice box. The yield was 4.3 g. and 4.5 g., respectively, in two experiments. After one recrystallization from alcohol, the sugar showed $[\alpha]_D^{25} = +80.1$, and 2 g. of it yielded 2.15 g. of *d*-galactose phenylosazone, which was purified by two recrystallizations from 50% alcohol and one from absolute alcohol. The pure osazone melted with decomposition at 195° and gave the initial (+0.77) and final (+0.32) rotations in a mixture of six volumes of 95% alcohol and four volumes of pyridine (1-dcm. tube, 1 g. of osazone per 100 cc. of solvent, sodium light) that are characteristic of the osazone of galactose.[9] The other hexose that results from the hydrolysis of neolactose was identified as *d*-altrose by its conversion to *d*-altrose phenylosazone and its oxidation to *d*-talomucic acid, as follows.

***d*-Altrose Phenylosazone.**—The sirupy mixture of *d*-altrose and *d*-galactose which was obtained as the mother liquor from a crystallization of *d*-galactose (4.5 g.), as described above, was dissolved in 100 cc. of a solution containing 12 cc. of pure phenylhydrazine in 12 cc. of 50% acetic acid and the mixture heated for three hours at 98°. The hot solution was decanted from the precipitated solids and oil and filtered while still warm; Fraction A (0.2 g.) separated therefrom. The solids and oil were extracted with 800 cc. of hot water (80°), and from this solution, after filtration, Fraction B (1 g.) separated. A second extraction with 500 cc. of hot water (85°) yielded Fraction C (0.3 g.). The residue was dissolved in 50 cc. of 50% hot alcohol and, on cooling, Fraction D (0.6 g.) separated. Fraction B contained most of the *d*-altrose phenylosazone (initial rotation, −0.28; final, −0.24, expressed in the units mentioned previously in the measurement on galactose osazone). Fraction A was of about the same composition, Fraction C was dark colored and was discarded and Fraction D contained mostly *d*-galactose phenylosazone. Fraction B was extracted with cold ether, petroleum ether was added until the solution became slightly turbid and the filtered solution soon yielded crystals of *d*-altrose phenylosazone. After one recrystallization from ether and petroleum ether, the substance melted with decomposition at 168–170° and showed initial and final levorotations of −0.37 and −0.30, respectively. The melting point is somewhat lower than that which Levene and La Forge found (178°), but the rotations agree with their measurements.

***d*-Talomucic Acid.**—Fischer's directions[10] for preparing this acid have been followed.

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[8] Levene and Jacobs, *Ber.*, **43,** 3141 (1910).

[9] Neuberg, *Ber.*, **32,** 3386 (1900). Levene and La Forge, *J. Biol. Chem.*, **20,** 429 (1915).

[10] Fischer, *Ber.*, **24,** 3622 (1891).

The mother liquor after the crystallization of galactose (4.3 g.) following the acid hydrolysis of neolactose (see under that heading) was evaporated under reduced pressure to a sirup and this was oxidized with 40 cc. of nitric acid (d., 1.15) by evaporation to dryness on the steam-bath during constant stirring, adding water and evaporating again. The solution of the residue in 50 cc. of cold water slowly deposited about 0.1 g. of mucic acid. The filtrate therefrom was diluted to 75 cc., heated for two hours on the steam-bath with calcium carbonate in excess, purified with active carbon, and from the filtered hot solution a calcium salt crystallized as a yellow powder. The mother liquor from this was concentrated to 50 cc. and heated with calcium hydroxide and, on filtering and cooling, a second crop of calcium salt was obtained. Further crops were produced similarly and the total yield was 3 g. of crude calcium salt. This was added gradually to 50 cc. of a hot aqueous solution containing 1.6 g. of oxalic acid, the solution treated with decolorizing carbon, the excess of oxalic acid quantitatively precipitated with lime water, and the filtrate concentrated under reduced pressure to a sirup. The extraction of this residue with acetone left behind about 0.3 g. of mucic acid. The acetone solution of *d*-talomucic acid was evaporated to a sirup from which 1.2 g. of pure calcium *d*-talomucate was obtained by the procedure already described. Its calcium content was found to be 15.93% (0.1223 g. of salt, dried at 105°, yielded 0.0662 g. of calcium sulfate) while that calculated is 16.13%. One g. of the pure calcium salt was quantitatively decomposed by oxalic acid and, by Fischer's acetone extraction procedure, 0.2 g. of pure, crystalline *d*-talomucic acid was obtained; m. p., 158°; $[\alpha]_D^{22} = +29.2$ (initial), +24 (after one day) and +19 (after three days). These data agree well with Fischer's observations on *d*-talomucic acid.

**The Approximate Rotation of Neolactose in Water.**—By the de-acetylation of recrystallized α-neolactose octa-acetate according to the directions already given, the free sugar was obtained in sirupy form, and in aqueous solution it showed dextrorotation, $[\alpha]_D^{24} = +34.7$ (sugar from 1.099 g. of octa-acetate, 50 cc. of aqueous solution, 6-dcm. tube, 2.125 rotation). Under the same conditions pure lactose octa-acetate gave the value $[\alpha]_D^{24} = +54.4$ for its parent sugar (correct value for lactose, +55), which proves that the method is reliable. The equilibrium rotation of neolactose in water appears, therefore, to be approximately $[\alpha]_D = +35$.

**The Approximate Rotation of *d*-Altrose in Water.**—Some sirupy neolactose that was obtained by the de-acetylation of twice-recrystallized α-neolactose octa-acetate, as above described, was heated for two and one-half hours at 98° with 20 cc. of *N* hydrochloric acid and the cooled solution was made up to 50 cc. with water. The mixture of *d*-galactose and *d*-talose contained therein showed levorotation; $[\alpha]_D^{24} = -8.95$ (sugars from 1.0551 g. of octa-acetate, 50 cc. of solution, 6-dcm. tube, −0.601° rotation). Correcting for the equilibrium rotation of the galactose (+80) that of *d*-altrose is found to be approximately $[\alpha]_D = -98$. Evidently, this hexose is very strongly levorotatory, even more so than is *d*-fructose. The observed value is near that of *d*-arabinose (−105°, equilibrium) which is closely similar in configuration to *d*-altrose.

## Summary

It is shown that the component hexoses of neolactose are *d*-galactose and *d*-altrose and that the new disaccharide is a *d*-galactosido-*d*-altrose. Neolactose results from a stereochemical rearrangement of the glucose portion of lactose (*d*-galactosido-*d*-glucose) brought about by the action of aluminum chloride upon lactose octa-acetate. This rearrangement is a new type of isomerization in the sugar group.

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