

[CONTRIBUTION FROM THE POLARIMETRY SECTION, NATIONAL BUREAU OF STANDARDS,
UNITED STATES DEPARTMENT OF COMMERCE]¹

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE
IN THE SUGAR GROUP. XV.² CONVERSION OF LACTOSE
TO ANOTHER DISACCHARIDE, NEOLACTOSE. THE
CHLORO-HEPTA-ACETATE AND TWO OCTA-ACETATES
OF NEOLACTOSE

BY ALFONS KUNZ AND C. S. HUDSON

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It was stated in a recent article³ dealing with the revision of the rotation of α -chloro-acetyl lactose that "on one occasion, upon the addition of petroleum ether to the mother liquor of an original crystallization of α -chloro-acetyl lactose, which had been prepared from 20 g. of crude lactose octa-acetate, there slowly separated about 1 g. of a crystalline substance in the form of prisms which showed $[\alpha]_D^{25} = +71.7$ in chloroform, melted at 160° and gave a strong chlorine reaction with silver nitrate. It seems to be a new chlorine derivative of lactose octa-acetate and is being examined further." The results of this examination are now presented.

The substance proves to be a chloro-hepta-acetate of a disaccharide (di-hexose) and is thus an isomer of chloro-acetyl lactose. It has been transformed by reactions in which its chlorine atom was exchanged for the acetyl radical to two crystalline isomeric pure octa-acetates of a disaccharide, which are different from all known acetates of the sugar group. The saponification of these acetates has yielded a sirupy sugar which has not crystallized when seeded with lactose and on re-acetylation regenerates these acetates. Since no difficulty was found in preparing crystalline lactose in high yield by the saponification of lactose octa-acetate, it seems reasonably certain that the new substances are derivatives of a disaccharide that is different from lactose. The sugar will provisionally be named neolactose, pending the determination of its structure. Its identity with known disaccharides is excluded in all cases but one by the properties of its two octa-acetates. It may prove to be the 5-galactosido-mannose which Bergmann⁴ prepared from the oxidation of lactal but, since acetates of 5-galactosido-mannose are not described, further experimental work will be required to decide the question whether neolactose is Bergmann's 5-galactosido-mannose.^{4a}

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

² Article XIV was published in *THIS JOURNAL*, **48**, 1434 (1926).

³ Hudson and Kunz, *ibid.*, **47**, 2052 (1925).

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

^{4a} Note added May 10, 1926. Subsequent results, which will soon be published, have shown that neolactose is different from 5-galactosido-mannose and is indeed a new sugar. It is dextrorotatory but after acid hydrolysis the solution is levorotatory, which distinguishes it definitely from 5-galactosido-mannose. The structure of neolactose has been found to be that of *d*-galactosido-*d*-altrose.

The new chlorine derivative, which will be designated *α-chloro-acetyl neolactose*, was originally obtained in low yield by Skraup and Kremann's⁵ chlorination process, which consists in boiling the chloroform solution of a sugar acetate, here lactose octa-acetate, with phosphorus pentachloride and a small amount of anhydrous aluminum chloride as catalyst. A systematic study of this reaction was made in the hope of finding a way to increase the yield of chloro-acetyl neolactose and it led to a surprising result. The aluminum chloride is not only a catalytic agent with respect to the chlorination by phosphorus pentachloride but it chlorinates the octa-acetate in the absence of the pentachloride and gives the desired good yield of chloro-acetyl neolactose. It has been found as the result of numerous trials, however, that commercial sublimed aluminum chloride is not suitable for use in this case. It is necessary to employ highly reactive aluminum chloride such as is required for the Friedel-Crafts reaction. This material has been prepared by Stockhausen and Gattermann's⁶ method, which consists in leading a stream of hydrogen chloride over red-hot aluminum (iron-free), and by its use chloro-acetyl neolactose has been prepared in regular and satisfactory yield. A large excess of aluminum chloride is required. It appears that the reaction involves an absorption of the lactose octa-acetate at the surface of the insoluble aluminum chloride, a conclusion which is drawn from the facts, (1) that the acetate is largely removed from the chloroform solution by the solid aluminum chloride and (2) that finely divided aluminum chloride is more reactive than coarse lumps. The solid phase in the reaction mixture goes through a series of color changes. It first becomes cherry-red, then lilac and finally dark gray. At this stage the mixture is to be cooled and shaken with ice; the dark color then disappears and the solid sugar acetate derivative of aluminum chloride decomposes to yield *α-chloro-acetyl lactose* and *α-chloro-acetyl neolactose*, which dissolve in the chloroform layer, and aluminum chloride, which dissolves in the water. When the reaction is stopped when the color is cherry-red *α-chloro-acetyl lactose* results in good yield (about 60%) but only a small quantity of the neolactose derivative is obtained. Evidently, *α-chloro-acetyl lactose* is formed first and is then transformed to the isomeric neolactose compound. This conclusion was verified by substituting *α-chloro-acetyl lactose* for the octa-acetate in one experiment and obtaining a normal yield of *α-chloro-acetyl neolactose* from it. During the chlorination reaction there is a slow decomposition and in order to obtain the best yield of the neolactose derivative the reaction must be stopped at the empirically determined stage already mentioned, which we find to result in a yield of about 30% of *α-chloro-acetyl lactose* and 20% of *α-chloro-acetyl neolactose*, based on the weight of the original lactose

⁵ Skraup and Kremann, *Monatsh.*, **22**, 375 (1901).

⁶ Stockhausen and Gattermann, *Ber.*, **25**, 3521 (1892).

octa-acetate. The two substances are readily separated and purified and their physical properties (see Table I) show unmistakably that they are different substances.

TABLE I
ROTATIONS IN CHLOROFORM OF LACTOSE AND NEOLACTOSE DERIVATIVES

Substance	$[\alpha]_D^{20-5}$	$[\alpha]_{578}^{20}$	$[\alpha]_{546}^{20}$	$[\alpha]_{436}^{20}$	M. p., °C.
α -Chloro-acetyl lactose	+84	120
α -Chloro-acetyl neolactose	+71.2	+75.6	+84.5	+147	182
α -Octa-acetyl lactose	+53.9	152
α -Octa-acetyl neolactose	+53.4	+56.0	+63.1	+112	178
β -Octa-acetyl lactose	- 4.3	90
β -Octa-acetyl neolactose	- 7.1	- 7.9	- 9.2	- 16.2	148

α -Chloro-acetyl neolactose yielded two isomeric crystalline octa-acetates of neolactose, according to the method used in replacing the chlorine atom by the acetyl radical. When a solution of the substance in acetic anhydride was warmed on the steam-bath with silver carbonate or with sodium acetate, a good yield of a neolactose octa-acetate of $[\alpha]_D$ about +44-46 was obtained. Under like conditions α -chloro-acetyl lactose did not react with sodium acetate, and with silver carbonate it yielded β -lactose octa-acetate ($[\alpha]_D = -2$). At the boiling point of acetic anhydride it reacted with sodium acetate to give the same octa-acetate ($[\alpha]_D = -4$). When an acetone solution of α -chloro-acetyl neolactose was gently boiled with silver carbonate and the resulting substance, which is presumably a hepta-acetate of neolactose, was then acetylated by heating it with acetic anhydride and sodium acetate there was obtained in good yield a neolactose octa-acetate of $[\alpha]_D$ about -0.5. The low-rotating form will be designated *neolactose β -octa-acetate* and the strongly dextrorotatory form *neolactose α -octa-acetate*, in conformity with the names of the well-known α - and β -octa-acetates of lactose. In a similar manner chloro-acetyl neolactose will be designated as an alpha form because of its high dextrorotation in conformity with the previous naming of α -chloro-acetyl lactose from similar considerations.⁷

The transformations of the α -chloro-acetates of lactose and neolactose to octa-acetates by various reactions are summarized in Table II, from which it is seen that the chemical reactivities of the chloro-acetates of the two sugars are markedly different. α -Chloro-acetyl lactose yields only lactose β -octa-acetate by the reactions employed, while α -chloro-acetyl neolactose yields neolactose α -octa-acetate in most cases but its β -octa-acetate results from one of the reactions. Certainly, in the case of α -chloro-acetyl lactose or neolactose there has been realized a Walden reversal on Carbon 1 during the passage to an octa-acetate.

The molecular rotations of the carefully purified neolactose derivatives

⁷ Hudson, THIS JOURNAL, 46, 462 (1924).

TABLE II
SOME REACTIONS OF α -CHLORO-ACETYL LACTOSE AND NEOLACTOSE

Reagent and solvent	α -Chloro-acetyl lactose yields octa-acetate showing $[\alpha]_D$ as follows:	α -Chloro-acetyl neolactose yields octa-acetate showing $[\alpha]_D$ as follows:
Silver acetate in glacial acetic acid on the steam-bath	(Lactose β -octa-acetate according to literature ⁸)	+46.8 (Neolactose α -octa-acetate)
Silver carbonate in acetic anhydride on the steam-bath	-1.7 (Lactose β -octa-acetate)	+43.7 (Neolactose α -octa-acetate)
Sodium acetate in acetic anhydride on the steam-bath	No reaction	+46.3 (Neolactose α -octa-acetate)
Sodium acetate in acetic anhydride at boiling temperature	-3.5 (Lactose β -octa-acetate)	(Neolactose α -octa-acetate)
Silver carbonate in aqueous acetone, followed by acetylation with acetic anhydride and sodium acetate	+6.1 (Lactose β -octa-acetate)	-0.5 (Neolactose β -octa-acetate)

are recorded in Table III, and from them values of the rotations of the end asymmetric carbon atom (A_{Ac} and A_{Cl}) have been obtained in the usual way.⁷ The value of A_{Ac} is normal, conforming fairly closely with the coefficient of the lactose series, which indicates that the two neolactose octa-acetates are a true α , β pair having like ring structure, though the position of the ring is unknown. The value of A_{Cl} is much smaller than that found for α -chloro-acetyl lactose. Whether this difference is to be attributed to the possession of another type of ring by α -chloro-acetyl neolactose or to other influences must be left for further investigation. Possibly the determination of the hexoses of which neolactose is composed will throw light upon this question.

TABLE III
VALUES OF THE COEFFICIENTS A_{Ao} AND A_{Cl} IN THE NEOLACTOSE SERIES

Neolactose	Mol. wt.	$[\alpha]_D$ in $CHCl_3$	$[M]_D$	Rotation of end carbon	A for correspond- ing derivative of lactose
α -Octa-acetyl	678	+53.4	+36,200	$A_{Ao} =$	19,700 ⁹
β -Octa-acetyl	678	-7.1	-4,810	+20,500	
α -Chloro-acetyl	655	+71.2	+46,700	$A_{Cl} =$	38,100 ⁸
				+31,000	

Experimental Part

Preparation of α -Chloro-acetyl Neolactose.—One hundred g. of crude lactose octa-acetate⁸ was dissolved in a liter of absolute chloroform; the solution was filtered and to it 200 g. of Stockhausen and Gattermann's powdered aluminum chloride was added,

⁸ Bodart, *Monatsh.*, **23**, 5 (1902).

⁹ Hudson and Dale, *THIS JOURNAL*, **40**, 993 (1918).

the mixture being kept at 65° during two hours in a 2-liter, round flask with a reflux condenser closed by a calcium chloride drying tube. The color changes that were observed during the progress of the reaction have been described. The aluminum chloride remains undissolved and the lactose octa-acetate, originally in the chloroform solution, disappears therefrom and unites with the solid phase (aluminum chloride). Whether it unites before or after a transformation has not been determined. The reaction mixture is next cooled, and the solution and dark colored suspended solids are poured into a 2-liter separatory funnel half filled with cracked ice. The coarse pieces of aluminum chloride remaining in the flask were not removed but were rinsed with 100 cc. of chloroform, and the suspension of finer solids was poured into the funnel. The chloroform suspension was shaken thrice with ice water, during which treatment the aluminum chloride dissolved in the water while the acylated sugar compounds dissolved in the chloroform. The chloroform solution was then dried with calcium chloride and evaporated under reduced pressure to a sirup which was dissolved in 750 cc. of ether and the solution was kept in an ice box until crystallization was complete, which required about five days. The yield was 50 g. of a mixture of the α -chloro-acetates of lactose and neolactose. The crystals of the lactose compound are needles arranged in radiating clusters while those of the neolactose derivative are well formed stout prisms and the two substances are readily distinguished by the naked eye. They were easily separated by extraction with 100 cc. of cold ethyl acetate which dissolved the lactose compound but left the α -chloro-acetyl neolactose; the yield of the latter was 20 g. of crude substance. This was recrystallized by dissolving in 200 cc. of hot ethyl acetate, decolorizing with carbon and adding 200 cc. of ether; yield, 18 g. of pure α -chloro-acetyl neolactose. Another recrystallization did not change its specific rotation; *m. p.*, 182° with decomposition; $[\alpha]_D^{25} = +71.2$ in pure chloroform (0.5115 g. of subst., 50 cc. of chloroform solution; 4.37° rotation to the right, 600-mm. tube). The chlorine percentage was 5.45 from the reaction of the substance in aqueous acetone solution with silver nitrate, 5.42 to 5.47 by C. W. Bacon's method¹⁰ and 5.38 by the Carius method, in comparison with 5.41 calculated for a chloro-hepta-acetyl disaccharide (di-hexose), $C_{26}H_{35}O_{17}Cl$.

The acetyl determination was made by a new method which gives accurate results and is quite simple to carry out.¹¹ One-half g. of the substance was dissolved in 50 cc. of pure acetone, the solution was cooled in an ice-and-salt mixture, 100 cc. of 0.1 *N* aqueous potassium hydroxide solution was added drop by drop and the solution kept for two hours below 0° in the mixture. The excess alkali was then titrated with 0.2 *N* hydrochloric acid. A control test was made to determine the slight amount of alkali that is neutralized by the acetone. Measurements with chloro-acetyl lactose showed the method to be reliable; 0.5189 g. of the substance was equivalent to 31.6 cc. of 0.2 *N* hydrochloric acid in two hours' action (calculated, 31.6 cc.), and another sample after 16 hours' action still gave the calculated percentage. By this method 0.4884 g. of chloro-acetyl neolactose was found to be equivalent to 29.7 cc. of 0.2 *N* hydrochloric acid in comparison with 29.8 cc. calculated. Chloro-acetyl neolactose is very soluble in chloroform, benzene and acetone, slightly soluble in cold ethyl acetate or alcohol, but considerably so in the hot solvents, slightly soluble in ether and insoluble in petroleum ether and water. It reduces Fehling's solution on heating. It is a stable substance and can even be kept in an open bottle for long periods.

Preparation of Neolactose α -Octa-acetate.—Ten g. of α -chloro-acetyl neolactose and 10 g. of anhydrous sodium acetate were heated with 50 cc. of acetic anhydride on the steam-bath for one hour and the solution was poured into ice water. The pre-

¹⁰ Bacon, THIS JOURNAL, 31, 49 (1909).

¹¹ This method was devised by Dr. Kunz (C. S. H.).

cipitated oil quickly crystallized and the yield of octa-acetate was nearly quantitative. It was decolorized with carbon and purified by recrystallization from hot alcohol. After four recrystallizations, using 300 cc. of alcohol each time, and four using 200 cc., 1.6 g. of the substance was obtained melting at 178°; $[\alpha]_D^{26} = +53.2$ in chloroform. The values were not appreciably changed by five additional recrystallizations, and the value $[\alpha]_D^{24} = +53.4$ (0.4626 g. of the subst., 50 cc. of chloroform solution; 2.964 degrees to the right, 600mm. tube) obtained after the thirteenth recrystallization is chosen for the value of neolactose α -octa-acetate in chloroform. The acetyl estimation was made by the previously mentioned method; 0.5725 g. of the substance was found to be equivalent to 33.5 cc. of 0.2 *N* hydrochloric acid, the reaction time being six hours, in comparison with 33.7 cc. calculated for a di-hexose octa-acetate. The substance is quite soluble in chloroform, acetone, ethyl acetate and benzene, slightly soluble in cold alcohol and insoluble in ether, petroleum ether and water. It is conveniently recrystallized from hot alcohol, 1 g. requiring about 50 cc. of solvent. On cooling, the acetate crystallized in plates grouped in star form. It reduces Fehling's solution on heating.

Preparation of Neolactose β -Octa-acetate.—A mixture of 15 g. of α -chloro-acetyl neolactose, 10 g. of freshly prepared silver carbonate, 100 cc. of pure acetone and 5 cc. of water was refluxed for one hour, filtered after the addition of a little decolorizing carbon and the filtrate evaporated to dryness under reduced pressure. The crystalline residue, which was presumably a neolactose hepta-acetate, was acetylated by heating for one-half hour on the steam-bath with 50 cc. of acetic anhydride (96%) and 5 g. of anhydrous sodium acetate. When the solution was poured into ice water a crude octa-acetate quickly crystallized in nearly quantitative yield. After two recrystallizations from 200 cc. of hot alcohol, using some decolorizing carbon in the first, 12.5 g. of the substance crystallizing in beautiful plates was obtained; $[\alpha]_D^{23} = -0.8$; m. p., 145°. It is a mixture of about 90% of the β - and 10% of the α -octa-acetate of neolactose, judging from its rotation. The freeing of the beta form from its alpha isomer required 17 recrystallizations from alcohol. The $[\alpha]_D^{26}$ value was then -7.03 in chloroform. After the nineteenth recrystallization, $[\alpha]_D^{25} = -7.16$ and the melting point 148°; and after the twenty-first, $[\alpha]_D^{23} = -7.04$ (0.5102 g. of substance, 50 cc. of pure chloroform solution; 0.431° to the left, 600mm. tube). The melting point 148° and the value $[\alpha]_D^{24} = -7.1$ in chloroform are chosen for the pure neolactose β -octa-acetate. It is readily soluble in chloroform, acetone, ethyl acetate and benzene, soluble in hot alcohol from which it crystallizes on cooling, only slightly soluble in ether and hot water, insoluble in petroleum ether and cold water. It reduces Fehling's solution on heating.

An acetyl estimation was made by the new method, using the previously mentioned product showing $[\alpha]_D^{23} = -0.8$; 0.5162 g. of the substance was equivalent to 30.1 cc. of 0.2 *N* hydrochloric acid; the value calculated for an octa-acetyl di-hexose is 30.4. The duration of the treatment was here six hours. In another test lasting for 16 hours the same result was obtained.

Summary

It is found that lactose octa-acetate in chloroform solution can be chlorinated with highly reactive aluminum chloride. The first product is the well known α -chloro-acetyl lactose but on longer duration of the reaction a crystalline chloro-hepta-acetate of a disaccharide isomeric with lactose, which will be designated provisionally neolactose, is obtained in about 20% yield. This α -chloro-acetyl neolactose has been transformed to an α - and a β -octa-acetate of neolactose, crystalline substances which are

different from all known disaccharide octa-acetates. The difference of their molecular rotations has the normal value for an α , β pair of sugar acetates, and they have been named in accordance with this fact. The structure of neolactose and the applicability of the new chlorinating method to other sugar derivatives will be investigated.

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WASHINGTON, D. C.

[CONTRIBUTION FROM THE DYE LABORATORY OF THE CHEMISTRY DIVISION OF THE NATIONAL BUREAU OF STANDARDS, UNITED STATES DEPARTMENT OF COMMERCE]

THE ABSORPTION SPECTRA OF BENZENE-AZOBENZENE¹

BY WALLACE R. BRODE

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In the decade between 1905 and 1915 a number of workers² made rather extensive studies of the absorption spectra of benzene-azobenzene but during the past decade little or no work has been done on this remarkable colored compound. Benzene-azobenzene may well be considered the parent substance of all azo dyes and as such a more accurate determination of its absorption spectra should be welcome. With the development of more accurate apparatus and the general adoption of the method of relative transmission for the determination of absorption spectra, the curve for this compound can be determined with greater accuracy than was previously possible.

The results of this investigation show that the curve is not so simple as was previously thought, but that the principal band of this substance the simplest of azo dyes, is in reality composed of at least two overlapping bands. Repeated observations in various kinds of solvents have confirmed this fact beyond any doubt.

The apparatus used in making these measurements included a Hilger sector photometer and quartz spectrograph.³ (For a detailed description of the apparatus see Ref. 4 c.) A slit 0.1 mm. wide, which was the equivalent of 2.5 f units at a frequency of 750 f and 2.0 f at a frequency of 1200 f , was used in making these observations. The unit of frequency^{4c} (f)

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² (a) Hartley, *J. Chem. Soc.*, **51**, 152 (1887). (b) Baly and Tuck, *ibid.*, **89**, 982 (1906). (c) Tuck, *ibid.*, **91**, 449 (1907). (d) Hantzsch, *Ber.*, **42**, 2129 (1909). (e) Purvis and McClelland, *J. Chem. Soc.*, **101**, 1514 (1912). (f) Hantzsch, *Ber.*, **46**, 1537 (1913). (g) Purvis, *J. Chem. Soc.*, **105**, 590 (1914). (h) Baly and Hampson, *ibid.*, **107**, 248 (1915).

³ Placed at the author's disposal by the Colorimetry Section of the Optics Division of the National Bureau of Standards, U. S. Department of Commerce.