

with nitric acid (sp. gr. 1.59) at 100°. When crystallized from benzene it melts at 192–193°.

*Anal.* Calcd. for  $C_{12}H_6N_3O_6Br$ : Br, 21.72. Found: Br, 22.08.

The same product was also obtained by the nitration of IX or of the mixture III.

**3,3',5-Trinitro-4-aminobiphenyl (X).**—Ten grams of 3,3'-dinitro-4-acetaminobiphenyl was added gradually to 60 cc. of nitric acid (sp. gr. 1.5) keeping the temperature below 8°. On pouring the solution into water and recrystallizing from acetone-alcohol, the acetylated base was obtained yielding 4.5 g., m. p. 241–242°. The pure product melts at 242–243°.

*Anal.* Calcd. for  $C_{14}H_{10}N_4O_7$ : N, 16.19. Found: N, 15.83.

This product could also be obtained in a lower state of purity by direct nitration of 4-acetamino-3'-nitrobiphenyl.

The free base was obtained by hydrolysis of the acetamino compound with dilute sulfuric acid at 120°. On crystallization from glacial acetic acid it melted at 233°.

*Anal.* Calcd. for  $C_{12}H_8N_3O_6$ : N, 18.43. Found: N, 18.19.

**Deamination of 3,3',5-Trinitro-4-aminobiphenyl.**—The deamination of 3,3',5-trinitro-4-aminobiphenyl by the simple method used to prepare 2,3'-dinitro-4-bromobiphenyl proved ineffective in this case, as the base was recovered unchanged. The following procedure was therefore adopted: 3.5 g. of the trinitro base, dissolved in 30 cc. of concd. sulfuric acid, was treated at 0° with a solution of 1.5 g. of sodium nitrite in 20 cc. of concd. sulfuric acid. The mixture was treated at 2° with a solution of 70 cc. of phosphoric acid, and then with 40 cc. of 20% oleum, at 15–20°. The resulting solution was poured into 250 cc. of boiling ethanol, and refluxed for one hour. After pouring into ice-water, the resulting solid was filtered and crystallized from methanol-acetone, yielding 1 g. of a product melting at 167–168°. This was further purified by treatment with chromic acid which appeared to remove traces of unchanged base. The melting point of the pure product was 177–178°.

*Anal.* Calcd. for  $C_{12}H_7N_3O_6$ : N, 14.54. Found: N, 14.48.

**3,3',5-Trinitrobiphenyl (XI).**—A mixture of 12 g. of 3,5-dinitroiodobenzene, 7.5 g. of *m*-nitroiodobenzene and 8 g. of copper powder was heated for an hour at 270°. From the ether extract it was possible to obtain by repeated crystallization from alcohol-acetone a small amount of a solid melting at 173–174° and unchanged when mixed with the deamination product of 3,3',5-trinitro-4-aminobiphenyl.

**3,3',5-Trinitro-4-bromobiphenyl (XII).**—This was prepared from the corresponding base by Schoutissen's method. From 3.5 g. of base was obtained 3 g. of pure product, melting at 222–223° (from alcohol-acetone).

*Anal.* Calcd. for  $C_{12}H_8N_3O_6Br$ : Br, 21.72. Found: Br, 22.04.

**3,3',5,5'-Tetranitrobiphenyl.**—A mixture of 13 g. of 3,5-dinitroiodobenzene and 8.5 g. of copper powder was heated with stirring at 270° for one and one-half hours. The acetone extract, after evaporation, was crystallized from toluene, yielding 0.7 g. of a solid melting at 228–229°.

*Anal.* Calcd. for  $C_{12}H_6N_4O_8$ : N, 16.77. Found: N, 16.52.

The nitrogen analyses were carried out by Messrs. Richard Schock and Chester White.

### Summary

1. The nitration of 4-bromobiphenyl according to Mascarelli is shown to yield 3,4'-dinitro-4-bromobiphenyl and 2',3-dinitro-4-bromobiphenyl.
2. Depending on conditions, the following substances have been obtained from the nitration of 4-bromo-3'-nitrobiphenyl: 2,3'-dinitro-4-bromobiphenyl, 3,3'-dinitro-4-bromobiphenyl, 2,3',4'-trinitro-4-bromobiphenyl and 3,3',4'-trinitro-4-bromobiphenyl.
3. Proof of the structures of the above substances is given.

PHILADELPHIA, PENNSYLVANIA RECEIVED MAY 11, 1942

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## Syntheses of Epi-lactose and Lactose

BY W. T. HASKINS, RAYMOND M. HANN AND C. S. HUDSON

In continuation of our use of acetone-D-mannosan  $\langle 1,5 \rangle \beta \langle 1,6 \rangle^1$  for the synthesis of disaccharides<sup>2</sup> having their linkage at carbon atom 4, we have now combined this substance with acetobromo-D-galactose and thereby produced, first, the epimer of lactose (4- $\beta$ -D-galactopyranosido]-D-mannose), which we shall designate epi-lactose for brevity, and, second, lactose (4- $\beta$ -D-galacto-

pyranosido]-D-glucose) itself. Bergmann, Schotte and Rennert<sup>3</sup> discovered epi-lactose by the epimerization of lactose through the reactions *lactose*  $\rightarrow$  *acetobromolactose*  $\rightarrow$  *hexaacetyl lactal*  $\rightarrow$  *lactal*  $\rightarrow$  *epi-lactose*. The reverse transformation of epi-lactose to lactose, which has now assumed importance, has been accomplished readily by the reactions *epi-lactose*  $\rightarrow$  *acetobromo-epi-lactose*  $\rightarrow$  *hexaacetyl lactal*  $\rightarrow$  *lactose hexaacetate*  $\rightarrow$  *lactose octa-*

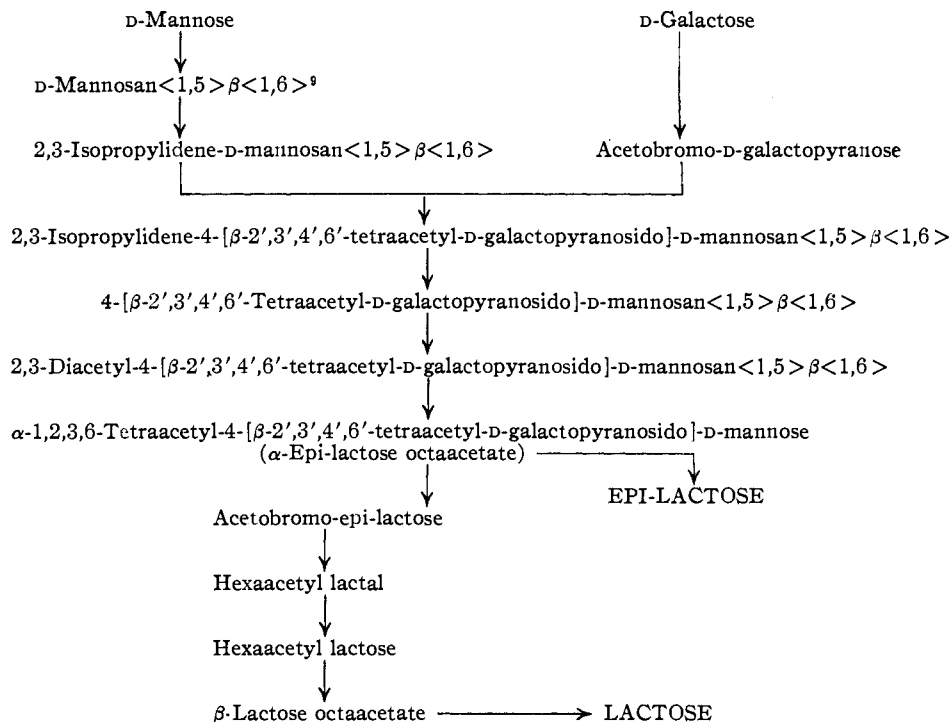
(1) Knauf, Hann and Hudson, *THIS JOURNAL*, **63**, 1447 (1941).

(2) Haskins, Hann and Hudson, *ibid.*, **63**, 1724 (1941); **64**, 1490 (1942).

(3) Bergmann, Schotte and Rennert, *Ann.*, **434**, 94 (1923).

acetate  $\rightarrow$  lactose, following the general method of Levene and Tipson<sup>4</sup> in the oxidation of the glycol hexaacetate, as used by us<sup>2</sup> recently in the transformation of epi-cellobiose to cellobiose. Several of the essential designations in the structure of lactose were established by Haworth and Long<sup>5</sup> when they identified the products of the acid hydrolysis of fully methylated lactobionic acid as 2,3,4,6-tetramethyl-D-galactopyranose and 2,3,5,6-

present syntheses. The syntheses also represent total syntheses since such have been accomplished previously for D-mannose and D-galactose.<sup>8</sup> Lactose, the unique disaccharide of mammalian life, is producible in the chemical laboratory, by structurally definitive reactions of organic chemistry, from the elements carbon, hydrogen and oxygen. A flow diagram of the synthetic steps, beginning with D-mannose and D-galactose, is shown.



tetramethyl-D-gluconic acid. The  $\beta$ -configuration of the galactose moiety has been inferred in the past from the fact that the enzyme lactase, which hydrolyzes lactose, likewise hydrolyzes  $\beta$ -methyl-D-galactopyranoside, but is without action on the  $\alpha$ -modification; and also from a comparison of the rotatory powers of the methyl esters of the octamethyl derivatives of lactobionic, cellobionic and maltobionic acids, before and after acid hydrolysis.<sup>6</sup> The  $\beta$ -configuration of the galactose moiety is now conclusively established from the fact that acetobromo-D-galactose, which yields  $\beta$ -methyl-D-galactopyranoside when treated with methyl alcohol and silver carbonate by the Koenigs-Knorr reaction,<sup>7</sup> was employed in the

The synthesis of the enantiomorph of lactose, namely, 4-[ $\beta$ -L-galactopyranosido]-L-glucose, unquestionably could be made by parallel reactions provided sufficient supplies of the rare L-mannose and L-galactose should become available.

**Historical Note.**—A formation of lactose by the direct combination under catalytic conditions of supposedly pure glucose and galactose was reported in 1879 by Demole.<sup>10</sup> Pictet and Vogel<sup>11</sup> also reported such a formation. The reality of these reported formations of lactose remains in grave doubt; in any event, they have not contributed any new knowledge concerning the structure of lactose. In 1902 Emil Fischer and

(4) Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931).

(5) Haworth and Long, *J. Chem. Soc.*, 544 (1927).

(6) Haworth, "The Constitution of Sugars," Edward Arnold & Co., London, 1929 edition, pp. 59, 65.

(7) Levene and Sobotka, *J. Biol. Chem.*, **67**, 771 (1926); Dale and Hudson, *THIS JOURNAL*, **52**, 2537 (1930).

(8) Fischer, *Ber.*, **23**, 2114 (1890); Fischer and Ruff, *ibid.*, **33**, 2142 (1900).

(9) Montgomery, Richtmyer and Hudson, *THIS JOURNAL*, **64**, 1483 (1942).

(10) Demole, *Ber.*, **12**, 1935 (1879); cf. Berthelot, *Bull. soc. chim.*, [2] **34**, 82 (1880).

(11) Pictet and Vogel, *Helv. Chim. Acta*, **11**, 209 (1928).

E. F. Armstrong<sup>12</sup> reported a synthesis of a galactosido-glucose from acetochloro-D-galactose and sodium glucosate ("glucose-natrium"); a crystalline osazone, which was thought by them to be possibly melibiose osazone, was described; a later re-investigation of the subject by Schlubach and Rauchenberger<sup>13</sup> suggested that the osazone may be that of lactose. Biochemical syntheses of lactose from its constituent hexoses through the enzymotic activity of mammary gland tissue have been reported.<sup>14</sup>

### Experimental

**Acetobromo-D-galactose.**—The general method of Levene and Raymond<sup>15</sup> was followed. A solution of 25 g. of  $\beta$ -D-galactopyranose pentaacetate (m. p. 140–142°;  $[\alpha]^{20}_D +24.0^\circ$  in chloroform)<sup>16</sup> in 50 cc. of glacial acetic acid was cooled to 0° and saturated with gaseous hydrobromic acid; after standing two hours at 5°, the solution was diluted with 100 cc. of toluene and concentrated *in vacuo* at 45° to a thin sirup; three successive 100-cc. portions of toluene were added and removed in the same manner; the residual sirup was dissolved in 25 cc. of warm ether and as the solution cooled the acetobromogalactose crystallized. The yield was 23.6 g. (90%). The substance was recrystallized by solution in two parts of ether and the addition of one part of isopentane; it formed fine needles which melted at 84–85° (cor.) and showed a rotation of +217° (*c*, 1.2)<sup>17</sup> in chloroform and +242° (*c*, 1.2) in benzene. Fischer and Armstrong<sup>18</sup> report a melting point of 82–83° and a specific rotation  $[\alpha]^{20}_D$  of +236.4° in benzene for acetobromogalactose; Ohle, Marecek and Bourjau<sup>19</sup> record a melting point of 85° for the compound, but did not measure its rotation. Apparently the rotation in chloroform has not been recorded previously; its value is now found to be near that which was predicted some years ago.<sup>20</sup>

*Anal.* Calcd. for  $C_{14}H_{19}O_9Br$ : Br, 19.4. Found, Br, 19.7.

**2,3-Diacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan<1,5> $\beta$ <1,6>**.—A solution of 10.1 g. (0.05 mole) of 2,3-isopropylidene-D-mannosan

(12) Fischer and Armstrong, *Ber.*, **35**, 3146 (1902).

(13) Schlubach and Rauchenberger, *ibid.*, **58**, 1184 (1925); **59**, 2102 (1926).

(14) Grant, *Biochem. J.*, **30**, 2027 (1936); Peterson and Shaw, *Science*, **86**, 398 (1937).

(15) Levene and Raymond, *J. Biol. Chem.*, **90**, 247 (1931).

(16) Hudson and Parker, *THIS JOURNAL*, **37**, 1589 (1915); Hudson and Johnson, *ibid.*, **38**, 1224 (1916).

(17) All of the crystalline compounds described in the experimental part were recrystallized to constant melting point and specific rotation  $[\alpha]^{20}_D$ ; *c* is the concentration in grams in 100 cc. of solution; the tube length was 4 dm. The microchemical analyses reported were performed by Dr. A. T. Ness to whom we express our appreciation.

(18) Fischer and Armstrong, *Ber.*, **35**, 837 (1902).

(19) Ohle, Marecek and Bourjau, *ibid.*, **62**, 833 (1929).

(20) Hudson, *THIS JOURNAL*, **46**, 462 (1924). The difference between the molecular rotations in the pyranose pair, acetobromo-D-glucose and  $\beta$ -D-glucose pentaacetate, is +81,400 – 1,500 = 79,900, and in the similar pair, acetobromo-D-galactose and  $\beta$ -D-galactose pentaacetate, it is now found to be 89,200 – 9,000 = 80,200. According to the isorotation rules an equality of these differences, which are ( $A_{Br} + A_{Ac}$ ) in each case, is to be expected.

<1,5> $\beta$ <1,6> in 100 cc. of dry alcohol-free chloroform, 50 g. of "Drierite," 25 g. of silver oxide and 35 g. of glass beads (4 mm. diameter) was placed in a 500-cc. brown glass stoppered bottle and agitated on a machine for one hour to ensure drying of the reagents; 2.5 g. of solid iodine and a solution of 20.6 g. (0.05 mole) of acetobromo-D-galactose in 50 cc. of pure chloroform were then added and shaking was resumed for seven days at room temperature (24°). The solid reaction products and excess of reagents were removed by filtration, and the filtrate (which gave no test for ionizable halogen) containing the 2,3-isopropylidene-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan<1,5> $\beta$ <1,6>, was concentrated *in vacuo* to a sirup. A solution of the sirup in 200 cc. of 80% acetic acid was heated on the steam-bath until the rotation became constant (two hours), to remove the isopropylidene residue; the solvent was removed by concentration *in vacuo*, and the residual sirup, containing the partly acetylated 4- $[\beta$ -D-galactopyranosido]-D-mannosan<1,5> $\beta$ <1,6>, was heated for one hour on the steam-bath with 2.5 g. of fused sodium acetate and 25 cc. of acetic anhydride. The reaction mixture was poured over crushed ice and the gum which precipitated was washed with water and dissolved in 10 cc. of warm alcohol; the new crystalline material (2,3-diacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan <1,5> $\beta$ <1,6>) which deposited as the solution cooled was recrystallized from 15 parts of absolute alcohol and obtained in the form of elongated prisms which melted at 193–194° (cor.) and showed a rotation of –62.7° (*c*, 0.8) in chloroform. The yield was 8.8 g. (30%).

*Anal.* Calcd. for  $C_{24}H_{32}O_{16}$ : C, 50.00; H, 5.60;  $CH_3CO$ , 44.8. Found: C, 50.11; H, 5.62;  $CH_3CO$ , 44.7.

**$\alpha$ -1,2,3,6 Tetraacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannose ( $\alpha$ -Epi-lactose Octaacetate).**—A solution of 12.5 g. of 2,3-diacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan<1,5> $\beta$ <1,6> in 100 cc. of an acid acetylating solution (prepared by adding 2 cc. of concentrated sulfuric acid dropwise to an ice-cold mixture of 70 cc. of acetic anhydride and 30 cc. of glacial acetic acid) changed in rotation from +50.8°, after fifteen minutes, to a constant value of +55.1° in two hours at 20°. The reaction mixture was poured over crushed ice and the crystalline precipitate which formed was recrystallized from 3 parts of absolute alcohol. The yield was 14.5 g. (99%). The new substance ( $\alpha$ -epi-lactose octaacetate) crystallized as elongated prisms which melted at 96–97° (cor.) and rotated +41.2° (*c*, 0.8) in chloroform.

*Anal.* Calcd. for  $C_{28}H_{38}O_{19}$ : C, 49.56; H, 5.64;  $CH_3CO$ , 50.8. Found: C, 49.44; H, 5.66;  $CH_3CO$ , 50.5.

**4- $[\beta$ -D-Galactopyranosido]-D-mannose (Epi-lactose).**—To an ice-cold solution of 5.0 g. of epi-lactose octaacetate in 50 cc. of absolute methyl alcohol, 5 cc. of 0.5 *N* barium methylate solution was added and the mixture was allowed to stand at 5° for sixteen hours; the barium was precipitated by the addition of an equivalent amount of 0.1 *N* sulfuric acid and, following the removal of the barium sulfate, the solution was concentrated to a sirup, which was dissolved in 10 cc. of warm methyl alcohol. The  $\beta$ -form of the disaccharide deposited from the solution as it cooled; it was recrystallized by solution in 1 part of warm water and the addition of 5 parts of methyl alcohol and was obtained

in the form of fine needles which melted at 195–196° (cor.); an aqueous solution (*c.* 1.2) of the substance exhibited a rotation of +18.0° three minutes after preparation and its equilibrium rotation was +27.2°. The unimolecular coefficient of the mutarotation at 20° was 0.0151, using minutes and decimal logarithms; the initial rotation of this  $\beta$ -form of the sugar is thus approximately +17°, in good agreement with the value (+16°) which Haworth, Hirst, Plant and Reynolds<sup>21</sup> obtained indirectly, through solubility measurements of the  $\alpha$ -form. Bergmann, Schotte and Rennert<sup>3</sup> report a melting point of 196–197° and an equilibrium rotation of +30.0° for  $\beta$ -epi-lactose, and Haworth and co-workers<sup>21</sup> record an equilibrium value of +27° for anhydrous epi-lactose (calculated from that of  $\alpha$ -epi-lactose monohydrate). It may be summarized for reference purposes that the initial rotations of the  $\alpha$ - and  $\beta$ -forms of epi-lactose are +38°<sup>21</sup> and +17°, respectively, by direct measurements, and that the equilibrium rotation is +27°.

*Anal.* Calcd. for C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>: C, 42.10; H, 6.48. Found: C, 42.00; H, 6.53.

**Lactal Hexaacetate from Epi-lactose Octaacetate.**—To a solution of 10.0 g. of synthetic epi-lactose octaacetate in a mixture of 10 cc. of glacial acetic acid and 5 cc. of acetic anhydride, 40 g. of a 30% solution of hydrobromic acid in glacial acetic acid was added. The reaction mixture was allowed to stand at 5° overnight and after dilution with 50 cc. of chloroform, it was poured into 250 cc. of ice-cold water. The aqueous solution was extracted with chloroform in the usual manner, and the washed and dried chloroform extract was concentrated *in vacuo* to a sirup. A solution of the sirup, containing the acetobromo-epi-lactose, in 100 cc. of 50% acetic acid was cooled to 0° and 20 g. of zinc dust and 2 drops of a 0.5% solution of chloroplatinic acid in 50% acetic acid were added and the reaction mixture was stirred vigorously for two hours; the zinc was removed by filtration and the filtrate was poured into 200 cc. of ice-cold water; the crystalline lactal hexaacetate (4.1 g.; 50%) which precipitated was separated by filtration and recrystallized by solution in 3 parts of warm alcohol and the addition of 7 parts of water, being deposited in the form of needles which melted at 114° (cor.) and rotated –18.0° (*c.* 0.8) in chloroform. A mixed melting point with authentic lactal hexaacetate (from acetobromolactose) showed no depression. Haworth, Hirst, Plant and Reynolds<sup>21</sup> record a melting point of 114° and a specific rotation of –18° for lactal hexaacetate.

*Anal.* Calcd. for C<sub>24</sub>H<sub>32</sub>O<sub>16</sub>: C, 51.43; H, 5.75; CH<sub>3</sub>CO, 46.1. Found: C, 51.62; H, 5.69; CH<sub>3</sub>CO, 46.2.

**Lactose Octaacetate from Lactal Hexaacetate.**—A solution of 5.0 g. of lactal hexaacetate in a mixture of 30 cc. of ethyl acetate, 5 cc. of water and 30 cc. of a 0.3335 *M* ether solution of perbenzoic acid (1.12 molecular equivalents) was agitated for twenty hours at 25°; at the expiration of this period titration of a one cubic centimeter aliquot indicated that 0.9 of a molecular equivalent of perbenzoic acid had been consumed in the oxidation. The reaction mixture was agitated with 10 cc. of water and 4.0 g. of sodium bicarbonate until the ether-ethyl acetate layer was

neutral, and the aqueous layer was separated and extracted with chloroform; the combined ether and chloroform extracts were dried and concentrated *in vacuo* to a thick sirup. The sirup, which presumably contained lactose hexaacetate, was acetylated with acetic anhydride and fused sodium acetate and yielded 3.8 g. (58%) of  $\beta$ -lactose octaacetate. The product was recrystallized from 1.5 parts of alcohol and obtained as prisms which melted at 88–90° (cor.) and showed a rotation of –4.5° (*c.* 0.8) in chloroform solution, in good agreement with the reported values<sup>22</sup> of 90° and –4.7°, respectively, for  $\beta$ -lactose octaacetate. A mixed melting point with authentic  $\beta$ -lactose octaacetate showed no depression.

*Anal.* Calcd. for C<sub>36</sub>H<sub>58</sub>O<sub>19</sub>: C, 49.56; H, 5.64; CH<sub>3</sub>CO, 50.8. Found: C, 49.60; H, 5.58; CH<sub>3</sub>CO, 50.9.

**Lactose Monohydrate from  $\beta$ -Lactose Octaacetate.**—A solution of 4.0 g. of  $\beta$ -lactose octaacetate in 100 cc. of methyl alcohol was deacetylated by barium methylate in the usual manner. The disaccharide monohydrate (2.0 g., 94%) was obtained in the form of prisms which melted with decomposition at 202° (cor.); a mixed melting point determination with authentic lactose monohydrate showed no depression; an aqueous solution of the substance exhibited initial and final rotations of +81° and +52.7° (*c.* 2.0), respectively, with a mutarotation rate of 0.0042 at 20°. Isbell and Pigman<sup>23</sup> report an initial rotation of +85.0° (*c.* 7.6), a final rotation of +52.6° and a mutarotation rate of 0.0047 for  $\alpha$ -lactose monohydrate. The over-all yield of lactose monohydrate from epi-lactose octaacetate was 27% and from acetobromo-D-galactose it was 8%.

*Anal.* Calcd. for C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>·H<sub>2</sub>O: C, 40.00; H, 6.71; H<sub>2</sub>O, 5.00. Found: C, 39.99; H, 6.80; H<sub>2</sub>O, 5.04.

### Summary

The condensation of 2,3-isopropylidene-D-mannosan <1,5> $\beta$ <1,6> with acetobromo-D-galactopyranose yields a product from which, after removal of its acetone residue followed by acetylation of the two hydroxyl groups thus liberated, there was obtained crystalline 2,3-diacetyl-4-[ $\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan <1,5> $\beta$ <1,6> in 30% yield. The action of an acid acetylating mixture upon this substance opened its 1,6-anhydro ring and produced the  $\alpha$ -octaacetate of epi-lactose, from which epi-lactose was obtained by deacetylation. Epi-lactose octaacetate was converted by customary procedures to the known lactal hexaacetate, which, upon oxidation at the double bond with perbenzoic acid, generated the new hydroxyl group mainly in a position *trans* to the acetylated hydroxyl group on carbon three, since it was found that the product, upon complete acetylation, gave a 58% yield of the crystalline  $\beta$ -octaacetate of lactose, from which lactose was obtained by de-

(22) Hudson and Johnson, *ibid.*, **37**, 1270 (1915).

(21) Haworth, Hirst, Plant and Reynolds, *J. Chem. Soc.*, 2644 (1930); see also Hudson and Watters, *THIS JOURNAL*, **52**, 3472 (1930).

(23) Isbell and Pigman, *J. Res. Natl. Bur. Standards*, **18**, 158 (1937).

acetylation. The over-all yield of lactose, based upon the acetobromo-D-galactose used in its synthesis, was 8%. The results constitute the structurally definitive syntheses of epi-lactose and lactose from D-mannose and D-galactose. They are total syntheses, since such syntheses of these hexoses were accomplished by Emil Fischer. The new

crystalline substances that are described are 2,3-diacetyl-4- $[\beta$ -2',3',4',6'-tetraacetyl-D-galactopyranosido]-D-mannosan $\langle$ 1,5 $\rangle\beta$  $\langle$ 1,6 $\rangle$  and  $\alpha$ -octaacetyl-epi-lactose; some other new substances, intermediates in the syntheses, were not isolated in crystalline form.

BETHESDA, MARYLAND

RECEIVED MAY 22, 1942

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 879]

## Isomerization of $\beta$ -Carotene. Isolation of a Stereoisomer with Increased Adsorption Affinity

BY A. POLGÁR AND L. ZECHMEISTER

In spite of the fact that the phenomenon of carotenoid isomerization was first detected for  $\beta$ -carotene,<sup>1</sup> our knowledge in this special case is still very incomplete. These authors crystallized an isomer which they termed pseudo- $\alpha$ -carotene, and which appears immediately below  $\beta$ -carotene on the Tswett column. Gillam first assumed that the chromatographic process itself was responsible for the partial conversion, but it was shown later that the phenomenon occurs spontaneously and independently of a chromatographic experiment.<sup>2</sup> This conclusion was confirmed by Carter and Gillam.<sup>3</sup>

Early experiments in our laboratory showed that the reversible isomerization, for the explanation of which *trans-cis* shifts were suggested, can either increase or decrease the adsorptive power as compared with that of the respective all-*trans* carotenoid.<sup>4</sup> It was stated<sup>5</sup> as a rule that carotenoids with at least two free hydroxyl groups yield isomers which are adsorbed at higher sections of the column than the starting pigment. This result is in accordance with observations on the hydroxy-ketones capsanthine and capsorubine<sup>6</sup> as well as with investigations of Strain<sup>7</sup> concerning leaf xanthophylls. In the case of the monohydroxy-compound cryptoxanthin and especially of the hydrocarbons C<sub>40</sub>H<sub>56</sub> the opposite behavior was observed; to this rule, however, the present paper provides an exception.

(1) A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **30**, 1935 (1936).

(2) L. Zechmeister and P. Tuzson, *ibid.*, **32**, 1305 (1938).

(3) G. P. Carter and A. E. Gillam, *ibid.*, **33**, 1325 (1939).

(4) L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939).

(5) L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678, 2039 (1939).

(6) L. Zechmeister and L. Cholnoky, *Ann.*, **543**, 248 (1940).

(7) H. H. Strain, "Leaf Xanthophylls," Carnegie Inst. Washington, No. 490, Washington (1938); *cf.* also F. W. Quackenbush, H. Steenbock and W. H. Peterson, *THIS JOURNAL*, **60**, 2937 (1938).

In systematic experiments now being carried out in our laboratory with a series of carotenoids mainly four methods of isomerization are applied, *viz.*, refluxing the pigment solution, iodine catalysis, hydrochloric acid catalysis (both at room temperature), and melting the crystals.<sup>8</sup> The first two procedures have already been described.<sup>2,4,5</sup> As a convenient method for the acid catalysis, a mechanical shaking of the petroleum ether solution with concentrated hydrochloric acid is used. The catalysis is here mainly effective at the continuously shifting intersurface of the two liquids.

It was assumed and confirmed by experiment that stereoisomers of a carotenoid which are formed and are present in the melt can be separated by rapid cooling and chromatography. Such a treatment, which is much milder than the so-called "thermal decomposition" of carotenoids,<sup>9</sup> is best carried out in a sealed tube, in the absence of oxygen. Under suitable conditions no carbonization occurs. The melting of  $\beta$ -carotene gave us four main types of products, *viz.*, unchanged starting material, reversibly formed stereoisomers of  $\beta$ -carotene, pigments with a much shorter chromophore than that of carotene, and finally colorless, strongly fluorescent substances with very low adsorption affinity. The ratio of these types depends on the conditions, especially on the temperature. A solution of a crude melt was found to show about one-half the initial color intensity, whereas "thermal decomposition" as practiced earlier leads to complete bleaching.

(8) It is intended to report on photochemical isomerization later.

(9) (a) J. F. B. van Hasselt, *Rec. trav. chim.*, **30**, 1 (1911); **33**, 192 (1914). (b) R. Kuhn and A. Winterstein, *Helv. chim. acta*, **11**, 427 (1928). *Ber.*, **65**, 1873 (1932); **66**, 429, 1733 (1933). L. Zechmeister and L. Cholnoky, *Ann.*, **478**, 95 (1930).