mates corresponds to a *cis* configuration of the two carboxyl groups relative to the lactone ring was unsuccessful.

Experimental[4-6]

Monopyridine Salt of *dl*-Alloisocitric Lactone.—Ten grams of *dl*-isocitric lactone[1b] was dissolved in 20 ml. of redistilled pyridine and 10 ml. of water and heated at 140–150° in a sealed tube for three hours. One hundred ml. of 95% ethanol was added. Upon standing and seeding, 7.00 g. of crystals, m.p. 138–140° formed. Recrystallization from 50 ml. of 95% ethanol and treatment with Norite yielded 5.42 g. of colorless crystals, m.p. 139.6–141.8°.

Anal. Calcd. for $C_{11}H_{11}O_6N$: C, 52.17; H, 4.38. Found: C, 52.59; H, 4.46.

***dl*-Alloisocitric Lactone.**—A solution of the recrystallized pyridine salt in 50 ml. of 1.5 *N* sodium hydroxide was boiled to dryness *in vacuo*. Eight ml. of 12 *N* hydrochloric acid was added and the mixture was boiled to dryness and heated for one-half hour at 100° *in vacuo* to close the lactone ring.[1b] The residue was extracted with five 20-ml. portions of hot ethyl acetate. After treatment with Norite the combined extracts were evaporated to dryness *in vacuo* leaving an oil which crystallized on scratching. After drying on a porous plate the product weighed 3.18 g. and melted at 146–155°. Recrystallization from 25 ml. of ethyl acetate and 20 ml. of 60–70° petroleum ether yielded 1.98 g. of *dl*-alloisocitric lactone, m.p. 157–158.5°. A m.p. of 135–141° was observed when this material was mixed with *dl*-isocitric lactone, m.p. 161–163°. A second crop of 0.54 g., m.p. 155–158°, was obtained from the mother liquors, a total yield of 68% from the pyridine salt.

An analytical sample crystallized from the same solvents melted at 158.2–159.4°.

Anal. Calcd. for $C_6H_6O_6$: C, 41.39; H, 3.48. Found: C, 41.08; H, 3.62.

By direct titration a neutralization equivalent of 86.8 was observed, calculated 87.1. At the end-point excess alkali was added and the solution boiled to open the lactone ring. Back titration indicated an equivalent weight of 175.3 for the lactone, calculated 174.1.

The mother liquors from the original isolation of the pyridine salt contained additional quantities of *dl*-alloisocitric acid. Lactone prepared from them resembled the impure fractions described by Pucher and Vickery[1b] although it was possible by fractional crystallization to isolate small additional quantities of *dl*-alloisocitric lactone relatively free of *dl*-isocitric lactone.

Di-(*p*-bromophenacyl) Ester of *dl*-Alloisocitric Lactone.—By the method of Pucher and Vickery[1b] 292 mg., 45%, of an ester, m.p. 163–165.5°, was prepared from 200 mg. of *dl*-alloisocitric lactone. An analytical sample from absolute ethanol melted at 166.8–167.2°.[7]

Anal. Calcd. for $C_{22}H_{16}O_8Br_2$: C, 46.50; H, 2.84. Found: C, 46.92; H, 2.91.

(4) A grant from the Research Committee, Santa Barbara College, is gratefully acknowledged.

(5) Corrected melting points were taken in an electrically heated copper block.

(6) Microanalyses for carbon and hydrogen by Elek Microanalytical Laboratories, Los Angeles, California.

(7) Pucher and Vickery prepared an ester, m.p. 153–154°, from their impure fraction containing *dl*-alloisocitric lactone. Melting points as low as this were observed in the present work when highly impure *dl*-alloisocitric lactone was used; however, in such cases the melting point could be raised to 166°.

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New Disaccharide from the Acid Reversion of D-Galactose

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Recently, considerable attention has been focused on condensation polymerization[1] and acid reversion products of sugars[2] and in the case of the acid treatment of D-glucose, the disaccharides produced have been largely characterized.[3,4] In the present study the effect of hydrochloric acid on D-galactose has been examined, and preliminary experiments indicated that optimum polymerization, as measured by fall in reducing power, occurred in a 1.0 *M* solution of D-glactose in 37% hydrochloric acid (Fig. 1). Under these conditions equilibrium was reached after 24 hr. when the reducing power had fallen to 67% of its original value and paper chromatographic examination revealed the presence of two disaccharides, a trisaccharide and a smaller proportion of higher saccharides in addition to D-galactose. The disaccharide having the lower R_f value (referred to as "disaccharide A") was present in greater amount than the other disaccharide (disaccharide B). The mixture of disaccharides was freed from D-galactose and higher saccharides by chromatography on charcoal.[5] Repeated fractionation of this mixture on charcoal progressively reduced the proportion of disaccharide B present until it could no longer be detected by paper chromatography. The pure disaccharide A was obtained as an amorphous, slightly deliquescent powder which had $[\alpha]^{20}D$ +149° (*c* 0.725, water). Attempts at crystallization failed.

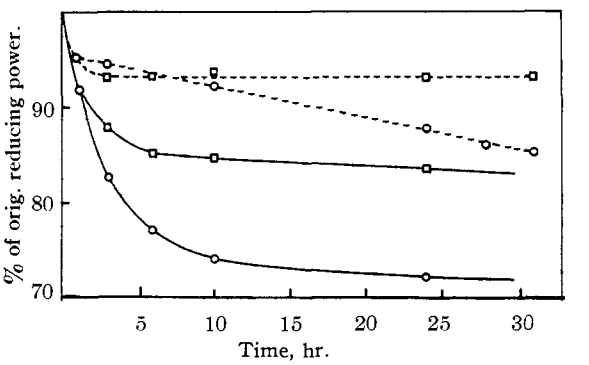

Fig. 1.—Polymerization of D-galactose by hydrochloric acid; □, 0.5 *M* D-galactose; O, 1.0 *M* D-galactose, ------ 25% hydrochloric acid and — 37% hydrochloric acid.

Disaccharide A showed reducing power and the sole product of its acid hydrolysis was D-galactose which was characterized as the phenylosazone. Methylation of the sugar followed by hydrolysis of the methylated product yielded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose which were separated by large-scale paper chromatography and characterized as their anilides. Oxidation of the biose with bromine water yielded a stable lactone which was methylated to give the octa-*O*-methyl methyl ester of the bionic acid. Hydrolysis of this ester gave 2,3,4,6-tetra-*O*-methyl-D-galactose, which was again characterized as the anilide, and a tetra-*O*-methyl acid (I) which was oxidized with nitric acid. The product of this

(1) E. Pacsu and P. T. Mora, THIS JOURNAL, **72**, 1045 (1950).

(2) W. R. Fetzer, E. K. Crosby, C. E. Engel and L. C. Kirst, *Ind. Eng. Chem.*, **45**, 1075 (1953).

(3) A. Thompson, M. L. Wolfrom and E. J. Quinn, THIS JOURNAL, **75**, 3003 (1953).

(4) A. Thompson, K. Anno, M. L. Wolfrom and M. Inatome, *ibid.*, **76**, 1309 (1954).

(5) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

oxidation was esterified to give a methyl ester which was identified as tetra-*O*-methyl dimethyl mucate, showing that the tetra-*O*-methyl acid (I) was 2,3,4,5-tetra-*O*-methyl-D-galactonic acid. When disaccharide A was treated with sodium metaperiodate, 6 moles of oxidant per mole of sugar were consumed rapidly (Fig. 2). The above data proved that disaccharide A had a 6-glycosidic linkage. That this linkage had an α-configuration was suggested by the disaccharide's high positive rotation and this was confirmed by the fact that the disaccharide was readily hydrolyzed by an α-galactosidase.[6]

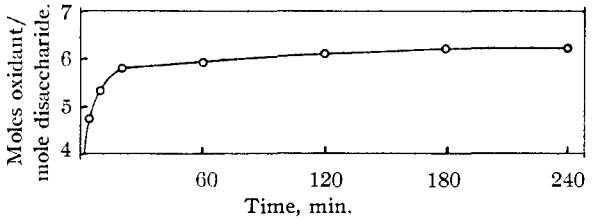

Fig. 2.—Periodate oxidation of disaccharide A.

Hence disaccharide A was shown to be 6-*O*-α-D-galactopyranosido-D-galactopyranose. It is interesting to note that the preferential formation of 6-glycosidic linkages occurred during the acid reversion of D-glucose.[3,4]

Acetylation of disaccharide A yielded only a small amount of crystalline material (10%) and it was found that the same yield of this crystalline material was obtained irrespective of the temperature of acetylation or of the nature of the catalyst (sodium acetate, zinc chloride or sulfuric acid). The high rotation of the crystalline product ($[\alpha]^{20}D$ +186°) suggested that it was an α-acetate and this view was supported by the fact that heating with acetic anhydride and zinc chloride produced no change in the rotation of the compound; the original material was recovered in 65% yield. A β-acetate would have been converted to the α-form rapidly under such conditions. The non-crystalline material which was isolated from the acetylation mixtures had $[\alpha]^{20}D$ + 111°. In contrast to the crystalline material it restored the color to the Schiff reagent and gave a positive result with the alkaline permanganate reagent used by Pacsu[7] as a test for open-chain acetates. As was expected, hydrogenation of the non-crystalline material yielded a product that responded to neither of these tests. This product was also non-crystalline.

Experimental

Isolation of Disaccharide A.—D-Galactose (45 g.) was dissolved in 37% hydrochloric acid (500 cc.) and the solution was kept at 20°. At intervals aliquot portions were withdrawn and the reducing power was determined by the method of Kline and Acree.[8] After 48 hr., when the reducing power had fallen to 67% of its initial value, the solution was diluted and neutralized with lead carbonate. The lead salts were removed by filtration at 0° and the filtrate was passed successively through two columns (25 × 7.5 cm.) packed with a mixture of charcoal (2 parts) and Celite (1 part). The first column retained the trisaccharide and the higher polymers whilst the disaccharides (6.7 g.) were adsorbed on the second column, from which they were eluted with 20% ethanol. The disaccharide mixture was fractionated on a third similar column using 3% ethanol as eluant. Three further fractionations yielded disaccharide A in a pure form.

Acid Hydrolysis of Disaccharide A.—The disaccharide (50 mg.) was treated with *N* hydrochloric acid (5 cc.) at 100° for 45 min. The solution was neutralized with 2 *N* sodium hydroxide and the sugar was converted to the phenylosazone, which was recrystallized from aqueous ethanol. It had m.p. 183–185° (undepressed by admixture with authentic D-galactose phenylosazone).

Methylation of Disaccharide A.—Disaccharide A (1.0 g.) in water (1.0 cc.) was treated with dimethyl sulfate (0.7 cc.) at 30–35°. The solution was kept just alkaline to phenolphthalein by the addition of 20% sodium hydroxide. After two such methylations the solution showed no reducing power and was methylated four further times at 40°. Two more methylations were carried out at 50° and from the reaction mixture a sirup (0.6 g.) was extracted with chloroform. The residual aqueous solution again was methylated four times and the material extractable with chloroform was combined with that previously obtained to give a total of 0.87 g. of sirup. The residual reaction solution was treated with sodium hydroxide (3.0 g.) at 100° for 30 min., cooled and diluted by an equal volume of ethanol. The mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The solid was extracted with ethanol and the extracted material was methylated further. In all a total of 1.1 g. of chloroform-soluble material was obtained. This sirup was thoroughly dried and treated with methyl iodide (8.0 cc.) and silver oxide (1.0 g.). A second methylation with these reagents was required to bring the methoxyl content of the sirup up to 50.3 (calcd. for fully methylated derivative 54.6). The final yield of sirup was 0.79 g.

Hydrolysis of Methylated Disaccharide A and Identification of Products.—Methylated disaccharide A (0.78 g.) was treated with 5% hydrochloric acid (40 cc.) at 100° until there was no further change in rotation (8 hr.). The solution was neutralized with barium carbonate and evaporated to dryness under reduced pressure. The residue was extracted with boiling ether and the extracts were evaporated to give a clear sirup (0.46 g.), which was dissolved in water (4.4 cc.). The solution was put onto paper chromatograms so that a chromatogram 23 cm. wide (Whatman No. 1 paper) carried 20 mg. of methylated sugar. The chromatograms were irrigated with a butanol–ethanol–water–ammonia mixture[9] for 18 hr. and then dried. The separated methylated sugars were located and the paper strips bearing them were cut out and eluted with water. Evaporation of the aqueous solutions under reduced pressure yielded a sample of tetra-*O*-methyl-D-galactose (225 mg.) and one of tri-*O*-methyl-D-galactose (180 mg.). The tetra-*O*-methyl-D-galactose was converted to the anilide[10] which recrystallized from ethyl acetate as pure white needles (92 mg.) with m.p. 195–195.5° (undepressed by admixture with 2,3,4,6-tetra-*O*-methyl-D-galactose anilide). The anilide of the tri-*O*-methyl-D-galactose was prepared likewise and recrystallized from ethyl acetate to give a pure sample (86 mg.) which showed m.p. 167.5–168.5° (undepressed by admixture with 2,3,4-tri-*O*-methyl-D-galactose anilide).

Oxidation of Disaccharide A.—The disaccharide (2.0 g.) was dissolved in water (15 cc.) and stirred with an excess of bromine (1.2 cc.) and lead carbonate (3.6 g.) for seven days. The calcium salt of the bionic acid was isolated according to the method of Glattfeld and Hanke[11] and the free acid was liberated by treatment with oxalic acid.

Methylation of the Bionic Acid.—A sample of the acid (2.0 g.) in water (1.0 cc.) was treated with dimethyl sulfate (2.7 cc.) and a solution of sodium hydroxide (2.55 g.) in water (5.4 cc.) over a period of 2.5 hr. During the methylation the temperature was raised slowly from 50 to 70 and finally to 100°. Sodium sulfate was removed by precipitation with ethanol and acetone at *p*H 3 and after distilling off the organic solvents, the partly methylated product

(6) M. Adams, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 1369 (1943).

(7) E. Pacsu, *ibid.*, **54**, 3649 (1932).

(8) G. M. Kline and S. F. Acree, *Bur. Standards, J. Research*, **5**, 1063 (1930).

(9) S. M. Partridge, *Nature*, **158**, 270 (1946).

(10) W. N. Haworth, J. V. Loach and C. W. Long, *J. Chem. Soc.*, 3146 (1927).

(11) J. W. E. Glattfeld and M. T. Hanke, THIS JOURNAL, **40**, 973 (1918).

was extracted from the reaction solution with chloroform. The material remaining in the aqueous solution was treated as above seven times in all when the total material extracted by chloroform weighed 850 mg. This sirup was methylated with methyl iodide and silver oxide four times when the product (800 mg.) had a methoxyl content of 55.9 (calcd. for octa-*O*-methyl methyl bionic ester "OMe" 57.6).

Hydrolysis of Methylated Bionic Acid and Identification of Products.—Disaccharide ester (750 mg.) was hydrolyzed with 7% hydrochloric acid (20 cc.) for 3 hr. at 80° during which time the rotation had fallen from $[\alpha]^{20}D$ +86° to a constant value of +54°. The hydrolysate was neutralized with barium carbonate, adjusted to *p*H 7.5 with barium hydroxide and evaporated to dryness. The solid was carefully dried and extracted with anhydrous ether to collect the tetra-*O*-methyl-D-galactose. The barium salt of the tetra-*O*-methyl-D-galactonic acid was extracted from the residue with chloroform.[12]

The ether extracts were combined and evaporated to dryness. The residual sirup (311 mg.) was converted to the anilide which was recrystallized from ethyl acetate to give pure crystals (183 mg.) with m.p. 195–196° (undepressed by admixture with 2,3,4,6-tetra-*O*-methyl-D-galactose anilide).

The chloroform extract was evaporated to a sirup (325 mg.) which was dissolved in concentrated nitric acid (2.5 cc.). The solution was heated slowly to 100° on a water-bath and maintained at that temperature for 5 hr. Water was added and the solution was distilled under reduced pressure. Distillation and continuous addition of water were carried on until all but a slight trace of nitric acid had been removed. A little methanol was added and the solution was concentrated to a sirup (259 mg.) which was dried, dissolved in dry methanol and neutralized with silver carbonate. Excess methyl iodide (0.2 cc.) was added and after the solution had stood for 1 hr. it was filtered and the solid was washed with methanol. The washings and filtrate were combined and evaporated to a sirup which crystallized. The product was recrystallized from an acetone, ether and benzine mixture to give colorless crystals (156 mg.) which had m.p. 111–112° (undepressed by admixture with authentic 2,3,4,5-tetra-*O*-methyl dimethyl mucate).

2,3,4,5-Tetra-*O*-methyl Dimethyl Mucate.—The direct methylation of mucic acid with dimethyl sulfate and sodium hydroxide[13] was found to be unsatisfactory due to the insolubility of the sodium salt of mucic acid. A more indirect approach was adopted using 2,3,4-tri-*O*-methyl-D-galactose which had been prepared as a reference compound by the method of Smith.[14]

The 2,3,4-tri-*O*-methyl-D-galactose was oxidized to tri-*O*-methylmucic acid which was esterified with methanol as described by Smith.[14] The 2,3,4-tri-*O*-methyl dimethyl mucate (2.0 g., m.p. 101.5–102.5°) was dissolved in methyl iodide (20 cc.) and silver oxide (10 g.) was added. After standing for 24 hr. the mixture was filtered, the solid was washed with methyl iodide and the combined filtrate and washings were evaporated to a sirup. The sirup was methylated again as above. The product was distilled in high vacuum when the first fraction distilling over crystallized. The crystalline solid was sucked free of sirup and recrystallized from acetone to give a product (1.1 g.) with m.p. 111.5–112.5° (depressed to 89–94° by mixing with 2,3,4-tri-*O*-methyl dimethyl mucate) and "OMe" 62.6, calcd. for $C_{12}H_{22}O_8$ 63.3. *Anal.* Calcd. for $C_{12}H_{22}O_8$: C, 48.90; H, 7.52. Found: C, 49.07; H, 7.61.

Karrer and Peyer[13] reported m.p. 103° for the product obtained from the direct methylation of mucic acid.

Periodate Oxidation of Disaccharide A.—A dry sample of disaccharide A (98.7 mg.) was dissolved in water and the solution was diluted to exactly 50 cc. Aliquot portions (5 cc.) were taken and treated with 0.2 *M* periodic acid adjusted to *p*H 4.5–5.0 with sodium hydroxide. After suitable intervals the residual periodate was determined using 0.10 *N* sodium arsenite. "Blanks" were run simultaneously. Six moles of periodate per mole of disaccharide were used up rapidly (Fig. 2).

Hydrolysis of Disaccharide A by an α-Galactosidase.[4]—A 0.8% solution of disaccharide A (10 cc.) in acetate buffer of *p*H 5.0 was treated with a highly active solution (0.1 cc.) of an invertase preparation called "Brewer's A."[4,15] The hydrolysis of the disaccharide at 20° was followed by determining the change in the rotation of the solution with a polarimeter. The sugar solution was allowed to mutarotate to a constant rotation before treatment with the enzyme solution. As references the hydrolyses of melibiose and lactose were carried out under the same conditions (Fig. 3).

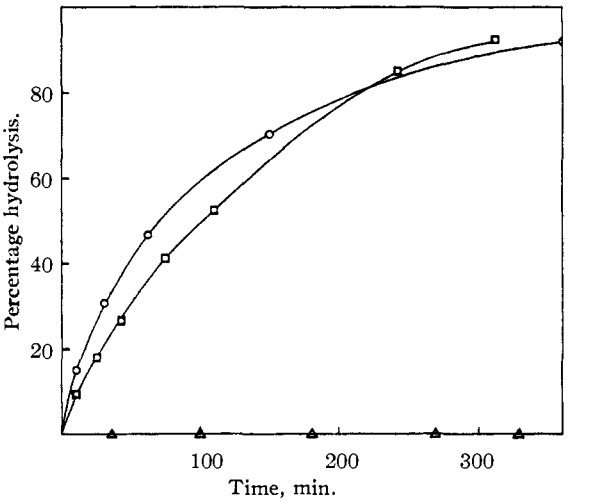

Fig. 3.—Hydrolysis of disaccharide A with an α-galactosidase: O, disaccharide A; □, melibiose; Δ, lactose.

Acetylation of Disaccharide A.—Anhydrous sodium acetate (50 mg.), disaccharide A (100 mg.) and acetic anhydride (1.0 cc.) were heated together at 100° for 30 min. The product was isolated in the usual manner and crystals which had m.p. 223–227° and $[\alpha]^{20}D$ +186° (*c*, 0.5 chloroform) were obtained in 10% yield from ethanol solution. An amorphous material was obtained from the mother liquors in 80% yield and it had $[\alpha]^{20}D$ +111° (*c* 0.5, chloroform). The same products in approximately the same yields were obtained when the temperature of the above acetylation was increased and also when standard acetylation procedures using zinc chloride or sulfuric acid as catalysts were employed.

Anal. Calcd. for $C_{28}H_{38}O_{19}$ (crystalline octaacetate): C, 49.51; H, 5.65. Found: C, 49.46; H, 5.64.

Hydrogenation of the Aldehydoacetate.—A sample of the amorphous material from the previous experiment (150 mg.) was dissolved in glacial acetic acid (10 cc.) and shaken with hydrogen at 4 atmospheres pressure for 24 hr. (platinum oxide catalyst, 50 mg.). The sirupy material obtained was non-reducing to Fehling solution and gave a negative Schiff test.

Acknowledgment.—The authors wish to express their appreciation to E. I. du Pont de Nemours and Co., Wilmington, Del., for a research grant that made this investigation possible.

(12) *Cf.* W. N. Haworth and S. Peat, *J. Chem. Soc.*, 3094 (1922).

(13) P. Karrer and J. Peyer, *Helv. Chim. Acta*, **5**, 577 (1922).

(14) F. Smith, *J. Chem. Soc.*, 1724 (1939).

(15) The authors are indebted to Dr. N. K. Richtmyer of the National Institutes of Health, Bethesda, Md., for the gift of a sample of this enzyme solution.

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Bisdehydro-carotenes

BY L. ZECHMEISTER AND F. J. PETRACEK

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Two years ago a deeply colored dehydrogenation product of β-carotene, "bisdehydro-β-carotene," $C_{40}H_{52}$, was prepared in our laboratory,[1] and the

(1) L. Zechmeister and L. Wallcave, THIS JOURNAL, **75**, 4493 (1953).