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.[15]—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."[14,15] 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).

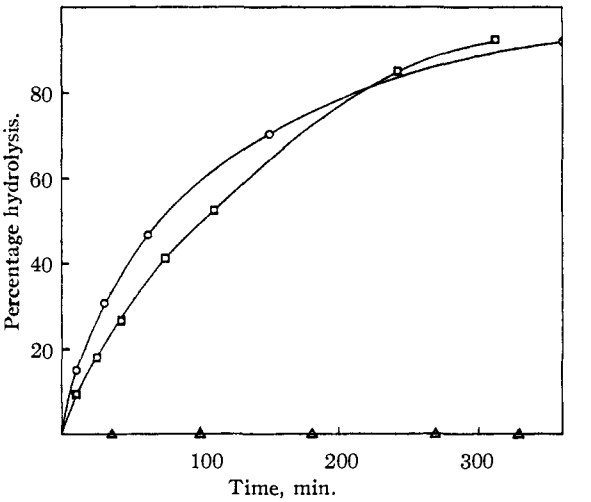

Fig. 3.—Hydrolysis of disaccharide A with an α-galactosidase: O, disaccharide A; □, melibiose; △, lactose.

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.

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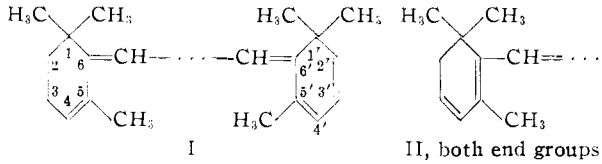
Two years ago a deeply colored dehydrogenation product of β-carotene, "bisdehydro-β-carotene," $C_{40}H_{52}$, was prepared in our laboratory,[1] and the

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

structural possibilities for it were narrowed down to a symmetrical (normal) II and a non-symmetrical (retro) formula I. Since recently, Inhoffen and Raspé[2] have obtained II, *i.e.*, 3,4,3′,4′-bisdehydro-β-carotene by total synthesis and have found it different from our "bisdehydro-β-carotene," the structure I should now be adopted for the latter.



I II, both end groups

It seems to be of some theoretical interest that, in contrast to II, compound I, now termed, retro-bisdehydro-carotene, shows the following characteristics: the retro structure increases the adsorbability; it involves extensive fine structure in the main spectral band; and upon iodine catalysis it produces a stereoisomeric mixture that shows no marked *cis* peak, although *cis* forms are preponderant in it.

Inhoffen's synthetic compound mentioned has, however, now been identified with a minor product of the dehydrogenation of either α- or β-carotene,[3] *viz.*, "dehydrocarotene III" ($E^{mol}_{1\,cm.}$ 12.6 × 10^4 at λ_{max} 471 mμ, in hexane). Hence, both I and II may result from a direct dehydrogenating attack on carotenes.

(2) H. H. Inhoffen and G. Raspé, *Ann.* (in press; communicated to us by Prof. Inhoffen who also kindly sent us a sample of his synthetic preparation).

(3) G. Karmakar and L. Zechmeister, THIS JOURNAL, **77**, 55 (1955).

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Synthesis[1] of L-Iduronic Acid and an Improved Production[1] of D-Glucose-6-C^{14}

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In the synthesis of D-glucose-6-C^{14} described by Sowden,[2] the main step is the introduction of the radioactive label by condensation of 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose with C^{14}-labeled sodium cyanide followed by the isolation of 1,2-*O*-isopropylidene-D-glucofuranuronic-6-C^{14} acid from the reaction mixture by hydrolysis of the cyanohydrins, decationization with exchange resin and extraction from the aqueous solution with ethyl acetate. We experienced some difficulty in isolating the above product in this manner and found it desirable to reinvestigate the reaction.

Preparation of 1,2-*O*-isopropylidene-D-glucofuranuronic acid is complicated by the possible impurities of the sirupy starting material, the decomposition of the products during hydrolysis, sensitivity of the isopropylidene group to acid hydrolysis, and finally by the formation of the two epimeric *O*-isopropylidene derivatives of D-glucuronic acid and L-iduronic acid as well as their lactones. We failed to crystallize 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose, but modified its purification. The alkaline hydrolysis of the cyanohydrins was conducted at a low temperature according to the general conditions outlined by Isbell and co-workers,[3] and the use of an ion exchange resin for generating the free acids was abandoned in favor of the method described by Mehltretter and associates[4] which is simpler and enables the extraction of the products from a more concentrated solution. 1,2-*O*-Isopropylidene-D-glucofuranuronic acid readily crystallizes from the evaporated extract, which also contains 1,2-*O*-isopropylidene-L-idurono-γ-lactone and some 1,2-*O*-isopropylidene-D-glucurono-γ-lactone; but the purification of the latter by recrystallization is a wasteful process and efficient recovery of the remaining material in the mother liquors as D-glucurono-γ-lactone-6-C^{14}, according to the method of Sowden,[2] requires isotopic dilution. To avoid this it was found possible to by-pass the isolation of 1,2-*O*-isopropylidene-D-glucofuranuronic acid by converting the mixture of the epimeric products to the corresponding lactones, which could be separated by chromatography on clay according to the general technique of Lew, Wolfrom and Goepp.[5] In this way a clear-cut separation of the two products was achieved without isotopic dilution and the resulting pure 1,2-*O*-isopropylidene-D-glucofuranurono-γ-lactone-6-C^{14} was converted readily to D-glucose-6-C^{14} by reduction with lithium aluminum hydride according to Roseman.[6]

The identity of the second product as 1,2-*O*-isopropylidene-L-idofuranurono-γ-lactone was proved by the reduction of this compound to 1,2-*O*-isopropylidene-L-idofuranose, the constants of which were in good agreement with those recorded by Meyer and Reichstein,[7] who prepared this substance by a different method. Mild acid hydrolysis of the isopropylidene group yielded crystalline L-iduronic acid. To our knowledge, this is the first recorded uronic acid in the idose series.

Isbell and co-workers[3] have shown that the proportion of the two epimeric aldonic acids formed in the cyanohydrin synthesis involving the glycosidic group depends in part on the conditions under which the reaction takes place. This is also true for the formation of D-glucuronic and L-iduronic acids, since in the presence of sodium hydrogen carbonate and excess carbon dioxide a higher proportion of the L-iduronic derivative could be isolated, while the presence of sodium carbonate in the reaction mixture reversed the proportion. However, in the latter case, the over-all yield of the epimeric products was found to be lower.

These variations readily provide an improved yield of 1,2-*O*-isopropylidene-D-glucofuranurono-γ-lactone-6-C^{14} as well as the corresponding L-idose

(1) This work was carried out under contract (DA-33-019-ord-1476; supervising agency, Ballistic Research Laboratories, Aberdeen Proving Ground, Maryland) between the Ordnance Corps and The Ohio State University Research Foundation (Project 591).

(2) J. C. Sowden, THIS JOURNAL, **74**, 4377 (1952).

(3) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952).

(4) C. L. Mehltretter, B. H. Alexander, R. L. Mellies and C. E. Rist, THIS JOURNAL, **73**, 2424 (1951).

(5) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *ibid.*, **68**, 1149 (1946).

(6) S. Roseman, *ibid.*, **74**, 4467 (1952).

(7) A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).