

models of known structure, showed that these three methods each measured only one sugar, and interference produced by equimolar amounts of the others was less than 10%. The Harding and MacLean modification of the ninhydrin reaction was used²⁷; this measures amino nitrogen alpha to carboxyl or carbonyl but not other amines or ammonia.

The hydroxamic acid reaction, under conditions previously described, *et. al.*²⁸ gave low (30–40%) and variable color yields with sugar esters; as modified here (decreased pH, increased hydroxylamine concentration) it gave molar yields for model sugar esters and a lactone²⁹ which

(27) V. J. Harding and R. M. MacLean, *J. Biol. Chem.*, **24**, 503 (1916).

(28) S. Hestrin, *ibid.*, **180**, 249 (1949).

(29) We wish to thank Dr. Harold Markowitz for a specimen of α -methylgalacturonide methyl ester, used as a standard, and Mr. Eugene

were concordant within 10% and were about 90% those of ethyl acetate or acetylcholine. In the modified procedure, 0.20 ml. of solution containing 0.3–3 μ eq. of ester was mixed with 0.4 ml. of reagent freshly prepared from equal volumes of 8.0 *M* hydroxylamine hydrochloride solution and 1.0 *M* glycine in 8.5 *M* sodium hydroxide solution. After 2–3 hr. at room temperature, 2.50 ml. of 1.00 *M* hydrochloric acid and 6.0 ml. of 0.1 *M* ferric chloride in 0.01 *M* hydrochloric acid were added, and the optical density at 540 *m* μ was measured at once.

Davidson for preparation of methyl *D*-galacturonate and chondrosin methyl ester hydrochloride, as well as performance of some of the colorimetry. Other models were *D*-glucuronolactone, methyl α -*D*-glucuronate tetraacetate and β -*D*-glucose pentaacetate.

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The Acetolysis of Some Carbohydrate Benzyl Ethers

BY R. ALLERTON¹ AND HEWITT G. FLETCHER, JR.

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The acetolysis of the benzyl ethers of 1,3:4,6-di-*O*-methylene-*D*-mannitol, 2,4:3,5-di-*O*-methylene-*L*-iditol, 1,6-anhydro- β -*D*-glucopyranose and 1,4:3,6-dianhydro-*D*-mannitol has been studied in mixtures of acetic acid, acetic anhydride and sulfuric acid. In each case the benzyl group was readily cleaved. The results appear to indicate that primary-secondary methylene groups are cleaved more readily than secondary benzyl groups while secondary-secondary methylene groups are more stable toward acetolysis than primary benzyl groups. The acetolysis of 1,4:3,6-dianhydro-2,5-di-*O*-benzyl-*D*-mannitol is a slow and complex reaction which gave an amorphous sulfur-bearing material. Treatment of this latter with lithium aluminum hydride afforded 1,4-anhydro-*D*-mannitol in low yield.

Burton and Praill² have recently shown that a variety of aryl benzyl ethers may be acetolyzed with acetic anhydride in the presence of perchloric or sulfuric acid and, since the benzyloxy group is widely used in the synthesis of carbohydrate derivatives, it becomes of interest to enquire, first, whether the benzyloxy group in carbohydrate derivatives may be removed by acetolysis and, second, if this proves to be true, how readily such a removal takes place in comparison with some other substituents which are frequently cleaved from carbohydrate moieties by acetolysis.

Although more stable than their isopropylidene and benzylidene analogs methylene bridges between primary and secondary carbons are known to acetolyze with ease³ and therefore the first substance tested was 2,5-di-*O*-dibenzyl-1,3:4,6-di-*O*-methylene-*D*-mannitol, a substance which could be obtained in pure but amorphous form from the readily accessible, crystalline 1,3:4,6-di-*O*-methylene-*D*-mannitol.^{4,5} When this benzyl ether was dissolved in a mixture of acetic anhydride and glacial acetic acid containing about 0.09% (v./v.) of sulfuric acid, a very rapid dextrorotation took place followed by a slower levomutarotation. When the reaction was halted at the inflection point and the product deacetylated with methanolic hydrogen chloride,⁶ a crystalline di-*O*-benzyl-

hexitol was obtained in 18% yield. Its structure as 2,5-di-*O*-benzyl-*D*-mannitol was confirmed by the fact that it consumed but one mole of periodate. The same substance was obtained in 11% yield by the action of hydrochloric acid in aqueous ethanol on 2,5-di-*O*-benzyl-1,3:4,6-di-*O*-methylene-*D*-mannitol. It was characterized further through its tetra-*p*-nitrobenzoate and tetratosylate.

When 2,5-di-*O*-benzyl-1,3:4,6-di-*O*-methylene-*D*-mannitol was dissolved in an acetolysis mixture containing more sulfuric acid (2.4% v./v.) and the reaction allowed to go to completion, the product, after deacetylation with methanolic hydrogen chloride, was *D*-mannitol, the benzyl groups as well as the methylene groups having been cleaved. It is apparent, then, that in this particular substance the primary-secondary methylene bridges are cleaved with somewhat greater ease than the benzyl groups under these conditions.

Secondary-secondary methylene bridges are much more resistant to acetolysis than are primary-secondary ones.⁷ How does the acetolysis of a benzyl group compare with that of a secondary-secondary methylene group? To answer this

(1) Chemical Foundation Fellow, 1952–1953.

(2) H. Burton and P. F. G. Praill, *J. Chem. Soc.*, 522 (1951).

(3) A. T. Ness, R. M. Hann and C. S. Hudson, *THIS JOURNAL*, **65**, 2215 (1943).

(4) W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **65**, 67 (1943).

(5) H. G. Fletcher, Jr., and H. W. Diehl, *ibid.*, **74**, 3797 (1952).

(6) It is presumed that the initial product here is 3,4-di-*O*-acetoxymethyl-1,6-di-*O*-acetyl-2,5-di-*O*-benzyl-*D*-mannitol. Fletcher and Diehl (ref. 5) found methanolic hydrogen chloride a useful reagent for the removal of acetyl and acetoxyethyl groups.

(7) The acetolysis of secondary-secondary methylene groups is not unknown. R. M. Hann, J. K. Wolfe and C. S. Hudson [*THIS JOURNAL*, **66**, 1898 (1944)] found that the 3,5-methylene group (but not the 2,4-methylene group) of 2,4:3,5-di-*O*-methylene-*D*-glucitol could be acetolyzed, scission taking place at position 5. It should be remembered, however, that in contrast to the usual stability of secondary-secondary methylene groups to acetolysis, they may, in some cases at least, be removed by hydrolysis. M. L. Wolfson, B. W. Lew and R. M. Goepf, Jr. [*ibid.*, **68**, 1443 (1946)] were able to effect the complete hydrolysis of 1,3:2,4:5,6-tri-*O*-methylene-*D*-glucitol and other methylated glycolols by heating in aqueous acid with phloroglucinol as a formaldehyde acceptor. The acetolysis of 1,3:2,5,4,6-tri-*O*-methylene-*D*-mannitol which contains the rare *C*₆O₂ dioxepane ring produces 2,5-*O*-methylene-*D*-mannitol while hot aqueous hydrochloric acid gives 1,3:4,6-di-*O*-methylene-*D*-mannitol as well as *D*-mannitol itself (ref. 5).

question, 2,4:3,5-di-*O*-methylene-L-iditol, a readily accessible substance whose structure has been well demonstrated,^{8,9,10} was converted to its crystalline 1,6-dibenzyl derivative and the acetolysis of this latter studied. In an acetolysis mixture containing 2% (v./v.) of sulfuric acid mutarotation slackened markedly after 12 minutes at 20° and 1,6-di-*O*-acetyl-2,4:3,5-di-*O*-methylene-L-iditol⁸ was isolated in 32% yield. In this substance, therefore the benzyl groups are acetylated more rapidly than the methylene groups. It should be noted, however, that these benzyl groups are attached to primary carbons while those in the first example tested were linked to secondary ones.

In one experiment the acetolysis of 1,6-di-*O*-benzyl-2,4:3,5-di-*O*-methylene-L-iditol was carried out with 2% sulfuric acid at 20° for 74 hours and benzyl acetate was isolated by distillation *in vacuo*. The reaction mixture also yielded L-iditol hexaacetate, showing that prolonged acetolysis may even remove these relatively stable secondary-secondary methylene groups.

The acetolysis of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucose to an equilibrium mixture of the anomeric D-glucopyranose pentaacetates was first reported by Freudenberg and Soff¹¹ and the general nature of the reaction has since been demonstrated by the acetolysis of various other acetylated glycosans. Zemplén, Csürös and Angyal¹² have described the acetolysis of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucose which, under relatively mild conditions (0.22% sulfuric acid, three minutes), gave a mixture of the anomeric forms of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucose in which the α-form predominated. It is evident, therefore, that scission of the 1,6-anhydro ring proceeds more rapidly than acetolysis of the benzyl groups. That the latter may also be cleaved under more drastic conditions has now been demonstrated. An acetolysis mixture containing 2% sulfuric acid was used and after mutarotation had ceased (18 hours) α-D-glucopyranose pentaacetate was isolated in 29% yield.

As a further test substance having secondary benzyl groups, the dibenzyl ether of 1,4:3,6-dianhydro-D-mannitol ("isomannide"^{13,14}), a superbly crystalline substance, was prepared. The mutarotation of this compound in an acetolysis mixture containing 2% sulfuric acid proved to be complex and at 20° was completed only after 84 hours. The product was a neutral, chloroform-soluble sulfur compound which could not be induced to crystallize. Desulfurization with lithium aluminum hydride afforded 1,4-anhydro-D-mannitol in small yield, demonstrating that the acetolysis mixture had removed the benzyl groups and cleaved at least one of the tetrahydrofuran rings in the original ether.¹⁵

The identity of the 1,4-anhydro-D-mannitol which was obtained in the above sequence of reactions was confirmed by conversion to its tetrabenzoate, a crystalline substance which proved to be identical with the tetrabenzoate prepared from 1,4-anhydro-2,6(or 3,6)-di-*O*-benzoyl-D-mannitol.¹³

The preparation of 1,4:3,6-dianhydro-2,5-di-*O*-benzyl-D-glucitol as a distillable sirup is also described although its acetolysis was not studied.

Experimental¹⁶

2,5-Di-*O*-benzyl-1,3:4,6-di-*O*-methylene-D-mannitol.—

A suspension of 1,3:4,6-di-*O*-methylene-D-mannitol^{4,5} (67.6 g.) and finely powdered potassium hydroxide (73.5 g., 4 moles) in benzyl chloride (377 ml., 10 moles) was stirred and heated under anhydrous conditions at 130–140° for 3 hours. After cooling, the mixture was diluted with 200 ml. of water and 300 ml. of chloroform and then shaken thoroughly. The aqueous layer was extracted with more chloroform and the combined chloroform extracts were washed with water. After moisture was removed with sodium sulfate the solution was concentrated *in vacuo* at 100° and the residue fractionally distilled. The first fraction (b.p. 125–200° (0.05 mm.), 24.8 g.) consisted largely of benzyl alcohol; the second fraction was obtained at 234–250° and 0.05 mm. and consisted of the pure dibenzyl derivative (89.4 g., 74%). Attempts to obtain the clear, colorless sirup in crystalline form failed. It showed n_D^{20} 1.5446 and rotated –64.1° in absolute ethanol (c 0.82) and –54.9° in chloroform (c 1.57).

Anal. Calcd. for $C_{22}H_{26}O_6$: C, 68.37; H, 6.78. Found: C, 68.23; H, 6.88.

2,5-Di-*O*-benzyl-D-mannitol (a) by Acidic Hydrolysis of

2,5-Di-*O*-benzyl-1,3:4,6-di-*O*-methylene-D-mannitol.—A solution of 2,5-di-*O*-benzyl-1,3:4,6-di-*O*-methylene-D-mannitol (1.42 g.) in a mixture of ethanol (40 ml.), water (6 ml.) and concentrated hydrochloric acid (4 ml.) was heated in a sealed tube at 100° for 9 hours. After cooling, the reaction mixture was poured on ice (*ca.* 300 g.) and then extracted with chloroform. The combined chloroform extracts were washed successively with aqueous sodium bicarbonate and water, dried over sodium sulfate and concentrated to a crystalline residue. Two recrystallizations from a small quantity of absolute ethanol afforded small, colorless needles (155 mg., 11%) melting at 119–120° and rotating –7° in absolute ethanol (c 0.63).

Anal. Calcd. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.37; H, 7.12.

A sample of the 2,5-di-*O*-benzyl-D-mannitol was treated with sodium metaperiodate in aqueous solution and found to consume 1.045 molar equivalents of oxidant.

(b) By Partial Acetolysis of 2,5-Di-*O*-benzyl-1,3:4,6-di-*O*-methylene-D-mannitol.—2,5-Di-*O*-benzyl-1,3:4,6-di-*O*-methylene-D-mannitol (7.08 g.) was dissolved in a mixture of 15 ml. of glacial acetic acid and 35 ml. of acetic anhydride. The solution was cooled to 0°, concentrated sulfuric acid (1 drop, *ca.* 0.05 ml.) added, and the well-mixed solution observed in a 1-dm. tube at 20°. From an initial value of –8.76° the observed rotation rose in 50 min. to +4.26° and remained constant at this value for 2 min. The mixture was then quickly poured into 600 ml. of ice-water and, after being agitated for 1 hour, was extracted with chloroform. The combined chloroform extracts were washed with aqueous sodium bicarbonate and water, dried with sodium sulfate and concentrated *in vacuo* to a mobile sirup (9.8 g.).

ever, Raney nickel, which has been found to cleave sulfonic esters [G. W. Kenner and M. A. Murray, *J. Chem. Soc.*, S178 (1949)], did not remove sulfur from this sirup. Lithium aluminum hydride also cleaves sulfonic esters [H. Schmidt and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949)] and does not attack benzyloxy groups [K. E. Hamlin and F. E. Fischer, *THIS JOURNAL*, **73**, 5007 (1951); M. E. Speeter, R. O. Heinzelmann and D. I. Weisblat, *ibid.*, **73**, 5514 (1951)]. This latter fact as well as the fact that the tetrahydrofuran rings in 1,4:3,6-dianhydro-2,5-di-*O*-benzyl-D-mannitol are stable to lithium aluminum hydride was confirmed by a "blank" reduction. After treatment with an excess of lithium aluminum hydride in boiling tetrahydrofuran for six hours the dibenzyl ether was recovered in 82% yield.

(16) Melting points are corrected. Rotations are specific rotations for the D line of sodium at 20° unless otherwise specified.

- (8) R. M. Hann and C. S. Hudson, *THIS JOURNAL*, **67**, 602 (1945).
- (9) W. G. M. Jones and L. F. Wiggins, *J. Chem. Soc.*, 364 (1944).
- (10) H. G. Fletcher, Jr., and H. W. Diehl, *THIS JOURNAL*, **74**, 3799 (1952).
- (11) K. Freudenberg and K. Soff, *Ber.*, **69**, 1245 (1936).
- (12) G. Zemplén, Z. Csürös and S. Angyal, *ibid.*, **70**, 1848 (1937).
- (13) R. C. Hockett, H. G. Fletcher, Jr., E. L. Sheffield, R. M. Goepf, Jr., and S. Soltzberg, *THIS JOURNAL*, **68**, 930 (1946).
- (14) H. G. Fletcher, Jr., and R. M. Goepf, Jr., *ibid.*, **68**, 939 (1946).
- (15) Presumably the sulfur compound was a neutral sulfate. How-

Deacetylation was accomplished by solution in 50 ml. of 1.109 *N* methanolic hydrogen chloride. When mutarotation had ceased (25 hr. at 20°) the reaction mixture was poured into 50 ml. of water and the product extracted with chloroform. The combined chloroform extracts, washed with aqueous sodium bicarbonate and water, and dried over sodium sulfate, were concentrated *in vacuo* to give a crystalline residue. Recrystallized from chloroform the 2,5-di-*O*-benzyl-*D*-mannitol (1.17 g., 18%) melted at 117–119°. Mixed with the product prepared in (a) above it melted at 116–119°.

2,5-Di-*O*-benzyl-1,3,4,6-tetra-*O*-*p*-nitrobenzoyl-*D*-mannitol.—2,5-Di-*O*-benzyl-*D*-mannitol was treated with *p*-nitrobenzoyl chloride in pyridine to give a yellow solid which, recrystallized from absolute ethanol, melted at 75–79° and showed a rotation of +104° in chloroform (*c* 1.19).

Anal. Calcd. for $C_{48}H_{38}O_{18}N_4$: C, 60.12; H, 3.99; N, 5.84. Found: C, 60.06; H, 4.08; N, 5.81.

2,5-Di-*O*-benzyl-1,3,4,6-tetra-*O*-*p*-tolylsulfonyl-*D*-mannitol.—2,5-Di-*O*-benzyl-*D*-mannitol was tosylated in the usual fashion to give a product which, after recrystallization from absolute ethanol, melted at 157–159° and showed a rotation of +40.1° in chloroform (*c* 0.34).

Anal. Calcd. for $C_{48}H_{50}O_{14}S_4$: C, 58.88; H, 5.15; S, 13.10. Found: C, 58.62; H, 5.29; S, 13.06.

Complete Acetolysis of 2,5-Di-*O*-benzyl-1,3,4,6-di-*O*-methylene-*D*-mannitol.—The *D*-mannitol derivative (897 mg.) was dissolved in 7.5 ml. of glacial acetic acid and the cooled solution diluted to 25.0 ml. with a cooled mixture prepared from 1 ml. of concentrated sulfuric acid and 34 ml. of acetic anhydride. The rotation of the resulting solution was observed at 20° in a 4-dm. tube. A very rapid change in the positive direction was followed by a slow change in the negative one. After two hours the observed rotation became constant at +4.00° and the reaction mixture was then poured into ice-water (200 ml.) and the solution kept stirred at 0° for 45 min. to decompose the excess acetic anhydride. The product was extracted with chloroform and the combined extracts were washed with aqueous sodium bicarbonate and water and then dried over sodium sulfate. Removal of the solvent *in vacuo* at 40° yielded a mobile sirup, presumably crude 3,4-di-*O*-acetoxymethyl-1,2,5,6-tetra-*O*-acetyl-*D*-mannitol since it liberated formaldehyde when treated with methanolic hydrogen chloride. Deacetylation was carried out by solution in 25 ml. of 0.55 *N* methanolic hydrogen chloride. At 20° the rotation of the mixture fell to 0° after 5.5 hours. After removal of acid and methanol there was obtained, from absolute ethanol, 150 mg. (35%) of *D*-mannitol melting at 165°. Mixed with authentic *D*-mannitol it melted at 165–166°.

1,6-Di-*O*-benzyl-2,3,5-di-*O*-methylene-*L*-iditol.—1,6-Di-*O*-acetyl-2,4,3,5-di-*O*-methylene-*L*-iditol⁸ (8.0 g., m.p. 219–221°) was dissolved in 64 ml. of redistilled benzyl chloride and 37 g. of powdered potassium hydroxide added. With good stirring the mixture was heated at 130–140° for 3 hours. To the cooled reaction mixture 200 ml. of water was added and the product extracted with chloroform. The extract, washed with water and dried over sodium sulfate was concentrated *in vacuo*, finally at 100° and 0.7 mm. The residue crystallized spontaneously on cooling; from absolute alcohol there was obtained 9.18 g. (86%) of product melting at 117–120°. Recrystallized from hot, absolute ethanol the 1,6-di-*O*-benzyl-2,4,3,5-di-*O*-methylene-*L*-iditol was obtained as glistening flat needles melting at 121–122° and rotating in chloroform +42.5° (*c* 1.35). The substance is readily soluble in chloroform, acetone and hot alcohol.

Anal. Calcd. for $C_{22}H_{28}O_6$: C, 68.37; H, 6.78. Found: C, 68.56; H, 6.90.

Partial Acetolysis of 1,6-Di-*O*-benzyl-2,4,3,5-di-*O*-methylene-*L*-iditol.—The dibenzyl derivative (545 mg.) was dissolved in a mixture of 7.5 ml. of glacial acetic acid and 17.5 ml. of acetic anhydride and the solution cooled. A cold, freshly prepared mixture of 1 ml. of concentrated sulfuric acid, 17.5 ml. of acetic anhydride and 7.5 ml. of glacial acetic acid was then added and the mutarotation of the resulting solution observed in a 1-dm. tube at 20°. After 12 min. the rotation became constant at an observed value of +0.02° and the mixture was poured into 500 ml. of ice-water to form a suspension which was gently agitated with a stream of air for 4 hours. The product was then extracted with methylene dichloride and the combined extracts were

washed with aqueous sodium bicarbonate and water. After drying with sodium sulfate the solution was concentrated *in vacuo* at 40° to yield a crystalline residue which, crystallized from absolute ethanol, gave 130 mg. (32%) of colorless needles melting at 217–219° and showing in chloroform a rotation of –4.06° (*c* 1.48). Mixed with authentic 1,6-di-*O*-acetyl-2,4,3,5-di-*O*-methylene-*D*-mannitol it melted at 218–220°. Hann and Hudson⁸ reported 1,6-di-*O*-acetyl-2,4,3,5-di-*O*-methylene-*L*-iditol to have a rotation in chloroform of –3.9° (*c* 2.01).

Complete Acetolysis of 1,6-Di-*O*-benzyl-2,4,3,5-di-*O*-methylene-*L*-iditol.—The dibenzyl ether (1.059 g.) was dissolved in a mixture of 7.5 ml. of glacial acetic acid and 17.5 ml. of acetic anhydride and the solution cooled. To this was added a cooled mixture of 1 ml. of concentrated sulfuric acid, 7.5 ml. of glacial acetic acid and 17.5 ml. of acetic anhydride and the resulting reaction mixture observed polarimetrically at 20° in a 1-dm. tube. After 74 hours the observed rotation had become constant (–0.41°) and the reaction mixture was poured into ice-water. The whole was gently agitated for 5.5 hours and the product then extracted with methylene dichloride. The combined extracts, washed with aqueous sodium bicarbonate and water and dried with sodium sulfate, were concentrated *in vacuo*, finally being held at 55° and 0.01 mm. pressure for 1 hour.¹⁷ The resulting sirup (1.53 g.) crystallized on standing at 0°; after two recrystallizations from aqueous ethanol the product (68 mg., 5.7%) melted at 120–122°; mixed with authentic *L*-iditol hexaacetate it melted at 121–122°. In chloroform (*c* 0.22) the product showed a rotation of –25.0°; *L*-iditol hexaacetate rotates⁸ –25.5° in this solvent (*c* 3.36).

Complete Acetolysis of 1,6-Anhydro-2,3,4-tri-*O*-benzyl- β -*D*-glucose.—1,6-Anhydro-2,3,4-tri-*O*-benzyl- β -*D*-glucose was obtained in 81% yield from 2,3,4-tri-*O*-acetyl-1,6-anhydro- β -*D*-glucose using a modification of the method of Zemplén, Csűrös and Angyal.¹² The clear, stout needles melted at 90–91° and showed in chloroform a rotation of –31.8° (*c* 2.18); the authors cited above reported m.p. 91° and $[\alpha]^{20}_D$ –29.5° ($CHCl_3$).

The 1,6-anhydro-2,3,4-tri-*O*-benzyl- β -*D*-glucose (575 mg.) was dissolved in a mixture of 7.5 ml. of glacial acetic acid and 17.5 ml. of acetic anhydride. The solution was cooled and stirred well while 0.5 ml. of concentrated sulfuric acid was slowly added. Polarimetric observation of the reaction mixture at 20° showed the reaction to be complete after 18 hours, and it was then poured on ice. After agitation for 1 hour the mixture was extracted with chloroform. The combined chloroform extracts, washed with aqueous sodium bicarbonate and water and dried with sodium sulfate, were concentrated *in vacuo* to give a sirup which crystallized on seeding with α -*D*-glucopyranose tetraacetate. Recrystallization from absolute ethanol afforded 150 mg. (29%) of α -*D*-glucopyranose pentaacetate melting at 111° and showing +102° in chloroform (*c* 0.5). Mixed with authentic α -*D*-glucopyranose pentaacetate the product melted at 112–113°.

1,4:3,6-Dianhydro-2,5-di-*O*-benzyl-*D*-mannitol.—Five grams of 1,4:3,6-dianhydro-*D*-mannitol¹³ was dissolved in 78 ml. of redistilled benzyl chloride and 38 g. of powdered potassium hydroxide added to the resulting solution. The stirred reaction mixture was heated slowly to 130–140° and held at that temperature for 2 hours. More potassium hydroxide (38 g.) was then added and the heating continued for two more hours. Water (200 ml.) was added to the cooled reaction mixture and the whole extracted with chloroform. The combined extracts, washed with water and dried over sodium sulfate, were concentrated *in vacuo*, the benzyl alcohol being removed at 100° and 0.2 mm. pressure. When cooled in Dry Ice-acetone the residue crystallized. From 70 ml. of warm hexane there was obtained 9.65 g. (87%) of product melting at 66–68°. Recrystallized from ethanol and then from methanol the pure product was obtained as needles: m.p. 66–67°, $[\alpha]^{20}_D$ +154° ($CHCl_3$, *c* 0.86).

Anal. Calcd. for $C_{20}H_{22}O_4$: C, 73.60; H, 6.80. Found: C, 73.54; H, 6.83.

(17) In a parallel experiment the sirup produced in this manner was held at 160–170° (bath) and ca. 15 mm. pressure to give a colorless distillate which, fractionated in a microcolumn, boiled at 213–214° (Emich tube, atm. pressure) and showed n^{20}_D 1.5035. Benzyl acetate boils at 217° and shows n^{20}_D 1.5200 [G. S. Gardner and J. E. Brewer, *Ind. Eng. Chem.*, **29**, 179 (1937)].

Acetolysis of 1,4:3,6-Dianhydro-2,5-di-O-benzyl-D-mannitol.—Pure, crystalline 1,4:3,6-dianhydro-2,5-di-O-benzyl-D-mannitol (4.46 g.) was dissolved in a mixture of 70 ml. of acetic anhydride and *ca.* 20 ml. of glacial acetic acid. The solution was cooled slightly, 2 ml. of concentrated sulfuric acid added, and the whole diluted with glacial acetic acid to 100 ml. After 84 hours at 20° mutarotation had ceased and the mixture was poured on 300 g. of ice. When the ice had melted and the resulting solution had stood at room temperature for one hour, the product was extracted with chloroform. The combined chloroform extracts, washed successively with a suspension of barium carbonate in water and with water, were dried with sodium sulfate and concentrated *in vacuo* to yield a neutral, sulfur-containing sirup which could not be induced to crystallize. Dissolved in 60 ml. of pure tetrahydrofuran, the material was slowly added to a suspension of 5 g. of lithium aluminum hydride in 50 ml. of tetrahydrofuran. After refluxing for 6 hours the reaction mixture was cooled and the excess reagent decomposed by the cautious addition of 50 ml. of absolute ethanol and then *ca.* 300 ml. of water. The precipitate was removed by filtration on Hyflo Super-Cel and the filtrate deionized by passage through Amberlite IR-120¹⁸ (45 × 512 mm. column). After filtration through a thin bed of carbon the solution was concentrated to a sirup which was dissolved in 100 ml. of absolute ethanol. After another treatment with a small amount of carbon the solvent was removed and the product dissolved in 4 ml. of 1-butanol. After 2 days at +5° the solution gave 223 mg. (10%) of crude 1,4-anhydro-D-mannitol. Recrystallized twice from 1-butanol the product melted at 145–148° either alone or in admixture with authentic material.¹³ In water (*c* 0.81) the product showed a rotation of –24°. Valentin¹⁹ reported 1,4-anhydro-D-mannitol to rotate –23.8° in water.

A parallel acetolysis of a quantity (2 g.) of 1,4:3,6-dianhydro-D-mannitol equivalent to the dibenzyl ether used above yielded 312 mg. of 1,4-anhydro-D-mannitol. The combined aqueous washings proved to be optically active and were therefore freed of barium by treatment with Amberlite IR-120.¹⁸ After removal of the water the residue was reduced with lithium aluminum hydride as described above and gave 55 mg. more of 1,4-anhydro-D-mannitol, raising the total yield to 16%.

(18) A product of Rohm and Haas Co., Philadelphia, Pa.

(19) F. Valentin, *Collection Czechoslov. Chem. Commun.*, **8**, 35 (1936).

Benzoilation of a sample of 1,4-anhydro-D-mannitol prepared in the above acetolyses afforded the corresponding tetrabenzoate in 65% yield. It melted at 123–124°. When mixed with 1,4-anhydro-2,3,5,6-tetra-O-benzoyl-D-mannitol, prepared from authentic 1,4-anhydro-2,6(or 3,6)-di-O-benzoyl-D-mannitol as described below, it melted at 124–125°.

1,4-Anhydro-2,3,5,6-tetra-O-benzoyl-D-mannitol.—Authentic 1,4-anhydro-2,6(or 3,6)-di-O-benzoyl-D-mannitol¹⁸ (403 mg.) was benzoilated with benzoyl chloride in pyridine and the product, freed of excess reactants in the usual manner, crystallized from alcohol as stubby needles. After recrystallization from absolute ethanol it melted at 124–125°, rotated in chloroform (*c* 1.02) –157.5° and in absolute ethanol (*c* 0.29) –114.3°; yield 299 mg., 48%.

Anal. Calcd. for C₃₄H₂₈O₉: C, 70.34; H, 4.86. Found: C, 70.38; H, 5.05.

1,4:3,6-Dianhydro-2,5-di-O-benzyl-D-glucitol.—Ten grams of 1,4:3,6-dianhydro-D-glucitol²⁰ was dissolved in 64 ml. of benzyl chloride and 38 g. of powdered potassium hydroxide added to the solution. With good stirring the mixture was warmed to 140° and then held at 130–140° for 3 hours. The cooled reaction mixture was diluted with 200 ml. of water and extracted with chloroform. The chloroform extract, washed with water and dried over sodium sulfate, was concentrated *in vacuo*, the residue being held finally at 100° and 0.4 mm. pressure. Under a pressure of 0.05 mm. the product distilled at 210–230° (bath) as a pale yellow, viscous liquid which could not be obtained in crystalline form. It rotated +75.7° in chloroform (*c* 1.02) and showed *n*_D²⁰ 1.5544.

Anal. Calcd. for C₂₀H₂₂O₄: C, 73.60; H, 6.80. Found: C, 73.87; H, 6.77.

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(20) R. C. Hockett, H. G. Fletcher, Jr., E. L. Sheffield and R. M. Goepf, Jr., *THIS JOURNAL*, **68**, 927 (1946).

BETHESDA, MARYLAND

[CONTRIBUTION FROM RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Path of Carbon in Photosynthesis. XXI. The Cyclic Regeneration of Carbon Dioxide Acceptor¹

By J. A. BASSHAM, A. A. BENSON, LOREL D. KAY, ANNE Z. HARRIS, A. T. WILSON AND M. CALVIN

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Photosynthesizing plants have been exposed to C¹⁴O₂ for short periods of time (0.4 to 15 sec.) and the products of carbon dioxide reduction analyzed by paper chromatography and radioautography. Methods have been developed for the degradation of ribulose and sedoheptulose. These sugars, obtained as their phosphate esters from the above C¹⁴O₂ exposures and from other experiments, have been degraded and their distribution of radiocarbon determined. The distribution of radiocarbon in these sugars, and other data, indicate that sedoheptulose phosphate and ribulose diphosphates are formed during photosynthesis from triose and hexose phosphates, the latter being synthesized, in turn, by the reduction of 3-phosphoglyceric acid. Further evidence has been found for the previously proposed carboxylation of ribulose diphosphate to phosphoglyceric acid. Free energy calculations indicate this step would proceed spontaneously if enzymatically catalyzed. The efficiency of this cycle for reduction of CO₂ to hexose would be 0.9 if the reduction of each molecule of PGA requires the concurrent conversion of one molecule of ATP and one of DPN (red) to ADP, inorganic phosphate and DPN (ox.).

Previously reported tracer studies of the path of carbon in photosynthesis² led to the conclusion that carbon is incorporated by a carboxylation re-

action leading to phosphoglyceric acid (PGA)³ which is then reduced and condensed to fructose

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission. This paper was presented before the Division of Biological Chemistry, American Chemical Society, at the 124th National Meeting, Chicago, Illinois, September, 1953.

(2) M. Calvin, "The Harvey Lectures," Charles C Thomas Publishing Company, Springfield, Ill., 1950–51, p. 218.

(3) The following abbreviations will be used throughout this paper: PGA, phosphoglyceric acid; DHAP, dihydroxyacetone phosphate; FMP, fructose monophosphate; GMP, glucose monophosphate; SMP, sedoheptulose monophosphate; RDP, ribulose diphosphate; ADP, adenosine diphosphate, ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide (Coenzyme I), oxidized form; DPN[H₂], diphosphopyridine nucleotide, reduced form.