washed with water (yield 0.6 g.) and recrystallized from alcohol; m.p. 222–223°, undepressed by an authentic sample of apoxanthoxyletin.[6] IV gave a yellow solution in dilute sodium hydroxide, a red ferric chloride reaction, a yellow color with concentrated sulfuric acid and an orange deposit with p-phenylenediamine in alcohol.

Anal. Calcd. for $C_{11}H_8O_5$: C, 60.0; H, 3.6. Found: C, 59.8; H, 3.5.

A solution containing IV (0.2 g.), phenylhydrazine hydrochloride (0.6 g.), acetic acid (20 ml.), sodium acetate (0.4 g.) and water (2 ml.) was refluxed for 20 minutes and then cooled to 0°. The phenylhydrazone of IV separated as yellow crystals, which were recrystallized from alcohol; m.p. 251°, reported m.p. for the phenylhydrazone of apoxanthoxyletin 251°.[6]

Anal. Calcd. for $C_{17}H_{14}O_4N_2$: C, 65.9; H, 4.5; N, 9.0. Found: C, 66.2; H, 4.7; N, 8.8.

5,7-Dimethoxy-6-hydroxy-2-methylchromone (VI).— Five ml. of a 30% solution of hydrogen peroxide were added to a suspension of 1 g. of V in 100 ml. of 20% sulfuric acid. The mixture was kept at room temperature for 6 hours with occasional shaking. When 100 ml. of water was added and the mixture cooled in ice, VI was precipitated. It was recrystallized from hot water as colorless needles; m.p. 222–223° (unchanged by sublimation). VI developed a brown color gradually with ferric chloride and dissolved in both 4% sodium hydroxide and concentrated sulfuric acid with a yellow color.

Anal. Calcd. for $C_{12}H_{12}O_5$: C, 61.1; H, 5.1. Found: C, 61.3; H, 5.0.

5,6-Dihydroxy-7-methoxy-2-methylchromone (VIIc).— VI (0.5 g.) was refluxed with 50 ml. of 18% hydrochloric acid for 90 minutes. The solution was then diluted with water and cooled. The colorless crystals of VIIc separated, m.p. 234°; with ferric chloride in alcohol it gave a blue color changing to olive green. VIIc showed the typical reactions of a catecholic phenol, e.g., when one drop of 1% sodium hydroxide solution was added to a solution of the substance in alcohol containing one drop of ferric chloride solution, a deep red color was produced.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5. Found: C, 59.2; H, 4.7.

6,7-Dihydroxy-5-methoxy-2-methylchromone (VIIa).— When IIc (4 g.), dissolved in 30 ml. of a 4% sodium hydroxide solution was treated with 10 ml. of a 30% hydrogen peroxide in an ice-bath, a copious white precipitate was formed after a few minutes. The mixture was left in the ice-bath with occasional shaking for 6 hours and was then neutralized with acetic acid. The precipitate was filtered and recrystallized from hot water, in which it was fairly soluble, to give colorless silky needles of VIIa, m.p. 229°. It gave a greenish black ferric chloride reaction and dissolved in 4% sodium hydroxide solution and concentrated sulfuric acid, giving a yellow color in both cases. VIIa displayed the reactions characteristic of a catechol derivative described for VIIc. Also, addition of a lead acetate solution to an alcoholic solution of the substance gave a yellow precipitate; addition of an aqueous titanium trichloride solution gave a red-brown deposit.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5. Found: C, 59.2; H, 4.5.

Demethylation of VIIa.— VIIa (1 g.) was demethylated under the conditions described for VI to yellowish plates of 5,6,7-trihydroxy-2-methylchromone (VIIb) which gave a green-brown ferric chloride reaction; m.p. 280–282°, reported m.p. 284–286°.[7]

Anal. Calcd. for $C_{10}H_8O_5$: C, 57.7; H, 3.8. Found: C, 57.5; H, 4.1.

Methylation of VIIa.— VIIa (1 g.) was refluxed for 8 hours with a mixture of 80 ml. of acetone, 5 ml. of methyl iodide and 5 g. of anhydrous potassium carbonate. The solvent was evaporated under reduced pressure, and the residue was acidified with dilute acetic acid and extracted with chloroform. The chloroform layer was washed with 4% sodium hydroxide solution and evaporated to dryness. The residue consisting of 5,6,7-trimethoxy-2-methylchromone (VIId) crystallized from water in colorless prisms; m.p. 100°, reported m.p. 99–100°.[7] VIId had a negative ferric chloride reaction; with potassium hydroxide pellets it gave the red color characteristic of 2-methylchromones having no free phenolic group.[10]

Anal. Calcd. for $C_{13}H_{14}O_5$: C, 62.4; H, 5.6. Found: C, 62.2; H, 5.5.

VIId is appreciably soluble in water (1 part in 150 at 24° and 1 part in 10 at 70°); 5,8-dimethoxy-2-methylchromone has been described as a water-soluble compound.[11]

Acidification of the alkaline washings from the above experiment yielded a small amount of a phenolic substance (m.p. 222°) which was identified as VI.

Anal. Calcd. for $C_{12}H_{12}O_5$: C, 61.1; H, 5.1. Found: C, 60.9; H, 4.8.

The yield of VI was considerably increased at the expense of the completely methylated product (VIId) when the time of the reaction was cut down to 4 hours.

(11) L. Vargha and M. Rados, *Acta Chim. Acad. Sci. Hung.*, **3**, 223 (1953).

Cairo, Egypt

Transformation of D-Gulose to 1,6-Anhydro-β-D-gulopyranose in Acid Solution[1,2]

By Laura C. Stewart and Nelson K. Richtmyer

D-Gulose is transformed by hot dilute acid to the extent of at least 43% to a crystalline anhydride whose structure has been established through periodate oxidation methods as 1,6-anhydro-β-D-gulopyranose.

In continuation of our studies on the formation of monomeric non-reducing anhydrides from reducing sugars under the influence of acids, and particularly from those with the *gulo* configuration, we now wish to describe the behavior of D-gulose (I) itself. When that sugar is heated with dilute acid

it suffers a considerable loss of reducing power. After a suitable period of time has been allowed for establishing equilibrium and the unchanged D-gulose then destroyed by heating with alkali, an anhydro-D-gulose, melting at 154–155° and showing $[\alpha]^{20}D$ +50.4° in water, can be isolated without difficulty. At least 43% of the D-gulose is estimated to be converted to anhydride in this manner. The anhydride has been characterized further through its tri-O-acetyl, benzoyl, and tosyl derivatives; it has
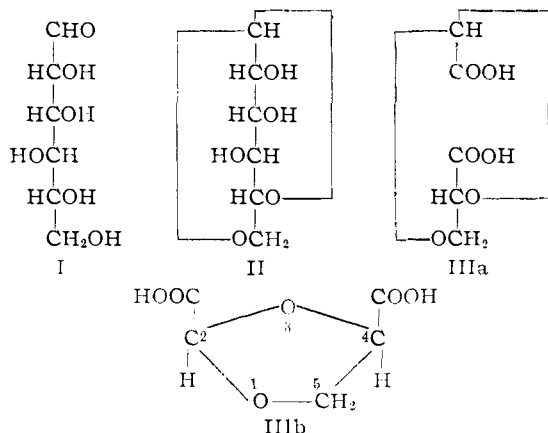
been transformed also by simultaneous hydrolysis and oxidation to D-gulonic 1,4-lactone and by acetolysis to α-D-gulose pentaacetate.

The structure of the anhydride was determined through its oxidation with sodium metaperiodate. The consumption of two molar equivalents of oxidant and the liberation of one molar equivalent of formic acid, but no formaldehyde, indicated the presence of three contiguous secondary hydroxyl groups in the molecule. The compound must have, therefore, either the unknown 1,2:1,6 combination of rings or the 1,5:1,6 combination already established in the 1,6-anhydro-β-D-pyranose derivatives of glucose, altrose, mannose, galactose and idose. Of these two possibilities, the 1,6-anhydro-β-D-gulopyranose structure (II) is indicated immediately because the rotation of the expected dialdehyde in the periodate oxidation mixture is in good agreement with that observed in the similar oxidation of the first four of the other anhydrohexoses mentioned above.[3] Oxidation of the dialdehyde with hypobromite then yielded a dibasic acid (III) whose crystalline strontium salt agreed in its properties, including its characteristic hydration, rotation in water and in excess hydrochloric acid, and X-ray powder diffraction pattern, with the strontium salt described previously.[3] Furthermore, the strontium salt was converted to the di-p-phenylphenacyl ester and identified by melting point and mixed melting point, as well as by rotation, with the di-p-phenylphenacyl ester derived from levoglucosan by a similar procedure. The dibasic acid, written in the form IIIa, was named L'-oxy-D-methylenediglycolic acid by Jackson and Hudson.[3a] It also may be written as the 1,3-dioxolane IIIb, in which the carbon atom at position 4 has the same configuration that it had as C₅ in D-gulose, and is then called 4-D-glycero-1,3-dioxolane-2,4-cis-dicarboxylic acid.

In summary, we can state that D-gulose, like D-altrose and D-idose, is converted by acid, and in a relatively large amount, to the 1,6-anhydro-β-D-pyranose form. The new anhydride, in contrast to the other known 1,6-anhydro-β-D-hexopyranoses, is dextrorotatory ([α]²⁰D +50.4°); in this respect, however, it is similar to the other anhydrides with the D-gulo configuration, namely, 2,7-anhydro-β-D-gulo-heptulopyranose ([α]²⁰D +39.7°, by reversal of sign of the known L-enantiomorph[4]), 1,6-anhydro-D-glycero-β-D-gulo-heptopyranose ([α]²⁰D +52.9°)[5] and 1,7-anhydro-D-glycero-β-D-gulo-heptopyranose ([α]²⁰D +60.0°).[2]

Experimental

1,6-Anhydro-β-D-gulopyranose (II).—A preliminary experiment with 1.1 g. of an old, impure, levorotatory D-gulose sirup showed that heating with acid, followed by treatment with alkali to destroy the unchanged sugar, and deionization, yielded a non-reducing, dextrorotatory solution; concentration of the solution left a very small amount of sirup that crystallized after several months in a desiccator.

Quantitative data next were obtained by heating 20 g. of crystalline methyl α-D-gulopyranoside tetraacetate[6] ([α]²⁰D +96.9°) in 200 ml. of 0.5 N sulfuric acid. After 30 minutes on the steam-bath the product had dissolved as a consequence of deacetylation, and the rotation of the solution, [α]²⁰D +91.7° calcd. as the methyl glycoside, corresponded roughly to the value +109.4° reported for methyl α-D-gulopyranoside monohydrate.[6] The solution was then refluxed for 1.5 hours; the rotation became negative ([α]²⁰D −10.8° calcd. as hexose) and indicated the presence of mostly free D-gulose (final rotation calcd. as −26.4° for the D-gulose constituent of α-D-gulose·CaCl₂·H₂O⁷). After 13 hours, the equilibrium rotation between sugar and anhydride appeared to have been reached at [α]²⁰D +15.3° (calcd. as hexose) and further heating seemed to cause a slight drop in rotation, presumably due to the continuing destruction of the sugar, inasmuch as the solution was already quite dark in color. In preparation for isolating the anhydride, the unchanged D-gulose was first destroyed by adding excess barium hydroxide and heating the alkaline solution in an open beaker for several hours on the steam-bath. The non-reducing mixture was freed from barium ions by precipitation with sulfuric acid and the filtered solution finally was deionized completely by passage through Amberlite IR-120 and Duolite A-4 ion-exchange resins. From the rotation of this solution and the specific rotation of the anhydride as determined later we estimated 3.85 g. of anhydride to be present. This value corresponds to a 43% conversion of D-gulose to its 1,6-anhydride, but it is undoubtedly low because of unavoidable mechanical losses; actually, the value may be as high as 55% if we base the calculations on the equilibrium rotation of [α]²⁰D +15.3° that was noted above. The deionized solution was concentrated in vacuo to a sirup that crystallized spontaneously in the distilling flask. The product was dissolved in hot ethanol from which, on cooling, it separated as prisms in a total yield of 3.0 g. After several recrystallizations the 1,6-anhydro-β-D-gulopyranose melted at 154–155° and showed [α]²⁰D +50.4° in water (c 2.8).

Anal. Calcd. for C₆H₁₀O₅: C, 44.44; H, 6.22. Found: C, 44.51; H, 6.14.

Additional material was obtained by reducing 140 g. of D-gulonic lactone with 2.5% sodium amalgam in the usual manner and deionizing and concentrating the resulting solution to sirupy D-gulose in a yield of 95 g. The solution of the sirup in 500 ml. of 0.5 N sulfuric acid showed [α]²⁰D −19.9°, a value that changed with gentle boiling for 20 hours to [α]²⁰D +11.3° (constant) based on the original weight of the sirup. Unconverted sugar was destroyed with baryta as described above and the resulting non-reducing solution had a rotation equivalent to 30 g. of anhydride. From that solution a total of 26.5 g. of crystalline 1,6-anhydro-β-D-gulopyranose was recovered.

2,3,4-Tri-O-acetyl-1,6-anhydro-β-D-gulose.—Acetylation of 1 g. of the anhydride II with acetic anhydride and pyridine yielded 1.1 g. of triacetate; the compound was recrystallized twice from acetone-n-pentane and then from chloroform-n-pentane, furnishing shiny plates with m.p. 114–115° and [α]²⁰D +22.1° in chloroform (c 1.5).

CHO
HCOH
HCOH
HOCH
HCOH
CH₂OH

I

II

IIIa

IIIb

(3) (a) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **62**, 958 (1940); (b) N. K. Richtmyer and C. S. Hudson, *ibid.*, **62**, 961 (1940); (c) A. E. Knauf, R. M. Hann and C. S. Hudson, *ibid.*, **63**, 1447 (1941); (d) R. M. Hann and C. S. Hudson, *ibid.*, **64**, 2435 (1942).

(4) L. C. Stewart, N. K. Richtmyer and C. S. Hudson, *ibid.*, **74**, 2206 (1952).

(5) E. M. Montgomery, N. K. Richtmyer and C. S. Hudson, *ibid.*, **65**, 1848 (1943).

(6) H. S. Isbell, *Bur. Standards J. Research*, **8**, 1 (1932).

(7) H. S. Isbell, *ibid.*, **5**, 741 (1930).

Anal. Calcd. for C₁₂H₁₆O₈: C, 50.00; H, 5.59. Found: C, 50.28; H, 5.52.

1,6-Anhydro-2,3,4-tri-O-benzoyl-β-D-gulose.—Benzoylation of 1 g. of the anhydride II with benzoyl chloride and pyridine overnight at room temperature gave a practically quantitative yield of the tribenzoate. After four recrystallizations from aqueous acetone and one from chloroform–pentane, the product—fluffy needles—melted at 158–159° and showed [α]²⁰D +214° in chloroform (c 2.0).

Anal. Calcd. for C₂₇H₂₂O₈: C, 68.35; H, 4.67. Found: C, 68.28; H, 4.81.

1,6-Anhydro-2,3,4-tri-O-p-tolylsulfonyl-β-D-gulose.—Tosylation of 1 g. of the anhydride II with 15 g. (12.6 molar equivalents) of p-toluenesulfonyl chloride in 50 ml. of anhydrous pyridine for 3 days at room temperature afforded a practically quantitative yield of the tri-O-tosyl derivative. The product crystallized from acetone–pentane as prisms melting at 133–134° and from methanol as needles melting at 143–144°; a mixture of the two forms sintered at 134° and melted at 141–143°. Nearly identical rotations of [α]²⁰D +36.6° and +36.8° in chloroform (c 1.3) were observed for the prisms and needles, respectively.

Anal. Calcd. for C₂₇H₂₈O₁₁S₃: C, 51.91; H, 4.52; S, 15.40. Found: (prisms) C, 52.08; H, 4.60; S, 15.22; (needles) C, 51.90; H, 4.49.

In a preliminary experiment, 1 g. of the anhydride II was allowed to react with 3.9 g. (3.3 molar equivalents) of p-toluenesulfonyl chloride in 10 ml. of anhydrous pyridine for 24 hours at room temperature. Decomposition of the mixture with ice-water and isolation of the product with chloroform in the usual way yielded a sirup. Solution of the sirup in acetone and dilution with n-pentane resulted in a gel that crystallized readily upon the addition of a small amount of methanol. Recrystallization from a mixture of methanol–ether–pentane gave 1.2 g. of a compound that melted at 149–150°; two recrystallizations from methanol raised the m.p. to 151–152° and the fluffy needles showed [α]²⁰D +51.0° in chloroform (c 2.0). The same product was isolated even when the reaction was carried out with 6.3 molar equivalents of tosyl chloride for 3 days at room temperature, and in each of these preliminary experiments the tri-O-tosyl derivative described above was isolated from the mother liquors. This new product was found to be a di-O-tosyl derivative and may, by analogy with the tri-O-tosyl derivatives of the 1,6- and 1,7-anhydro-D-glycero-β-D-gulo-heptopyranoses,[5,2] be designated tentatively as **1,6-anhydro-2,3-di-O-p-tolylsulfonyl-β-D-gulopyranose.**

Anal. Calcd. for C₂₀H₂₂O₉S₂: C, 51.05; H, 4.71; S, 13.63. Found: C, 51.00; H, 4.71; S, 13.44.

Oxidation of 1,6-Anhydro-β-D-gulopyranose (II) with Sodium Metaperiodate.—The reaction of 2.9 g. of the anhydride II with a small excess of 0.48 M sodium metaperiodate at 20° showed the consumption of 1.93 and 1.96 molar equivalents of oxidant and the liberation of 0.96 and 0.98 molar equivalent of formic acid at the end of 3 and 24 hours, respectively. The rotation of the resulting solution was [α]²⁰D −14.2° (calcd. as the expected dialdehyde), in good agreement with the average value of −14.3° obtained from the similar oxidations of the 1,6-anhydro-β-D-pyranose forms of glucose, altrose, mannose and galactose.[3] Aqueous barium chloride was added to precipitate most of the iodate and periodate ions, and to the clear filtrate were added 140 g. of powdered barium carbonate and 5 ml. of bromine. The mixture, kept in the dark at 20°, was shaken from time to time during the day and then left overnight. Excess bromine was removed by aeration, halide ions with silver carbonate, silver ions with hydrogen sulfide, and the remaining cations by passage of the solution through Amberlite IR-120. The solution was concentrated *in vacuo* to about 200 ml., neutralized with strontium hydroxide to the phenolphthalein end-point, and concentrated further with the bath temperature kept below 40°. A strontium salt began to crystallize spontaneously; filtered, washed with ice-water, and dried for several hours in the air at room temperature, it weighed 3.0 g. The weight remained constant for about 16 hours, and then the product began to lose weight slowly in the manner noted earlier for strontium L'-oxy-D-methylenediglycolate pentahydrate.[3b] The product was recrystallized by dissolving it in hot water, filtering, and concentrating *in vacuo* as before. The 1.8 g. of salt thus obtained became constant in weight after standing 5 hours in the air

at room temperature and did not change during the next 12 hours. It had the approximate composition of the expected pentahydrate (*Anal.* Calcd. for C₅H₄O₆Sr·5H₂O: C, 17.78; H, 4.18; Sr, 25.94; H₂O, 26.67. Found: C, 18.18; H, 4.31; Sr, 24.73; H₂O, 25.47). The rotation, [α]²⁰D +27.0° in water (c 1), was slightly lower than the values +28.1° and +27.7° reported previously for the pentahydrate.[3b,d]

A second recrystallization of the pentahydrate under the same conditions yielded chalky crystals that had the approximate composition of the monohydrate (*Anal.* Calcd. for C₅H₄O₆Sr·H₂O: C, 22.60; H, 2.28; Sr, 32.98. Found: C, 23.03; H, 2.36; Sr, 32.38). The rotation, [α]²⁰D +36.1° in water (c 0.4), was slightly lower than the average value +36.7° that had been reported previously,[3a] while the rotation of the free organic acid (c 0.4), obtained by dissolving the strontium salt in an excess of dilute hydrochloric acid, was [α]²⁰D +8.1° in good agreement with the average of +7.9° reported previously[3] for L'-oxy-D-methylenediglycolic acid. A comparison of the X-ray powder diffraction patterns of the strontium salt monohydrate derived from our anhydro-D-gulose with that derived from levoglucosan (= 1,6-anhydro-β-D-glucopyranose)[3a,b] likewise confirmed the identity of the two salts.

Di-p-phenylphenacyl 4-D-glycero-1,3-Dioxolane-2,4-cis-dicarboxylate (= Di-p-phenylphenacyl L'-Oxy-D-methylenediglycolate). (a) From 1,6-Anhydro-β-D-glucopyranose.— In order to obtain a derivative of the periodate oxidation product from the 1,6-anhydro-β-D-hexopyranoses that could be identified more readily than the strontium salt of the dibasic acid, which has no characteristic m.p. and which has been reported not only as a mono- and a pentahydrate but also as a tri- and a tetrahydrate,[3c] we decided to try to make an ester with p-phenylphenacyl bromide, the reagent for identifying organic acids that was first prepared and recommended by Drake and Bronitsky.[8] Accordingly, to an aqueous solution containing 0.53 g. (0.002 mole) of the strontium salt monohydrate derived from levoglucosan was added 0.25 g. (0.002 mole) of sodium carbonate monohydrate. The precipitated strontium carbonate was removed by filtration and the aqueous solution of the sodium salt of the organic acid was concentrated *in vacuo* to about 4 ml. The solution was made barely acid to litmus with hydrochloric acid, 8 ml. of ethanol and 1.10 g. (0.004 mole) of p-phenylphenacyl bromide were added, and the mixture was refluxed for 2 hours. The character of the solid phase changed noticeably during the heating. After cooling, the crystalline material was filtered, washed with aqueous ethanol, and dried; wt. 1.1 g. The product was recrystallized from absolute ethanol–acetone, twice from acetone–ether, and finally from chloroform–pentane. The resulting small acicular prisms of the p-phenylphenacyl ester melted at 148–149° and showed [α]²⁰D +4.0° in chloroform (c 0.8).

(b) From 1,6-Anhydro-β-D-gulopyranose.—In the same manner, 0.2 g. of the strontium salt derived from the anhydro-D-gulose was converted to a di-p-phenylphenacyl ester. The crude material melted at 144–146°; two recrystallizations from absolute ethanol raised the m.p. to 147–148°; and a mixture with the product from levoglucosan melted also at 147–148°. The rotation was [α]²⁰D +4.2° in chloroform (c 2.5).

Anal. Calcd. for C₃₃H₂₈O₈: C, 71.99; H, 4.76. Found: C, 72.18; H, 4.67.

Simultaneous Hydrolysis and Oxidation of 1,6-Anhydro-β-D-gulopyranose to D-Gulonic 1,4-Lactone.—A solution containing 1 g. of the anhydride II, 1 ml. of 40% aqueous hydrogen bromide and 40 drops of bromine in 40 ml. of water in an all-glass 1.5-dm. polarimeter tube had an observed rotation of α²⁰D +5.9°S. The mixture was placed in an oven at 80°, small amounts of bromine were added from time to time as the solution became colorless, and the changing rotation was followed until the observed value reached α²⁰D −3.4°S. after 3 days and did not change significantly during the next 4 days. Bromine and bromide ions were removed in the usual manner and the resulting solution was estimated from its rotation to contain 0.31 g. of D-gulonic lactone. The solution was concentrated *in vacuo* to a sirup that was taken up in ethanol and seeded to yield 0.25 g. of crystalline D-gulonic lactone, which was identified by m.p., mixed m.p. and rotation.

(8) N. L. Drake and J. Bronitsky, THIS JOURNAL, **52**, 3715 (1930).

Acetolysis of 1,6-Anhydro-β-D-gulopyranose.—The reaction of 1 g. of the anhydride II with 50 ml. of acetic anhydride and 0.5 ml. of concentrated sulfuric acid at 20° led to a constant rotation of $[\alpha]^{20}$D +15.3° (calcd. as D-gulose pentaacetate) at the end of 16 hours, with no further change during the next 6 hours. The product, isolated in the usual manner, was a sirup that could not be induced to crystallize.

The acetolysis of a second 1-g. portion of anhydride, in 25 ml. of acetic anhydride and 0.5 ml. of sulfuric acid, was carried out for 4 days at −5° in the hope that at the lower temperature a larger amount of the crystalline α-anomer might be formed and its rearrangement to the unknown β-anomer be retarded. Although no rotation was taken to confirm those expectations, the final result was the isolation of about 0.2 g. of the known α-D-gulose pentaacetate.[9] Re-

crystallized from ether–pentane, the product—large acicular prisms—melted at 106–107° and showed no depression of m.p. when mixed with an authentic sample furnished through the courtesy of Drs. Frush and Isbell. The rotation, $[\alpha]^{20}$D +86.6° in chloroform (c 0.24), was in good agreement with the value +86.2° reported by those authors.

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(9) H. L. Frush and H. S. Isbell, J. Research Natl. Bur. Standards, **34**, 111 (1945).

Isolation and Identification of Planteose from Tobacco Seeds[1]

BY DEXTER FRENCH

A crystalline trisaccharide prepared from tobacco seeds is found to be identical with planteose, O-α-D-galactopyranosyl (1 → 6)-O-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside.

Recently Wada and Yamazaki reported[2] the existence of a trisaccharide isomeric with raffinose in seeds of tobacco, *Nicotiana tabacum*. This trisaccharide differed from raffinose in its paper chromatographic behavior and in its cleavage by mild acid hydrolysis to D-glucose and a ketose disaccharide of D-fructose and D-galactose. These properties are similar to those recorded for planteose,[3,4] a trisaccharide from seeds of various *Plantago* species.

In order to make a more detailed examination of the trisaccharide, it was prepared on a macroscopic scale and its properties were compared with those of planteose (Table I). The close similarities of the characteristic properties of planteose from *Plantago* seeds and tobacco seed trisaccharide, together with the identical appearance of the X-ray diffraction powder patterns, point to the identity of the trisaccharides from these two rather distantly related plants.

Experimental

Four hundred grams of tobacco seeds, variety Yellow Mammoth,[5] was ground overnight in a ball mill to remove the seed coat and partially break down the seed structure. The seeds tended to pack in the mill because of the high apparent oil content and after brief extraction with diethyl ether the seeds were returned to the ball mill for a second overnight grinding.

The entire batch of ground seeds was extracted in a large Soxhlet apparatus, first for 24 hours with diethyl ether,

then for 72 hours with 99% methanol. The methanolic extract was concentrated to about 200 ml. by evaporation in a warm air stream and treated with 200 ml. of petroleum ether and 200 ml. of acetone. The upper phase was discarded and the lower phase was again treated with 200 ml. of acetone and 300 ml. of petroleum ether. All was discarded except a gummy precipitate which had appeared during the concentration and extraction of the methanol extract.

The gummy precipitate was washed briefly with methanol and acetone and dissolved in 125 ml. of water. Paper chromatography[6] showed that it contained sucrose and trisaccharide in a ratio of 3 or 4 to 1, and measurement of the optical rotation indicated the presence of about 2.3 g. of material, assuming that it consisted only of sucrose and planteose with an average specific rotation for the mixture of +81.

Charcoal Separation of Tobacco Seed Sugars.—The entire sugar solution was diluted to 1 liter and placed upon a 40-g. charcoal column.[7] One liter eluates with dilute ethanol were examined for total optical rotation and for qualitative composition by paper chromatography (Table II).

Crystallization and Identification of Tobacco Seed Sucrose.—The 2% ethanol eluate from the charcoal column was evaporated to a sirup and purified by large scale paper chromatography. Crystals which formed from ethanol-acetic acid were removed by filtration, washed briefly with 85% ethanol and dry propanol and air-dried; 0.24 g., specific rotation[8] +67.1 ± 1.6 (accepted[9] value for sucrose, +66.5).

The tobacco seed sucrose (0.2 g.) was acetylated using an equal weight of anhydrous sodium acetate and 2 ml. of acetic anhydride by heating to boiling on a hot-plate until the sucrose was completely dissolved. The reaction product was evaporated to dryness overnight in a warm air stream and the residue was extracted with benzene. The filtered extract was evaporated to dryness and taken up in hot butanol. The crystals which formed on cooling were removed

(1) Journal Paper No. J-2596 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1116. Supported in part by a grant from the Corn Industries Research Foundation. Technical assistance was furnished by Miss Ana Pellecer.

(2) E. Wada and K. Yamazaki, J. Agr. Chem. Soc. Japan, **24**, 398 (1951).

(3) N. Wattiez and M. Hans, Bull. Acad. roy. med. Belg., **8**, 386 (1943).

(4) D. French, G. M. Wild, B. Young and W. J. James, THIS JOURNAL, **75**, 709 (1953).

(5) Purchased from T. W. Wood and Sons, Richmond, Virginia.

(6) A. Jeanes, C. S. Wise and R. J. Dimler, Anal. Chem., **23**, 415 (1951); D. French, D. W. Knapp and J. H. Pazur, THIS JOURNAL, **72**, 5150 (1950).

(7) The procedure of R. L. Whistler and D. F. Durso was followed, except that no Celite was used in preparing the column; R. L. Whistler and D. F. Durso, THIS JOURNAL, **73**, 4189 (1951).

(8) Specific rotations reported are for dilute aqueous solutions (<4%) using sodium light.

(9) F. J. Bates, "Polarimetry, Saccharimetry and the Sugars," Circular C440 of the National Bureau of Standards, Washington, 1942.