Anal. Calcd. for $C_{24}H_{34}O_7$: C, 66.34; H, 7.89. Found: C, 66.00; H, 8.08.

It was found that purification of intermediates was not necessary in the preparation of IX from IV.

Thus starting with 5.0 g. of IV and carrying the material through the hydrolysis, bromination, acetoxylation and oxidation steps without isolation, there was obtained 3.37 g. of IX, m.p. 194–200°. One crystallization from acetone–hexane yielded 2.58 g., m.p. 209–212°.

11β,17α,21-Trihydroxypregnane-3,20-dione 21-Acetate (X).—A solution of 0.50 g. of IX in 20 ml. of C.P. methanol was combined with 6 ml. of water and 11.5 ml. of C.P. methanol containing 0.115 g. of sodium hydroxide, all under an argon atmosphere. The solution was allowed to stand for 18 hours at 27°, the excess alkali was neutralized with acetic acid, water added and the methanol removed under reduced pressure. Filtration yielded 0.21 g., m.p. 143–170°; this was acetylated with acetic anhydride and pyridine, and chromatographed on Florisil to give 30 mg. of X, m.p. 189–195°. Its infrared spectrum was identical with that of an authentic sample.[8]

4-Bromo-11β,17α,21-trihydroxypregnane-3,20-dione 11-Formate 21-Acetate (XI).—A solution of 3.0 g. of IX in 30 ml. of methylene chloride and 30 ml. of *t*-butyl alcohol at 25° was brominated by the dropwise addition of 1.14 g. of bromine in 25 ml. of methylene chloride. The bromine color discharged after standing 4 hours. The methylene chloride was removed under reduced pressure and the remaining solution poured into water. Filtration of the crystalline precipitate gave 3.76 g., m.p. 163–169° dec. Recrystallization from aqueous acetone gave 1.75 g. of XI, m.p. 185–187° dec., $[\alpha]D$ +86.3° (acetone).

Anal. Calcd. for $C_{24}H_{33}O_7Br$: Br, 15.57. Found: Br, 15.37.

Debromination of the mother liquor gave 1.07 g. of X, m.p. 213–221°.

11β,17α,21-Trihydroxy-Δ⁴-pregnene-3,20-dione 11-Formate 21-Acetate (XII).—A suspension of 2.65 g. of XI in 53 ml. of *t*-butyl alcohol and 40 ml. of C.P. chloroform, stirred in an argon atmosphere, was treated with 0.81 g. of semicarbazide and stirring was continued for 2 hours. The reaction was concentrated under reduced pressure to *ca.* half-volume, water added and the distillation continued to the precipitation of solid. Filtration yielded 2.65 g., which was taken up in 25 ml. of acetic acid, combined with a solution of 1.22 g. of 94% pyruvic acid and 1.05 g. of sodium acetate in 7.5 ml. of water, and refluxed for 5 minutes. After the addition of 100 ml. of hot water, the mixture was chilled to precipitate 1.91 g. of XII, m.p. 190–200°. The analytical sample, crystallized several times from acetone–hexane, melted at 199–201°, $[\alpha]D$ +176.1°, ϵ_{max} 15,100 at 239 mμ (95% EtOH).

Anal. Calcd. for $C_{24}H_{32}O_7$: C, 66.65; H, 7.46. Found: C, 66.46; H, 7.22.

11β,17α,21-Trihydroxy-Δ⁴-pregnene-3,20-dione 11-Formate (XIII).—A solution of 0.50 g. of XII in 5 ml. of C.P. chloroform and 17.5 ml. of C.P. methanol was chilled and combined with 1.0 ml. of concentrated hydrochloric acid in 1.8 ml. of water. The reaction was allowed to stand for 48 hours at 25°, diluted with water, and extracted with methylene chloride. The organic extracts were washed with dilute sodium bicarbonate solution and water, dried and evaporated. The resinous residue, 0.48 g., was taken up in benzene and chromatographed on Florisil. The fraction (0.17 g.) eluted with 1% methanol in methylene chloride crystallized on trituration with ether, m.p. 168–178°. Recrystallization from acetone–hexane yielded 0.08 g. of XIII, m.p. 184–187°, $[\alpha]D$ +162.7°, ϵ_{max} 15,700 at 238 mμ (95% EtOH).

Anal. Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.63; H, 7.92.

17α-Hydroxycorticosterone 21-Acetate (XIV).—Under an argon atmosphere, a mixture of 0.50 g. of XII in 30 ml. of C.P. methanol and 0.116 g. of sodium hydroxide in 6 ml. of water was allowed to react 20 hours at 25°. The excess alkali was neutralized with acetic acid, water was added and the methanol removed under reduced pressure. Only a small amount of solids separated, so the aqueous residue was extracted several times with methylene chloride, the extracts combined, dried and evaporated to give 0.37 g. This was acetylated with acetic anhydride and pyridine, then crystallized from acetone to give 0.11 g., m.p. 215–218°. Its infrared spectrum was identical with that of an authentic sample of XIV.

BLOOMFIELD, N. J.

(8) Kindly supplied by Merck and Co., Inc.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

6-O-β-Maltosyl-α-D-glucopyranose Hendecaacetate

BY A. THOMPSON[1] AND M. L. WOLFROM

RECEIVED JANUARY 13, 1955

The reaction mixture[2] obtained by treating 6-O-β-maltosyl-β-D-glucopyranose hendecaacetate successively with titanium tetrachloride, mercuric acetate and acetic anhydride–pyridine, has been investigated chromatographically and found to contain 6-O-β-maltosyl-α-D-glucopyranose hendecaacetate, β-maltose octaacetate and β-D-glucopyranose pentaacetate, in addition to the starting material. A proof of the identity of 6-O-β-maltosyl-α-D-glucopyranose hendecaacetate is presented.

The trisaccharides of D-glucose containing mixed (1 → 4)-α-D and (1 → 6)-α-D linkages are of interest in studies on the structure of amylopectin. Of the three possible trisaccharides, containing these linkages, that should be preformed in amylopectin, panose[3] (4-O-α-isomaltopyranosyl-D-glucose)[4] has been isolated from amylopectin.[5] An impure preparation of a second member of this group, 6-O-α-maltosyl-D-glucose hendecaacetate, has been reported by Asp and Lindberg[2] as resulting from the action of titanium tetrachloride on 6-O-β-maltosyl-β-D-glucopyranose hendecaacetate[6] with subsequent treatment with mercuric acetate and reacetylation with acetic anhydride and pyridine. Such a transformation of a (1 → 6)-β-D-glucosidic to the (1 → 6)-α-D form has been established by Lindberg[7] for the conversion of β-gentiobiose octaacetate to β-isomaltose octaacetate and has been verified in this Laboratory.

In the hope that we could obtain 6-O-α-maltosyl-β-D-glucopyranose hendecaacetate in a state of purity, we have made a chromatographic study of the reaction mixture obtained by Asp and Lindberg, at the stage in which all products appear as

(1) Corn Industries Research Foundation Associate.

(2) L. Asp and B. Lindberg, *Acta Chem. Scand.*, **5**, 665 (1951).

(3) S. C. Pan, A. A. Andreasen and P. Kolachov, *Science*, **112**, 115 (1950); S. C. Pan, L. W. Nicholson and P. Kolachov, THIS JOURNAL, **73**, 2547 (1951).

(4) M. L. Wolfrom, A. Thompson and T. T. Galkowski, *ibid.*, **73**, 4093 (1951).

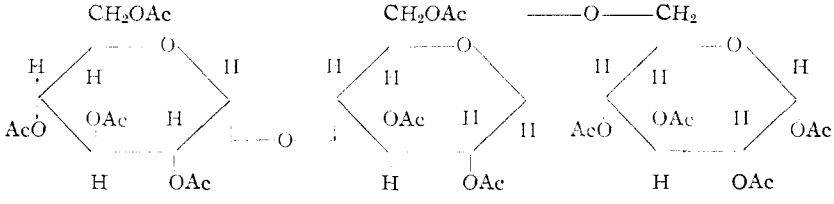
(5) A. Thompson and M. L. Wolfrom, *ibid.*, **73**, 5849 (1951).

(6) S. H. Nichols, Jr., W. L. Evans and H. D. McDowell, *ibid.*, **62**, 1754 (1940).

(7) B. Lindberg, *Acta Chem. Scand.*, **3**, 1355 (1949).

fully acetylated sugars. In this mixture, besides unchanged starting material, we found 6-*O*-β-maltosyl-α-D-glucopyranose hendecaacetate, β-maltose hendecaacetate and β-D-glucopyranose pentaacetate. A small amount of deacetylation occurred during the reaction which was later corrected by reacetylation with pyridine and acetic anhydride, and this could account for the presence of the acetate of the α-D-anomer of the starting material. The maltose and D-glucose acetates obviously arose from a rupture of the (1 → 6)-β-D linkage in the starting material. While we failed to isolate a crystalline hendecaacetate of the desired trisaccharide, our results do not disprove its possible presence in the reaction mixture.

To demonstrate the anomeric relationship of the new acetate to the starting material, 6-*O*-β-maltosyl-β-D-glycopyranose hendecaacetate, and incidentally to provide a better source of the substance, we subjected the 6-*O*-β-maltosyl-β-D-glucopyranose hendecaacetate to an adaptation of the reaction with zinc chloride in acetic anhydride which has been used effectively by Hudson and associates[8] to convert β-D- to α-D-anomers in the acetates of numerous sugars. Since, like titanium tetrachloride, zinc chloride is a strong Lewis acid, this reaction could not be considered as conclusive evidence that only a simple anomeric shift had occurred. The new acetate was accordingly subjected to deacetylation, partial hydrolysis and reacetylation. The resultant reaction product was separated into its components by chromatographic techniques and these were found to be β-D-glucopyranose pentaacetate, β-gentiobiose octaacetate and 6-*O*-maltosyl-β-D-glucopyranose hendecaacetate. The fact that the unchanged portion of the trisaccharide reacetylated to the original 6-*O*-β-maltosyl-β-D-glucopyranose hendecaacetate and that the isolated disaccharide portion was found to be β-gentiobiose octaacetate proves that the new acetate is 6-*O*-β-maltosyl-α-D-glucopyranose hendecaacetate.



O-α-D-Glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-α-D-glucopyranose hendecaacetate (6-*O*-β-maltosyl-α-D-glucopyranose hendecaacetate).

Experimental

Treatment of 6-*O*-β-Maltosyl-β-D-glucopyranose Hendecaacetate with Titanium Tetrachloride.—Following Asp and Lindberg,[2] 10 g. of 6-*O*-β-maltosyl-β-D-glucopyranose hendecaacetate,[6] dissolved in 140 ml. of absolute chloroform, was treated with a solution of 12 ml. of titanium tetrachloride in 100 ml. of absolute chloroform. A yellow precipitate formed and this heterogeneous mixture was refluxed for 5 hr. The mixture was then poured into 200 ml. of ice and water, the chloroform layer was separated and the aqueous layer was further extracted with chloroform. The combined chloroform extracts were washed with water until free of acid, dried with anhydrous sodium sulfate and evaporated under reduced pressure to a sirup. This sirup was dissolved in 80 ml. of acetic acid containing 8 g. of mercuric acetate and allowed to stand at room temperature for 2 hr. The solution was diluted with 100 ml. of chloroform and washed with water until free of acid. The chloroform solution was dried with anhydrous sodium sulfate and evaporated under reduced pressure to a sirup. This material crystallized from 600 ml. of ethanol and yielded 6.1 g. of unchanged material. The mother liquor was evaporated to a sirup and treated with 10 ml. of pyridine and 20 ml. of acetic anhydride overnight at room temperature to correct some incidental deacetylation which had occurred. The reaction mixture was poured into 100 ml. of ice and water and extracted with chloroform. The chloroform extract was washed several times with water. To remove the traces of pyridine, it was then shaken with a saturated aqueous solution of cadmium chloride and filtered, repeating the procedure until no more precipitate formed. The resulting chloroform solution was washed with water, dried with anhydrous sodium sulfate and evaporated to a sirup (3.3 g.) which upon solution in ethanol yielded 0.65 g. of unchanged trisaccharide acetate and upon evaporation of the mother liquor left an amorphous residue; yield 2.6 g. This material, dissolved in 20 ml. of benzene, was placed upon a column (50 mm., diam., × 250 mm.) of Magnesol[9]–Celite[10] (5:1 by wt.) and developed with 4000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). When the column was extruded and streaked with indicator (0.1 g. of potassium permanganate, 1.0 g. of sodium hydroxide and 10 ml. of water) a zone appeared 60 to 130 mm. from the top of the column. The crystalline material in this zone was recovered by acetone elution; yield 460 mg., m.p. 150–160°. Pure material was obtained on four recrystallizations from ethanol; yield 260 mg., m.p. 173–176° cor., $[\alpha]^{30}D$ +81.3° (*c* 3.6, chloroform). This substance was identical with 6-*O*-β-maltosyl-α-D-glucose hendecaacetate further described in the following paragraph. The benzene effluent was evaporated to a sirup under reduced pressure and rechromatographed in two equal portions, each on a column (35 mm., diam., × 180 mm.) of Magnesol–Celite (5:1 by wt.) by developing with 600 ml. of benzene–*t*-butyl alcohol (125:1 by vol.). Two zones appeared upon application of the indicator. The combined upper zones were eluted with acetone and evaporated to a sirup which crystallized from ethanol; yield 330 mg., m.p. 154–157° cor., undepressed on admixture with authentic β-maltose octaacetate, $[\alpha]^{30}D$ +61° (*c* 4.3, chloroform), in good agreement with accepted values for β-maltose octaacetate. The combined lower zones were eluted with acetone and upon evaporation of the eluting solvent the residue was crystallized from ethanol; yield 60 mg., m.p. 127–129° cor., unchanged on admixture with authentic β-D-glucopyranose pentaacetate, $[\alpha]^{30}D$ +4.8°.

O-α-D-**Glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-α-D-glucopyranose** (6-*O*-β-Maltosyl-α-D-glucopyranose) **Hendecaacetate.**—6-*O*-β-Maltosyl-β-D-glucopyranose hendecaacetate (10 g.) was dissolved in 30 ml. of acetic anhydride containing 0.3 g. of freshly fused zinc chloride. The mixture was agitated mildly in a water-bath at 80° for 50 min. at which time the solid had all dissolved. The solution was poured into 150 ml. of ice and water and stirred occasionally for 2 hr. The water and residue was extracted with chloroform and the extract was washed with water, saturated sodium bicarbonate solution, again with water, dried with anhydrous sodium sulfate and evaporated to a sirup. The sirup was dissolved in 75 ml. of hot ethanol. The unchanged material crystallized and was filtered from the hot solution; yield 1.4 g., m.p. 227–232° cor. Upon cooling, another product crystallized which was recrystallized once from ethanol; yield 3.2 g., m.p. 163–168°. Pure material was obtained by chromatographic treatment. The crude material (1 g.) was placed on a Magnesol–Celite column (45 mm., diam., × 220 mm.) and developed with 2500 ml. of benzene–*t*-butyl alcohol. The carbohydrate

(8) C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1280 (1915).

(9) A product of the Westvaco Chemical Division of Food Machinery and Chemical Corp., South Charleston, W. Va.

(10) A siliceous filter-aid produced by Johns-Manville Co., New York, N. Y.

material was eluted from the single zone with acetone and, after evaporation of the eluate, the residue was crystallized from ethanol; m.p. 174–176° cor., $[\alpha]^{22}D$ +80.4° (*c* 3.7, chloroform), X-ray powder diffraction data: 13.35[11] w[12], 12.24 vw, 11.09 vs, 9.91 w, 8.82 w, 8.34 w, 6.59 m, 6.24 vw, 6.58 s, 5.34 s, 5.01 m, 4.81 s, 4.55 w, 4.27 m, 4.12 w, 3.81 m, 3.69 m, 3.54 vw, 3.46 vw, 3.38 m.

Anal. Calcd. for $C_{18}H_{21}O_{16}(CH_3CO)_{11}$: C, 49.69; H, 5.63; CH_3CO, 11.38 ml. of 0.1 *N* sodium hydroxide per 0.1 g.; mol. wt., 966.83. Found: C, 49.87; H, 5.52; CH_3CO, 11.33; mol. wt., 957 (Rast).

Partial Hydrolysis of 6-*O*-β-Maltosyl-α-D-glucopyranose Hendecaacetate.—6-*O*-β-Maltosyl-α-D-glucopyranose hendecaacetate (3 g.) was dissolved in 40 ml. of 0.05 *N* sodium methoxide in methanol. After standing at room temperature for 5 min., a precipitate was formed which was dissolved by the addition of a small amount of water. The solution was deionized by passing successively through columns (2.5 mm. diam., × 15 mm.) of Amberlite 120[13] and Duolite A-4.[14] The effluent was evaporated under reduced pressure to a sirup which was dissolved in 75 ml. of 0.05 *N* sulfuric acid and refluxed for 7 hr. The sulfuric acid was removed by passing the solution again through the column of Duolite A-4, and the effluent was evaporated to dryness under reduced pressure; yield 1.47 g. This material was acetylated by boiling for 1 min. with 0.7 g. of anhydrous sodium acetate and 10 ml. of acetic anhydride. The reaction mixture was poured into 100 ml. of ice and water and stirred occasionally for 2 hr. The mixture was then extracted with four 25-ml. portions of chloroform. The combined chloroform extracts were washed with water, dried with anhydrous sodium sulfate and evaporated to a sirup. The sirup was dissolved in benzene and placed on a column (75 mm., diam., × 275 mm.) of Magnesol–Celite and developed with 3000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). Upon extrusion and application of the indicator, one large zone appeared in the middle of the column (the effluent will be discussed below). The carbohydrate material was eluted from the zone with acetone and the residue obtained on solvent removal was fractionally crystallized from ethanol; yield 300 mg. of 6-*O*-β-maltosyl-β-D-glucopyranose hendecaacetate, m.p. 229–231° cor., $[\alpha]^{23}D$ +39.4° (*c* 4.2, chloroform). The second fraction was impure and was rechromatographed on a column (45 mm. diam., × 220 mm.) of Magnesol–Celite (5:1 by wt.) by developing with 700 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). The principal zone, near the top of the column, was sectioned and the acetone-eluted material was crystallized from ethanol; yield 200 mg., m.p. 193–195° cor., $[\alpha]^{23}D$ −3.0° (*c* 2.5, chloroform), in agreement with accepted values for β-gentiobiose octaacetate.

The above mentioned effluent from the first column was evaporated to dryness and rechromatographed on a column (50 mm. diam., × 225 mm.) of Magnesol–Celite by developing with 800 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). The acetone-eluted material of the one zone which appeared was crystallized from ethanol; yield 400 mg. of β-D-glucopyranose pentaacetate, m.p. 128–130° cor., $[\alpha]^{30}D$ +4.6° (*c* 2.5, chloroform).

COLUMBUS 10, OHIO

(11) Interplanar spacing, Å., CuK_α radiation.

(12) Relative intensity, estimated visually; vs, very strong; s, strong; m, medium; w, weak; vw, very weak.

(13) A product of the Resinous Products and Chemical Co., Philadelphia, Penna.

(14) A product of the Chemical Process Co., Redwood City, Calif.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of the Hemicellulose of the Straw of Flax (Linum Usitatissimum Sp.). I. Identification of 2-*O*-(4-*O*-Methyl-D-glucuronosyl)-D-xylose

BY J. D. GEERDES AND F. SMITH[1]

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The acid component of the hemicellulose obtained from flax-straw (*Linum Usitatissimum* Sp.) has been isolated as an aldobiouronic acid (I) and this has been identified as 2-*O*-(4-*O*-methyl-D-glucopyruronosyl)-D-xylose.

Flax hemicellulose obtained from the delignified straw by extraction with alkali gave upon hydrolysis a mixture of an aldobiouronic acid (I), D-xylose and a small amount of L-rhamnose. The acidic component (I), which forms the subject of this communication, was separated from the hydrolysate by the use of an anion-exchange resin. The methoxyl content of I and its equivalent weight, indicated that it was composed of a methoxy uronic acid and a pentose sugar, a deduction further substantiated by the observation that, upon vigorous hydrolysis, I afforded 4-*O*-methyl-D-glucuronic acid and D-xylose as indicated by paper chromatography.

Cleavage of the aldobiouronic acid (I) with 8% methanolic hydrogen chloride at 115°, followed by treatment of the cleavage products with ammonia, yielded the crystalline amide of methyl 4-*O*-methyl-α-D-glucuronoside.[2] After removal of this uronic acid derivative, hydrolysis of the neutral sugar glycoside gave crystalline D-xylose.

When the methyl ester of the methyl glycoside of I was reduced with lithium aluminum hydride[3,4] to the corresponding disaccharide, methyl *O*-(4-*O*-methyl-D-glucopyranosyl)-D-xyloside, and the latter hydrolyzed with dilute acid, the cleavage products so formed were found by chromatographic analysis to be D-xylose and 4-*O*-methyl-D-glucose.[2,5]

The point of attachment of the 4-*O*-methyl-D-glucuronic acid unit to the D-xylose residue was derived from a study of the methylated aldobiouronic acid (II) formed when methylated flax straw hemicellulose was subjected to the action of boiling 2% methanolic hydrogen chloride.[6] Cleavage of II with 8% methanolic hydrogen chloride at 115° and treatment of the resulting glycosides with methanolic ammonia yielded the crystalline amide of methyl 2,3,4-tri-*O*-methyl-α-D-glucuronoside.[7] When the neutral component of the methylated aldobiouronic acid, namely, methyl mono-*O*-methyl-D-xyloside, was hydrolyzed and the free sugar purified by paper chromatography, there was isolated crystalline

(1) Paper No. 3204, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota. Extracted from a thesis presented by J. D. Geerdes to the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. (1953). This paper was presented at the 125th A.C.S. meeting in Kansas City, 1954.

(2) F. Smith, *J. Chem. Soc.*, 2646 (1951).

(3) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950).

(4) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(5) R. Shinle, *Ber.*, **65**, 315 (1932).

(6) J. D. Geerdes and F. Smith, THIS JOURNAL, **77**, 3572 (1955).

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