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The Constitution of a Glucomannan from Wheat Stem Rust (*Puccinia graminis tritici*) Urediospores[1]

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A glucomannan has been extracted from urediospores of mixed races of *Puccinia graminis tritici* with alkali and shown to be composed of equal numbers of D-glucose and D-mannose residues. Methylation and periodate oxidation studies have demonstrated that the average repeating unit of the glucomannan consists of approximately 6 terminal non-reducing residues, (3 of D-glucose and 3 of D-mannose), 104 non-terminal D-mannose residues (52 residues linked through C_1 and C_4 and 52 through C_1 and C_3) and 4 branching residues probably consisting of D-glucose units joined through C_1, C_2 and C_6. The glycosidic linkages are of the β-D-type. An aqueous extract of the urediospores is shown to contain glycerol, D-arabitol and D-mannitol.

The pathogen causing wheat stem rust is the fungus, *Puccinia graminis tritici*, which requires for its life cycle two hosts, the common barberry (*Berberis vulgaris*) and one of several grasses, notably wheat (*Triticum vulgare*) and barley (*Hordeum vulgare*).[2,3] Five types of spores are associated with the life cycle. In the uredial stage of the life cycle the familiar red-brown pustules containing urediospores develop on the stems and leaves of the cereal plant. This paper is concerned with the constitution of a glucomannan, previously recognized[4] in the mixed races of *Puccinia graminis tritici* urediospores grown on wheat by the identification of D-glucose and D-mannose in a hydrolyzate of the spores. The general structure features of the glucomannan have been ascertained by methylation and periodate-oxidation studies. The identification of the polyhydric alcohols glycerol, D-arabitol and D-mannitol present in this type of spore has been established.

The glucomannan, $[\alpha]_D$ $-80°$ (water), isolated by extracting the urediospores with alkali, was purified by acetylation. Methylation of the glucomannan acetate, $[\alpha]_D$ $-74°$ (chloroform), with methyl sulfate and sodium hydroxide in the usual manner and completion of the methylation with silver oxide and methyl iodide afforded the corresponding methylated glucomannan, $[\alpha]_D$ $-69°$ (chloroform).

The methylated glucomannan gave upon hydrolysis the following methylated sugars which were separated by chromatography on a column composed of a mixture of hydrocellulose and cellulose[5]: (a) 2,3,4,6-tetra-*O*-methyl-D-mannose (3 moles), (b) 2,3,4,6-tetra-*O*-methyl-D-glucose (3 moles), (c) 2,3,6-tri-*O*-methyl-D-mannose (52 moles), (d) 2,4,6-tri-*O*-methyl-D-mannose (52 moles) and (e) an unidentified di-*O*-methyl ether of D-glucose or D-mannose (4 moles). Components a and b were identified by paper chromatography and by demethylation[6] to D-mannose and D-glucose, respectively. The 2,3,6-tri-*O*-methyl-D-mannose (component c) readily gave a crystalline 1,4-di-*p*-nitrobenzoate[7] while the 2,4,6-tri-*O*-methyl-D-mannose (component d) crystallized as the characteristic monohydrate identical with that obtained from 2,4,6-tri-*O*-methyl-D-glucose by epimerization.[8] Component e was not identified, but it must be a di-*O*-methyl derivative of either D-glucose or D-mannose or possibly a mixture of the two in view of the composition of the original polysaccharide.[4,9] Chromatographic and electrophoretic examination on paper showed that component e did not correspond to any of the following: 2,3-, 2,4-, 2,6-, 3,6-, 4,6-di-*O*-methyl-D-glucose or 3,4-di-*O*-methyl-D-mannose.

The results of these methylation studies do not permit the postulation of a precise formula for the glucomannan of the urediospores, but the main structural features of the polysaccharide are readily apparent. Out of about every 114 sugar residues which comprise the average repeating unit of the glucomannan, 6 residues occupy terminal non-reducing positions and of these 3 are D-mannose and 3 are D-glucose residues. The linear, doubly linked, non-terminal residues, about 104, are composed entirely of D-mannose, half of them being joined by 1→4- and the other half by 1→3-glycosidic linkages. The di-*O*-methylhexose component of the hydrolyzate of the methylated glucomannan arises from the residues which constitute the branch points in the polymer. The methylation studies also reveal that the ratio of terminal to non-terminal residues is 1:18.

Support for the above interpretation of the methylation results was provided by the observation that the polysaccharide consumed 0.54 mole of periodate per hexose residue, and one mole of formic acid was produced for every 21 residues. A polymer composed of 6,terminal, non-reducing residues (each reacting with 2 molecular proportions of periodate and giving 1 molecular proportion of formic acid), 52 periodate-stable residues joined by 1→3-linkages, 52 residues joined by 1→4-linkages (each reacting with 1 molecular proportion of periodate) and 4 triply linked residues (each reacting with 1 molecular proportion of periodate (see below)), would consume 0.53 mole of periodate per hexose residue, and liberate 1 mole of formic acid per 19 hexose residues.

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The presence of the 1→3-linked D-mannose residues was also demonstrated by the fact that both the periodate-oxidized glucomannan, the polyaldehyde and the corresponding polyalcohol formed by sodium borohydride reduction of the polyaldehyde,[10,11] gave D-mannose upon acid hydrolysis.

Further analysis[10] of the hydrolyzate of the polyalcohol from the glucomannan showed that it contained glycerol as well as D-mannose in the molar ratio of 1:4.5. The non-terminal D-mannose residues joined by 1→4- and 1→3-linkages clearly will not give rise to glycerol and if the non-reducing terminal residues are the sole source of the glycerol, the above general structure requires that the molar ratio of glycerol to D-mannose would be 1:8.7. On the other hand, if the residues forming the branch points also yield glycerol, the ratio of glycerol to D-mannose would be 1:5.2, a result that is in fairly good agreement with the experimentally observed value. This evidence indicates that the residues involved in branching are attacked by periodate between C_3 and C_4 and hence these residues must be joined through C_1, C_2 and C_6. If this tentative conclusion be correct, it follows that the di-*O*-methylhexose component of the hydrolyzate of the methylated glucomannan is a 3,4-di-*O*-methyl derivative, and since chromatographic evidence clearly showed that it was not 3,4-di-*O*-methyl-D-mannose, it seems likely that the unidentified di-*O*-methyl component is 3,4-di-*O*-methyl-D-glucose.

Polyhydric alcohols have been shown to be present in a variety of micro-organisms. Thus glycerol is produced by several *Mucor* and *Fusaria*,[12] erythritol has been found in spores of *Ustilago zeae*,[13] in the mycelium of *Penicillium brevi-compactum* and of *P. cyclopeum*,[14] D-arabitol occurs commonly in lichens[15-23] and D-mannitol is produced by a number of fungi[12] such as *Pleurotus ostreatus*.[24] Aqueous extractions of the urediospores of the wheat stem rust used in these investigations was found to contain glycerol, D-arabitol and D-mannitol, all of which were identified by the preparation of characteristic crystalline derivatives.

Experimental

Isolation of the Acetylated Glucomannan.—A suspension of about 0.5 g. of wheat stem rust (*Puccinia graminis tritici*) urediospores (mixed races) in 60% potassium hydroxide (40 ml.) solution was boiled under reflux in a platinum vessel for 4 hr. The solution was diluted with water and neutralized with acetic acid. The resulting clear light brown solution was poured with stirring into ethanol (10 volumes), whereupon the crude glucomannan appeared as a flocculent precipitate.

The crude glucomannan, prepared in the above manner from 30 g. of spores, was centrifuged, washed with ethanol, and divided into 3 equal portions. Each of the three portions of the precipitate, while still containing some ethanol, was dispersed in formamide (300 ml.) and stirred at about 38° for 8 hr. Portions of pyridine (10 ml.) and of acetic anhydride (20 ml.) were added every 10 min. with stirring until a total of 130 ml. of pyridine and 300 ml. of acetic anhydride had been added. After the fourth addition of these reagents the temperature of the liquid had increased to 55° and thereafter slowly decreased to about 40°. After stirring for 24 hr., the solution was poured into ice-water (3 l.), whereupon the acetylated glucomannan appeared as a flocculent light brown precipitate. The suspension was thoroughly extracted with chloroform and the chloroform solution washed successively with *N* hydrochloric acid, saturated sodium bicarbonate solution, and finally with water. The chloroform solution of the acetate was dried with anhydrous magnesium sulfate, evaporated to about 75 ml. and poured with stirring into petroleum ether (10 vols.). The acetylated glucomannan appeared as a flocculent light brown precipitate which was removed by centrifuging, washed with petroleum ether and dried. The product so obtained from the three acetylations was dissolved in chloroform (50 ml.) and reprecipitated with petroleum ether as before, yield 6.2 g., $[\alpha]^{22}D$ −68.6° in chloroform (*c*, 0.5). *Anal.* Calcd. for $C_{12}H_{16}O_8$: $COCH_3$, 44.8. Found: $COCH_3$, 43.7.

Fractional Precipitation of the Glucomannan Acetate.—The acetylated glucomannan (6.2 g.) was dissolved in chloroform (200 ml.) and fractionally precipitated in the usual way by the gradual addition with stirring of petroleum ether (b.p. 40–60°). Each fraction was dissolved in chloroform (50–60 ml.) and precipitated as a white amorphous powder by pouring this solution into petroleum ether (800 ml.). The individual fractions were washed with petroleum ether and dried *in vacuo*. The yield of the fractions and their specific optical rotation in chloroform were: (1) 0.143 g., $[\alpha]^{25}D$ not observable; (2) 0.196 g., −64°; (3) 0.396 g., −68°; (4) 0.587 g., −72°; (5) 0.904 g., −74°; (6) 1.026 g., −74°; (7) 0.675 g., −73°; (8) 0.404 g., −75°; (9) 0.373 g., −74°; (10) 0.360 g., −74°; (11) 0.338 g., −73°; (12) 0.276 g., −73°; (13) 0.098 g., −40°. The specific rotation of the fractions indicate that the major proportion of the glucomannan acetate is essentially homogeneous. Fractions 4 to 12 inclusive were combined (wt. 4.8 g.) and used for the subsequent constitutional studies.

Anal. (Fraction 7): Calcd. for $C_{12}H_{16}O_8$: $COCH_3$, 44.8; Found: $COCH_3$, 44.5.

Deacetylation of the Glucomannan Acetate.—To a solution of the acetate (1.5 g.) in acetone (30 ml.), 20% sodium hydroxide (20 ml.) was added and the mixture was heated for 30 min. in a platinum vessel with stirring at 60°. The acetone layer was removed and the aqueous alkaline layer neutralized with acetic acid and poured with stirring into absolute ethanol (10 vols.). The flocculent, cream-colored precipitate was centrifuged, washed successively with ethanol, diethyl ether and dried, yield 1.1 g., $[\alpha]^{25}D$ −80° in water (*c* 1).

Hydrolysis of the Glucomannan.—A solution of the glucomannan (38.6 mg.) in *N* sulfuric acid (4.0 ml.), which showed $[\alpha]^{25}D$ −77°, was boiled under reflux. After 3.5 hr., when the rotation had become constant ($[\alpha]^{25}D$ +11°), the solution was neutralized with barium carbonate, filtered and the filtrate evaporated *in vacuo*. Paper electrophoretic examination[25] of the sirupy residue revealed the presence of a small proportion of D-glucose and a large proportion of D-mannose. A third component, probably a mannose oligosaccharide which showed little or no movement, was also detected.

Methylation of the Glucomannan.—To a solution of the acetylated glucomannan (3.0 g.) in acetone (75 ml.), 30% sodium hydroxide (150 ml.) and dimethyl sulfate (50 ml.) were added in ten portions over a period of 1 hr., the reaction mixture being stirred vigorously at 50° during the addition of the reagents and for 1 hr. thereafter. The mixture

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was heated on a boiling water-bath for 45 min. The mixture was neutralized with 6 *N* sulfuric acid and water was added to dissolve the precipitated sodium sulfate. Some methylated glucomannan, which had precipitated, was separated by decantation. The decanted liquid was extracted with chloroform, and ethanol (2 vols.) was added to the aqueous solution to precipitate most of the inorganic salt. The chloroform and the filtered aqueous ethanolic solution were evaporated and the residue from each was combined with the methylated glucomannan removed from the methylation mixture and a second methylation carried out as before. At the completion of the second methylation the methylated product separated and coalesced to form a gum-like mass. This was removed and the remainder of the reaction treated as for the first methylation to recover any partially methylated polysaccharide. Two more methylations were performed in the same manner. After the last methylation, the methylated product was extracted with chloroform, the solution was dried with magnesium sulfate, and evaporated; yield 2.1 g. (Found: OMe, 39). The methylated product (2.1 g.) was treated with methyl iodide (25 ml.), silver oxide (2.0 g.) and calcium sulfate (2.0 g.) under reflux with stirring for 15 hr. The product, recovered by extraction with chloroform, was subjected to a second Purdie methylation as before; yield 2.0 g.

Fractional Precipitation of the Methylated Glucomannan.—The methylated glucomannan (2.0 g.) was dissolved in chloroform (25 ml.) and a series of fractions obtained by adding increasing amounts of petroleum ether in the usual manner. Each fraction was dissolved in chloroform and precipitated by pouring with stirring into petroleum ether. The results of the fractionation were: fraction 1, 0.129 g.; 2, 0.205 g., $[\alpha]^{25}D$ ($CHCl_3$, *c*, 0.6) −69°, (OCH_3, 45.9); 3, 1.07 g., −69°, (OCH_3, 46.4); 4, 0.224 g., −69°, (OCH_3, 44.2); 5, 0.069 g., −59°, (OCH_3, 44.1).

Hydrolysis of the Methylated Glucomannan.—The methylated glucomannan (0.203 g., fraction 3) was dissolved in 2% methanolic hydrogen chloride (3.5 ml.) and the solution, which showed $[\alpha]^{25}D$ −80°, was heated (sealed tube) for 10 hr. in a boiling water-bath. The final constant rotation of the cooled solution was $[\alpha]^{25}D$ +63°. The solution was neutralized with silver carbonate, filtered, and evaporated; yield 0.206 g.

A solution of the sirupy mixture of the methyl glycosides in *N* hydrochloric acid (7 ml.) was heated (sealed tube) for 7 hr. in a boiling water-bath. After heating for 4 hr. the specific rotation had become constant $[\alpha]^{25}D$ +4.5°. The solution was neutralized with silver carbonate, filtered, and evaporated *in vacuo* (yield 0.185 g.).

Separation of the Mixture of Methylated Sugars by Column Chromatography.—A preliminary analysis was made by paper chromatography using butanone–water azeotrope[26] as the solvent, the methylated sugars being located by spraying with *p*-anisidine.[27] Four components were observed with R_f values 0.79, 0.55, 0.46 and 0.23, indicating the presence of a tetra-*O*-methyl fraction, two tri-*O*-methyl fractions and a di-*O*-methyl fraction. For the quantitative separation of the components a cellulose–hydrocellulose column[5] was used.

The tetra-*O*-methyl fraction was shown to consist of a mixture of tetra-*O*-methyl-D-glucose and tetra-*O*-methyl-D-mannose, and the tri-*O*-methyl fractions consisted of 2,4,6-tri-*O*-methyl-D-mannose (R_f 0.55) and 2,3,6-tri-*O*-methyl-D-mannose (R_f 0.46). The di-*O*-methyl fraction was not identified. The results of the column chromatography are given in Table I.

From the amounts of the various components, calculated when necessary from the values of the specific rotations, the molar proportions were found to be: 2,3,4,6-tetra-*O*-methyl-D-glucose (3), 2,3,4,6-tetra-O-D-mannose (3) 2,4,6-tri-*O*-methyl-D-mannose (56), 2,3,6-tri-*O*-methyl-D-mannose (56) and di-*O*-methylhexose (4). Of 180 mg. of hydrolyzate 167.3 mg. was recovered from the eluate; the apparent low recovery (89%) was probably due to solvent impurity present in the sirupy mixture put on the column. In a duplicate experiment with 0.509 g. of hydrolyzate the respective molar ratios were 3:3:48:48:4. The average of the two experiments, namely, 3:3:52:52:4, was used in structural discussions.

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TABLE I

COLUMN CHROMATOGRAPHIC ANALYSIS OF THE METHYLATED GLUCOMANNAN HYDROLYZATE[5]

Tube	Component	Wt., mg.	$[\alpha]^{25}D$ (in water)
5–11	Tetra-*O*-methyl-D-glucose and tetra-*O*-methyl-D-mannose	8.9	+41°
23–41	2,4,6-Tri-*O*-methyl-D-mannose	73.1	+17
42–44	2,4,6-Tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose	4.7	± 0.0
45–48	2,4,6-Tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose	11.1	−11
49–80	2,3,6-Tri-*O*-methyl-D-mannose	64.4	−13
135–151	Di-*O*-methyl fraction	5.1	+14

Identification of the Components of the Hydrolyzate of the Methylated Glucomannan. (1) **2,3,4,6-Tetra-*O*-methyl-D-glucose and 2,3,4,6-Tetra-*O*-methyl-D-mannose.**—A portion (5.0 mg.) of the tetra-*O*-methyl fraction was dissolved in 48% hydrobromic acid (1 ml.) and heated (sealed tube) for 5 min. in a boiling water-bath. After cooling, the solution was neutralized with silver oxide, filtered, evaporated to a small volume, and investigated qualitatively by filter paper chromatography using pyridine–ethyl acetate–water (1:2.5:3.5)[28] as the irrigating solvent. Both D-glucose and D-mannose were present as well as products arising from partial demethylation. The rotation of the tetra-*O*-methyl fraction was $[\alpha]^{25}D$ +41° in water, indicating that it contained about equal proportions of 2,3,4,6-tetra-*O*-methyl-D-glucose, $[\alpha]D$ +84° (water)[29], and 2,3,4,6-tetra-*O*-methyl-D-mannose, $[\alpha]D$ +2.4° (water).[30]

(2) **2,4,6-Tri-*O*-methyl-D-mannose.**—Crystallized spontaneously as the crystalline monohydrate, m.p. and mixed m.p. 64°, $[\alpha]^{26}D$ +17° in water (*c*. 2.5), no mutarotation (after recrystallization from ethanol–light petroleum ether).[8] *Anal.* Calcd. for $C_9H_{18}O_6$; H_2O: C, 45.0; H, 8.3; OCH_3, 38.8; H_2O, 7.5. Found: C, 45.2; H, 8.0; OCH_3, 39.9; H_2O (from loss in weight on heating *in vacuo* at 56° over P_2O_5), 7.5.

(3) **2,3,6-Tri-*O*-methyl-D-mannose.**—To a portion (14.2 mg.) of the sirup obtained by evaporation of the solution from tubes 49–80 (Table I), *p*-nitrobenzoyl chloride (68.3 mg.) and dry pyridine (0.9 ml.) were added. The solution was kept at 65–70° for 30 min. and then allowed to stand at room temperature overnight. After addition of saturated sodium bicarbonate solution until effervescence ceased, water (2.0 ml.) was added and the solution extracted with three portions (2.0 ml.) of chloroform. The chloroform solution was dried with magnesium sulfate, and evaporated. The residue was dissolved in hot methanol (25 ml.) and the 2,3,6-tri-*O*-methyl-D-mannose 1,4-di-*p*-nitrobenzoate[7] allowed to crystallize in the cold. After recrystallization from methanol the product (19.7 mg.) showed m.p. and mixed m.p. 191°, and $[\alpha]^{25}D$ +32° (in chloroform, *c*, 1.0). *Anal.* Calcd. for $C_{34}H_{24}O_{12}N_2$: C, 53.0, H, 4.6; N, 5.4. Found: C, 53.3; H, 4.5; N, 5.4.

(4) **The Di-*O*-methyl Fraction.**—The di-*O*-methyl fraction was investigated by paper chromatography using butanone–water azeotrope and the upper layer of benzene–ethanol–water (200:47:15) as irrigating solvents, and by filter paper electrophoresis using the method of Briggs, *et al.*[25] The unknown di-*O*-methyl sugar did not correspond to any of the following: 2,3-di-, 2,4-di-, 2,6-di-, 3,6-di-, 4,6-di-*O*-methyl-D-glucose or 3,4-di-*O*-methyl-D-mannose.

Oxidation of the Glucomannan with Sodium Periodate.—The oxidation was carried out at 4° as described previously,[31] with 180 mg. of glucomannan dissolved in 200 ml. of 0.0898 *N* sodium periodate. After 24 hr. the periodate consumption

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(30) R. D. Greene and W. L. Lewis, THIS JOURNAL, **50**, 2813 (1928).

(31) M. Abdel-Akher and F. Smith, *ibid.*, **73**, 994 (1951).

was constant at 0.53 mole periodate per hexose residue and the formic acid production after 500 hr. was 1 mole for every 21 moles of hexose.

Examination of the Periodate-oxidized glucomannan.—To a portion (24 ml.) of the above periodate-oxidation solution, barium chloride (0.15 g.) was added to precipitate iodate as barium iodate. The solution was filtered and the precipitate was washed with water (3.0 ml.). To the filtrate sodium borohydride (50 mg. in 1.0 ml. water) was added and the reduction of the polyaldehyde allowed to proceed for 3.5 hr. at room temperature. After neutralization of the reaction mixture with acet c acid, the solution was evaporated to a small volume (2.0 ml.) and adjusted to 1 *N* with hydrochloric acid. The solution was heated (sealed tube) for 3 hr. in a boiling water-bath, cooled, and neutralized with silver oxide. After removal of the silver chloride, the solution was evaporated to a small volume and de-ionized by passing successively through a cation (Amberlite IR 120)[32] and an anion (Duolite A^4)[33] exchange resin. The eluate was evaporated to dryness *in vacuo* and dissolved in 0.1 ml. of water. For the determination of D-mannose, which was the only sugar detected in the hydrolyzate, the solution (75.3 μl.) was chromatographed using pyridine–ethyl acetate–water (1:2.5:3.5) as the irrigating solvent, and the D-mannose was determined by the phenol–sulfuric acid method.[34] The glycerol was determined by the chromotropic acid method[35] suitably modified.[36] In the 75.3 μl. of solution there were 2.08 μmoles of glycerol and 9.25 μmoles of D-mannose, the molecular ratio being 1:4.5.

The Isolation and Identification of D-Arabitol, Glycerol, D-Mannitol, D-Glucose and D-Mannose.—Urediospores (200 mg.) were shaken with water (25 ml.) at room temperature for 2 hr. The solution was filtered and evaporated to give a residue (32.7 mg.) which was dissolved in water (0.3 ml.). Paper chromatography, using three different solvents, namely, pyridine–ethyl acetate–water,[28] butanone–water azeotrope[25] and butan-1-ol–ethanol–water,[37] followed by spraying with ammoniacal silver nitrate revealed three spots whose R_f values corresponded to those of D-mannitol, D-arabitol and glycerol.

Identification of D-Mannitol and Glycerol.—(1) *D-Mannitol.*—To a sample of urediospores of mixed races (1.0 g.) in a Potter–Elvehjem homogenizer enough benzene was added to form a paste and the sample was found until no intact spores could be detected under the microscope. The sample was extracted with petroleum ether (100 ml.) and with boiling 75% ethanol (200 ml.). The spore debris was removed by centrifuging and washed with a further quantity of boiling 75% ethanol. The combined ethanol extract and washings were evaporated to dryness and the yellow residue was extracted with cold absolute ethanol (10 ml.). This cold ethanol extract (A) was retained for the isolation of glycerol.

The yellow residue was dissolved in a minimum amount of hot ethanol and allowed to crystallize in the cold. After two recrystallizations D-mannitol (17 mg.) was obtained, m.p. and mixed m.p. 116°. The D-mannitol (17 mg.) was dissolved in 1.0 ml. of a mixture of acetic anhydride (15 ml.) and sulfuric acid (1.0 ml.) and warmed for 15 min. at 60°. The reaction mixture was cooled and poured into ice-water (4.0 ml.) and the product was extracted with chloroform, and the chloroform solution was dried with magnesium sulfate, filtered and evaporated. The residue was dissolved in acetone (1.0 ml.) and precipitated by pouring into water (10.0 ml.). The precipitate was centrifuged, dried, and recrystallized from ethanol to give D-mannitol hexaacetate (23 mg.), m.p. and mixed m.p. 125–126°, $[\alpha]^{25}D$ +18.6° in benzene (*c*, 2.1). *Anal.* Calcd. for $C_{18}H_{26}O_{12}$: C, 49.8; H, 6.0. Found: C, 50.3; H, 6.3.

(2) **Glycerol.**—The cold ethanol extract (A) obtained during the isolation of D-mannitol described above was evaporated to a volume of 1.0 ml. and the glycerol isolated by paper chromatography using butanone–water azeotrope as the solvent, the chromatogram being developed for 15 hr. by the descending technique. Elution of the appropriate section of the paper with ethanol followed by evaporation gave crude glycerol (9.2 mg.) as a yellow sirup.

To the sirup (9.2 mg.) dissolved in pyridine (1.0 ml.), *p*-nitrobenzoyl chloride (186 mg.) was added. The solution was heated at 80–85° for 1 hr., cooled and treated with a few drops of water to hydrolyze the excess *p*-nitrobenzoyl chloride. After standing for 1 hr., pyridine (0.5 ml.) was added and the mixture was poured into a cold 10% sodium bicarbonate solution (5.0 ml.) whereupon the glycerol tri-*p*-nitrobenzoate precipitated. The precipitate was removed, washed first with water, and then with methanol. Recrystallization of the product successively from chloroform, ethyl acetate and acetone gave glycerol tri-*p*-nitrobenzoate 14.4 mg., m.p. and mixed m.p. 196°. *Anal.* Calcd. for $C_{24}H_{17}O_{12}N_3$: C, 53.4; H, 3.2; N, 7.8. Found: C, 53.6; H, 3.5; N, 8.2.

Identification of D-Mannose, D-Arabitol and D-Glucose.—A suspension of urediospores (1.8 g.) in 72% sulfuric acid (2 ml.) was kept at 4° for 24 hr. The reaction mixture was diluted with water to an acid concentration of 2 *N* and heated (sealed tube) for 16 hr. on a boiling water-bath. The cooled solution was extracted with petroleum ether to remove lipids, neutralized ($BaCO_3$), filtered and evaporated *in vacuo* to a sirup (0.707 g.). This sirup which was shown by paper chromatography using ethyl acetate–acetic acid–water (3:1:3)[28] to contain arabitol, glycerol, glucose, mannitol and mannose, was resolved into its components by sheet paper (8 sheets 9″ × 22″, Whatman No. 3) chromatography for 58 hr. using the same solvent. The components were located by cutting a quarter inch strip from each side and the center, and spraying the strips with Tollens reagent.

Extraction of the appropriate areas of the paper with 50% aqueous ethanol gave D-mannose as a sirup which was purified by extraction with ethanol (yield 244 mg.). A portion (11 mg.) of this sirup was treated with *p*-nitroaniline (13 mg.) in methanol (0.2 ml.) containing a trace of hydrochloric acid for 5 min. at 60° as previously described.[38] The crystalline *p*-nitro-*N*-phenyl-D-mannosylamine was filtered, washed with methanol and dried (yield 4 mg.), m.p. and mixed m.p., 220°, $[\alpha]^{25}D$ −404° in pyridine (*c*, 0.2) changing to −330°.

The appropriate sections of the paper containing the D-arabitol were eluted with 50% aqueous ethanol. Evaporation of the solvent gave a sirup (70.8 mg.) of which a portion (49 mg.) was dissolved in acetic anhydride (1 ml.) containing 0.15% (w./w.) sulfuric acid and warmed for 15 min. at 60°. The solution was cooled, poured into ice-water and the product extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and evaporated to give crystalline D-arabitol pentaacetate[4] m.p. and mixed m.p. 76°, $[\alpha]^{25}D$ +37.5° in chloroform (*c*. 3) (after recrystallization from ethanol). *Anal.* Calcd. for $C_{15}H_{22}O_{10}$: C, 49.7; H, 6.1. Found: C, 49.6; H, 6.1.

The sections of the paper containing the D-glucose were extracted with 50% aqueous ethanol as described above to give a sirup (46 mg.) which was found to be contaminated with mannose. Accordingly a further quantity of urediospores was hydrolyzed as described above from which a crude sirup (2 g.) was obtained. Separation of D-glucose as a sirup (111 mg.) was attained by chromatographing 1-g. portions on a cellulose column 3.5 cm. diameter × 60 cm. length using ethyl acetate–acetic acid–water (3:1:3)[28] as the developing solvent. Since a preliminary experiment had shown that the *p*-nitroaniline derivative could not be prepared directly, the sirupy product, after decolorizing with carbon, was acetylated[39] by treatment with a mixture of acetic anhydride (0.5 ml.) and acetic acid (0.5 ml.) to which was added 70% perchloric acid (0.1 ml.). The reaction mixture was kept for 15 min. at room temperature and then warmed for 5 min. at 50°. After standing overnight at room temperature the reaction mixture was poured with stirring into ice-water (4 ml.) and the D-glucose pentaacetate extracted with chloroform. The chloroform extract was washed with a saturated solution of sodium bicarbonate, water and dried ($MgSO_4$). Evaporation gave a sirup (25 mg.) which failed to crystallize. The sirupy acetate was purified by chromatography on a column of Magnesol–

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

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

(34) M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(35) Marguerite Lambert and A. C. Neish, *Can. J. Res.*, **B28**, 83 (1950).

(36) W. W. Luchsinger, L. S. Cuendet, P. D. Boyer and W. F. Geddes, *Cereal Chem.*, **32**, 395 (1955).

(37) S. M. Partridge and R. G. Westall, *Biochem. J.*, **42**, 238 (1948).

(38) F. Weygand, W. Perkow and P. Kuhner, *Ber.*, **84**, 594 (1951).

(39) J. D. Nicholas and F. Smith, *Nature*, **161**, 349 (1948).

Celite (5:1, 24 g.) as previously described[40]. This procedure yielded crystalline α-D-glucose pentaacetate (7 mg.), m.p. and mixed m.p. 108°, $[\alpha]^{26}D$ +105° in chloroform (*c*, 1), after recrystallization from ethanol.

(40) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

Acknowledgment.—The authors thank Dr. James G. Dickson, University of Wisconsin, and Dr. Helen Hart, University of Minnesota, for their interest in this work and for samples of urediospores.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Stereochemistry of Some Resin Acid Derivatives[1]

BY ERNEST WENKERT AND JAMES W. CHAMBERLIN

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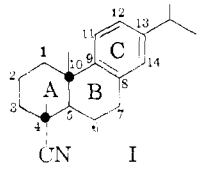
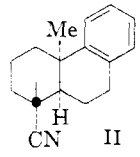
The stereochemistry of the minor product of the aluminum chloride-induced dealkylation of dehydroabietonitrile is presented. The oxidation products of both the major and minor deisopropyl compounds are discussed. The stereoconfiguration of C-13 in pimaric and isopimaric acids is portrayed and its biogenetic significance discussed.

Deisopropyldehydroabietonitrile.—A recent investigation of the aluminum chloride-induced dealkylation of dehydroabietonitrile (I) has shown that the major product of this reaction was 5-isodesoxypodocarponitrile enantiomer (II).[2] It became of interest to isolate and characterize the minor reaction products.

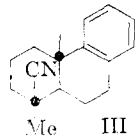


CN I

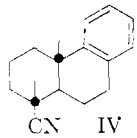


CN II

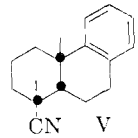
While attempted fractionation of the mother liquor from the crystallization of II failed, the physical behavior of the mixture suggested that it consisted of II and only one other compound. Precipitation from petroleum ether solution finally led to a new product, m.p. 104-105°, whose chemical and spectral analyses proved it to be a stereoisomer of II. Its non-identity with II and desoxypodocarponitrile (III)[2] indicated that it was either deisopropyldehydroabietonitrile (IV) or its 5-iso derivative V. Differentiation between these two possibilities appeared easy in view of the recently described diagnostic test for distinguishing A/B *trans* from A/B *cis* systems of the general structure of II–V.[2] Thus, the new deisopropyl product was exposed to a chromic acid oxidation under controlled conditions. The preponderant formation of a 7-keto product, and the absence of a 6,7-dione, indicated strongly the presence of an A/B *trans* configuration. Hence, the minor dealkylation product was considered to be the long-desired deisopropyldehydroabietonitrile (IV).



Me III



CN IV



CN V

(1) Parts of the first phase of this work were presented at the Ninth Annual Seminar in the Chemistry of Natural Products, University of New Brunswick, Fredericton, Canada, October 23–25, 1957. For a preliminary communication of the second phase *cf.* E. Wenkert and J. W. Chamberlin, THIS JOURNAL, **80**, 2912 (1958).

(2) E. Wenkert and B. G. Jackson, *ibid.*, **80**, 211 (1958).

Soon after starting an oxidative removal of the isopropyl group of dehydroabietonitrile (I)[3] as a means of confirming structure IV, we became aware of the work by Ohta and Ohmori on the deisopropylation of dehydroabietic acid,[4] which showed in most elegant and thorough manner that the reaction mixture consisted of two acids whose stereochemistry corresponded to our nitriles II and IV. A comparison of the physical properties of the hydrolysis product of nitrile IV with those of Ohta's minor acid constituent proved the identity of the dealkylation products.[5]

On the basis of a complete product analysis it now appears that the acid-catalyzed deisopropylation of a dehydroabietic system involves merely the rupture of the C(9)–C(10) bond, besides the cleavage of the isopropyl group, and a subsequent recyclization of the intermediate carbonium ion VI or its equivalent into an A/B *cis* or *trans* system. The amazing similarity in the product ratio of our dealkylation (43% *cis* and 9% *trans*) and that of Ohta (44% *cis* and 6% *trans*)[4] despite an appreciable difference in reaction conditions strongly suggests that the reaction is an equilibrium process and that the product ratio is a reflection of the slightly greater stability of the *cis* configuration in an octahydrophenanthrene of general structure I–V. This is in conformity with previously studied cases of similar structure.[6] Moreover, octahydrophenanthrene (VII) itself led exclusively to a *trans* compound on aluminum chloride-induced equilibration.[7] The presence of an angular methyl group thus strongly diminishes the difference of energy content between a *cis* and *trans* system.[8]

As part of the structural elucidation of II it has been demonstrated that its oxidation by chromic acid yielded a 7-keto compound, a 6,7-dione and an acid of unknown constitution.[2] Investigation of

(3) *Cf.* T. F. Sanderson (assigned to Hercules Powder Company) U. S. Patent 2,750,367 and 2,750,368.

(4) M. Ohta and L. Ohmori, *Pharm. Bull. (Japan)*, **5**, 91, 96 (1957).

(5) The authors are most grateful to Dr. Y. Suzuki of the Kowa Chemical Laboratories, Tokyo, for a mixed melting point determination.

(6) E. Wenkert and T. E. Stevens, THIS JOURNAL, **78**, 2318 (1956), and reference cited therein.

(7) J. W. Cook, N. A. McGinnis and S. Mitchell, *J. Chem. Soc.*, 286 (1944).

(8) *Cf.* R. B. Turner, THIS JOURNAL, **74**, 2118 (1952).