solution of 5.6 g. (0.10 mole) of potassium hydroxide in 5 ml. of water and 45 ml. of methanol was added and the mixture refluxed gently for 2 hr. After removal of the bulk of methanol under reduced pressure, the cooled solution was diluted with water, acidified and extracted with ether. Evaporation of ether and crystallization from methanol afforded 215 mg. (84%) of XVII as colorless needles, m.p. 174–176°. For analysis the product was recrystallized twice from methanol, m.p. 176–176.5°, $[\alpha]^{25}D$ +34° (*c* 0.32 in chloroform), λ^{KBr} 2.94 μ (3400 cm.$^{-1}$).

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 79.89; H, 12.17.

Cholestane-2β,3β-diol Acetonide (XVIII).—To 100 mg. of XVII, m.p. 174–176°, dissolved in 50 ml. of anhydrous acetone was added 5 ml. of acetone saturated with hydrogen chloride. The mixture was swirled 75 minutes, then poured into 200 ml. of 5% potassium carbonate solution. The oily precipitate was extracted with ether, washed with water, dried over magnesium sulfate and evaporated to dryness. Recrystallization from methanol yielded 91 mg. (84%) of the acetonide as colorless plates, m.p. 110–112°. Further recrystallization raised the melting point to 117–118°.

Anal. Calcd. for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 81.12; H, 11.55.

Diol (XIX).—A solution which was made of 94 mg. (0.0002 mole) of 3β-acetoxycholestane-2-one (XI), m.p. 144–145°, in 50 ml. of ether was added to a vigorously stirred solution of 150 ml. of liquid ammonia, 50 ml. of ether and 25 ml. of absolute methanol in a 500-ml. round-bottomed flask fitted with mechanical stirrer, dropping funnel and Dry Ice condenser. Cautiously, 3 g. of sodium was added in 0.2-g. portions over a period of 30 minutes, vigorous stirring being continued 30 minutes. After evaporation of liquid ammonia overnight, the mixture was acidified with 5% hydrochloric acid and the organic layer partitioned. The aqueous layer was extracted with ether, the combined ethereal layers washed with 5% sodium bicarbonate and water, dried over magnesium sulfate and evaporated to dryness. Recrystallization from methanol led to 74 mg. (86%) of XIX as colorless needles, m.p. 175–188°. A sample recrystallized from absolute methanol in needles, m.p. 182–183°, $[\alpha]^{25}D$ +37.2° (*c* 0.29 in chloroform), λ^{KBr} 2.94 μ (3400 cm.$^{-1}$).

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 79.91; H, 12.01.

A mixture of a sample of XIX with the *cis*-diol XVII, m.p. 174–176°, showed a marked depression in melting point, 150–165°.

Conversion of 3β-Acetoxycholestane-2-one (XI) to 3β-Hydroxycholestane-2-one (II).—A solution of 200 mg. (0.0001 mole) of the acetoxyketone XI, dissolved by heating in 100 ml. of absolute methanol, was cooled to room temperature and treated with 100 ml. of *N* potassium hydroxide solution. After storage for 36 hr. at room temperature, the mixture was diluted with water and acidified to litmus; the precipitated solid was taken up in ether, the ether washed with 5% sodium bicarbonate and evaporated to dryness. The residue, after recrystallization from aqueous methanol, yielded 47 mg. (26%) of colorless plates, m.p. 100–105°. After three rerystallizations from methanol the product melted at 104–105°, $[\alpha]^{25}D$ +64° (*c* 1.31 in chloroform).

A mixture of the product with a sample of the α-hydroxyketone II, m.p. 105–107°, from chromatography of acyloin VI gave no depression of m.p. 104–105°.

2-Acetoxycholestane-3-one (XXII).—A solution of 50 mg. of the α-ketol III, m.p. 120–125°, in 1 ml. of dry pyridine and 2 ml. of acetic anhydride was stored 12 hr. at room temperature. Lyophilization of the solvents and recrystallization of the residue from methanol afforded 35 mg. (65%) of the acetate XXII as colorless needles, m.p. 139–141°. A sample recrystallized from methanol for analysis melted at 147–149°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.17; H, 10.59.

A mixture of the acetate XXII with a sample of 3β-acetoxycholestane-2-one (XI), m.p. 144–145°, gave a marked depression in melting point, 125–137°.

2,3-Secocholestane-2,3-diol (XXI).—To a rapidly stirred slurry of 0.5 g. of lithium aluminum hydride in 200 ml. of anhydrous ether was added dropwise, over a period of 20 minutes, 500 mg. (0.001 mole) of the diester XV. Stirring was continued 30 minutes under a nitrogen atmosphere after final addition of the diester. Acidification and ether extraction led to 0.425 g. (98%) of XXI as colorless prisms, m.p. 155–156°, after recrystallization from methanol. A sample for analysis recrystallized from benzene–petroleum ether in fine needles, m.p. 155–156°, $[\alpha]^{24.9}D$ +5.2° (*c* 0.56 in chloroform), λ^{KBr} 2.99 μ (3300 cm.$^{-1}$).

Anal. Calcd. for $C_{27}H_{50}O_2$: C, 79.74; H, 12.39. Found: C, 79.89; H, 12.11.

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Galactomannan from Soy Bean Hulls[1,2]

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Water at 40° removes from acetone extracted soy bean hulls a galactomannan in 2% yield. The ratio of D-galactose units to D-mannose units is 2:3. Periodate analysis as well as examination of the products from hydrolysis of the methylated polysaccharide indicate the presence of a chain of 1 → 4 linked D-mannopyranose units with D-galactopyranosyl units joined to certain D-mannose units by 1 → 6 linkages. The structure is similar to guaran but is of lower molecular weight.

Large tonnages of crop residues are produced each year which are potential industrial raw materials. Thus a description of the amounts and of the chemical nature of polysaccharides present in crop residues would be helpful to prospective industrial users. This Laboratory has characterized some of the polysaccharides present in corn cobs and corn hulls, which are two readily available crop residues. Now attention is turned to soy bean hulls which are available at soy bean processing plants.

Acetone-extracted soy bean hulls contain 8% lignin, 64% alpha cellulose and 16% hemicelluloses extractable with alkaline solution. Of these hull hemicelluloses, one is found to be a galactomannan which is totally extractable in 2% yield by water at 40°. The galactomannans guaran and locust bean gum have attained significant industrial importance. Galactomannans from various sources exhibit quite different ratios[3] of D-galactose units to D-mannose units. Soy bean hull galactomannan has a D-galactose unit to D-mannose unit ratio of 2:3

(1) Presented before the Division of Carbohydrate Chemistry at the 131st Meeting of the American Chemical Society, Miami, Florida, in April, 1957.

(2) Journal Paper No. 1105 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

(3) R. L. Whistler and C. L. Smart, "Polysaccharide Chemistry," Academic Press Inc., New York, N. Y., 1953, p. 291; E. Anderson, *Ind. Eng. Chem.*, **41**, 2887 (1949).

and thus compares with guaran[3] which has a ratio of approximately 1:2 reported for the fractionated gum[4] and a ratio of 2:3 for the unfractionated gum.[5] Locust bean gum has a ratio[3,6] of about 1:4.

Soy bean galactomannan yields on acid hydrolysis only D-galactose and D-mannose which are chromatographically separated and obtained crystalline.

On periodate oxidation, the galactomannan consumes 1.2 moles of oxidant per mole of anhydrohexose unit and produces 0.37 mole of formic acid. These data support the view that the galactomannan structure, like guaran, consists of a principal chain of 1 → 4 linked β-D-mannopyranose units some of which have their primary hydroxyls linked with α-D-galactopyranosyl units. Such a molecule with a D-galactose unit to D-mannose unit ratio of 2:3 would be expected to consume 1.4 moles of periodate per anhydrohexose unit and produce 0.4 mole of formic acid per anhydrohexose unit.

Examination of the methylated soy bean galactomannan provides further evidence for its structural similarity to guaran. Hydrolysis of the fully methylated polysaccharide yields 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3-di-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose in a ratio of 2:2:1. Thus all of the D-galactose units are terminal and would appear as substituents on the mannan chain at positions C6 in such fashion as to form a one unit branch on the average for each 1.5 mannose units; however, this structural possibility is not the only one that is in agreement with the experimental findings. Partial hydrolysis of the underivified polysaccharide would be expected to yield the same oligosaccharides as obtained from guaran.[7,8]

The galactomannan has a low intrinsic viscosity and an osmotic molecular weight of 32,000.

Experimental

Isolation of the Galactomannan.—Commercial soy bean hulls were extracted with acetone for 24 hr. in a Soxhlet extractor and the hulls air dried. Lignin analysis showed the presence of 8.2% lignin. When the hulls were converted to holocellulose,[9,10] 21.5% was then extractable with a 10% solution of potassium hydroxide at 25°.

Acetone extracted hulls were extracted two successive times with fresh water (ratio of water to hulls 12:1) at *p*H 6.5 and at 40° for 16 hr. each. After filtration through cloth the combined extracts were acidified to *p*H 4.5 and centrifuged. The centrifugate was concentrated under reduced pressure to one-fourth the original volume, and the light yellow solution was poured into three volumes of absolute ethanol. The white precipitate was removed by centrifugation, resuspended four times in absolute ethanol and the final centrifuged precipitate freed of ethanol in a vacuum desiccator over calcium chloride, yield 2%. After hydrolysis in *N* sulfuric acid for 6 hr. at 100°, paper chromatography with ethyl acetate:pyridine:water (8:2:1 v./v.) as solvent and aniline phthalate as spray reagent showed the presence of galactose and mannose with trace amounts of arabinose and xylose.

Final purification of the polysaccharide was effected by two precipitations as the copper complex.[11] The white product contained 1.08% ash, showed a specific optical rotation of $[\alpha]^{25}D$ +65.0° (water, *c*, 1.0) and a limiting intrinsic viscosity in water of 0.35. On hydrolysis and paper chromatography, the only sugars indicated were galactose and mannose.

Isolation and Identification of Constituent Sugars.—Two grams of the polysaccharide were hydrolyzed 7.5 hr. in *N* sulfuric acid at 100°. During the hydrolysis, the optical rotation changed from $[\alpha]^{25}D$ +65.0°, initially, to a constant value of +41.4°. The final value corresponded to D-galactose and D-mannose mixed in the ratio of 2.1 to 3.0. After neutralization of the hydrolyzate with barium carbonate, the filtrate was passed through a column of cation exchange resin,[12] Amberlite IR 120. The neutral solution was evaporated to a colorless sirup, 2.14 g.

Eight hundred mg. of this sirup in 10 ml. of water was placed on a cellulose column (60 × 3 cm.) and irrigated with 2-propanol:water (9:1). Fractions were collected in an automatic collector.[13] Fractions were tested by paper chromatography, and those indicating the presence of one type of sugar were grouped and evaporated to sirups. The first sirup (0.488 g.) crystallized from ethanol to yield D-mannose; m.p. 129–130°; $[\alpha]_D^{25}$ + 15.2° (*c*, 1.0 equil. in water). When treated with phenylhydrazine crystalline D-mannose phenylhydrazone was obtained, m. p. 186–188°, with no change when mixed with an authentic specimen. The second sirup (0.275 g.) in ethanol produced crystals of D-galactose, m. p. 166–168°, unchanged on admixture with an authentic specimen. Its optical rotation was $[\alpha]^{25}D$ +79.2° (*c*, 1.0 equil. in water). Oxidation with nitric acid gave mucic acid, m.p. 216°.

An aliquot of the unchromatographed sirup from the above hydrolyzate was quantitatively analyzed[14] by paper chromatography. The paper was irrigated with ethyl acetate:pyridine:water and the sugars determined by the microferricyanide method of Hagedorn and Jensen.[15] D-Galactose was present to the extent of 39.6% and D-mannose to the extent of 60.4% (average of five separate determinations).

Periodate Oxidation.—Purified polysaccharide (0.52 g.) was dissolved in 25 ml. of water, and 50 ml. of sodium acetate–acetic acid buffer at *p*H 3.6 was added. Then there was added 50 ml. of 0.3 *M* sodium metaperiodate solution, and the mixture was allowed to stand in the dark at 25°. At regular intervals, 5 ml. aliquots were withdrawn and titrated for periodate. After 40 hr., the periodate consumption became constant at 1.15 moles per mole of anhydrohexose unit. After 130 hr., the excess of periodate was destroyed with ethylene glycol. The sample was dialyzed against distilled water for 24 hr., evaporated to dryness and hydrolyzed in *N* sulfuric acid for 7 hr. at 100°. The neutralized hydrolyzate was chromatographed on paper, but showed no evidence of either D-galactose or D-mannose.

A further sample of the galactomannan was oxidized by metaperiodate as described above. Formic acid liberated was determined by the iodometric method.[16,17] After 72 hr., no further formic acid was produced. At this point there was 0.368 mole of formic acid per mole of anhydrohexose units.

Galactomannan Acetate.—One gram of polysaccharide was acetylated[18] to produce an acetate (1.3 g.) softening at 235–237° and with an optical rotation of $[\alpha]^{25}D$ +55.1° (*c*, 1.0 in chloroform). Calcd.: CH_3CO, 44.8%. Found: CH_3CO, 44.5%.

Methylation of the Galactomannan.—Pure polysaccharide was methylated[19] by six successive treatments with potassium hydroxide and dimethyl sulfate and further methyl-

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ated by two successive treatments with methyl iodide and silver oxide in dimethylformamide,[20,21] The methylated galactomannan was fractionated from chloroform solution by gradual addition of hexane (b.p. 64–65°). The bulk of the material precipitated between hexane concentrations of 84 to 86%; $[\alpha]^{25}D$ +58° (*c*, 1.0 in chloroform); methoxyl content 44.1%.

Hydrolysis and Isolation of Methylated Sugars.—Two grams of methylated galactomannan were methanolized 10 hr. in methanol containing 5% hydrogen chloride to a constant optical rotation of $[\alpha]^{25}D$ +66.2°. The mixture was concentrated under reduced pressure to a sirup and after addition of water and boiling for 7 hr., again concentrated. The solution was neutralized with silver carbonate and deionized with Amberlite IR 120 H. The solution, free of silver and chloride ions, was concentrated to a sirup (2.0 g.). Chromatographed on paper with ethyl acetate:pyridine: water, as above, indicated three components with R_f values of 0.82, 0.77 and 0.57, respectively. An aliquot from the above hydrolyzate was quantitatively analyzed by paper chromatography. The paper was irrigated as above and the sugars determined with alkaline hypoiodite.[22] The results correspond to a ratio of tetra-*O*-methyl- to di-*O*-methyl- to tri-*O*-methyl-sugar of 2.1:2:1.

A portion (1.48 g.) of the methylated sugar sirup was separated on a cellulose column with benzene:ethanol:water (167:47:15 v./v.) as an irrigant and using an automatic fraction collector.[13] The first fraction of 0.604 g. with R_f of 0.82 and $[\alpha]^{25}D$ +101° (*c*, 1.0 in water) was 2,3,4,6-tetra-*O*-methyl-D-galactose. Calcd.: OCH_3, 52.5. Found: OCH_3, 52.5. When 25 mg. of the sirup was boiled with 15 mg. of distilled aniline in 10 ml. of absolute ethanol, there was obtained 28 mg. of N-phenyl-D-galactopyranosylamine 2,3,4,6-tetramethyl ether,[23] m.p. 192–193°. Calcd.: N, 4.5. Found: N, 4.6. The second effluent sirup of 0.257 g. with an R_f value of 0.77 had an optical rotation of $[\alpha]^{25}D$ +27.9° (*c*, 1.0 in ethanol) and was 2,3,6-tri-*O*-methyl-D-mannose. Calcd.: OCH_3, 41.8. Found: OCH_3, 42.7. On demethylation of 5 mg. in 1 ml. of 48% hydrobromic acid for 5 minutes at 100°, only mannose was obtained by paper chromatographic examination of the products. When 50 mg. of the methylated sirup was boiled with 0.18 of distilled aniline in 10 ml. of absolute ethanol, N-phenyl-D-mannopyranosylamine 2,3,6-trimethyl ether[24] was obtained; m.p. 128–130° unchanged when mixed with an authentic sample. When 0.1 g. of the methylated sirup was oxidized with 10 ml. of bromine water and the solution concentrated, 2,3,6-tri-*O*-methyl-D-mannonolactone was obtained as a sirup. This on boiling with phenylhydrazine in ethanol gave 2,3,6-tri-*O*-methyl-D-mannonic acid phenylhydrazide[25] (60 mg.), m.p. 130° unchanged on admixture with an authentic sample. Calcd.: N, 8.5. Found: N, 8.4.

The third effluent sirup of 0.453 g. with an R_f value of 0.57 had an optical rotation of $[\alpha]^{25}D$ +9.4° (*c*, 2.0 in ethanol) and was 2,3-di-*O*-methyl-D-mannose. Calcd.: OCH_3, 29.8. Found: OCH_3, 29.9. Oxidation with bromine water gave 2,3-di-*O*-methyl-D-mannonolactone,[26] m.p. 110–111°. Calcd.: OCH_3, 30.1. Found: OCH_3, 28.6. When the lactone was boiled with phenylhydrazine in ethanol, there was produced 2,3-di-*O*-methyl-D-mannonic acid phenylhydrazide,[26] m.p. 168–170°, and $[\alpha]^{25}D$ −22° (*c*, 0.5 in water). Calcd.: N, 8.9. Found: N, 9.0.

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LAFAYETTE, IND.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF WAYNE STATE UNIVERSITY]

Nitrogen Analogs of Ketenes. III. Formation of Hydroperoxides and Vinylamines by Reaction with Lithium Aluminum Hydride and Organometallic Reagents[1,2]

BY CALVIN L. STEVENS AND ROBERT J. GASSER[3]

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Reduction of diphenylketene-*p*-tolylimine (I) with lithium aluminum hydride gave the amino alcohol II by reaction *via* a hydroperoxide. The ketenimine I reacted with phenylmagnesium bromide to give a stable hydroperoxide III which could be reduced catalytically to the ketone imine IV or with lithium aluminum hydride to the amino alcohol V. Mesitylmagnesium bromide also yielded a stable hydroperoxide VI when allowed to react with I. Hydroperoxide III was cleaved by acid to give benzophenone (VII) and the toluide of benzoic acid (VIII), Mesitylphenylketene-*p*-tolylimine (IX) was reduced by lithium aluminum hydride to give the stable vinylamine X and reacted with mesityllithium to give the vinylamine XI.

Early in the investigation of the reactions of the nitrogen analogs of ketenes an attempt was made to characterize diphenylketene-*p*-tolylimine (I) by reduction with lithium aluminum hydride. The fact that the product was an amino alcohol prompted the present investigation on the course of this type of reaction and resulted in the isolation and characterization of the hydroperoxides III and VI and the stable vinylamines X and XI.

The reduction of the ketenimine I with lithium aluminum hydride in ether solution gave a 60% yield of crude amino alcohol II, which was characterized by analysis and an acetate derivative. The structure was established by independent synthesis from α-hydroxydiphenylacetic acid *p*-toluide by reduction with lithium aluminum hydride.

The introduction of an oxygen atom into the product suggested a probable reaction course involving reduction of the ketenimine linkage with one equivalent of hydride, followed by oxidation of the resulting metal salt of the vinylamine with molecular oxygen to form the hydroperoxide salt and then further reduction of the hydroperoxide linkage to the alcohol and of the ketone imine linkage to the amine.

The oxidation of an enolate salt formed from a ketone and a Grignard reagent to give a hydroperoxide has been studied by Kohler and Mydans.[4] Also, the alkoxide-catalyzed autoxidative cleavage

(1) Presented before the Organic Division at the 127th Meeting of the American Chemical Society, Cincinnati, Ohio, April, 1955, p. 13N.

(2) The preceding paper in this series was submitted by C. L. Stevens and J. C. French, THIS JOURNAL, **76**, 4398 (1954).

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