on nitric acid addition); rhamnose (Rosenthaler test[7]). All of these tests were negative.

The phenylosazone test was carried out with the hydrolyzed mucilage at 0° using freshly purified phenylhydrazine and glacial acetic acid. Pure D-mannose and D-glucose were used as a control. The hydrolyzed mucilage and D-mannose yielded a creamy white, crystalline precipitate. After standing for one hour in the cold the mannose phenylhydrazone was removed by filtration. The crystals, when examined under the microscope were homogeneous and had the same crystal form as stated by Hassid[8] for mannose phenylhydrazone. The phenylhydrazone of the mucilage hydrolyzate was recrystallized twice from 60% ethanol; m. p. 186–188°, unchanged on admixture with an authentic specimen of D-mannose phenylhydrazone.

After filtration of the mannose phenylhydrazone from the hydrolyzate, the solution was heated on the water-bath for thirty minutes and cooled to room temperature. At this point yellow colored crystals separated. These crystals had the same shape as a simultaneously prepared D-glucose phenylosazone; they were recrystallized twice from 60% ethanol; m. p. 205–206°, unchanged on admixture with an authentic specimen of D-glucose phenylosazone.

(7) R. Rosenthaler, *Z. anal. Chem.*, **48**, 165 (1909).

(8) W. Z. Hassid and R. M. McCready, *Ind. Eng. Chem., Anal. Ed.*, **14**, 683 (1942).

Quantitative Assay of Constituents.—Quantitative assays showed that essentially all of the sugars present in the mucilage hydrolyzate were aldoses with no fructose or other ketoses being present since the total reducing sugar as determined by the Hassid ferrocyanide method[9] was 87.80% and aldose sugars as determined by the Willstätter–Schudel[10] hypoiodite method was 89.0%. Uronic acid as determined by the method of Lefèvre and Tollens[11] was 2.37%.

Mannose was determined quantitatively by weighing the mannose phenylhydrazone (using freshly distilled phenylhydrazine and glacial acetic acid); 46.90% was found.

Summary

1. A mucin has been isolated from the medicinal plant *Aloe vera*. The substance has been shown to consist essentially of about equal parts of glucose and mannose together with a small amount of uronic acid.

(9) W. Z. Hassid, *ibid.*, **8**, 138 (1936); **9**, 228 (1937).

(10) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918); G. W. Pucher and M. W. Finch, *J. Biol. Chem.*, **76**, 331 (1928).

(11) K. V. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907).

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[CONTRIBUTION FROM THE DIVISION OF CHEMICAL ENGINEERING, UNIVERSITY OF MINNESOTA, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BIRMINGHAM, ENGLAND]

The Constitution of Carob Gum

BY F. SMITH

Carob gum is the galactomannan polysaccharide obtainable from the carob bean (*Ceratonia Siliqua L.*) by extraction of the seeds with water or aqueous alkaline solutions. It appears to be known also as Swine's bread, gum Hevo, gum Gatto, Jandagum, Lakoe gum, Lupogum, Luposol, Rubigum, Tragon and Tragasol. The bean is grown in the Mediterranean region and used as a food called St. John's bread. In Europe and the United States the gum from the seeds is used in the sizing of textiles, tanning of leather, in the paper industry and as a mucilage in pharmaceutical products. The nature of the polysaccharide carob gum forms the subject of this investigation and is one of a series directed to the study of plant gums and related substances.

The gum is shown herein to be composed of D-galactose (20%) and D-mannose (80%), the presence of which have already been established by Effront, Van Ekenstein, Bourquelot and Herissey and by Iglesias.[1,2,3,4] The polysaccharide resembles the plant mucilages in the manner in which it forms gels.[5] Like other mannan polysaccharides, for example yeast mannan,[6] carob gum forms a copper hydroxide complex when treated with Fehling solution; it appears also to be sensitive to borates.[5,7]

Compared with ivory nut mannan,[8] carob gum was found to be relatively easy to hydrolyze and from the cleavage products α-methyl-D-mannoside was isolated in good yield. For this reason it has been recommended as an excellent source of D-mannose.[9] The presence of D-mannose in the hydrolysis product of carob gum was also established by the production in good yield of the phenylhydrazone and the anilide of mannose; D-galactose can be identified as a constituent of the gum by nitric acid oxidation of either the acid hydrolysis products of the gum or of the gum itself.

Graded hydrolysis of the polysaccharide gum with 0.2 *N* sulfuric acid afforded D-galactose and a mixture of oligosaccharides which appeared to be of varying molecular size. There was no arrest point in the hydrolysis of the carob gum as is the case with plant gums such as gum arabic,[10] damson gum,[11] cherry gum[12] and mesquite gum,[13] and there appears to be no evidence to support the view that the rest of the molecular complex re-

(1) J. Effront, *Compt. rend.*, **125**, 38, 309 (1897).

(2) A. Van Ekenstein, *ibid.*, **125**, 719 (1897).

(3) E. Bourquelot and H. Herissey, *ibid.*, **129**, 228, 391 (1899).

(4) G. Iglesias, *Anales soc. espan. fis. quim.*, **33**, 114 (1935).

(5) R. Hart, *Ind. Eng. Chem., Anal. Ed.*, **2**, 329 (1930).

(6) W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 784 (1937).

(7) A. L. Williams, *Analyst*, **53**, 411 (1928).

(8) C. S. Hudson, "Organic Syntheses," Coll. Vol. I, 1946, p. 371.

(9) F. Smith, *J. Chem. Soc.*, in press.

(10) F. Smith, *ibid.*, 744 (1939).

(11) E. L. Hirst and J. K. N. Jones, *ibid.*, 1174 (1938).

(12) J. K. N. Jones, *ibid.*, 558 (1939).

(13) E. Anderson and Louise Otis, THIS JOURNAL, **52**, 4461 (1930).

mains unimpaired and contains only mannose units; indeed the degraded gum did not appear to be homogeneous and still contained galactose as well as mannose residues.[14]

Methylation of the gum can be effected directly or the gum may be converted first into its acetate and the latter then methylated with methyl sulfate and sodium hydroxide. Fractionation of that portion of the gum soluble in water and the methyl derivative indicated that both galactose and mannose residues were integral parts of the molecule.

When the methylated carob gum was subjected to methanolysis, there resulted a mixture consisting of the glycosides of 2,3,4,6-tetramethyl-D-galactose (1 part), 2,3,6-trimethyl-D-mannose (2–3 parts) and 2,3-dimethyl-D-mannose (1 part).

The structure of these cleavage fragments is based upon the following experimental facts. Hydrolysis of the methyl-tetramethylgalactoside afforded crystalline 2,3,4,6-tetramethyl-D-galactopyranose and this in turn yielded the characteristic crystalline anilide.[15]

The trimethyl-D-mannose obtained from the glycoside by acid hydrolysis gave a crystalline anilide which was identical with 2,3,6-trimethyl-D-mannose anilide.[16,17] The 2,3,6-trimethyl-D-mannose was further characterized by its transformation into a crystalline γ-lactone from which a crystalline amide and a crystalline phenylhydrazide[17] were derived. It was also established that oxidation of the trimethylmannono-γ-lactone with nitric acid gave rise to inactive dimethoxysuccinic acid identified as its methyl ester and amide.[18]

The methyl-dimethylglycoside was shown to be a derivative of methyl-D-mannopyranoside by reason of the fact that upon methylation it gave methyl-2,3,4,6-tetramethyl-D-mannoside, the latter being identified by hydrolysis and formation of crystalline 2,3,4,6-tetramethyl-D-mannose anilide. Further support for the view that the methyl-dimethylglycoside was a pyranoside was provided by the observation that it underwent relatively slow hydrolysis with *N* sulfuric acid. The dimethylmannose thus formed was smoothly converted into a crystalline γ-lactone from which it followed that a free hydroxy group must be present at C_4 in the dimethyl mannose. Since these observations demonstrated that positions 4 and 5 were not occupied by methyl groups it was deduced that the two methyl groups must be in positions 2,3, 2,6, or 3,6. The dimethyl-D-mannono-γ-lactone and the phenylhydrazide obtained from it were found to be identical with the dimethylmannono-lactone and the corresponding phenylhydrazide, the former of which was prepared by Goodyear and Haworth from a monoacetone-dimethyl-D-mannono-γ-lactone.[19] The structures of the dimethyl-monoacetone-mannono-γ-lactone and the dimethyl-mannono-γ-lactone derived from it were not established by these authors but it is apparent from the mode of acetone condensation with mannono-γ-lactone that the acetone group will engage either C_5 and C_6 or C_2 and C_3. It follows therefore that the methyl groups must be located either at C_2 and C_3 or C_5 and C_6. Positions 5 and 6 have already been precluded by the previous evidence and hence the conclusion was reached that the methyl groups must be at C_2 and C_3.

The main structural features of the galactomannan to be deduced from the isolation and characterization of 2,3,4,6-tetramethyl-D-galactose (1 part), 2,3,6-trimethyl-D-mannose (2–3 parts), and 2,3-dimethyl-D-mannose (1 part) are as follows: the formation of 2,3,6-trimethyl-D-mannose could be obtained from D-mannofuranose units joined by 1:5-glycosidic linkages or from D-mannopyranose residues joined by 1,4 linkages. The relative stability of the polysaccharide seems to favor the latter structure. Since this work was completed, support for the presence of 1:4 linkages in this gum and also in the galactomannan of guar seed *Cyamposis tetragonalaba* (*psoralioides*) has also been deduced by other workers from the results of periodate oxidation.[20] The 2,3,4,6-tetramethyl-D-galactose must arise from galactose units which form the end of the side chains present in what must be a highly branched structure. The 2,3-dimethyl-D-mannose will be formed from those mannopyranose units of the main mannose chain to which are attached the branching side-chains terminated by galactose units. This would then lead to the suggestion that the side chains are attached to position 6 of the mannose unit which gives rise to 2,3-dimethyl-D-mannose. Such a 1,6-linkage is present in the amylopectin fraction of starch,[21,22,23,24,25] and it is not unlikely that 1,6-linkages are also present in glycogen. The simplest structure for the repeating unit of carob gum is shown in formula (I). It consists of a chain of D-mannopyranose units joined by 1,4 glycosidic bonds (believed to be mainly of the β-variety in order to explain the low specific rotation of the polysaccharide and its methyl derivative) and to this mannose chain are attached side chains of D-galactopyranose units, each one separated from the next by 2–3 mannose residues.

(14) *Cf.* B. W. Lew and R. A. Gortner, *Arch. Biochem.*, **1**, No. 3, 325 (1943).

(15) J. C. Irvine and D. McNicoll, *J. Chem. Soc.*, **97**, 1449 (1910).

(16) W. N. Haworth, E. L. Hirst and H. R. L. Streight, *ibid.*, 1349 (1931).

(17) F. Klages, *Ann.*, **509**, 159 (1934); *ibid.*, **512**, 185 (1935).

(18) F. Smith, *J. Chem. Soc.*, 571 (1944).

(19) E. H. Goodyear and W. N. Haworth, *ibid.*, 3136 (1927).

(20) O. A. Moe, S. E. Miller and Marjorie H. Iwen, THIS JOURNAL, **69**, 2621 (1947).

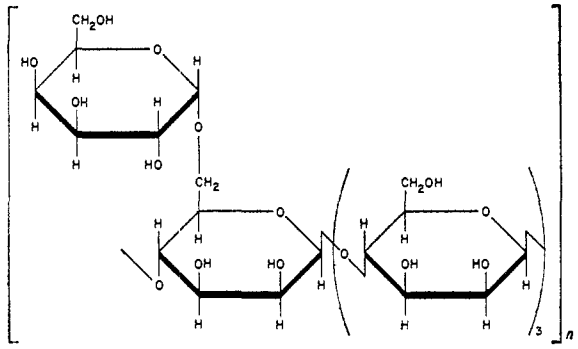
(21) C. C. Barker, E. L. Hirst and G. T. Young, *Nature*, **147**, 296 (1940).

(22) K. Freudenberg and H. Boppel, *Naturwiss.*, **28**, 2641 (1940).

(23) K. Myrbäck and K. Ahlborg, *Biochem. Z.*, **307**, 60 (1940).

(24) L. W. Georges, I. L. Miller and M. L. Wolfrom, THIS JOURNAL, **69**, 473 (1947).

(25) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **69**, 2249 (1947).



I

Variations of this structure are possible in which one or more of the mannopyranose units of the main chain, which give rise to 2,3,6-trimethyl-D-mannose, are interposed between the side chain galactose residue and that residue of the main chain which gives rise to the 2,3-dimethyl-D-mannose. It is also conceivable that the mannose residue at which branching occurs may be involved in union with a mannose unit through position 6 and with the galactose terminated side chains through position 4.

Experimental

The carob gum used in these experiments was a white powder (moisture content 9%) which dissolved in water giving a neutral mucilaginous solution containing some insoluble particles. The latter consisted of carbohydrate material and a nitrogenous substance which showed a positive test for proteins. The presence of galactose was established by the fact that the gum yielded mucic acid upon oxidation with nitric acid. Dialysis of an acidified (hydrochloric acid) solution of the gum against water for two days, followed by precipitation of the gum with alcohol, afforded a neutral product thus showing the absence of acid groups in the gum.

Hydrolysis of the gum (10 g.) by heating for two hours at 95° with *N* sulfuric acid afforded a mixture of reducing sugars ($[\alpha]^{18}D$ +22.5° in water) from which there was produced by the usual procedure D-mannose phenylhydrazone, m. p. 194° (10.7 g.). In two other experiments the mixture of sugars from 10 g. of carob gum yielded D-mannose anilide (5.5 g.) (m. p. 178°, $[\alpha]^{18}D$ −170° in pyridine (*c* 1.0)) and a α-methyl-D-mannopyranoside (5.1 g.) (m. p. 195°, $[\alpha]^{25}D$ +78° in water (*c* 0.8)), respectively. Fractional precipitation of that portion (65%) of the gum soluble in cold water by the addition of alcohol indicated that the material was essentially homogeneous. Each of the three fractions so obtained showed $[\alpha]^{20}D$ +9° in *N* sodium hydroxide and the mixture of sugars ($[\alpha]^{18}D$ +20° in water) obtained from each fraction contained both D-mannose (established by the preparation of the characteristic phenylhydrazone, anilide and α-glycoside) and D-galactose (mucic acid test). The portion (35%) of the gum insoluble in cold water which showed $[\alpha]^{20}D$ −4.5° in *N* sodium hydroxide contained D-mannose (the mixture of sugars $[\alpha]^{18}D$ +16° produced by hydrolysis with *N* sulfuric acid afforded D-mannose phenylhydrazone, m. p. 194°, $[\alpha]^{20}D$ +20° in pyridine), but repeated tests for galactose by the mucic acid test were negative.

Graded Hydrolysis of Carob Gum.—(a) The gum (20 g.) was hydrolyzed by heating for one hour at 80° with 0.35 *N* sulfuric acid. Neutralization (barium carbonate) and concentration to 500 cc. followed by the addition of methanol (1500 cc.) gave a degraded gum product (9 g.) which gave a copper hydroxide complex with Fehling solution and showed slight reducing activity. Removal of solvent from the aqueous methanolic mother liquor furnished a glassy residue (8.82 g., $[\alpha]^{20}D$ +17.5°, mol. wt. 590 by the hypoiodite method[26,27]) which reduced Fehling solution actively. A portion (4.5 g.) of this glassy solid gave upon methylation with methyl sulfate methyl-2,3,4,6-tetramethyl-D-galactoside 0.42 g., b. p. (bath temp.) 130–150° (0.02 mm.), $[n]^{27}D$ 1.4483, OCH_3 58.0, converted by hydrolysis to 2,3,4,6-tetramethyl-D-galactose (anilide, m. p. and mixed m. p. 200°, OCH_3, 38.6). The other portion (4 g.) of the glassy residue which did not show a positive test for mannose upon addition of phenylhydrazine in dilute acetic acid yielded α-methyl-D-mannoside (2 g.), m. p. and mixed m. p. 195° when boiled with 1% methanolic hydrogen chloride.

(b) Hydrolysis of the gum (6 g.) for seventy-five minutes at 95° with 0.2 *N* sulfuric acid (100 cc.)[14] followed by neutralization (barium carbonate), filtration and addition of ethanol (700 cc.) gave a precipitate of degraded gum (1.7 g., $[\alpha]^{25}D$ +10° in water (*c*, 1.0), after two reprecipitations from water by ethanol), which contained not only mannose (it gave α-methyl-D-mannoside 0.65 g. m. p. and mixed m. p. 196°, when boiled with methanolic hydrogen chloride) but also galactose residues (0.14 g. mucic acid m. p. 218° dec. was obtained from the product in the mother liquors after separation of the α-methyl-D-mannoside). Removal of solvent from the first aqueous ethanolic mother liquors (those obtained during the above reprecipitations was not utilized) gave a colorless glassy solid. To a solution of the latter in water (10 cc.) methanol (150 cc.) was added giving a precipitate (1.06 g.) of degraded gum which was discarded. Removal of solvent from the methanolic mother liquor furnished a glassy solid (2.05 g.); this strongly reducing product did not contain any free mannose (negative phenylhydrazine test) but when boiled with 3% methanolic hydrogen chloride it yielded α-methyl-D-mannoside (1.15 g.) m. p. 196°, $[\alpha]^{25}D$ +75° in water (*c*, 1.3). Oxidation of the product from the glycoside mother liquors with nitric acid gave mucic acid (0.315 g.), m. p. 215° dec.

Carob Gum Acetate.—A solution of carob gum (5 g.) in formamide (100 cc.) was added during one hour to a mixture of pyridine (100 cc.) and acetic anhydride (80 cc.).[28] After warming for three hours at 60° the viscous solution was kept overnight and poured with stirring into water. The precipitated acetate was filtered, washed with dilute hydrochloric acid, water and dried. The fibrous product was reacetylated by heating for two hours at 70° a solution of it in pyridine (100 cc.) with acetic anhydride (80 cc.), yield 6.9 g., $[\alpha]^{20}D$ +14° in acetone.

Methylated Carob Gum.—(a) The acetate (13.6 g.) was stirred with acetone (300 cc.) and methylated with methyl sulfate (140 cc.) and 30% sodium hydroxide (400 cc.) in the usual way. After completion of the methylation, acidification of the reaction mixture with 5 *N* sulfuric acid precipitated the methylated gum. The methylated gum was then subjected to two more methylations in each of which methyl sulfate (140 cc.) and sodium hydroxide (300 cc.) were employed. Completion of each of these methylations by heating the reaction mixture on the boiling water-bath resulted in the precipitation of the methylated gum as white nodules.

A solution of the crude methylated carob gum in a mixture of water (150 cc.) and acetone (40 cc.) was subjected to dialysis in cellophane tubes against water for two days. Evaporation of the solution gave the methyl gum as a tough glassy material (6.5 g.). The methyl gum dissolves slowly in formamide, acetone, chloroform and dioxane to give viscous solutions; it is insoluble in ether and light petroleum. (Found: OCH_3, 44.0). Fractional precipitation from acetone solution with ether showed the methyl gum to be essentially homogeneous, $[\alpha]^{18}D$ −4° in acetone (*c* 0.5), $[\alpha]^{18}D$ −2° in dioxane (*c*, 1.0), OCH_3, 44.1.

(26) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).

(27) M. Bergmann and H. Machemer, *ibid.*, **63**, 316 (1930).

(28) J. F. Carson and W. D. Maclay, THIS JOURNAL, **68**, 1015 (1946).

(b) A well-stirred solution of carob gum (10 g.) in sodium hydroxide (400 cc. of 30%) was treated with methyl sulfate (140 cc.) during five hours; no heat was applied. The viscous reaction mixture was then heated to 60° while methyl sulfate (70 cc.) and sodium hydroxide (200 cc. of 30%) were added simultaneously during two hours. The reaction was completed and the product subjected to three further methylations as in (a) (yield 7 g., after purification by dialysis). The application of seven further methylations did not increase the methoxyl content of the methyl gum.

Methanolysis of Methylated Carob Gum.—Preliminary experiments demonstrated that complete cleavage could not be effected with 2% methanolic hydrogen chloride at room temperature ($[\alpha]^{20}D$ −7° changing to zero in three hours) whereas with boiling 1% methanolic hydrogen chloride the reaction was accomplished in two hours (approx.) (final $[\alpha]^{20}D$ +65°).

The methyl gum (9.48 g.) was transformed by methanolysis into a mixture of glycosides which, after being freed from hydrochloric acid (by silver oxide) and from solvent, gave upon fractional distillation: Fraction I (2.572 g.), b. p. (bath temp.) 125–128° (0.1 mm.), $n^{14.5}D$ 1.4550, $[\alpha]^{16}D$ +93° in water (*c*, 0.8), OCH_3 59.1; Fraction II (3.185 g.), b. p. (bath temp.) 140° (0.1 mm.), $n^{14.5}D$ 1.4630, $[\alpha]^{16}D$ +29.5° in water (*c*, 0.6), OCH_3 51.7; Fraction III (1.245 g.), b. p. (bath temp.) 140–170° (0.1 mm.), $n^{14.5}D$ 1.4655, OCH_3 51.4; Fraction IV (1.12 g.), b. p. (bath temp.) 170–190° (0.1 mm.), $n^{14}D$ 1.4750, OCH_3 43.6; Fraction V (from a small distilling flask) (0.09 g.), b. p. (bath temp.) 170° (0.1 mm.), $n^{17}D$ 1.4770. There was an undistillable residue of 0.42 g.

Fractions I and II were recombined (5.66 g.) and slowly distilled giving: Fraction VI (1.915 g.), b. p. (bath temp.) 112–114° (0.1 mm.), $n^{19}D$ 1.4515, $[\alpha]^{18}D$ +107° in water (*c*, 1.2), OCH_3 60.2; Fraction VII (0.772 g.), b. p. (bath temp.) 120° (0.1 mm.), $n^{19}D$ 1.4605, $[\alpha]^{18}D$ +36° in water (*c*, 1.0), OCH_3 53.1; Fraction VIII (2.57 g.), b. p. (bath temp.) 120° (0.1 mm.), $n^{19}D$ 1.4616, $[\alpha]^{18}D$ +26° in water (*c*, 0.9), OCH_3 52.6. The residue in the flask (0.33 g.) had $n^{19}D$ 1.4685 (OCH_3 46.7).

Assuming that the refractive indices of the methyl tetra-, methyl tri-, and methyl dimethyl-glycosides are $n^{19}D$ 1.4515, 1.4616 and 1.4730, respectively, the above fractions (III, IV, V, VI, VII, VIII) and the residue (0.33 g.) contained methyl 2,3,4,6-tetramethyl-D-galactoside (1.999 g.), methyl 2,3,6-trimethyl-D-mannoside (4.382 g.) and methyl-2,3-dimethyl-D-mannoside (1.661 g.) approx. The mole ratio of the methyl tetramethyl-, methyl trimethyl- and methyl-dimethyl-glycosides was 1:2.3:0.95. The amount of methyl-2,3,4,6-tetramethyl-D-galactoside corresponded to 23.6% of the methylated gum from which it followed that the unmethylated gum contained 21% (approx.) of galactose; the rest (79%) of the complex was composed of mannose residues. These figures approximate to those previously reported.[3,4,29] In another experiment the mole ratio of tetra-, tri- and dimethyl glycosides was found to be 1:2.7:0.6 approximately and the amount of methyl 2,3,5,6-tetramethyl-D-galactoside corresponded to 23% of the methyl gum.

Identification of 2,3,4,6-Tetramethyl-D-galactose.—Hydrolysis of Fraction VI (1.1 g.), with *N* sulfuric acid in the usual way afforded 2,3,4,6-tetramethyl-D-galactose (0.939 g.), b. p. (bath temp.) 135–140° (0.1 mm.); $n^{20}D$ 1.4680, m. p. 74–5° (from ether), $[\alpha]^{18}D$ +102° changing to +83° equilibrium value in water (*c*, 0.8) (found: OCH_3, 52.6); anilide, m. p. and mixed m. p. 194°, $[\alpha]^{18}D$ −140° in pyridine (*c*, 0.5) (after recrystallization from ethyl alcohol) (Found: OCH_3, 40.0).

Identification of 2,3,6-Trimethyl-D-mannose.—Hydrolysis of the methyl trimethyl-D-mannoside (Fraction VIII, 2.5 g.) with 2 *N* sulfuric acid in the usual manner gave 2,3,6-trimethyl-D-mannose (2.3 g.), $[\alpha]^{18}D$ −6.5° in water (*c*, 0.6) (Found: OCH_3, 41.2). The trimethyl-D-mannose failed to give a crystalline osazone.

(29) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 28 (1944).

Two treatments of the trimethyl-D-mannose (0.45 g.) with silver oxide and methyl iodide afforded methyl-2,3,4,6-tetramethyl-D-mannoside (0.42 g.), b. p. (bath temp.) 130° (0.3 mm.), $n^{28}D$ 1.4460, $[\alpha]^{28}D$ −26° in water (*c*, 0.7) (Found: OCH_3, 60.1). Hydrolysis of this product with 1.3 *N* sulfuric acid gave 2,3,4,6-tetramethyl-D-mannose (0.27 g.), b. p. (bath temp.), 155° (0.5 mm.), $n^{25}D$ 1.4573, $[\alpha]^{25}D$ +24° in methyl alcohol (*c*, 1.1) (Found: OCH_3, 52.2); anilide, m. p. and mixed m. p. 145° (after crystallization from petroleum ether–ethyl alcohol) (Found: OCH_3, 39.8).

Treatment of the 2,3,6-trimethyl-D-mannose with ethyl alcoholic aniline (it is important to use only 1 mole of aniline) gave the anilide,[16,17] m. p. and mixed m. p. 131° (from ethyl alcohol or ethyl alcohol–petroleum ether), $[\alpha]^{18}D$ −155° changing to −39° in methyl alcohol (*c*, 0.3) (Found: C, 60.4; H, 7.8; N, 4.8; OCH_3, 30.8). One treatment of the anilide of 2,3,6-trimethyl-D-mannose with silver oxide and methyl iodide afforded 2,3,5,6-tetramethyl-D-mannose anilide, m. p. and mixed m. p. 144°.

When a solution of 2,3,6-trimethyl-D-mannose (1.5 g.) in water (4 cc.) was oxidized with bromine (2 cc.) at room temperature the corresponding lactone was produced. Isolation by the usual procedure gave 2,3,6-trimethyl-D-mannono-γ-lactone (1.0 g.), b. p. (bath temp.) 170–180° (0.1 mm.), m. p. 84–5°, (from ethyl acetate–ether) $[\alpha]^{18}D$ +65.5° in water (*c*, 1.0) changing in one hundred and twenty days to +38.5°. The corresponding acid, generated in aqueous solution by addition of the calculated amount of dilute sulfuric acid to the sodium salt, showed $[\alpha]^{18}D$ −19.5° changing in twenty-three days to +39°. (*Anal.* Calcd. for $C_9H_{16}O_6$: C, 49.1; H, 7.3; OCH_3, 42.2; equiv. 220. Found: C, 49.2; H, 7.3; OCH_3, 42.2; equiv. 217).

The 2,3,6-trimethyl-D-mannono-γ-lactone yielded a phenylhydrazide,[17] m. p. 131° (from ethyl alcohol) $[\alpha]^{17}D$ −21° in water (*c*, 1.0) (Found: N, 8.5; OCH_3, 28.3) and an amide, m. p. 125° (from dioxane or dioxane–ether), $[\alpha]^{18}D$ −16° in water (*c*, 0.5). (*Anal.* Calcd. for $C_9H_{19}O_6N$: C, 45.5; H, 8.1; N, 5.9; OCH_3, 39.2. Found: C, 45.65; H, 8.15; N, 5.6; OCH_3 38.2.)

Oxidation of the 2,3,6-trimethyl-D-mannono-γ-lactone (0.2 g.) with nitric acid followed by esterification of the acidic product with diazomethane yielded inactive methyl dimethoxysuccinate (0.07 g.), b. p. (bath temp.) 160–170° (40 mm.), $n^{21}D$ 1.4350, m. p. and mixed m. p. 69° (from ether), and this in turn afforded the corresponding amide m. p. and mixed m. p. 258° dec. (from water).[18]

By the above procedures Fractions III and VIII were also shown to be composed principally of 2,3,6-trimethyl-D-mannose.

Identification of 2,3-Dimethyl-D-mannose.—One treatment of the methyldimethyl-mannoside (0.29 g., Fraction V) with silver oxide and methyl iodide gave methyl-2,3,4,6-tetramethyl-D-mannoside which was hydrolyzed and converted into the characteristic anilide, m. p. and mixed m. p. 144° (from petroleum ether) (Found: OCH_3, 39.9).

When the methyl-dimethyl-mannoside (0.7 g.) was hydrolyzed for twenty-five hours at 95° with *N* sulfuric acid the rotation changed from $[\alpha]^{20}D$ +20° to −10°. Isolation of the product in the usual way afforded 2,3-dimethyl-D-mannose (0.63 g.) as a liquid, $[\alpha]^{18}D$ −9° in water (*c*, 2.0) (Found: OCH_3, 31.0).

Oxidation of the dimethyl mannose (0.6 g.) with bromine at room temperature yielded 2,3-dimethyl-D-mannono-γ-lactone (0.4 g.), b. p. (bath temp.) 160–170° (0.1 mm.), $n^{22}D$ 1.4640, which crystallized from ethyl alcohol, m. p. 111° alone or in admixture with the dimethyl-mannono-lactone (m. p. 110°) of Goodyear and Haworth,[19] $[\alpha]^{18}D$ +61.5° in water (*c*, 1.0) changing in twenty-eight days to +52.5° (mutarotation incomplete). The 2,3-dimethyl-D-mannonic acid generated in solution by warming (50°) the lactone with 0.1 *N* sodium hydroxide followed by addition of the calculated amount of dilute sulfuric acid showed $[\alpha]^{19}D$ −31° changing to zero in three days. (*Anal.* Calcd. for $C_8H_{14}O_6$: C, 46.6;

H, 6.9; OCH_3, 30.1. Found: C, 46.7; H, 6.85; OCH_3, 30.0.)

The dimethyl-mannonolactone gave a phenylhydrazide $[\alpha]^{18}D$ −25° in water (*c*, 0.5), m. p. 158° (from methyl alcohol–ether) which crystallized upon cooling and then remelted at 168°. (*Anal.* Calcd. for $C_{14}H_{22}O_6N_2$: N, 8.9; OCH_3, 19.7. Found: N, 8.8; OCH_3, 20.0.) The phenylhydrazide prepared from 5 mg. of the lactone of Goodyear and Haworth[19] had m. p. 158°, the melt crystallizing and remelting at 168–169°. It gave no depression of the m. p. when in admixture with the phenylhydrazide of 2,3-dimethyl-D-mannonic acid obtained above. The 2,3-dimethyl-D-mannono-γ-lactone could be regenerated from its phenylhydrazide by the usual procedure.

Examination of the glycosides derived from methylated carob gum in two other experiments confirmed the above results and enabled the material, corresponding to Fraction V, to be identified as mainly methyl-2,3-dimethyl-D-mannoside. The intermediate fractions which distilled between methyl-2,3,4,6-tetramethyl-D-galactoside and methyl-2,3,6-trimethyl-D-mannoside were shown to be composed only of these two substances.

Preparation of 2,3,6-Trimethyl-D-mannose and its Derivatives.—Crude ivory-nut mannan was methylated with methyl sulfate and sodium hydroxide. A sample of methylated mannan (10 g.) which, after four methylations, had $[\alpha]^{18}D$ −52° in water (*c*, 0.7); OCH_3, 44.2,[17] was subjected to methanolysis with 3% methyl alcoholic hydrogen chloride. The mixture of glycosides (10.84 g.) was subjected to fractional distillation. A portion of the methyl-2,3,6-trimethyl-D-mannoside (4.31 g., $n^{15}D$ 1.4620) thus produced was transformed into 2,3,6-trimethyl-mannose $[\alpha]^{15}D$ −5.5° in water (*c*, 2.0), OCH_3, 41.3. From this trimethyl sugar there were isolated by the methods already given above: (a) 2,3,6-trimethyl-D-mannose anilide, m. p. 129° (after crystallization from ethyl alcohol), $[\alpha]^{15}D$ −150° in methyl alcohol (*c*, 0.5) changing to −38°. (Found: C, 60.65; H, 7.85; N, 4.6; OCH_3, 30.8.) (b) 2,3,6-Trimethyl-D-mannono-γ-lactone, m. p. 83–84° (after recrystallization from ethyl acetate–ether), $[\alpha]^{15}D$ +69° initial value in water (*c*, 1.5) changing in one hundred and fifty days to +38° (constant value). The acid, obtained by addition of dilute sulfuric acid to a solution of the sodium salt formed by warming the lactone with dilute sodium hydroxide, showed $[\alpha]^{18}D$ −18.5 changing in twenty-three days to +40°. (Found: C, 49.25; H, 7.3; OCH_3, 42.3.) (c) The amide of 2,3,6-trimethyl-D-mannonic acid, m. p. 125° (after crystallization from dioxane). (*Anal.* Calcd. for $C_9H_{19}O_6N$: OCH_3, 39.2. Found: OCH_3, 39.8.) (d) The phenylhydrazide of 2,3,6-trimethyl-D-mannonic acid, m. p. 131°, $[\alpha]^{18}D$ −18° in water (*c*, 1.7) (after crystallization from ethyl alcohol–ether). (Found: N, 8.4; OCH_3, 28.3.)

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Summary

Carob gum, a polysaccharide, obtained from the seeds of the carob bean, is composed of D-galactose (20%) and D-mannose (80%). The methylated gum, obtained either directly or through the acetate by the agency of methyl sulfate and sodium hydroxide, gives upon methanolysis the glycoside of 2,3,4,6-tetramethyl-D-galactose (1 part,) 2,3,6-trimethyl-D-mannose (2–3 parts) and 2,3-dimethyl-D-mannose (1 part). These constituents have been identified by the formation of crystalline derivatives. The structure of the galactomannan polysaccharide is discussed.

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The Papilionaceous Alkaloids. IV. *Baptisia perfoliata* (L.) R. Br.[1]

BY LÉO MARION AND FRANÇOIS TURCOTTE

Until recently the genus *Baptisia* has been in a confused state,[2] but *B. perfoliata* (L.) R. Br. is one of the well-established species and no doubt seems to have existed concerning its identity. However, no study of its alkaloid content had ever been made and the results of such an investigation are now reported. The plant contains at least six alkaloids, four of which, *i. e.*, *d*-sparteine, cytisine, N-methylcytisine and anagyrine are known, while the fifth, isolated in very small quantity, only, seems to be identical with alkaloid P_2, previously reported as occurring in *B. australis*.[3] The sixth, alkaloid P3, appears to be new and it is proposed to designate it as baptifoline; it is crystalline and forms easily crystallizable salts. Until further characterized it is best represented by $C_{15}H_{20}O_2N_2$.

Experimental

The plant used in this investigation was grown at the Dominion Experimental Farm, Ottawa, through the courtesy of Dr. H. A. Senn, whom we wish to thank. The dried and ground material (wt. 4285 g.) was extracted in soxhlets with methanol and the extract evaporated until the solvent was largely removed. The residue was diluted with water, made acid to congo red by the addition of hydrochloric acid and heated on the steam-bath for eight hours. The mixture was cooled, filtered with suction and the insoluble matter again heated with dilute hydrochloric acid, cooled and filtered. The combined filtrate was extracted repeatedly with ether (extract A). During this extraction a crystalline solid separated which was filtered and washed with water. The combined filtrate and washings was alkalized with ammonia and extracted with chloroform in a continuous liquid-liquid extractor (extract B).

Isolation of a Neutral Substance.—The solid substance that had separated was also obtained from ether extract (A) from which it crystallized on standing. After several recrystallizations from boiling methanol in which it is only sparingly soluble, the colorless, neutral substance

(1) Published as National Research Council Bull. No. 1731.
(2) Mary M. Larisey, *Ann. Missouri Botan. Gard.*, **27**, 119 (1940).
(3) L. Marion and J. Ouellet, THIS JOURNAL, **70**, 691 (1948).