[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Constitution of the Polysaccharide from Tamarind Seed[1]

BY E. V. WHITE AND P. S. RAO[1a]

RECEIVED JANUARY 15, 1953

The polysaccharide isolated from the seed kernel of *Tamarindus indica* has been converted into the methyl ether derivative and the latter subjected to methanolysis. The glycosidic components of the resulting sirup have been separated and identified as derivatives of 2,3,4-trimethyl-D-xylose (1.06 parts), 3,4-dimethyl-D-xylose (1.07 parts), 2,3,4,6-tetramethyl-D-galactose (1.02 parts), 2,3,6-trimethyl-D-glucose (0.86 part), and 2,3-dimethyl-D-glucose (1.96 parts). On the basis of these components consideration is given to structural arrangements which may describe the repeating unit of the macromolecule.

The flour produced from the seed kernel of the tamarind tree (*Tamarindus indica* Linn) has been used in India as a sizing agent for cotton and jute. It may have other commercial significance as, for example, in the manufacture of fruit spreads and as a low-grade foodstuff suitable both for human and animal consumption. In part, the flour is carbohydrate in character and yields a water-soluble polysaccharide[2] upon extraction; the residue is comprised of fat, protein and fiber. On the basis of its origin, the polysaccharide would normally be considered as a member of the mucilage group.[3,4] This classification, however, is not entirely satisfactory in that the molecule contains a large proportion of D-glucose residues, whereas most of the neutral mucilages thus far investigated appear to be constituted largely of sugar units other than D-glucose. Furthermore, the polysaccharide forms jellies with sugar concentrates under diverse conditions of hydrogen ion concentration,[5] a rather unusual property in the mucilage group. The name "Jellose"[6] has been suggested for the polysaccharide as being descriptive of both the jelly-forming property and its carbohydrate character.

Tamarind seed polysaccharide apparently contains no uronic acid residues since D-glucose, D-xylose and D-galactose have been identified as the only products of aqueous acid hydrolysis. The molecular ratio of these components, evaluated from the pentosan content and mucic acid produced upon oxidation, has been given as 3:2:1,[7] respectively.

The present study was designed to investigate the structural character of the polysaccharide and for this purpose the methyl ether derivative was prepared. This, upon treatment with methanolic hydrogen chloride in the usual manner, furnished a glycosidic sirup which was separated into its components by a combination of high-vacuum fractional distillation and chromatographic methods. The terminal units of the polysaccharide which, of course, are present in the ether derivative as completely alkylated units, were identified as those giving rise to 2,3,4-trimethyl-D-xylose (one part) and 2,3,4,6-tetramethyl-D-galactose (one part). The macromolecule must, therefore, be branched in character in order to provide side chains at two positions in the repeating unit structure. The origin of such branching is evidently to be found in certain of the glucose units and must take place at the 4- or 6-positions thereof, since 2,3-dimethyl-D-glucose (two parts) was isolated as the crystalline methyl glycoside from the methanolysis sirup. The remaining components of the sirup were found to be the glycosidic derivatives of 3,4-dimethyl-D-xylose (one part) and 2,3,6-trimethyl-D-glucose (one part). In the original polysaccharide the corresponding units giving rise to these compounds must have been united glycosidically with other parts of the molecule at the 2- and 4-positions, respectively. They described to a degree the linearity of the macromolecule. Presumably, the D-xylose moiety is located in a side chain terminated by D-xylopyranose, whereas the glucose unit may occupy a position in the main chain structure of the molecule. In this event, a 1,4-main chain linkage would seem probable.

On the basis of these five components, present in a molecular ratio closely approximating simple whole numbers, the repeating unit of the polysaccharide is limited to relatively few structural representations. Obviously, either all the glucose units are located in the primary chain of the molecule or, alternatively, some of these are resident in the branched system. In the former case, the repeating unit of the polysaccharide is of relatively simple structure comprising three glucose units presumably united glycosidically through the 1,4-positions to form the primary chain of the macromolecule with branched systems originating at position 6 in two of the units. One of the branched systems is a single, terminal unit of D-galactose while the second is composed of two units of D-xylose united by a 1,2-glycosidic linkage. On the other hand, if one or more of the glucose units are located in the branched systems other equally simple structures come under consideration. A suitable differentiation among these different structures necessitates the isolation of fission fragments such as may be obtained by partial hydrolysis or by enzymatic degradation of the polysaccharide.

Experimental Part

Extraction and Purification of Tamarind Seed Polysaccharide.—The starting material used in the present investigation was prepared at the Forest Research Institute, Dehra Dun, India, by one of us (P. S. R.), following a previously described procedure.[8] In brief, the tamarind seed was

(1) Presented at the Symposium on Hemicelluloses and Plant Gums, 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March 15-19, 1953.

(1a) Forest Research Institute, Dehra Dun, India.

(2) T. P. Ghose and S. Krishna, *J. Indian Chem. Soc., Ind. & News Ed.*, **5**, 114 (1942).

(3) A. G. Norman, "Biochemistry of Cellulose, Polyuronides, Lignin, Etc.," The Clarendon Press, Oxford, 1937, p. 134.

(4) J. K. N. Jones and F. Smith, *Advances in Carbohydrate Chem.*, **4**, 265 (1949).

(5) P. S. Rao, *J. Sci. Ind. Research (India)*, **7**, 89 (1948).

(6) P. S. Rao and S. Krishna, *Cur. Sci.*, **16**, 256 (1947).

(7) G. R. Savur and A. Sreenivsan, *J. Biol. Chem.*, **172**, 501 (1948).

(8) P. S. Rao, T. P. Ghose and S. Krishna, *J. Sci. Ind. Research (India)*, **4**, 705 (1946).

parched and the testa removed. The kernels were then broken to pea size, extracted with boiling water, and the combined extracts evaporated to a thin sirup under reduced pressure. The sirup was centrifuged to furnish a clear solution which was then treated with two volumes of ethanol. The resulting precipitate was removed, redissolved in water, and the solution again treated with alcohol. After several similar purifications, the product was filtered, washed successively with alcohol, ether and petroleum ether, dried at 60°, and ground to pass through an 80-mesh sieve.

Methylation of Tamarind Seed Polysaccharide.—Twelve and one-half grams of the polysaccharide was slurried to form a paste with 125 ml. of water in a three-necked flask equipped with an efficient sweep-stirring device and two graduated dropping funnels. Dimethyl sulfate (125 ml.) and 250 ml. of 30% sodium hydroxide were added dropwise and simultaneously over a period of four hours. The temperature of the reaction mixture was maintained at 25° by means of a water-bath. Acetone was added as necessary to facilitate stirring. After complete reaction of the reagents, the contents of the flask were dialyzed for two days against running water. The dialyzate was then filtered through Celite, concentrated under reduced pressure to a sirup, and remethylated under similar conditions. After the second treatment the partially methylated product became soluble in the acetone layer so that the aqueous layer containing the inorganic products of the reaction could be siphoned off and methylation continued. Five treatments furnished the fully methylated polysaccharide which was isolated as a precipitate upon warming a dialyzed solution thereof to 70°. The moist product was dissolved in acetone and dried at room temperature as a film on a glass surface: the yield was 10 g.; the product contained 42.5% OMe.

Methanolysis of Fully Methylated Tamarind Seed Polysaccharide and Fractional Distillation of the Glycosidic Sirup.—Ten grams of the fully methylated polysaccharide was dissolved in 400 ml. of absolute methanol and methanolic hydrogen chloride added slowly with swirling to 2% acid concentration. The reaction mixture was then heated under reflux for eight hours, cooled, the excess acidity was neutralized with silver carbonate, and the filtrate was concentrated to a sirup. The latter was dissolved in 500 ml. of anhydrous ethyl ether, filtered, and the solvent removed. The resulting sirup was distilled under vacuum (0.2 mm.) to furnish fraction I, b.p. 55–70° (2.28 g.); fraction II, b.p. 70–90° (2.70 g.); fraction III, b.p. 90–105° (1.21 g.); fraction IV, b.p. 105–130° (3.18 g.); and a still residue of 0.36 g.

Isolation of 2,3,4-Trimethyl-D-xylose.—Fraction I was redistilled and the major portion, b.p. 56–57° (0.2 mm.), collected. The distillate was then hydrolyzed with *N* sulfuric acid at 92° until the rotation of the solution became constant. The product, isolated in the usual manner, crystallized spontaneously upon distillation, b.p. 97° (0.2 mm.). Recrystallization from ethyl ether furnished 2,3,4-trimethyl-D-xylose,[9] m.p. 91°; $[\alpha]^{20}D$ 18° (*c* 2.5, equilibrium in water).

Anal. Calcd. for $C_8H_{16}O_6$: OMe, 48.5. Found: OMe, 49.0.

A sample of the compound, when treated with 1.1 moles of aniline dissolved in 3 ml. of absolute ethanol and heated under reflux for three hours, furnished the anilide of 2,3,4-trimethyl-D-xylose[10] upon removal of solvent and crystallization from ether; it melted at 102° alone or admixed with an authentic specimen.

Isolation of 3,4-Dimethyl-D-xylose.—Redistillation of fraction II provided a major portion with b.p. 77–80° (0.2 mm.). Five grams of the sirup was hydrolyzed with *N* sulfuric acid under reflux for five hours. The reaction mixture was then neutralized with barium carbonate and the product isolated in the usual manner. An examination of the sirup by paper chromatography indicated the presence of two compounds and further exploratory experiments demonstrated that these could be separated from each other on a column of Magnesol. Accordingly, 2.05 g. of the hydrolyzate was dissolved in 50 ml. of chloroform and chromatographed in 25-ml. portions on a No. 3 column of acid-washed Magnesol[11] using 175 ml. of 25:1 chloroform–ethanol as the developer in each instance. The columns were extruded, streaked, sectioned and eluted with acetone. Because of the tailing character of the two compounds a broad interzone was sectioned and this was rechromatographed under similar conditions. By combining the appropriate fractions there were obtained fraction II_t (0.83 g.); fraction II_m (0.43 g.); and fraction II_b (0.81 g.).

Fraction II_t was distilled under high vacuum to provide a colorless reducing sirup with b.p. 110° (0.2 mm.). Upon oxidation with 1 ml. of bromine in 10 ml. of water for 24 hours and isolation of the product by the usual methods, a non-reducing sirup was obtained. This was warmed under reduced pressure at 80° and extracted with anhydrous ether. The lactone of 3,4-dimethyl-D-xylonic acid[12] crystallized readily from the concentrated ether solution; m.p. 68°, $[\alpha]^{20}D$ −56° → −25° (*c* 3.71, equilibrium in water).

Anal. Calcd. for $C_7H_{12}O_5$: OMe, 35.2. Found: OMe, 35.2.

Isolation of 2,3,4,6-Tetramethyl-D-galactose.—Fraction II_b (0.81 g.) from the chromatographic column was distilled under high vacuum, b.p. 105° (0.2 mm.). The resulting sirup, $[\alpha]^{20}D$ 118° (*c* 3.36, equilibrium in water), was chromatographically pure but failed to crystallize. One hundred mg. of the sirup was treated with 1.1 moles of aniline in 3 ml. of absolute ethanol and heated under reflux for three hours. The product crystallized upon removal of solvent and recrystallization from ethanol furnished the anilide of 2,3,4,6-tetramethyl-D-galactose,[13] m.p. 196° alone or admixed with an authentic specimen.

Anal. Calcd. for $C_{16}H_{25}O_5N$: OMe, 39.9. Found: OMe, 39.7.

Fraction II_m when examined on the paper chromatogram proved to be a mixture of the compounds identified in fractions II_b and II_t.

Isolation of 2,3,6-Trimethyl-D-glucose.—Fraction III was redistilled and the major portion of the product collected at 98–102° (0.2 mm.). The resulting sirup (1.0 g.) was then dissolved in 10 ml. of *N* sulfuric acid and heated under reflux for five hours. After cooling, the solution was neutralized with barium carbonate, filtered, and evaporated to a sirup which was extracted with ethyl ether, treated with norite, and filtered. After removal of excess solvent the solution deposited crystals. Recrystallization from ethyl ether furnished 2,3,6-trimethyl-α-D-glucose,[14] m.p. 123° and unchanged upon admixture with an authentic specimen; $[\alpha]^{20}D$ 64° (*c* 2.0, equilibrium in water).

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 41.8.

The sirupy mother liquor (0.5 g.) was treated with 1 ml. of bromine in 10 ml. water for 24 hours at room temperature. The product, isolated in the usual manner and distilled, boiled at 110° (0.2 mm.) but failed to provide either the crystalline γ- or δ-lactone. When 0.1 g. of the distillate was treated in the usual manner with 1.1 moles of phenylhydrazine, the hydrazide of 2,3,6-trimethyl-D-gluconic acid[15] was obtained which, upon recrystallization from ether–methanol, melted at 145°.

Isolation of 2,3-Dimethyl-D-glucose.—Fraction IV crystallized spontaneously and recrystallization from ethyl ether furnished the well characterized α-methyl-2,3-dimethyl-D-glucopyranoside,[16] m.p. 85°, $[\alpha]^{20}D$ 145° (*c* 2.1, water).

One gram of the crystalline product was dissolved in 15 ml. of *N* hydrochloric acid and heated on a steam-bath until the rotation of the solution became constant. Silver carbonate was then added to neutralize the hydrochloric acid and, after filtering, silver ion was removed with hydrogen sulfide. The solution was then filtered, evaporated to a sirup under reduced pressure, and the product was extracted with ethyl acetate. Upon standing overnight, the concentrated solution deposited crystalline 2,3-dimethyl-α-D-glucose[16] with m.p. 121°, $[\alpha]^{20}D$ 50° (*c* 2.0, equilibrium in water).

Anal. Calcd. for $C_8H_{16}O_6$: OMe, 29.8. Found: OMe, 29.7.

Estimation of the Molecular Ratio of the Glycosidic Components Obtained by Methanolysis of Fully Methylated Tamarind Seed Polysaccharide.—A portion of the undis-

(9) F. P. Phelps and C. B. Purves, THIS JOURNAL, **51**, 2443 (1929).

(10) R. A. Laidlaw and E. G. V. Percival, *J. Chem. Soc.*, 1600 (1949).

(11) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **73**, 863 (1951).

(12) Sybil P. James and F. Smith, *J. Chem. Soc.*, 739 (1945).

(13) J. C. Irvine and D. McNicoll, *ibid.*, **97**, 1449 (1910).

(14) J. C. Irvine and E. L. Hirst, *ibid.*, **121**, 1213 (1922).

(15) H. C. Carrington, W. N. Haworth and E. L. Hirst, THIS JOURNAL, **55**, 1084 (1933).

(16) J. C. Irvine and J. P. Scott, *J. Chem. Soc.*, **103**, 575 (1913).

tilled methanolysis sirup (2.0 g.) was dissolved in 15 ml. of *N* sulfuric acid and heated for 10 hours on a boiling water-bath. The solution was then neutralized with barium carbonate, filtered, evaporated under reduced pressure to a sirup, and extracted with acetone. The components of the latter solution were separated by paper partition chromatography using the strip technique with ethyl acetate, acetic acid and water (9:2:2) as the developer. The appropriate sections cut from three separate chromatograms were combined, eluted, and the solution analyzed by the alkaline iodine method.[17] The average values from five determinations, calculated on the basis of six molar parts, were as follows: 2,3,4-trimethyl-D-xylose (1.06 parts), 3,4-dimethyl-D-xylose (1.07 parts), 2,3,4,6-tetramethyl-D-galactose (1.02 parts), 2,3,6-trimethyl-D-glucose (0.86 part) and 2,3-dimethyl-D-glucose (1.96 parts).

(17) A. E. Flood, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1679 (1948).

Acknowledgment.—One of us (P. S. R.) wishes to express his thanks to The Institute of Paper Chemistry for a Research Fellowship and to the Government of the United States for a Fulbright Travel Grant.

APPLETON, WIS.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

Crystalline Tetrabenzoyl-β-D-fructopyranosyl Bromide and its Reduction by Lithium Aluminum Hydride to 1,5-Anhydro-D-mannitol and 1,5-Anhydro-L-gulitol

BY ROBERT K. NESS AND HEWITT G. FLETCHER, JR.

RECEIVED JANUARY 23, 1953

Treatment of the known 1,3,4,5-tetrabenzoyl-β-D-fructose with hydrogen bromide in glacial acetic acid has led to the isolation of tetrabenzoyl-β-D-fructopyranosyl bromide in a crystalline form containing one quarter mole of acetic acid. The behavior of this substance in dioxane–methanol and in aqueous dioxane has been studied and supports the configuration assigned. Reduction of the halide with lithium aluminum hydride afforded a very small yield of 1,5-anhydro-D-mannitol and demonstrated that the bromine was at least in part replaced without inversion of configuration at carbon 2. The major product of the reduction, however, was a new hexitan, 1,5-anhydro-L-gulitol, which was produced by Walden inversion at carbon 2 upon replacement of the bromine by hydrogen. This new anhydride was characterized through its 4,6-benzylidene and 2,3-dibenzoyl-4,6-benzylidene derivatives. Its identity was confirmed by the synthesis of authentic corresponding derivatives of the D-series *via* the reduction of tetraacetyl-D-gulopyranosyl bromide with lithium aluminum hydride.

The relative instability of tetraacetyl-β-D-fructopyranosyl bromide and chloride[1] suggests that, as in the D-ribose series,[2,3] a more stable benzoyl analog might offer advantages for synthetic and other purposes. For the preparation of such an analog, the crystalline 1,3,4,5-tetrabenzoyl-β-D-fructopyranose (I), which Brigl and Schinle[4] prepared through the direct benzoylation of D-fructose, was employed. When this substance was treated with hydrogen bromide in glacial acetic acid there was obtained in 89% yield a crystalline tetrabenzoylhexosyl bromide containing one quarter mole of acetic acid of crystallization. Attempts to obtain the product in crystalline, solvent-free form were unsuccessful. By analogy with the known tetraacetyl-β-D-fructopyranosyl halides[1,5] the new bromide may be assigned structure III, tetrabenzoyl-β-D-fructopyranosyl bromide. Evidence adduced below confirms this assignment.

As with other benzoylated glycosyl halides previously studied in this Laboratory,[6] it was of interest to examine the behavior of this new bromide with methanol in the absence of an acid acceptor. Polarimetric observations of a solution of the halide in 1:9 (v./v.) dioxane–methanol disclosed the reaction to be of an approximately pseudo-unimolecular nature and yielded an average rate constant (minutes, decadic logs) of 0.057. Comparison of this rate with values previously obtained in a similar manner for other series may be made through examination of Table I where such constants are arranged in the order of decreasing magnitude. Consider first the benzoylated aldopyranosyl bromides. Both of those which possess a benzoyloxy group on carbon 2 *trans* to the halogen (β-D-ribose and α-L-rhamnose) and may thus react by a neighboring group mechanism[3] are more rapid than any of those halides which have a *cis* relationship on carbon 2 and presumably react by simple inversion only. While there is not a sharply defined gap between the two groups, the β-D-fructosyl halide clearly belongs in the more rapid one. Inspection of formula III shows that while the benzoyloxy group attached to carbon 3 is *cis* to the bromine and therefore would

TABLE I

RATES OF REACTION OF BENZOYLATED GLYCOPYRANOSYL BROMIDES WITH 1:9 DIOXANE–METHANOL AT 20° IN THE ABSENCE OF AN ACID ACCEPTOR

Series	*k* (min., decadic logs)	Benzoyloxy group available for participation in mechanism?
β-D-Ribose	0.076[a]	Yes
β-D-Fructose	.057	Yes
α-L-Rhamnose	.016[b]	Yes
α-D-Xylose	.013[c]	No
β-D-Arabinose	.011[c]	No
α-D-Ribose	.004[a]	No
α-D-Glucose	.00031[d]	No

[a] Ref. 3. [b] Ref. 8. [c] H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, 72, 4173 (1950). [d] Ref. 6.

(1) D. H. Brauns, THIS JOURNAL, 45, 2381 (1923).

(2) R. Jeanloz, H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, 70, 4055 (1948).

(3) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, 73, 959 (1951).

(4) P. Brigl and R. Schinle, *Ber.*, 66, 325 (1933). These authors assigned the structure given (I) since the substance gave methyl β-D-fructopyranoside tetrabenzoate (II) when methylated and showed a high negative rotation (−164.9°) similar to that of the previously known 1,3,4,5-tetraacetyl-β-D-fructose (−92.3°).

(5) C. S. Hudson, THIS JOURNAL, 46, 477 (1924).

(6) See R. K. Ness and H. G. Fletcher, Jr., *ibid.*, 74, 5344 (1952) and the references cited therein.