Monosaccharides were extracted by soaking and stirring appropriate sections in 5 ml. of water for 20 minutes and filtering the supernatant liquor into test-tubes. Papers were washed with 7 ml. of water and the washings combined with the supernatant liquors. This recovered 95–96.8% of the original material. Analysis with potassium ferricyanide[16] and comparison with known values indicated the composition of the polysaccharide (Table III).

Other Polysaccharides.—Water-soluble polysaccharides were isolated from the pulp, the mucus seed cover, and the seed placenta in yields of 0.17, 0.10 and 0.01% of the total fruit, respectively (dry weights). Hydrolysis of the polysaccharides produced the same monosaccharides as found in the husk and seed polysaccharide. In contrast to the seed polysaccharide, the placenta and mucus also contained traces of xylose.

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(16) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923).

TABLE III

COMPOSITION OF POLYSACCHARIDES

Monosaccharides	Seed polysaccharides, %	Husk polysaccharides, %
Rhamnose	36.29	37.97
Arabinose	28.81	13.27
Mannose (plus trace glucose)	12.02	9.98
Galactose	22.86	38.77

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Fractionation of Gum Arabic by Chemical and Immunological Procedures[1]

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Gum arabic, recovered from its specific precipitate with Type II antipneumococcus serum, contains only one-third to one-fifth as much rhamnose as does the native gum. Gum arabic is therefore a mixture, and no single over-all structural formula can properly be given. Several attempts at chemical fractionation of the gum were less successful and less specific than the serological method. In the chemical fractions the ratio of D-glucuronic acid to L-rhamnose was remarkably constant, but was much higher in the precipitates with antibody because of the loss of L-rhamnose. The Type II-precipitable portion of the gum does not differ greatly from the remainder with respect to its content of L-arabinose, D-galactose and D-glucuronic acid. In accord with this is the failure of Type XIV antipneumococcus serum to fractionate the gum. Precipitation in this instance is due to D-galactose residues in the gum.

Although the composition of gum arabic (GA), like that of all natural gums, has been known to vary within certain limits, definite evidence of inhomogeneity was lacking until recently. Beiser, Kabat and Schor[3] precipitated an acid-degraded sample of the gum with Type II antipneumococcus (anti-Pn) horse serum, noted that only a small portion of the rhamnose in the sample was to be found in the specific precipitate, and concluded that the material consisted "of a mixture of at least two substances, one containing methylpentose and unrelated to the specific polysaccharide of Type II pneumococcus (S II) and the other lacking methylpentose and yet cross reacting with anti-S II." It will be recalled that S II consists of L-rhamnose, D-glucose and D-glucuronic acid,[3–5] while GA contains L-rhamnose, L-arabinose, D-galactose and D-glucuronic acid.[6] The cross reaction between GA and Type II anti-Pn serum[7] has now been studied quantitatively and shown[8] to be due mainly to multiple recurrences of D-glucuronic acid in the native and degraded gums. With larger quantities of specific precipitate than used by Beiser, *et al.*,[3] it was found that one-third to one-fifth of the rhamnose in the native gum and roughly two-thirds of the residual rhamnose in the degraded gum appears in the specific precipitate. Native GA, therefore, is also a mixture, and hitherto published formulas are uncertain to that extent.

Although the power of immunochemical techniques was thus once more demonstrated, it was considered advisable to attempt fractionation of native GA by chemical methods as well. These were less successful, but effected sufficient separation to show that GA is indeed a mixture of substances of rather similar composition.

Experimental

Materials and Methods.—The GA used was a commercial sample. It was dissolved in 0.9% sodium chloride solution up to concentrations of 200 mg. per ml., with neutralization as necessary and centrifugation to remove insoluble matter. Acid-degraded gum was prepared as in reference 7. The Type II anti-Pn horse serum was kindly supplied by the Bureau of Laboratories, New York City Department of Health, and the Type XIV anti-Pn horse serum by the Division of Laboratories, New York State Department of Health.

For the quantitative estimations of antibody nitrogen[9]

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(2) Institute of Microbiology, Rutgers University, New Brunswick, N. J.

(3) S. M. Beiser, E. A. Kabat and J. M. Schor, *J. Immunol.*, **69**, 297 (1952).

(4) B. R. Record and M. Stacey, *J. Chem. Soc.*, 1561 (1948).

(5) K. Butler and M. Stacey, *ibid.*, 1537 (1955).

(6) F. Smith, *ibid.*, 1724 (1939); 1035 (1940); J. Jackson and F. Smith, *ibid.*, 74 (1940); E. L. Hirst, *ibid.*, 70 (1942).

(7) M. Heidelberger, O. T. Avery and W. F. Goebel, *J. Exp. Med.*, **49**, 847 (1929).

(8) M. Heidelberger and J. Adams, *ibid.*, **103**, 189 (1956).

(9) M. Heidelberger and F. E. Kendall, *ibid.*, **61**, 559 (1935); R. Markham, *Biochem. J.*, **36**, 790 (1942); E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," C. C. Thomas, Springfield, Ill., 1948.

TABLE I

PROPERTIES AND ANALYSES OF FRACTIONS OF GUM ARABIC

Substance	Yield, %	Sedimentation const., S_{20}	L-Arabinose, %	D-Galactose, %	D-Glucuronic acid, %	L-Rhamnose, %	Ratios: Gal arab	Gal glucur	Gal rham	Arab glucur	Glucur rham
Alc. fract. A	11	9.7	28[a]	49	17	13	1.8	2.9	3.8	1.6	1.3
B	48	8.7[a]	31[b]	52	15	12	1.7	3.5	4.3	2.1	1.3
C	24	7.3[a]	35[b]	49	13	10	1.4	3.8	4.9	2.7	1.3
Acetic fract. D	35		28	45	14	11	1.6	3.2	4.1	2.0	1.3
E	55		31	49	14	10	1.6	3.5	4.9	2.2	1.4
F	8		36[a]	46[a]	11	8[a]	1.3	4.2	5.8	3.3	1.4

The sum of the percentages of the sugars in each fraction is 107, 110, 107, 98, 104, and 101, resp.; calcd., 111.
[a] Mean of two determinations. [b] Mean of four determinations.

TABLE II

ANALYSES OF SPECIFIC PRECIPITATES FROM 150 MG. OF FRACTIONS OF GUM ARABIC AND 1.5 ML. OF TYPE II ANTIPNEUMOCOCCUS HORSE SERUM 1054, C-ABSORBED,[a] 1 → 2

GA Fraction	Antb. N pptd., μg.	Amt. gum pptd.,[b] μg.	Ratio antb N gum pptd.	L-Arabinose, μg.	D-Galactose, μg.	D-Glucuronic acid, μg.	Rhamnose, μg.	Ratios: Gal arab	Gal glucur	Gal rham	Arab glucur	Glucur rham
A	292	599	0.5		274[c]	85[d]	17[c]		3.2	16		5.0
B	249	485	.5		237[c]	54[d]	11[c]		4.4	22		4.9
	251				159[d]		11[d]			14		
	244	350[e]	.7	140[d]	165	45	11	1.2	3.7	15	3.1	4.1
C					143[d]	33	5[d]		4.3	29		6.6
A supernatant	273[f]	485[g]	.6	153	225	66	14	1.5	3.4	16	2.3	4.7

[a] After absorption with the C-polysaccharide of pneumococcus. [b] Calcd. as 10/6 × sum of galactose and glucuronic acid in ppt. [c] Mean of three determinations. [d] Mean of two determinations. [e] Sum of separate sugar estimations minus 10% = 325 μg. [f] Specific precipitate from 1.5 ml. more antiserum + 3.0 ml. of supernatant from first precipitation by antiserum. [g] Sum of separate sugar estimations minus 10% = 412 μg.

reaction mixtures were allowed to stand in a bath at 0° for 8 days to three weeks before they were centrifuged, washed twice with chilled saline and analyzed.

Precipitates which were to be analyzed for their content of sugars were given the second wash with 1.2% sodium sulfate solution, as NaCl interfered with some of the color reactions used. The gum in the washed precipitates was dissociated from the antibody with 5% trichloroacetic acid solution.[10] Galactose and rhamnose were estimated simultaneously by the 3-minute cysteine reaction.[11] Carbazole was used for the determination of D-glucuronic acid,[12] with a correction for the slight color given by D-galactose. A variation of the cysteine method[13] was used for the analysis of L-arabinose. Since this had been tested only on nucleotides and nucleic acids and not on polysaccharides it is described in full. That this procedure yielded correct values for L-arabinose is indicated by the fact that when these values were added to the sum of the amounts of the other constituent sugars the total in terms of anhydro residues was very nearly 100%. The procedure was as follows: to 1 ml. of the solution containing 10 to 20 μg. of pentose per ml. is added, with cooling under tap water, 4 ml. of concentrated, C.P. H_2SO_4 as uniformly as possible for every sample. The mixture of acid and water is shaken immediately after the addition of acid as uniformly as possible in each case, and immersed again in tap water. The mixtures are allowed to stand, with repeated shaking, for 1–2 hours and 0.1 ml. of a 3% aqueous solution of cysteine HCl is added with shaking. A standard solution of L-arabinose and D-galactose and a blank containing all the reagents and 1 ml. of water instead of pentose solution are also run. Readings are carried out 15 min. after the addition of cysteine in the Beckman spectrophotometer at two wave lengths: namely, 390 mμ, which corresponds to the absorption maximum of pentose, and at 424 mμ at which the galactose standard showed an optical density identical with that at 390 mμ. The difference, $D_{390} - D_{424}$ of the unknown divided by the same difference obtained with the standard, multiplied by 100 gives the concentration of L-arabinose in per cent. of the standard solution. It had previously been shown by direct measurements that the two other constituents in GA, namely, L-rhamnose and D-glucuronic acid, did not give significant values for $D_{390} - D_{424}$ in this procedure.

Results and Discussion

Attempts at Fractionation of Gum Arabic.—A 10% solution of GA in 1% aqueous sodium acetate was precipitated in such a way with isopropyl alcohol that relatively small first and last fractions (A and C) were obtained, so that differences from the principal fraction, B, would be accentuated. Chemical and physical properties of the fractions are given in Table I. The fractions differed little in composition, but the most readily precipitable, A, contained somewhat more L-rhamnose and D-glucuronic acid, showed a higher sedimentation constant, and precipitated more antibody than the others. The immunochemical data with Type II anti-Pn sera, given and discussed in detail in reference 8, are only briefly summarized here in Table II. It will be noted that only a small proportion of the gum added to antiserum was actually precipitated, and that a second portion of antiserum added to the supernatant from the first precipitation gave practically the same amount of precipitate of not greatly different composition.

It is apparent from the ratios of rhamnose to the other sugars in the specific precipitates with Type II antibodies that not more than one-third to one-fifth of the expected amount of rhamnose is carried down, and that, accordingly, a further fractionation of the material in each fraction is achieved in the immunological cross reaction. A similar fractionation does not occur in Type XIV anti-Pn serum, in

(10) M. Heidelberger, Z. Dische, W. B. Neely and M. L. Wolfrom, THIS JOURNAL, **77**, 3511 (1955).
(11) Z. Dische and L. B. Shettles, *J. Biol. Chem.*, **175**, 595 (1948).
(12) Z. Dische, *ibid.*, **167**, 189 (1947).
(13) Z. Dische, *ibid.*, **181**, 379 (1949); Z. Dische, "Methods of Biochemical Analysis," Vol. II, David Glick, ed., Interscience Publishers, New York, N. Y., 1955, p. 350.

which precipitation is mediated by multiple units of D-galactose[14] instead of D-glucuronic acid. The specific polysaccharide of Type XIV pneumococcus is composed of D-galactose, D-glucose and N-acetylglucosamine.[15] It is therefore probable that the D-galactose-containing portion of GA is much the same in both the rhamnose-poor fraction precipitated by Type II antiserum and in the residual gum. Since the proportion of D-glucuronic acid to L-arabinose and D-galactose is not greatly changed by precipitation with either antiserum, it is probable that the rhamnose-poor fraction precipitated by Type II serum contains the D-glucuronic acid in a steric arrangement more closely resembling the as yet only partially elucidated positions of the acid units in SII[5] than is characteristic of the D-glucuronic acid in the unprecipitated portion. This interpretation would indicate isomeric differences as well as differences in composition among the components of GA, but further chemical studies are clearly necessary to establish its validity. At any rate, the immunochemical data serve to indicate what directions such studies might take. Immunochemical tests of any fractions obtained would also be helpful in judging the validity of assigned structures.

(14) M. Heidelberger, THIS JOURNAL, **77**, 4308 (1955), esp. Table II.

(15) W. F. Goebel, P. B. Beeson and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939); M. Heidelberger, S. A. Barker and M. Stacey, *Science*, **120**, 781 (1954).

Since glacial acetic acid is sometimes useful in the fractionation of polysaccharides,[16] 2 g. of gum was dissolved in 10 ml. of water, with neutralization, and precipitated with 16 ml. of glacial acetic acid in the cold. The precipitate, fraction D, was centrifuged off in the cold and the gum in the supernatant (fraction E) precipitated with chilled alcohol. Both fractions were redissolved in cold water, neutralized, and precipitated with alcohol; yields: D, 0.7 g.; E, 1.1 g. Analyses are given in Table I. As the differences in composition were minor, most of another portion of gum was precipitated by a larger proportion of acetic acid. Eight per cent. of the amount taken was recovered from the supernatant by precipitation with alcohol. This fraction, F, showed appreciable deviations from the composition of the larger fractions, as also noted in Table I. Both L-rhamnose and D-glucuronic acid were low in this fraction, while L-arabinose tended to be high in all of the more soluble fractions

Attempts to split off labile L-arabinose and L-rhamnose in 45% acetic acid at room temperature, 20–28°, for three weeks failed completely, nor was there any change in composition after another three weeks at 37°.

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(16) M. Heidelberger and F. E. Kendall, *J. Exp. Med.*, **53**, 625 (1931).

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The Synthesis of 3-Hexuloses. Part 1. 2-O-Methyl-L-xylo-3-hexulose[1]

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The preparation of 2-O-methyl-L-xylo-3-hexulose from L-ascorbic acid is described.

1-Desoxy-D-xylo-3-hexulose was prepared by bacterial oxidation of L-fucitol some years ago.[2] This was the only known member of the 3-hexuloses. The present paper describes a synthesis of 2-O-methyl-L-xylo-3-hexulose (I) and a general procedure for the preparation of isomeric 3-hexulose derivatives. These isomeric ketoses are important as possible intermediates in the breakdown of hexoses by alkalies. The 3-pentuloses are possible intermediates in the biological fixation of carbon dioxide.[3]

The starting material in this synthesis of I was L-ascorbic acid (II) which was converted first to its 2,3-di-O-methyl ether (III) and thence by rearrangement to the well characterized crystalline amide of the methyl glycoside of a 3-hexulonic acid (IV).

The amide was then converted to the acid and thence to the lactone[4] V which was reduced with lithium aluminum hydride to the glycoside VI. This material was very labile to acids and gave on hydrolysis compound I, characterized as the 2,5-dichlorophenylhydrazone. The structure of the ketose was confirmed when it was oxidized by periodate to formic acid and an ester VII. This ester on methylation with silver oxide and methyl iodide gave a product which yielded, with alcoholic ammonia, glycolamide (VIII) and the amide[5] of 2,3-di-O-methyl D-glyceronic acid (glyceric acid) (IX). The glycolamide arises probably from the oxidation by silver oxide of the substituted glycolaldehyde derivative VII. 2-O-Methyl D-glyceronamide (X) resulted when VII was boiled with methanolic hydrogen chloride and the resultant ester was treated with alcoholic ammonia. The isolation of this amide, which was identical with a synthetic specimen,[6] and of the amide IX proved the configuration of C_2 in I and thus showed for the first time that the amide IV is a derivative of L-xylose. The configuration of the glycosidic methoxyl group is as yet unknown, but it is probably α-glycosidic.

When D-arabo-ascorbic acid was methylated

(1) Paper presented before the Division of Carbohydrate Chemistry at the 128th Meeting of the American Chemical Society in Minneapolis, Minnesota, September, 1955.

(2) L. E. Stewart, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2206 (1952).

(3) A. T. Wilson and M. Calvin, *ibid.*, **77**, 5948 (1955).

(4) W. N. Haworth, E. L. Hirst, F. Smith and W. J. Wilson, *J. Chem. Soc.*, 829 (1937).

(5) P. F. Frankland and N. L. Gebhard, *ibid.*, **87**, 866 (1905).

(6) J. K. N. Jones, *Can. J. Chem.*, **34**, 310 (1956).