(methyl-2,3,4-tri-*O*-methyl-D-glucopyranosid uronate)-3,4-di-*O*-methyl-D-xylopyranoside (2.0 g.), b.p. 175–180° (0.01 mm.), $n^{24}D$ 1.4638, $[\alpha]^{22}D$ +105° in methanol (*c*, 4). *Anal.* Calcd. for $C_{18}H_{32}O_{11}$: OCH_3, 51.2; equiv. wt., 424. Found: OCH_3, 48.8; equiv. wt. (by saponification), 438.

Methanolysis of the Methylated Aldobiouronic Acid and Isolation of 2,3,4-Tri-*O*-methyl-D-glucuronic Acid and 3,4-Di-*O*-methyl-D-xylose.—A portion (0.27 g.) of the methylated aldobiouronic acid, obtained in the previous experiment, was dissolved in 10% methanolic hydrogen chloride (3 ml.) and the solution heated (sealed tube) for 24 hr. on a boiling water-bath. Neutralization (Ag_2CO_3), filtration and concentration gave a sirup (92 mg.). The low yield of product is probably due to decomposition during methanolysis.

The sirupy methanolysis product, consisting of a mixture of methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid-uronate) and methyl 3,4-di-*O*-methyl-D-xylopyranoside, was heated for 1 hr. at 80° with 0.3 *N* barium hydroxide (10 ml.); the solution was neutralized (Dry Ice), filtered and passed successively over the cation and the anion resins. The neutral eluate was concentrated to give sirupy methyl 3,4-di-*O*-methyl-D-xyloside (22 mg.) which was heated for 20 hours with *N* sulfuric acid (1 ml.) on a boiling water-bath. Neutralization ($BaCO_3$), filtration and concentration gave 3,4-di-*O*-methyl-D-xylose, $[\alpha]^{23}D$ +22° in methanol (*c* 1.0). This di-*O*-methyl-D-xylose was readily distinguished from the 2,3- and the 3,5-di-*O*-methyl isomers by paper chromatography using methyl ethyl ketone:water azeotrope. Oxidation of the di-*O*-methyl-D-xylose (18 mg.) in water (1 ml.) with bromine (0.1 ml.) for 4 days at room temperature gave 3,4-di-*O*-methyl-D-xylonic acid. Aeration to remove the excess of the bromine, neutralization (Ag_2CO_3), passage of the filtered solution through the cation exchange resin followed by concentration gave sirupy 3,4-di-*O*-methyl-D-xylonic acid. Purification by extraction with ether and heating *in vacuo* at 50° gave crystalline 3,4-di-*O*-methyl-D-xylono-δ-lactone[9] (17 mg.) which after sublimation had m.p. and mixed m.p. 66°, $[\alpha]^{23}D$ −55°, initial value in water (*c* 0.2) changing to −19° (equilibrium value).

The methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid uronic acid was displaced from the anion resin column with 0.5 *N* sodium hydroxide and recovered by passing the alkaline eluate through the cation resin. Concentration gave a sirup which was dissolved in ether and treated with ethereal diazomethane. Removal of solvent and treatment of the methyl ester with methanolic ammonia afforded the crystalline amide[8] of methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside uronic acid, m.p. and mixed m.p. 183°, $[\alpha]^{22}D$ +140° in water (*c* 0.4); literature[8] m.p. 183°, $[\alpha]D$ +138° in water.

D-Glucuronic Acid *p*-Nitroanilide.—D-Glucurone (1 g.) and *p*-nitroaniline (1.05 g.) were dissolved in the minimum of methanol containing 0.01% hydrochloric acid[18] and the solution refluxed for 15 minutes. On allowing to stand, the *p*-nitroanilide of D-glucuronic acid separated in the form of plates (0.7 g.), m.p. 129–130°, $[\alpha]^{23}D$ +265° in pyridine (*c* 0.4) (after filtration and washing with ice-cold methanol).

Crystallization of the plates from methanol furnished needles, m.p. 129–130°, $[\alpha]^{23}D$ +261° in pyridine (*c* 0.5). *Anal.* Calcd. for $C_{17}H_{12}O_7N_2.H_2O$: C, 46.8; H, 4.5; N, 8.9. Found (for the plates): C, 46.2; H, 5.0; N, 8.9; Found (for the needles): C, 46.8; H, 4.9; N, 8.8.

When either the plates or the needles of the *p*-nitroanilide were crystallized from hot ethanol, cubic crystals of the anhydrous *p*-nitroanilide of D-glucuronic acid were obtained, m.p. 160–163° dec., $[\alpha]^{23}D$ +272° in pyridine (*c* 0.2). *Anal.* Calcd. for $C_{12}H_{12}O_7N_2$: C, 48.7; H, 4.1; N, 9.5. Found: C, 48.8; H, 4.2; N, 9.5.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Carbohydrates of Gramineae. IX. The Constitution of a Glucofructan of the Endosperm of Wheat (*Triticum vulgare*)[1,2]

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The constitution of a glucofructan from wheat flour has been investigated by methylation studies. The methylated glucofructan gives upon hydrolysis 2,3,4,6-tetra-*O*-methyl-D-glucose (1 mol. prop.), 1,3,4,6-tetra-*O*-methyl-D-fructose (3 mol. props.), 1,3,4-tri-*O*-methyl-D-fructose (2 mol. props) and 3,4-di-*O*-methyl-D-fructose (2 mol. props.). The structural significance of these findings, which are different from those reported by other investigators for a wheat glucofructan, is discussed.

The glucofructans of wheat represent a group of non-reducing compounds which extend in molecular size from sucrose to polysaccharides with molecular weights of around 2000.[3–6]

The present paper is concerned with the determination of the structure of one member of this homologous series of compounds.

The glucofructans of wheat flour were isolated by extraction with 70% ethanol after preliminary inactivation of the enzymic constituents with boiling 82% ethanol. Further purification was achieved by precipitation of the glucofructans as the barium hydroxide complexes, which, after liberation of the free carbohydrates with sulfuric acid, were acetylated and the complex mixture subjected to fractional precipitation. In this manner 12 fractions of acetate were obtained varying in optical rotation in chloroform from $[\alpha]D$ − 22° to $[\alpha]D$ + 46° with three principal fractions showing $[\alpha]D$ + 3°, $[\alpha]D$ + 26° and $[\alpha]D$ + 31° (Table I). Deacetylation of the main fraction, $[\alpha]D$ + 3°, afforded the corresponding free carbohydrate as a hygroscopic, amorphous solid, $[\alpha]D$ − 21° in water, which was non-reducing to Fehling solution; these properties were similar to those de-

(1) This paper, No. 3568, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, is part of a report of research done under contract over the period July, 1951, to December, 1953, with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service. Presented at the 128th national meeting of the A. C. S., Minneapolis, September, 1955.

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scribed by earlier workers,[7,8] for a wheat glucofructan. Upon hydrolysis, the polysaccharide gave D-glucose (12.5%) and D-fructose (87.5%), the ease of hydrolysis indicating that the fructose residues were in the furanose form. In periodate oxidation studies, 1.03 moles of periodate was consumed per mole of anhydrohexose residue and one mole of formic acid was concomitantly produced per 9 to 10 moles of hexose. The resulting glucofructan polyaldehyde after reduction with sodium borohydride followed by acid hydrolysis of the polyalcohol so produced, gave a hydrolyzate which was shown by chromatographic analysis to contain neither D-glucose nor D-fructose.[9] It was apparent, therefore, that the glycosidic bonds connected the sugar residues so that all these residues possessed adjacent hydroxyl groups and were susceptible to periodate oxidation.

In order to determine the mode of union of the component sugars, the glucofructan acetate (fraction 5, Table I) was methylated[10,11] with methyl sulfate and sodium hydroxide to give the tri-*O*-methylglucofructan as a viscous liquid. Fractional precipitation studies indicated that the methyl derivative was not homogenous (see Table II) and that more than one member of the glucofructan series of compounds was present. However, preliminary investigation of these fractions indicated that upon hydrolysis[11] all of them gave rise to the same cleavage fragments as revealed by paper chromatography. It was concluded therefore that the fractions of methylated material differed only in the relative proportions of these methylated sugars and that they were structurally similar.

After hydrolysis of the methylated derivative, fraction 2, Table II, $[\alpha]_D$ − 36° in acetone, OMe 45.3%, the mixture of methylated sugars was resolved by chromatography on a cellulose–hydrocellulose column[12] into three fractions which were shown to consist of 3,4-di-*O*-methyl-D-fructose, 1,3,4-tri-*O*-methyl-D-fructose and a mixture of 1,3,4,6-tetra-*O*-methyl-D-fructose and 2,3,4,6-tetra-*O*-methyl-D-glucose (see Table III). The tetra-*O*-methylhexoses were separated by the preferential conversion of 1,3,4,6-tetra-*O*-methyl-D-fructose to its methyl furanoside with 0.5% methanolic hydrogen chloride leaving the 2,3,4,6-tetra-*O*-methyl-D-glucose principally unchanged. The resulting mixture was then separated on a cellulose–hydrocellulose column[12] (Table IV).

The 1,3,4,6-tetra-*O*-methyl-D-fructose was identified by oxidation with nitric acid to give 3,4,6-tri-*O*-methyl-D-fructofuranuronic acid which was characterized as its crystalline amide.[13] In a similar way the 3,4-di-*O*-methyl-D-fructose was identified by the formation of the crystalline diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid.[14,15]

The 1,3,4-tri-*O*-methyl-D-fructose was isolated in crystalline form and was further characterized by oxidation with nitric acid and conversion of the acid so formed to the crystalline diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid. A careful search failed to reveal the presence of either tri-*O*-methylaldohexose or 3,4,6-tri-*O*-methyl-D-fructose in this fraction.

The 2,3,4,6-tetra-*O*-methyl-D-glucose was characterized by the formation of its crystalline anilide. It was also obtained as 2,3,4,6-tetra-*O*-methyl-D-gluconolactone by oxidation of the methylated glucofructan hydrolyzate with bromine and separation of the methylaldonic acid on an anion-exchange resin, from which the acid was eluted with sodium hydroxide and converted to the free acid by the use of a cation-exchange resin. The resulting lactone had the same optical rotation and mobility on filter paper as an authentic specimen of 2,3,4,6-tetra-*O*-methyl-D-gluconolactone.

In order to determine the ratios of the cleavage fragments of the methylated glucofructan, the tetra-*O*-methyl-D-glucose was removed by bromine oxidation[16] as described above and the methylfructoses were separated by chromatographic analysis on a cellulose–hydrocellulose column[12] (Table V). Taking into consideration the mutarotation of the sirupy fractions of the methylfructose cleavage fragments[17] the weights of the three components were found to correspond to 1,3,4,6-tetra-*O*-methyl-D-fructose (3 mol. props.), 1,3,4-tri-*O*-methyl-D-fructose (3 mol. props.), 3,4-di-*O*-methyl-D-fructose (2 mol. props.) and 2,3,4,6-tetra-*O*-methyl-D-glucose (1 mol. prop.). These results agreed with those from periodate oxidation whereby it was found that 1 molar proportion of formic acid (from the D-glucose end-residue) was produced for every 10 sugar units (1 glucose and 9 fructose units). Since the glucofructan is non-reducing, one possible structure that will account for these facts is shown in formula I. The glycosidic linkages are believed to be principally of the β-type since the substance displays a negative rotation.

Inspection of formula I shows that the Gβ1—2 Fru*f* portion of the molecule, enclosed by dotted lines, is a sucrose residue whose presence in the glucofructan is supported by the chromatographic identification of sucrose in the products formed by autohydrolysis,[18–20] of the glucofructans. Additional support for this general type of structure has been accumulating as a result of both enzymatic

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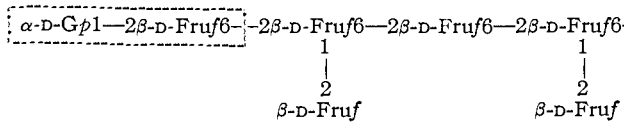

and chemical investigations similar to that described above.[21]

In a similar study of a wheat-flour glucofructan, called "sitosin," other investigators[6] report the isolation of 3,4,6-tri-*O*-methyl-D-fructose as the tri-*O*-methyl cleavage fragment of the methylated polysaccharide. This tri-*O*-methyl-D-fructose is different from that found in the present study and indicates an inulin type of structure for the glucofructan which is radically different from that shown in formula I in that the principal glycosidic linkage would be of the 1 → 2 rather than the 2→6 type. No trace of 3,4,6-tri-*O*-methyl-D-fructose was detected in the cleavage fragments of the methylated glucofructan in this investigation. Furthermore, inasmuch as "sitosin" acetate showed $[\alpha]_D$ − 10°, the glucofructan acetate fractions of the present study having a negative rotation (fractions 1, 2, 3 and 4, Table I) were combined, methylated and hydrolyzed as before, but no 3,4,6-tri-*O*-methyl-D-fructose was detectable by paper chromatographic analysis.

Such a difference between the glucofructan reported in the present paper and "sitosin," both isolated in essentially the same manner, is as yet unexplained. It is conceivable that different glucofructans are produced by different varieties of wheat, or by the different climatic conditions prevailing in Europe and America, or else there are two different types of glucofructan in wheat. Some support for the latter view is provided by the suggestion, based on chromatographic evidence, that there are two homologous series of glucofructans in wheat.[5]

Experimental

Extraction of Glucofructans.—Unbleached, Southwestern baker's patent, wheat flour (674 g., 13% moisture) was stirred under reflux with 82% ethanol (1850 ml.) for 30 to 60 min. after which time the alcohol was diluted to 70% and stirring continued for 4 to 7 hr. at room temperature. The mixture was centrifuged and the solid residue extracted again with 70% ethanol. The second extract was used to extract a fresh batch of flour. The two extracts of the flour were combined and evaporated under reduced pressure, precipitated protein being removed as the evaporation proceeded. The concentration was continued until the volume was 50 to 100 ml., at which point the solution was centrifuged and the supernatant liquid diluted with 95% ethanol until a slight turbidity was produced. To this turbid solution was added a hot solution of barium hydroxide octahydrate (20 g.) in water (75 ml.) and the solution diluted to 2000 ml. with 95% ethanol. After standing overnight, a second addition of barium hydroxide solution was made as before. The mixture was centrifuged to give a supernatant E and the insoluble barium "salts" of the wheat glucofructans. The glucofructan–barium hydroxide complex was suspended in ice-cold water and the barium was precipitated with sulfuric acid and removed (centrifuge). The supernatant solution was deionized by successive treatment with "Amberlite IR 120"[22] and "Duolite A4"[23] ion-exchange resins, concentrated to about 40 ml., centrifuged and added to ethanol–acetone (1:1 v./v. 400 ml.). The precipitated glucofructans, P, were collected (centrifuge), washed successively with ethanol, ethyl ether, light petroleum ether and dried *in vacuo;* yield 3.5 g., $[\alpha]^{25}_D$ −6° in water (*c* 2.2). The mother liquor from this last precipitation was evaporated to dryness yielding a colorless sirup, S (2.9 g.), $[\alpha]^{25}_D$ +21° in water (*c* 14.5).

The supernatant liquor E (see above) was deionized by passage through "Amberlite IR 120" and "Duolite A4" ion-exchange resins. The resulting sugar solution was evaporated to a sirup (0.17 g.) which by chromatographic analysis was shown to be composed of a mixture of fructose, glucose, maltose, sucrose and low molecular weight glucofructans.[3]

From the extraction of 3820 g. of wheat flour there was obtained 24.2 g. of precipitated glucofructans, P, which represents 0.7% of wheat flour on a dry-weight basis. The total glucofructan content of the flour including the material S above, which represents 0.3% of wheat flour, amounts to 1.0% on a dry-weight basis.

Acetylation of the Glucofructans. (a).—To a solution of glucofructan, P (19 g.) in formamide (50 ml.) and pyridine (100 ml.), was added with cooling, acetic anhydride (80 ml.) in 10 ml. aliquots. After addition of the acetic anhydride, a slight turbidity was produced and the reaction mixture was maintained at room temperature for 60 hr. The reaction mixture was poured with stirring into ice-water, and the precipitate washed with water and dissolved in chloroform. The chloroform solution was dried ($MgSO_4$) and evaporated to a sirup (31 g.). The sirup was dissolved in chloroform (400 ml.) and fractionated by adding petroleum ether (b.p. 30–60°) in the usual manner.

(b).—Acetylation of material S (10.6 g. from 3344 g. of wheat flour) as described above, gave a water-insoluble acetate (14 g.) which was fractionally precipitated from acetone solution by petroleum ether.

Refractionation of Glucofructan Acetate Fractions.—Repeated fractionation of the fractions of the acetates of the glucofructan material P and S and combination of intermediate subfractions having similar optical rotations gave the final fractions as summarized in Table I.

TABLE I

FRACTIONATION OF GLUCOFRUCTAN ACETATE

Fraction	Wt. (g.)	$[\alpha]^{23}_D$ in $CHCl_3$	Fraction	Wt. (g).	$[\alpha]^{23}_D$ in $CHCl_3$
1	1.6	−22°	7	9.1	+18°
2	0.7	−14	8	13.0	+26
3	0.7	− 3	9	11.7	+31
4	0.6	− 1	10	2.0	+40
5	19.3	+ 3	11	2.5	+42
6	0.7	+ 9	12	4.9	+46

Deacetylation of Fraction 5, Table I.—A portion (4.1 g.) of acetate, fraction 5, Table I, was dissolved in absolute ethanol (200 ml.) to which solution was added a small amount of sodium. The solution became turbid after a few minutes and a precipitate soon appeared. After standing overnight at room temperature the mixture was centrifuged and both the supernatant solution and the solid residue (0.7 g.) were subjected to a second deacetylation as described above. The glucofructan precipitated from the reaction solution and was isolated by centrifugation, as a hygroscopic, colorless powder (1.8 g.), showing $[\alpha]^{20}_D$ −21° in water (*c* 2.0); R_g (mobility relative to that of D-glucose) 0.04 using 1-butanol:ethanol:water (4:1:5). Movement of the carbohydrate on paper would appear to indicate that it is of relatively small molecular weight. Hydrolysis with 0.1 *N* sulfuric acid for 6 hr. on a boiling water-bath gave chromatographic evidence for D-glucose and D-fructose and a quantitative analysis in triplicate using the phenol–sulfuric acid procedure[24] showed D-fructose 87.5% and D-glucose 12.5%.

In periodate oxidation studies 1.03 molar proportions of sodium periodate were consumed per anhydrohexose unit and 1 molar proportion of formic acid was generated for every 9.7 anhydrohexose units. The polyaldehyde so

(21) For references see L. A. Boggs and F. Smith, THIS JOURNAL, **78**, 1880 (1956).

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

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

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

formed was reduced with sodium borohydride[9] and the resulting polyalcohol was treated with methanolic hydrogen chloride to remove borate[25] and then hydrolyzed with 0.1 *N* hydrochloric acid. The hydrolyzate was deionized with "Amberlite IR 120" cation- and "Duolite A-4" anion-exchange resins and the aqueous solution evaporated to dryness *in vacuo*. Partition chromatography of the residue showed that neither D-glucose nor D-fructose was present.

A sample of the glucofructan (100 mg.) was dissolved in distilled water (10 ml.) and the solution, $[\alpha]^{26}D$ −14°, *p*H 5.6, heated (sealed tube) on a boiling water-bath for 36 hr., when it showed $[\alpha]^{26}D$ −36°. Partition chromatography of the resulting solution using pyridine:ethyl acetate:water (1:2.5:3.5) indicated the presence of D-fructose (*R*g 1.17), D-glucose (*R*g 1.00), sucrose (*R*g 0.74), and four other components, *R*g 0.86 (probably fructobiose), *R*g 0.49, 0.26, and 0.18.

Methylation of Glucofructan.—A portion (7.3 g.) of the acetate (fraction 5, Table I) in acetone (150 ml.) was methylated in the usual way[10,11,14] with 30% sodium hydroxide (275 ml.) and methyl sulfate (90 ml.), the reagents being added during 2 hr. at 55° (approx.); acetone was added as required to keep the partially methylated product in solution. The methylglucofructan, isolated by extraction with chloroform, was subjected to five more methylations in the same way giving nearly the theoretical yield (5.3 g.) of a mobile, brown sirup, $[\alpha]^{22}D$ −28° in acetone (*c* 2.0).

The methylglucofructan was subjected to fractional precipitation from acetone solution with petroleum ether (b.p. 30–60°) to give fractions whose properties are summarized in Table II. A small amount of colored material which precipitated initially was discarded.

TABLE II

FRACTIONATION OF METHYLATED GLUCOFRUCTAN

Fraction	Wt. (g.)	$[\alpha]^{26}D$ in acetone, degree	OMe (%)
1	0.1	−26 (*c* 0.5)	..
2[a]	2.5	−36 (*c* 1.3)	45.3
3	0.6	−32 (*c* 0.9)	44.5
4 (from mother liquor)	1.6	−16 (*c* 1.5)	45.6

[a] After remethylation with silver oxide and methyl iodide.

Hydrolysis of Methylated Glucofructan.—The methylated glucofructan (fraction 2, Table II, 1.40 g.) was heated with methanol (50 ml.) and water (16 ml.) containing oxalic acid (0.67 g.) under reflux for 18 hr. The methanol was then removed *in vacuo* at room temperature, the volume of solution being maintained constant by the addition of water. The resulting aqueous solution which was maintained at 75°, showed $[\alpha]^{23}D$ −4° after 2 hr., constant for a further 5 hr. After neutralization ($CaCO_3$), the solution was filtered and evaporated to a sirup (1.27 g.) *in vacuo* at 25–30°. Examination of the sirup by paper chromatography, irrigating with ethyl methyl ketone:water azeotrope, indicated the presence of 1,3,4,6-tetra-*O*-methylfructofuranose, 2,3,4,6-tetra-*O*-methylglucose, 1,3,4-tri-*O*-methylfructofuranose and 3,4-di-*O*-methylfructofuranose with R_f values 0.85, 0.77, 0.66 and 0.33, respectively.

Separation of Methyl Sugars.—The sugars obtained above were separated on a hydrocellulose–cellulose column[12] using ethyl methyl ketone:water azeotrope as the developing solvent. The results are summarized in Table III.

The Separation of 1,3,4,6-Tetra-*O*-methyl-D-fructofuranose and 2,3,4,6-Tetra-*O*-methyl-D-glucopyranose.—Fraction A, Table III, was dissolved in 0.5% methanolic hydrogen chloride (42 ml.) and the solution kept at room temperature for 8 hr., $[\alpha]^{27}D$ +47°, constant final value. The solution was neutralized (Ag_2CO_3), filtered and evaporated *in vacuo* at room temperature to a sirup (0.57 g.) which was subjected to fractionation on a cellulose–hydrocellulose column[12] as before. When a drop of the eluate in each tube was put on paper and tested with *p*-anisidine,[26] the faster moving methyl 1,3,4,6-tetra-*O*-methyl-D-fructofuranoside gave a yellow color, as distinct from the darker color of the 2,3,4,6-tetra-*O*-methyl-D-glucose. On this basis, the tubes were combined to give the results summarized in Table IV.

TABLE III

CHROMATOGRAPHIC SEPARATION OF METHYLATED GLUCOFRUCTAN CLEAVAGE FRAGMENTS

Fraction	Component	Tube no.	Wt. (g.)	$[\alpha]^{27}D$, degree
A	1,3,4,6-Tetra-*O*-methyl-D-fructose 2,3,4,6-Tetra-*O*-methyl-D-glucose	6–27	0.559	+26 (EtOH)
B	1,3,4-Tri-*O*-methyl-D-fructose	28–104	.324	− 4 (MeOH)
C	3,4-Di-*O*-methyl-D-fructose	105–130	.305	−22 (MeOH)

TABLE IV

CHROMATOGRAPHIC SEPARATIONS OF METHYL 1,3.4.6-TETRA-*O*-METHYL-D-FRUCTOFURANOSIDE AND 2,3,4,6-TETRA-*O*-METHYL-D-GLUCOSE FROM FRACTION A, TABLE III

Fraction	Component	Tube no.[a]	Wt. (g.)[b]	$[\alpha]^{26}D$ (MeOH), degree
A 1	Methyl 1,3,4,6-tetra-*O*-methyl-D-fructofuranoside	1–10	0.429	..
A 2	2,3,4,6-Tetra-*O*-methyl-D-glucose	11–19	.186	+43
A 3	2,3,4,6-Tetra-*O*-methyl-D-glucose	20–40	.031	+60

[a] Collection time for tubes 1 to 12 was 10 minutes and thereafter it was 5 minutes per tube. [b] In order to avoid loss of these volatile components in these fractions, exhaustive drying *in vacuo* was avoided.

Examination of the Cleavage Products of Methylated Glucofructan. (a) **1,3,4,6-Tetra-*O*-methyl-D-fructose.**—Fraction A 1, Table IV, was dissolved in 0.2 *N* sulfuric acid (10 ml.) containing methanol (5 ml.) and the solution heated on a boiling water-bath for 6 hr., $[\alpha]D$ + 21°, constant after 4 hr. The acid was neutralized ($BaCO_3$) and after filtration the solution was evaporated *in vacuo* to give 1,3,4,6-tetra-*O*-methyl-D-fructose as a sirup (0.42 g.), OMe 49.1%. A portion (0.07 g.) of the sirup in nitric acid (3 ml., d. 1.42) was heated for 80 min. at 60–63° and then for 30 min. at 95–100°. The mixture was allowed to cool, diluted with water (10 ml.) and evaporated *in vacuo* with periodic additions of methanol to remove nitric acid. The resulting sirup was subjected to two methylations with silver oxide and methyl iodide in the usual way and the product (0.07 g.) distilled to give methyl (methyl 3,4,6-tri-*O*-methyl-D-fructofuranoside)-uronate, b.p. (bath temp.) 135–140°, 0.005 mm. Treatment with methanolic ammonia in the usual way, followed by removal of the solvent *in vacuo*, left a crystalline residue (0.06 g.) which upon recrystallization from acetone–light petroleum ether yielded methyl 3,4,6-tri-*O*-methyl-D-fructofuranosiduronamide,[13] m.p. and mixed m.p. 100–101°.

(b) **2,3,4,6-Tetra-*O*-methyl-D-glucose.**—A portion of fraction A 2, Table IV, (0.06 g.) was treated with aniline (0.06 g.) in boiling ethanol (5 ml.) for 3 hr., followed by removal of the solvent *in vacuo*. The residue crystallized and was recrystallized from ethyl acetate–light petroleum ether to yield 2,3,4,6-tetra-*O*-methyl-D-glucose anilide,[27] m.p. and mixed m.p. 122–123°.

(c) **1,3,4-Tri-*O*-methyl-D-fructose.**—Fraction B, Table III (0.324 g.) was dissolved in water (25 ml.) and treated with bromine (0.5 ml.) in the presence of barium carbonate (1 g.) for 3 days in the dark at room temperature. The excess bromine was removed by aeration and the reaction mixture evaporated to dryness under reduced pressure in the presence of sodium bicarbonate (1 g.). The solid residue was extracted three times with boiling chloroform. A careful search was made for a methylated gluconic acid in the insoluble residue but none was detected. The com-

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(27) J. C. Irvine and A. M. Moodie, *ibid.*, **93**, 95 (1908).

bined chloroform extracts were evaporated *in vacuo* to give a sirup (0.297 g.) showing $[\alpha]^{27}D$ −13° in methanol (*c* 3.0) and OMe, 36.6%. The sirup crystallized to give 1,3,4-tri-*O*-methyl-D-fructose,[14,28] m.p. 76–77°, undepressed on admixture with an authentic specimen, $[\alpha]^{25}D$ −31° in water (*c* 4.0) after 3 min., changing in 120 min. to −55° (equilibrium value). The mother liquor from the above crystals showed $[\alpha]^{26}D$ −8° in methanol and was treated with acetone containing sulfuric acid as described before[21] but no 1,2-*O*-isopropylidine-3,4,6-tri-*O*-methyl-D-fructose was obtained. The component of the sirup which did not form an isopropylidine derivative (0.16 g.) was oxidized with nitric acid and the resulting acid converted to the amide *via* the methyl ester as described above. In this way there was obtained the diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid,[14,15] m.p. and mixed m.p. 191–192°.

(d) **3,4-Di-*O*-methyl-D-fructose.**—A portion of fraction C, Table III (0.05 g.), OMe 24.2%, was oxidized with nitric acid as described above and the acid converted to the corresponding amide in the usual way. There was isolated the diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid,[14,15] m.p. and mixed m.p. with an authentic specimen 191–192°, $[\alpha]^{22}D$ −72° in water (*c* 0.4).

Quantitative Analysis of the Cleavage Fragments of Methylated Glucofructan.—A portion (1.100 g.) of fraction 2, Table II, was hydrolyzed as described above and the aqueous hydrolyzate concentrated to 50 ml. *in vacuo* at 25–30°. To this solution was added barium carbonate (3 g.) and bromine (1 ml.) and the oxidation continued at room temperature in the dark for 3 days. The excess bromine was removed by aeration, the solution filtered and the residue washed well with water. The combined filtrate and washings were freed from barium ions with sulfuric acid and then the aqueous solution treated with silver carbonate to remove the hydrochloric acid produced. After filtration the aqueous solution was passed through "Amberlite IR 120" cation- and "Duolite A4" anion-exchange resins and the neutral eluate evaporated *in vacuo* to a sirup (0.863 g.) which was separated into the component methyl-fructoses by column chromatography as described above.

The results were as follows: 1,3,4,6-tetra-*O*-methyl-D-fructose (0.379 g.), $[\alpha]^{23}D$ +13° in ethanol (*c* 4); 1,3,4-tri-*O*-methyl-D-fructose (0.230 g.), m.p. 77°, $[\alpha]^{23}D$ −7° → +16° in methanol (*c* 4.5); and 3,4-di-*O*-methyl-D-fructose (0.190 g.), $[\alpha]^{13}D$ −25° → +28° in methanol (*c* 2). The R_f values of the three component sugars were 0.85, 0.66 and 0.33, respectively, using ethyl methyl ketone:water azeotrope and Whatman No. 1 filter paper.

The acidic material absorbed by the "Duolite A4" anion-exchange resin was eluted with 4% sodium hydroxide and the free acids immediately regenerated by passing the alkaline eluate through "Amberlite IR 120" cation-exchange resin. The acidic eluate from the latter column was concentrated to about 10 ml. under reduced pressure and the aqueous solution extracted five times with chloroform. The combined chloroform extracts were evaporated *in vacuo* to give 2,3,4,6-tetra-*O*-methyl-D-gluconolactone as a sirup (0.091 g.), $[\alpha]^{21}D$ +98° in ethanol (*c* 0.3), equiv. wt., 214 (calcd. for $C_{10}H_{18}O_6$; equiv. wt., 234), R_f 0.90 with ethyl methyl ketone:water azeotrope (an authentic sample showed R_f 0.90).

The molecular ratios of the four cleavage fragments deduced from the above results are as follows: 2,3,4,6-tetra-*O*-methyl-D-glucose (1); 1,3,4,6-tetra-*O*-methyl-D-fructose (3), 1,3,4,-tri-*O*-methyl-D-fructose (2) and 3.4-di-*O*-methyl-D-fructose (2).

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(28) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 676 (1934).

[CONTRIBUTION FROM THE GOVERNMENT FOREST EXPERIMENT STATION, JAPAN]

Flavonoids of Various *Prunus* Species. V. The Flavonoids in the Wood of *Prunus verecunda*

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From the wood of *Prunus verecunda* Koehne, pinocembrin, pinocembrin-5-glucoside, genistein, prunetin, isosakuranetin, isosakuranin, naringenin, genkwanin, eriodictyol and taxifolin were isolated. The name "verecundin" was proposed for the second-named new glycoside.

This report deals with the flavonoids of the wood of *Prunus verecunda* Koehne, which is distributed in the mountainous region of middle Japan. The flavonoid pattern of this tree differs somewhat from any of those described before[1–4] for other *Prunus* species.

The flavonoid compounds which were isolated are pinocembrin-5-glucoside (5,7-dihydroxyflavanone 5-glucoside), genistein (5,7,4′-trihydroxyisoflavone), prunetin (5,4′-dihydroxy-7-methoxyisoflavone), pinocembrin (5,7-dihydroxyflavanone), isosakuranetin (5,7-dihydroxy-4′-methoxyflavanone), isosakuranin (5,7-dihydroxy-4′-methoxyflavanone 7-glucoside), naringenin (5,7,4′-trihydroxyflavanone), genkwanin (5,4′-dihydroxy-7-methoxyflavone), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone), taxifolin (3,5,7,3′,4′-pentahydroxyflavanone), and among these substances, the first four are the new additions to the *Prunus* constituents.

(1) M. Hasegawa and T. Shirato, THIS JOURNAL, **74**, 6114 (1952).
(2) M. Hasegawa and T. Shirato, *ibid.*, **76**, 5559 (1954).
(3) M. Hasegawa and T. Shirato, *ibid.*, **76**, 5560 (1954).
(4) M. Hasegawa and T. Shirato, *ibid.*, **77**, 3557 (1955).

Of these, the first-named glycoside is new. It is hydrolyzed by dilute mineral acids to give one mole each of pinocembrin[5] and glucose, and the analysis agreed with the formula $C_{21}H_{22}O_9$. It gives no coloration with ferric chloride showing that the 5-hydroxyl group of pinocembrin is not present owing to glycoside formation. It gives merely an orange coloration when reduced in methanol with magnesium powder and concd. hydrochloric acid. By methylation with diazomethane, one hydroxyl group is methylated. We wish to propose the name "verecundin" for this glycoside.

Experimental

Isolation of Flavonoids.—Wood chips (500 g.) of *Prunus verecunda* prepared from a stem of 7.5 cm. diameter were twice extracted with 3-l. portions of methanol for 3 hours. The filtered methanol extracts were concentrated to a sirup. A total of 2.4 kg. of wood chips were thus treated. The combined sirup was extracted repeatedly with ether and then with ethyl acetate.

Ether-soluble Portion.—After evaporation of the ether, the residue was twice extracted with 300 ml. of hot benzene

(5) H. Erdtman, *Svensk Papperstidn.*, **46**, 226 (1943); *C. A.*, **37**, 5862 (1943).