196. *The Quantitative Determination of Galactose, Mannose, Arabinose, and Rhamnose.*

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Methods are described for the quantitative analysis of mixtures of galactose, mannose, arabinose, and rhamnose, which may occur amongst the hydrolysis products of plant gums. Galactose is estimated as its phenylmethylhydrazone, mannose as its phenylhydrazone, and arabinose as its benzoylhydrazone. The methods are accurate to within 9 mg. on weights above 0·1 g. Rhamnose can be determined as the benzoylhydrazone with an accuracy of ±30 mg. on weights above 0·25 g.

The plant gums are complex polysaccharides which contain a variety of sugar residues and amongst the products of hydrolysis galactose, mannose, arabinose, and rhamnose frequently occur. The quantitative determination of these sugars is a matter of some difficulty, the more so when they are admixed with other sugars and acids formed during the hydrolysis of the gums. We have carried out by the standard mucic acid method a large number of determinations of galactose in mixtures of sugars obtained from the hydrolysis of plant products, and have found the method inadequate, especially when only small quantities of material are available and when *d*-galacturonic acid and *l*-galactose are present. Other methods which have been described are (1) the fermentation method using special strains of yeast (Wise and Appling, *Ind. Eng. Chem. Anal.*, 1944, **16**, 28), and (2) a method in which the insoluble phenylmethylhydrazone of galactose is weighed. This latter method has been used by Neuberg (*Biochem. Z.*, 1907, **3**, 519), Lüdtke (*Biochem. Z.*, 1919, **212**, 419), Neuberg and Schweitzer (*Monatsh.*, 1937, **71**, 46), and Freeman, Challinor, and Willis (*Biochem. J.*, 1940, **34**, 316). These authors converted pure galactose into its phenylmethylhydrazone under standard conditions and used a factor for the conversion of the weight of derivative isolated into the weight of galactose in solution. This method, however, gives accurate results only if the amounts of galactose in the control and in the unknown solution are identical. To avoid the necessity of carrying out a control estimation on each occasion we have determined the yields, under standard conditions, of the phenylmethylhydrazone of galactose obtained from various amounts of galactose (Table I). From the results a graph was constructed which showed that the relationship between galactose and the yields of galactose phenylmethylhydrazone was linear even when glucose, xylose, rhamnose, and glucuronic acid were present. Mannose and arabinose interfere with the estimation, but the difficulties thus arising can be readily circumvented. Mannose can be estimated by the phenylhydrazine method (Bourquelot and Herissey, *Compt. rend.*, 1899, **129**, 339; see below) and can be removed by fermentation. Arabinose cannot be removed by fermentation but can be estimated by means of diphenylhydrazine (Neuberg, *Ber.*, 1900, **33**, 2243; Wise and Paterson, *Ind. Eng. Chem.*, 1930, **22**, 365) or by boiling with 12% hydrochloric acid and estimation of the furfural evolved. Since the precipitation of arabinose with phenylmethylhydrazine appears to be nearly quantitative in the presence of galactose, an estimate of the galactose present can be made after deducting the weight of phenylmethylhydrazone formed from the arabinose, the amount of which has been estimated by another method.

The quantitative estimation of arabinose in the presence of galactose is a matter of some difficulty since most reagents which give insoluble derivatives with arabinose also give insoluble derivatives with galactose, *e.g.*, phenylmethylhydrazine and phenylbenzylhydrazine. A reagent which does not possess this disadvantage is benzoylhydrazine. This reagent also has the advantage of ease of preparation and of stability (Fischer and Paulus, *Arch. Pharm.*, 1935, 83; Militzer, *J. Chem. Educ.*, 1941, 25). In this estimation the mixture of sugars containing

arabinose (50 mg. in 500 mg.) is dissolved in water (2 c.c.) and a solution of benzoylhydrazine in alcohol (10 c.c.) is added; after two days the insoluble derivative is filtered off, dried, and weighed. Of many sugars examined the only one which interfered with this estimation was rhamnose, when present in quantities of over 300 mg. The standardisation was carried out as described above (see Table II *a* and *b*), and gave a method of estimating arabinose accurate to within 9 mg. on 100 mg. when using weights of arabinose above 0·1 g. In the absence of arabinose this reagent can be used for the characterisation and quantitative estimation of rhamnose (see Table III).

The estimation of mannose with phenylhydrazine has already been described (Bourquelot and Herissey, *Compt. rend.*, 1899, **129**, 339). By adoption of the modified procedure described in the experimental section this method can be used for the estimation of mannose with an accuracy of approximately ±3 mg. on quantities of 100 mg. and over of mannose. The method was standardised in a manner similar to that described for galactose and arabinose (Table IV), and it was found that no interference was caused by glucose, galactose, rhamnose, or arabinose.

Experimental.

Galactose. Reagent and Method of Analysis.—1-Phenyl-1-methylhydrazine (25 g.) was mixed with absolute alcohol (100 c.c.) containing glacial acetic acid (3 c.c.). The reagent was kept in a tightly stoppered brown glass bottle at 0°.

To the sugar sample (containing not more than 1·4 g. of sugars) dissolved in water (10 c.c.) the above reagent (10 c.c.) was added and the mixture was kept in a tightly stoppered flask at 33° for 12 hours with occasional shaking. It was then cooled to 0° for 9 hours and the crystals (m. p. 186°) were collected in a tared Gooch crucible, washed with ice-cold ethyl alcohol (10 c.c.), dried at 100° for 30 minutes, and weighed. The yields of phenylmethylhydrazone obtained from various weights of galactose with and without the presence of other sugars are given in Table I. From these results it can be seen that the method is accurate to within ±3 mg. on amounts above 0·1 g.

Table I.

Galactose (g.).	Sugars added (g.).	Yield of phenylmethyl hydrazone (g.).	Galactose (from graph) (g.).	Error (g.).	Error calc. on original wt. of galactose (%).
0·037	—	0·039	0·039	+0·002	+ 5·4
0·077	—	0·096	0·077	nil	nil
0·126	—	0·177	0·132	+0·006	+ 4·8
0·260	—	0·367	0·263	+0·003	+ 1·2
0·426	—	0·615	0·433	+0·007	+ 1·7
0·711	—	1·036	0·719	+0·008	+ 1·1
1·394	—	2·042	1·403	+0·009	+ 0·7
0·184	Mannose, 0·500	1·020 [1]	0·178	−0·006 [1]	− 3·3
0·231	Arabinose, 0·500	1·030 [2]	0·204	−0·027 [2]	−12·0
0·225	Xylose, 0·500	0·320	0·231	+0·006	+ 2·6
0·202	Rhamnose, 0·500	0·281	0·205	+0·003	+ 1·5
0·232	Glucuronic acid, 0·500	0·320	0·231	−0·001	− 0·5
0·296	Glucose, 0·500	0·405	0·289	−0·007	− 2·4

[1] 0·245 G. of mannose phenylmethylhydrazone.
[2] 0·283 G. of *l*-arabinose phenylmethylhydrazone.

From these figures the equation $y = 0{\cdot}673x + 0{\cdot}013$ can be deduced where y corresponds to the weight of galactose giving a weight x of phenylmethylhydrazone.

Arabinose. Reagent and Method of Analysis.—Benzoylhydrazine, recrystallised from water and having m. p. 112—113° (25 g.), was dissolved in 95% alcohol (500 c.c.) and the solution was kept in a stoppered bottle until required. The sugar sample containing between 50 and 500 mg. of arabinose was dissolved in water (2 c.c.) in a 50 c.c. stoppered flask, and the reagent (10 c.c.) then added. The mixture was kept with occasional shaking at 20° for 24 hours and then at −3° for 22 hours. The crystalline residue, m. p. 186—190° (decomp.), was collected in a tared Gooch crucible, washed with ice-cold alcohol (10 c.c.), dried at 100° for ½ hour and weighed. It will be seen (Table II*a*) that of the sugars glucose, mannose, xylose, galactose, glucurone, and rhamnose, only the last named interfered with the estimation and then only when present in amounts above 300 mg. The relationship between the weight of arabinose and the yield of derivative was linear and fitted the equation $y = 0{\cdot}555x + 0{\cdot}022$ where y is the weight of arabinose corresponding to a weight x of derivative isolated.

d-Arabinose benzoylhydrazone was prepared, as described above, from *d*-arabinose and benzoylhydrazine, m. p. 186° (decomp.). *d*-Glucose gave with benzoylhydrazine a *derivative* {m. p. 176—179° (decomp.), $[\alpha]_D^{20°}$ $+15° \xrightarrow{17 \text{ hrs.}} -23°$ in water} which was much more soluble than the derivative of arabinose and rhamnose; it did not interfere with the estimation of these two sugars (Found: C, 52·4; H, 6·0; N, 9·6. $C_{13}H_{18}O_6N_2$ requires C, 52·4; H, 6·0; N, 9·4%).

In the second method of estimation of arabinose, an aqueous solution of benzoylhydrazine saturated at 20° (*ca.* 4·3 g./100 c.c. of water) was used as a reagent. The derivative is more soluble in this reagent

but separates in a more easily filtered form. Since the reagent contains no alcohol, alcohol-insoluble materials such as oligosaccharides and the barium salts of uronic acids remain in solution during the reaction and do not contaminate the precipitate of arabinose benzoylhydrazone. The dry, water-soluble product to be analysed, containing between 50 and 150 mg. of arabinose, is dissolved in the reagent (5 c.c.) and kept at 30° for 48 hours and then cooled to 0° for 2 hours. The crystalline precipitate is filtered off, washed with 10 c.c. of 95% alcohol, and dried at 100° to constant weight. If the residue contains between 150 and 400 mg. of arabinose, more of the reagent (10 c.c.) is used. The derivative is isolated and weighed as above. From the results obtained the equation $y = 0{\cdot}67x + 0{\cdot}010$ can be deduced where y is the weight of arabinose required to give a weight x of derivative (see Table II*b*).

TABLE II*a*.

Arabinose (g.).	Other sugars (g.).	Derivative of arabinose.	Arabinose from equation.	Error (g.).	Error calc. on wt. of arabinose taken (%).
0·212	—	0·310	0·194	−0·018	−8·5
0·234	Rhamnose, 0·087	0·362	0·223	−0·011	−5·0
0·206	Galactose, 0·071	0·297	0·187	−0·019	−9·2
0·215	Mannose, 0·108	0·319	0·199	−0·016	−7·4
0·294	—	0·478	0·288	−0·006	−2·1
0·344	—	0·574	0·341	−0·003	−0·9
0·402	—	0·683	0·401	−0·001	−0·3
0·243	Glucurone, 0·240	0·376	0·231	−0·012	−5·0
0·257	—	0·435	0·264	+0·007	+2·8
0·389	Rhamnose, 0·332	0·656	0·386	−0·003	−0·8
0·347	—	0·592	0·351	+0·004	+1·2
0·410	Galactose, 0·175	0·701	0·411	+0·001	+0·3
0·447	—	0·768	0·449	+0·002	+0·5
0·483	—	0·827	0·481	−0·002	−0·4
0·055	—	0·054	0·052	−0·003	−5·5
0·072	—	0·083	0·068	−0·004	−5·6
0·107	—	0·162	0·112	+0·005	+4·5
0·178	—	0·297	0·191	+0·013	+7·4
0·154	—	0·254	0·163	+0·009	+5·8
0·198	—	0·337	0·209	+0·011	+5·6
0·055	Rhamnose, 0·350	0·234	0·152	+0·097	Interferes
0·236	Xylose, 0·331; Rhamnose, 0·041; Mannose, 0·080	0·389	0·238	+0·002	+0·9
0·120	Glucose, 0·372	0·174	0·119	−0·001	−0·8
0·120	Glucose, 0·371	0·166	0·114	−0·006	−5·0
0·111	Mannose, 0·397	0·148	0·104	−0·007	−6·3
0·067	—	0·081	0·067	nil	nil
0·112	—	0·166	0·114	+0·002	+1·8
0·271	—	0·462	0·279	+0·008	+2·9
0·394	—	0·687	0·404	+0·010	+2·5
0·421	—	0·724	0·424	+0·003	+0·7
0·088	—	0·122	0·090	+0·002	+2·3
0·218	Mannose, 0·308	0·363	0·224	+0·006	+2·8
0·172	—	0·278	0·176	+0·004	+2·3
0·266	Rhamnose, 0·358	0·603	0·357	+0·091	Interferes
0·446	—	0·770	0·450	+0·004	+0·9
0·361	—	0·617	0·365	+0·004	+1·1
0·160	Xylose, 0·321	0·264	0·169	+0·009	+5·6
0·129	Galactose, 0·372	0·175	0·119	−0·010	−8·0

TABLE II*b*.

Arabinose (g.).	Sugar impurity (g.).	Benzoyl-hydrazone found (g.).	Arabinose (from equation) (g.).	Error (g.).	Error calc. on wt. of arabinose taken (%).
0·068		0·062	0·052	−0·016	−23·5
0·079	—	0·088	0·069	−0·010	−12·7
0·116	—	0·164	0·120	+0·004	+ 3·4
0·185	—	0·286	0·202	+0·017	+ 9·2
0·213	—	0·314	0·220	+0·007	+ 3·3
0·173	Rhamnose, 0·190	0·202	0·147	−0·026	−15·0
0·268	—	0·389	0·271	+0·003	+ 1·1
0·384	Xylose, 0·190	0·559	0·385	+0·001	+ 0·3
0·412	—	0·625	0·428	+0·016	+ 3·9

Rhamnose.—Rhamnose cannot be so successfully estimated as arabinose by the use of benzoylhydrazine, owing to the greater solubility of the rhamnose derivative and the interfering effect of small

quantities of arabinose. The sugar sample (250 to 600 mg. in 2 c.c. water) was mixed with saturated alcoholic benzoylhydrazone solution (10 c.c.) and the solution kept at 30° for 41 hours. After 17 hours the solution was seeded with a trace of the rhamnose derivative. After 41 hours the solution was cooled to −3° and the product filtered off and washed with 95% alcohol (30 c.c. in all). To avoid charring it was necessary to dry the product first in a desiccator and then at 90° for 1 hour. From the yields of derivative (see Table III) the equation $y = 0{\cdot}482x + 0{\cdot}217$ was deduced, where y is the weight of rhamnose hydrate which will give a yield, x, of rhamnose benzoylhydrazone. The rhamnose benzoylhydrazone separated as very fine white crystals which could be recrystallised from methyl alcohol. The derivative separated from methyl alcohol as long white needles, m. p. 180° (decomp.) (Found : C, 55·5; H, 6·4; N, 10·0. $C_{13}H_{18}O_5N_2$ requires C, 55·3; H, 6·4; N, 9·9%).

TABLE III.

l-Rhamnose hydrate taken (g.).	Sugar impurity (g.).	Yield of benzoyl-hydrazone (g.).	Rhamnose hydrate (from equation) (g.).	Error (g.).	Error calc. on wt. of rhamnose taken (%).
0·578	—	0·679	0·544	−0·034	− 5·9
0·451	—	0·529	0·471	+0·020	+ 4·5
0·349	—	0·351	0·386	+0·037	+10·7
0·210	—	0·026	0·230	+0·020	+ 9·6
0·270	—	0·191	0·309	+0·039	+14·4
0·322	—	0·271	0·347	+0·025	+ 7·8
0·370	—	0·326	0·374	+0·004	− 1·1
0·408	—	0·385	0·379	−0·029	− 7·1
0·470	—	0·471	0·444	−0·026	− 5·5
0·503	—	0·481	0·449	−0·054	−10·7
0·213	—	0·002	0·218	+0·005	+ 2·3
0·338	—	0·234	0·330	−0·008	− 2·4
0·256	—	0·086	0·258	+0·002	+ 0·8
0·251	—	0·162	0·295	+0·044	+17·5
0·310	Glucose, 0·470	0·257	0·341	+0·031	+10
0·464	—	0·467	0·442	−0·022	− 4·7
0·554	—	0·605	0·508	−0·046	− 8·3

Mannose. Reagent and Method of Analysis.—Phenylhydrazine (redistilled) (25 c.c.) was mixed with absolute alcohol (100 c.c.) containing glacial acetic acid (3 c.c.). The mixture was kept at 0° in a well-stoppered, dark bottle.

The sugar sample was dissolved in water (10 c.c.), the above reagent (10 c.c.) added, and the mixture kept in a well-stoppered flask at 32° for 15 hours, with occasional shaking. It was then cooled to 0° for 12 hours, and the phenylhydrazone was filtered off, washed with ice-cold alcohol (10 c.c.), dried at 100° for 30 minutes, and weighed. The yields of phenylhydrazone from various weights of mannose, together with the effect of various other sugars on the estimation, are given in Table IV.

TABLE IV.

Mannose (g.).	Sugar impurity (g.).	Yield of hydrazone (g.).	Mannose (from graph) (g.).	Error (g.).
0·026	—	nil	nil	−0·026
0·051	—	0·011	0·033	−0·018
0·100	—	0·114	0·100	nil
0·203	—	0·277	0·205	+0·002
0·401	—	0·573	0·400	−0·001
0·601	—	0·890	0·610	+0·009
1·001	—	1·488	1·003	+0·002
0·221	Glucose, 0·500	0·310	0·228	+0·007
0·260	Arabinose, 0·500	0·355	0·258	−0·002
0·214	Galactose, 0·500	0·300	0·220	+0·006
0·225	Rhamnose, 0·500	0·298	0·219	−0·006
0·205	Glucuronic acid, 0·500	0·280	0·207	+0·002

These figures fit the equation $y = 0{\cdot}652x + 0{\cdot}031$ where y is the weight of mannose giving a weight x of mannose phenylhydrazone.

From these results it can be seen that glucose, arabinose, galactose, rhamnose, and glucuronic acid do not interfere with the estimation of mannose, and that it is possible to carry out the estimation to within ±3 mg. on amounts above 100 mg.

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