QUANTITATIVE ESTIMATION OF SUGAR RESIDUES IN ACIDIC GUMS

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Abstract—The neutral and acidic sugar residue compositions of acidic gums from *Prunus* have been determined by a combination of methods including a simple but effective titrimetric procedure. The molar ratio of D-glucuronic acid to 4-*O*-methyl-D-glucuronic acid residues has been determined.

Many plant and microbial polysaccharides contain uronic acid residues either alone or in combination with neutral sugar residues. There are variations in the identity and in the proportion of such uronic acids according to the plant source. Commonly occurring acids in such polysaccharides include D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-mannuronic acid, D-galacturonic acid, and L-guluronic acid. D-Galacturonic acid residues are present in primary plant cell-wall material, notably in pectin, and D-glucuronic acid and its 4-*O*-methyl ether are found in the hemicelluloses of higher land plants and in gums [1, 2].

Formerly it was common to estimate uronic acid residues in polysaccharides by a procedure involving decarboxylation with 12% hydrochloric acid and measurement of the carbon dioxide released [3]. Such methods are non-selective since CO_2 is also released from neutral sugar residues under the conditions employed. It is usually also difficult to determine simultaneously the proportion of uronic acid and of adjacent neutral sugar residues in acidic polysaccharides such as gums or hemicelluloses. The glycosiduronic acid linkage in aldobiouronic acids is more stable than is the glycosidic linkage in the neutral disaccharide analogue [4]. Lower rates of hydrolysis of the former than of the latter are attributed to steric factors; the

nature of the linkage markedly affects the magnitude of the stabilizing effect due to the carboxyl group on C-5 [5]. Various studies show that the $\alpha(1 \rightarrow 2)$ linkage of either D-glucuronic, or 4-*O*-methyl-D-glucuronic, acid residues in many xylans leads to marked stabilization of the glycosiduronic linkage. The acidic conditions that lead to hydrolysis of these and other such linkages commonly also lead to the degradation of the uronic acid liberated. Perry and Hulyalkar [6] minimized this danger by carrying out the hydrolysis under conditions that led to the immediate reduction of the latent aldehydic group of the newly liberated uronic acid molecules. The derived glyconic acids were estimated by GLC determination of TMS-derivatives of derived 1,4-lactones.

Blake and Richards [7] concluded that base titrimetric uronic acid estimations gave better results than more complex methods involving estimation of acetates of lactones derived from uronic acid residues. Buchala and Wilkie [8] more recently introduced a procedure which led both to complete accountability of the uronic acid and of the adjacent neutral residue in acidic polysaccharides. The carboxylic acids were esterified in a two-stage procedure and the esterified uronic acid residues reduced with potassium borotritide. There were then no problems of hydrolysis or of degradation of uronic acids during hydrolysis; the latter were radiocounted.

The present studies involve the development of

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Table 1. Equivalent weights and methoxyl contents of peach gums and calculated weight of 4-O-methyl-D-glucopyranuronosyl residues in each equivalent

Gum from variety	Range of equivalent weights*	Equivalent weight	Methoxyl %	Weight of 4 MeGlcpA residues
Lednická žltá	1905–2010	1955	0·675	80·9
Elberta	2155–2190	2170	0·670	89·1
Champion	2180–2185	2185	0·670	89·7
Red Haven	2040–2065	2050	0·930	116·9
South Haven	1955–1980	1970	1·005	121·3
Mixed	1980–2070	2025	1·030	127·8
Mixed autohydrolysed	905	905	0·885	49·1

* Triplicate analyses.

a new method for the examination of acidic polysaccharides that has the merit of simplicity. The studies were carried out on gums from five varieties of 3-year-old peach trees (*Prunus persica* [L.] Batsch), namely, Lednická žltá (i.e. yellow), Elberta, Champion, Red Haven, and South Haven, and on a mixture of gums collected from older trees in a peach orchard nursery, and also on this last mixture after autohydrolysis [9].

The method involves three stages; firstly, the titrimetric estimation of the uronic acid residues (Table 1); secondly, estimation of the methoxyl content of the polysaccharide corresponding to one such acid equivalent and direct conversion of this value into the corresponding proportion of 4-O-methyl-D-glucuronic acid residues (Table 1); and lastly, the GLC determination of the neutral sugars in acid hydrolysates after conversion of the sugars to glycitol acetates (Table 2) [10]. Gums from species of *Prunus* have (1 → 6) glycosiduronic acid linkages involving D-glucuronic acid and its 4-O-methyl ether. It has been established that this linkage is comparatively acid-labile and the adjac-

ent aglyconic neutral sugar residue is liberated under normal hydrolysis conditions [11]. It is accordingly possible to estimate the neutral sugars in acid hydrolysates of such gums.

It is essential for the success of the method that the carboxylic acid groups in the gum be in the free, and not the salt, form [12]. Accordingly the polysaccharides were thoroughly washed with ethanol containing 1% hydrochloric acid, the conversion of the carboxylic acid groups being monitored by IR spectroscopy. The polysaccharides were then dissolved in water under a layer of toluene to avoid atmospheric contamination both at this stage and during the subsequent titration with sodium hydroxide to pH 7·5. The method has the dual advantage that it is not essential for the polysaccharide to be water-soluble, and the material can be recovered after titration for further studies. A second sample of the polysaccharide was hydrolysed with 0·5 M H_2SO_4 and the neutral and acidic sugars separated by ion-exchange chromatography. The neutral sugars were then reduced, converted to their acetates, and estimated by GLC

Table 2. Molar proportions of neutral and acidic residues in one acid equivalent of peach gums*

Gum from variety	4Me GlcA	GlcA	Gal	Man	Ara	Xyl	Rha
Lednická žltá	0·57	0·43	3·64	0·21	6·04	2·33	0·25
Elberta	0·53	0·47	4·13	0·31	6·42	2·66	0·40
Champion	0·53	0·47	3·84	0·41	6·13	3·46	0·28
Red Haven	0·39	0·61	3·69	0·31	6·58	2·21	0·32
South Haven	0·36	0·64	3·85	0·16	5·80	2·54	0·20
Mixed	0·33	0·67	4·27	0·05	6·74	1·69	0·25
Mixed autohydrolysed	0·74	0·26	1·97	2·40	0·12	0·02	

* See Table 1. Sugars estimated by GLC of derived glycitol acetates (corrected as from Table 3).

Table 3. Relative molar responses (RMR) of GLC detector to glycitol acetates

Glycitol acetate	Relative molar response	Peak area correction factor
Rhamnitol	1·046	0·96
Arabinitol	1·055	0·97
Xylitol	1·089	1
Mannitol	1·085	0·99
Galacitol	1·239	1·13

using pure glycitol acetates for preparing standard curves (Table 3).

The calculation of the molar proportions of acidic and neutral residues in each gum is dependent on the three separate sets of determinations; namely, the equivalent weights, methoxyl contents, and the proportion of neutral sugars in hydrolysates. The first two sets of values are shown in Table 1, together with the weight equivalent of 4-O-methyl-D-glucopyranuronosyl residues in each acid equivalent weight of gum on the assumption that the methoxyl residues were exclusively associated with the above acidic sugar residues.

The molar proportions of the parent sugar residues were determined and used in combination with the values for the two uronic acid residues, to give the proportions by weight of all the acidic and neutral sugars in one acid equivalent of each gum (Table 2).

EXPERIMENTAL

Isolation of gums. The various gums were isolated at the Institute of Chemistry, Slovak Academy of Sciences, Bratislava as described. Samples 1–5 were from botanically characterized peach-trees (*Prunus persica* L. Batsch) and sample 6 was a mixture of peach gums from a State Cultivation Station orchard at Solary, South Slovakia. Sample 7 was an autohydrolytically degraded polysaccharide from gum sample 6. The autohydrolysis was carried out by heating an aq soln of the sample 6 gum for 35 hr on a water bath at 100°. Sample 6 on hydrolysis yielded traces of arabinose and xylose. The vol of each gum soln was increased four-fold by the addition of EtOH which had been made just acid by the addition of 1% HCl. Immediate precipitation of each gum took place giving dispersions. Free carboxyl groups were determined by IR spectroscopy [12].

Determination of equivalent weights. An accurately weighed quantity (*ca* 100 mg) of each gum was dissolved in H_2O covered by a layer of toluene by stirring for 48 hr. Each soln was titrated in triplicate with 0·1 M NaOH using an automatic titrimeter. The end-point of each titration had been determined to be at pH 7·5 by drawing titration curves of titrant against pH. It was determined that equilibrium had been established within 6 hr and this period was allowed for all titrations.

Determination of methoxyl values. The percentages of OMe were determined by the method of Viebóck and Brecher [13].

Hydrolysis and acetylation of gums. A sample (*ca* 30 mg) of each gum was hydrolysed in a sealed tube using 0·5 M H_2SO_4 for 22 hr at 100°. The samples were neutralized ($BaCO_3$) and the soln was passed on to a column of Zerolit FFIP SRA 65 (standard grade, acetate form). The neutral sugars were eluted by dist H_2O until no coloration was given on examination of the eluate with $PhOH–H_2SO_4$. The acidic components were similarly eluted using 30% HOAc. There was no evidence of the presence of aldobiouronic acids or of higher oligouronic acids in the eluate when it was examined by PC (EtOAc–HOAc–HCOOH–H_2O, 18:3:1:4) using aniline hydrogen phthalate in Me_2CO for detection. H_2O was removed from each eluate at 40° by rotary evaporation under red pres.

Each mixture of neutral components was dissolved in H_2O (5 ml) and reduced over 18 hr with $NaBH_4$ (200 mg). The neutralized soln was concentrated under reduced pressure and the borate removed by co-distillation with successive vols (5 × 5 ml) of MeOH. Ac_2O (10 ml) was added, and each mixture was heated at 120° for 5 hr. After cooling, an aliquot (4·1 mg/ml) of an erythritol tetraacetate soln (1·414 × 10^{-2} mol) and H_2O (10 ml) were then added and the mixture was taken to dryness. H_2O (10 ml) was added to complete the hydrolysis of the Ac_2O and the solvents again removed. Each product was treated with CH_2Cl_2 (5 ml) and the organic layer was separated and reduced in vol to *ca* 1 ml. An aliquot (0·5 μl) was withdrawn and immediately examined by GLC on columns 2 m × 3 mm packed with 3% silicone OV225 (100–120 mesh), on Chromosorb W (AW-DMCS). The carrier gas, N_2, was at 12 p.s.i. and the columns were heated at 200°. Standard solns were prepared with the fully acetylated derivatives of the following glycitols in the respective proportions stated—galactitol, mannitol, arabinitol, xylitol, rhamnitol, and erythritol: 11·52, 8·53, 10·5, 8·01, 6·91 and 14·14 mmol.

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