Separation and Quantitative Determination of Aldonic Acids by Gas-Liquid Chromatography

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In connection with our work on the determination of carbohydrates in cellulosic materials by gas-liquid chromatography,[1] attempts have been made to separate various aldonic acids present in pulping liquors as monomers or occurring in pulp polysaccharides as terminal units. The experiments reported here were carried out with pure aldonic acids. The work was started by converting the aldonic acid lactones into the corresponding *O*-trimethylsilyl derivatives. Under appropriate conditions it was possible to achieve an excellent separation of gluconic, mannonic, xylonic, and arabinonic acid derivatives, but the separation of gluconic and galactonic acid derivatives was unsuccessful (Fig. 1).

Additional experiments were made following the same principle as has recently been used in the quantitative analysis of sugar mixtures.[1,2] Because monosaccharides can be determined quantitatively after reduction with sodium borohydride and subsequent chromatography of the corresponding alditol acetates (Table 1), the whole problem was to develop a method for the quantitative reduction of aldonic acid lactones to alditols. Several experiments were performed to establish the best conditions; however, the reduction was not quantita-

Table 1. Relative retention times of alditol acetates.

Column packing 2: A mixture containing 1.5 % ethylene glycol succinate and 1.5 % silicone oil (XF−1150) on 100−120 mesh Gas-Chrom P. *Temperature:* 180−220° (0.8°/min).

Sample	Relative retention time
Arabinitol pentaacetate	0.27
Xylitol pentaacetate	0.35
Mannitol hexaacetate	0.63
Galactitol hexaacetate	0.72
Glucitol hexaacetate	0.81
Iditol hexaacetate	1.00

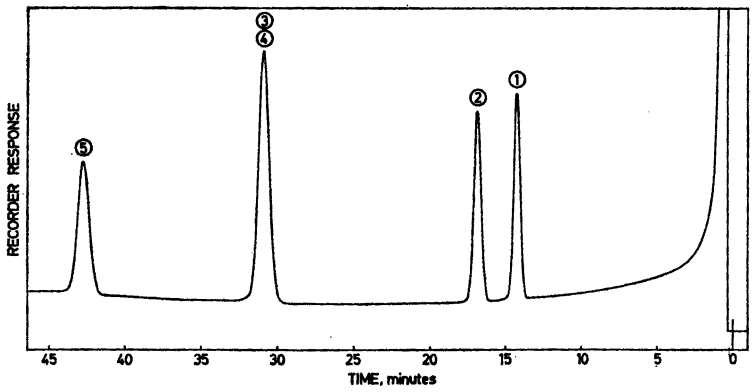

Fig. 1. Separation of aldonic acid lactones as trimethylsilyl derivatives. For preparation of derivatives, see Ref. 5.

Column packing 1: 5 % silicone oil (XF−1112) on 100−120 mesh Gas-Chrom P. *Temperature:* 155−195° (0.8°/min).
D-Arabinono-γ-lactone (1), D-xylonolactone (2), D-glucono-δ-lactone (3), D-galactono-γ-lactone (4), and D-mannono-γ-lactone (5).

tive, and the yields varied between about 60 and 90 % (*cf.* also Ref. 3).

The incomplete reduction obviously depends on the fact that the lactones are partially hydrolysed and the acids or their salts thus formed cannot be reduced. However, it was possible to overcome this difficulty by means of repeated sodium borohydride treatments. According to this principle the solution after the first sodium borohydride treatment is cation exchanged and evaporated to dryness to convert the remaining free acids into lactones. The sodium borohydride treatment is then repeated. The results, summarised in Table 2, show that the conversion into alditols is practically quantitative after three sodium borohydride treatments.

Experimental. Materials. The following commercial samples were used after recrystallisation: D-arabinono-γ-lactone, m.p. 97—100°, D-glucono-δ-lactone, m.p. 147—152°, and D-galactono-γ-lactone, m.p. 134—135°. D-Mannono-γ-lactone, m.p. 150—151° and D-xylonolactone, m.p. 99—103°, were prepared from the parent monosaccharides by hypoiodite oxidation followed by lactonisation.[4] L-Iditol hexaacetate, used as internal standard, was prepared from L-sorbose by reduction with sodium borohydride and subsequent acetylation and fractionation of the reaction mixture. The final (recrystallised) L-iditol hexaacetate had m.p. 122—123° and it contained 0.1 % D-glucitol hexaacetate.

Table 2. Analysis of aldonic acid (lactone) mixtures. The reduction was carried out in three consecutive steps. For chromatographic conditions, see Table 1 and Ref. 1.

Lactone of	Added, mg	Found,[a] mg	Recovery, %
Arabinonic acid	4.96	5.03	101
Xylonic acid	4.98	4.83	97
Mannonic acid	4.40	4.37	99
Galactonic acid	6.30	6.28	100
Gluconic acid	4.92	4.67	95

[a] Mean value of three separate determinations.

Reduction. An aqueous solution containing accurately weighed amounts of aldonic acid lactones (*ca.* 5 mg each) was evaporated to dryness under vacuum. The reduction and acetylation procedure was essentially carried out as described earlier,[1] but the reduction time was only 3 h and freshly prepared aqueous sodium borohydride solution (5 mg/ml) was added (5 ml). After removing the sodium ions by cation exchange, the solution was evaporated under vacuum to dryness. Methanol was then added to the residue and the boric acid was removed by evaporating the solution again to dryness. The reduction — cation exchange — evaporation procedure was repeated twice and the resulting alditol mixture was then acetylated. Finally, a known amount of iditol hexaacetate was added to the mixture (*ca.* 15 mg) after which 0.5—1 μl was injected into the gas chromatograph.

Chromatography. A Perkin Elmer gas chromatograph, model 880, equipped with a differential flame ionisation detector was used. The chromatographic conditions have been described in detail elsewhere.[1] The amounts of each component were calculated from the peak areas by taking into consideration the slightly different detector response of the various alditol acetates.[1] The detector response of iditol hexaacetate was found to be identical with that of glucitol hexaacetate.

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